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## CLINICAL SIGNS, PATHOMORPHOLOGICAL AND IMMUNOHISTOCHEMICAL FINDINGS IN THE VISCERAL ORGANS OF CHICKENS NATURALLY INFECTED WITH MOTILE *SALMONELLA* SEROTYPES IN LAGOS, OGUN AND OYO STATES, NIGERIA

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### Abstract

Study on the clinical signs, pathomorphological and immunohistochemical findings changes in the visceral organs of chickens infected naturally with some motile *Salmonella* serotypes was carried out to determine the pathogenicity of these serotypes infecting chickens in Lagos, Ogun and Oyo States, Nigeria. Clinical histories were obtained from clients that submitted carcasses of chickens for postmortem examination during outbreaks of salmonellosis in commercial poultry farms. Samples of the visceral organs from suspected cases were collected at postmortem in sterile sample bottles for bacterial isolation and identification; and also fixed in 10% buffered formalin for histopathology and immunohistochemical studies. The *Salmonella* isolates were confirmed by PCR and were inoculated into nutrient agar slope, freeze-dried and sent to the World Organization for Animal Health/OIE, Podava, Italy for serotyping using the Kauffman-white Scheme. Clinical signs were weakness (100%), anorexia (60%), yellowish diarrhea (100%), somnolence (80%) and mortality of 40 to 80%. Gross lesions were severe necrosis (70%) and yellowish nodules in the lungs (40%). There were pale foci of necrosis in the myocardium with misshapen and nodular enlargement of the heart (40%). The livers, spleens and kidneys were enlarged with subcapsular foci of necrosis (100%). The proventriculus, intestines and caeca contained large amount of mucus (70%) with hyperemic and necrotic mucosae (80%). Histopathological changes in visceral organs were necrosis and infiltration by lymphocytes, macrophages and heterophils (100%). Immunoreactions were observed in blood vessels and host cells. The *Salmonella* serotypes were *S. Kentucky*, *S. Herston*, *S. Nima*, *S. Teitelkebir*, *S. Colindale*, and *S. Tshiongwé*. To the best of our knowledge, the *Salmonella* serotypes identified in the present study have not been reported to cause high mortality, as well as severe pathological and immunohistochemical findings in the visceral organs of chickens. We recommend that in prevention, treatment and control of avian salmonellosis, attention should also be focused on *Salmonella* serovars that are not known to cause serious disease in chickens.

**Keywords:** *Salmonella*, infection, clinical signs, pathology, immunohistochemistry, chickens

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## SIGNES CLINIQUES, CHANGEMENTS PATHOMORPHOLOGIQUES ET IMMUNOHISTOCHIMIQUES DES ORGANES VISCÉRAUX DE POULETS NATURELLEMENT INFECTÉS PAR CERTAINS SÉROTYPES DE SALMONELLA MOBILE DANS LES ÉTATS DE LAGOS, OGUN ET OYO AU NIGERIA

### Résumé

La présente étude a examiné les signes cliniques, les changements pathomorphologiques et immunohistochimiques des organes viscéraux de poulets infectés naturellement par certains sérotypes de *Salmonella mobile* dans le but de déterminer la pathogénicité de ces sérotypes infectant les poulets dans les États de Lagos, Ogun et Oyo au Nigeria. Les antécédents cliniques ont été obtenus auprès de clients ayant soumis des carcasses de poulets à un examen post-mortem lors d'épidémies de salmonellose dans les exploitations avicoles commerciales. Des échantillons d'organes viscéraux provenant de cas suspects ont été prélevés en post-mortem dans des flacons d'échantillon stériles pour isolement et identification de bactéries, et ont également été fixés au formol tamponné à 10% pour des études histopathologiques et immunohistochimiques. Les isolats de *Salmonella* ont été confirmés par PCR et ont été inoculés dans une solution de gélose nutritive, lyophilisés et envoyés à l'Organisation mondiale de la santé animale (OIE), à Podava en Italie, pour sérotypage à l'aide du schéma de classification Kauffman-white. Les signes cliniques comprenaient la faiblesse (100%), l'anorexie (60%), une diarrhée jaunâtre (100%), une somnolence (80%) et une mortalité de 40 à 80%. Les lésions macroscopiques étaient une nécrose sévère (70%) et des nodules jaunâtres dans les poumons (40%). On a constaté des foyers pâles de nécrose dans le myocarde avec élargissement grossier et nodulaire du cœur (40%). Les foies, rates et reins ont été élargis avec des foyers de nécrose sous-capsulaire (100%). Le proventricule, les intestins et le caecum contenaient une grande quantité de mucus (70%), les muqueuses étant hyperémiques et nécrotiques (80%). Les modifications histopathologiques des organes viscéraux étaient la nécrose et l'infiltration par les lymphocytes, les macrophages et les hétérophiles (100%). Des immunoréactions ont été observées dans les vaisseaux sanguins et les cellules hôtes. Les sérotypes de *Salmonella* étaient S. Zega, S. Kentucky, S. Herston, S. Nima, S. Telelkebir, S. Colindale et S. Tshiongwe. A notre connaissance, les sérotypes de *Salmonella* identifiés dans la présente étude n'ont pas provoqué de fortes mortalités, ni de graves changements pathologiques et immunohistochimiques dans les organes viscéraux des poulets. Nous recommandons que dans la prévention, le traitement et le contrôle de la salmonellose aviaire, une attention soit également portée aux sérotypes de *Salmonella* non connus comme agents étiologiques de maladie grave chez les poulets.

**Mots-clés:** *Salmonella*, infection, signes cliniques, pathologie, immunohistochimie, poulets

### Introduction

Avian salmonellosis is considered one of the most common bacterial diseases in poultry industry world-wide (Majid *et al.*, 2010). *Salmonella* species are responsible for a variety of acute and chronic disease in poultry. The disease can result from infection with poultry specific serovars, *S. Gallinarum* and *S. Pullorum*, causing systematic infection in birds as well as other serotypes, including *S. Typhimurium* and *S. Enteritidis* in addition to many others that are cause of paratyphoid infection (Gast, 2003).

*Salmonella* organism is an intracellular bacterial pathogen capable of infecting a wide range of hosts, causing different forms

of disease syndromes, such as gastroenteritis, enteric fever, bacteraemia, and asymptomatic carriage (Gorvel and Merese, 2001; Hansen-Wester and Hensel, 2001).

Pathogenesis and immune response associated with *Salmonella* infections depend on the infecting *Salmonella* serotype, route of infection, infective dose, virulence of the infecting serovar and infected host factors, including breeds and genetic immunity (Cammie and Miller, 2000). Following ingestion of contaminated feed or water via the faecal-oral route in mammals, a proportion of the bacterial inoculums that survives the gastric acid environment then reach the intestinal tract. For effective invasion of tissues, efficient

adhesion to the epithelial layer is required, which is mediated by adhesin, including fimbriae (Bishop *et al.*, 2008). In poultry, oral infection with *Salmonella* results in the organisms passing through the crop, proventriculus and gizzard to the intestinal tract, where the bird caeca is the most preferred site of *Salmonella* adhesion and colonization (Clayton *et al.*, 2008). *Salmonella* species are tissue invasive and acquire multiple virulence genes for intercellular survival and production of Vi antigen for interaction and penetration of mucous epithelium for systemic infection (Hansen-wester and Hensel, 2001). Central to the pathogenesis of *Salmonella* infection is its ability to infect and survive inside host cells, including lymphoid tissues, epithelial cells and macrophages (Gorvel and Merese, 2001; Okamura *et al.*, 2005).

Gross lesions in avian salmonellosis varied considerably from complete absence of visible lesions to a septicaemic carcass in which the lung, heart, liver, spleen and kidneys are swollen and congested. There are unabsorbed yolk sacs in affected baby chicks, airsacculitis, enlarged and fibrinous liver, enlarged spleen, enlarged kidneys, and dehydration (Akhtar *et al.*, 2001). Histopathological lesions in visceral organs were severe vascular congestion (Hossain *et al.*, 2006), varying degrees of degeneration and necrosis. There are infiltration by *lymphocytes*, *heterophils*, and macrophages (Nwiyi and Omadamiro, 2012).

Diagnosis of avian salmonellosis is done based on clinical signs of weakness, anoraexia, somnolence, ruffled feathers, greenish to yellowish diarrhea, pasted vent and mortality ranging from 10 to 100%. Serologic test can also be carried out. Gross lesions of *pneumonia*, fibrinous pericarditis, white to yellowish nodules in the heart, necrosis in the liver, hepatomegaly, splenomegaly and enteritis and histopathological lesions characterized by necrosis of the mucosa and submucosa; and infiltration by *lymphocytes*, *heterophils* and macrophages are also used (Ibrahim *et al.*, 2003). Confirmation of the disease is done by isolation, identification and serotyping of *Salmonella* strains (Shivaprasad and Barrow, 2008).

Immunohistochemistry is a very sensitive and highly specific (100%) diagnostic tool for the diagnosis of prion diseases. However, its use for molecular study of tissue pathology in avian salmonellosis is rare. IHC allows co-localization of an antigen within lesions, thereby dramatically increasing diagnostic accuracy and understanding of pathogenesis (Jose *et al.*, 2008).

While *Salmonella* Kentucky is known to cause infection in poultry (Le Hello *et al.*, 2011), *Salmonella* Zega, *S. Nima*, *S. Herston*, *S. Colindale*, *S. Teitelkebir* and *S. Tshiongwé* are rarely reported to affect chickens, although there has been report on them causing diarrhea in human (Fain *et al.*, 1952; CMAJ, 1986; Traore *et al.*, 2015).

In the present work, there have been reports of disease outbreaks of unknown aetiology characterized with high mortality in commercial poultry farms in Lagos, Ogun and Oyo States, Nigeria. Postmortem examination suggested avian salmonellosis. The present work was therefore carried out to determine the *Salmonella* serotypes causing mortality in commercial poultry farms in the study area and described the clinical signs, pathomorphological and immunohistochemical changes in the visceral organs of chickens.

## Materials and Methods

Study on the morphopathological and immunohistochemical findings in the visceral organs of chickens naturally infected with some motile *Salmonella* serotypes were carried out to determine their pathogenicity. Carcasses of chickens from 36 natural outbreaks of salmonellosis in commercial poultry farms in Lagos, Ogun and Oyo states, within southwestern Nigeria were submitted for postmortem examination in the Department of Veterinary Pathology, Federal University of Agriculture, Abeokuta, over a period of 1 year (January to December, 2013). The flock sizes of the poultry farms ranged from 1,000 to 120,000 birds.

Clinical histories were obtained from the clients that submitted carcasses for

postmortem examination. Gross lesions in adult and young chicks were recorded. Samples of the lung, heart, liver, bile, spleen, kidney, proventriculus, intestine and caecum were collected from suspected cases of salmonellosis for bacterial culture and identification at the Department of Microbiology and Parasitology Laboratory, Federal University of Agriculture, Abeokuta; and also fixed in 10% buffered formalin for histopathology as described by (Avwioro, 2002); and immunohistochemistry, using Avidin Biotin Peroxidase Complex (ABC) method as described by (Rasmo-vera, 2005).

*Salmonella* species isolated from the visceral organs by bacterial culture and confirmed by PCR were innoculated into nutrient agar slope, freeze-dried and sent to the World Organization for Animal Health/OIE, Italian Reference Laboratory for *Salmonella*, *Istituto Zooprofilattico Sperimentale Dellevenzie, Podava*, Italy for serotyping using the Kauffman-white Scheme.

## Results

### *Clinical History associated with natural cases of avian salmonellosis*

The clinical signs recorded from the natural cases were acute (80%) and chronic (20%). The signs observed in the acute form included weakness (100%), *anorexia* (60%), somnolence (80%), yellowish diarrhea (100%) and mortality of 40 to 80 % in adult and young chicks. While in the chronic form, the disease was characterized by severe diarrhea, emaciation and mortality of 20 to 40%.

### *Non organ-specific findings:*

The most common findings in adult chickens included emaciation in 30% and 20% in adult birds and young chicks respectively, swollen hock joints, pale comb and wattle; pasted vents (70%), and unabsorbed yolk sacs in young chicks. Pasted vents were also common among chicks (60%). Cloudy air sacs were observed in 30% of the cases in adult and 10% in young chicks. Ruptured egg yolk, congested oviduct and egg yolk peritonitis were observed in 70% of the cases in laying hens; as

well as cystic, misshapened, pedunculated and congested ovarian follicles.

### *Bacteriology:*

Thirty seven *Salmonella* isolates were serotyped in which we have earlier reported seven serotypes identified to cause high mortality on poultry farms in three Southwestern States of Nigeria. They included *S. Zega*, *S. Kentucky*, *S. Herston*, *S. Nima*, *S. Teitelkebir*, *S. Colindale*, and *S. Tshiongwe*. The predominant serotype was *S. Zega* (n=13; 35.14%) followed by *S. Kentucky* (n=9; 24.32%), then *S. Herston* (n=6; 16.22%), *S. Nima* (n=4; 10.81%), *S. Teitelkebir* (n=3; 8.11%), *S. Colindale* (n=1; 2.70%), and *S. Tshiongwe* (n=1; 2.70%). Out of the 37 serotypes, 13.51% were isolated from Lagos State, 78.38% from Ogun State and 8.11% from Oyo State. All the *S. Hertson*, *S. Nima*, *S. Teitelkebir* as well as the *S. Tshiongwe* and *S. Colindale* serotypes were isolated from Ogun State (Mshelbwala et al., 2017).

### *Gross lesions in visceral organs of chickens infected naturally with motile Salmonella serotypes:*

The lungs had severe frothy exudates in the air ways; pulmonary congestion and oedema in 90% of the cases in adults and 50% in chicks. There were haemorrhages in the lung parenchyma, fibrin deposition on the pleural surfaces in adult (30%) and young chicks (50%). There was necrosis of the parenchyma and consolidation of the lung, along with yellowish nodules in the pleural surfaces in 50 % of the cases in young chicks infected with *Salmonella Zega* and in 30% of the



**Figure 1:** Photograph of lungs of a 1 week-old Isa brown pullet that died from natural *Salmonella Zega* infection in Lagos State, showing yellowish nodules in the pleural surfaces (arrows).



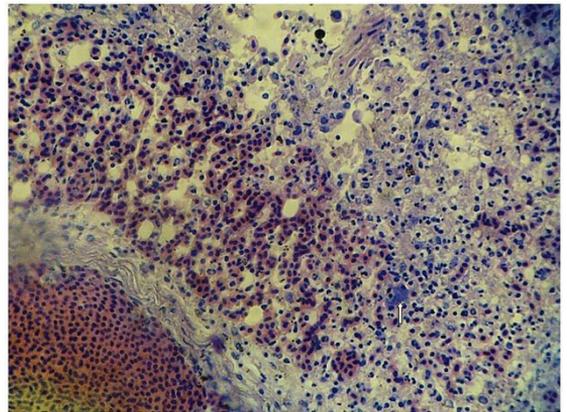
**Figure 2:** Photograph of the hearts of adult Isa brown layer chickens that died from natural *Salmonella* Telelkebir infection in Ogun State, presents as mishappened with pale necrotic foci (arrow heads) and nodular enlargement with distortion of the shape of the heart.



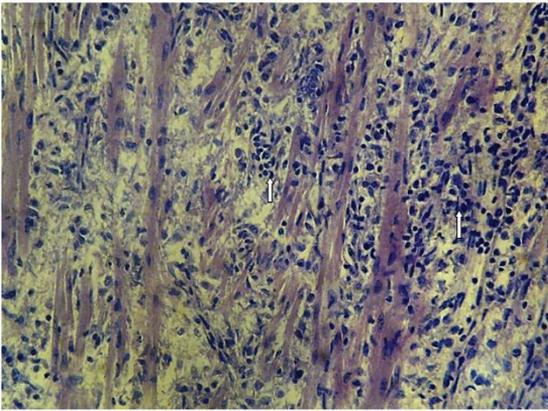
**Figure 3:** Photograph of the liver of a 1 week-old Isa brown pullet that died from natural *Salmonella* Tshiongwé infection in Ogun State, showing enlargement with multifocal subcapsular necrosis (arrows).

*Salmonella* Telelkebir and *S. Tshiongwé* infections (Figure 1). The lesions in the heart presented with fibrinous pericarditis in adult (30%) and young chicks (60%), congestion of coronary blood vessels and haemorrhagic epicarditis (20%). There were pale foci of necrosis in the myocardium and nodular enlargement of the heart (40%). In some cases, nodules were big in the myocardium causing distortion of the heart (Figure 2). The lesions in the liver were congestion, subcapsular pale foci of necrosis with hepatomegaly in both adult and young chickens. In some cases, there were bronze discolouration with fibrinous perihepatitis in the adult chickens, while multifocal and diffuse

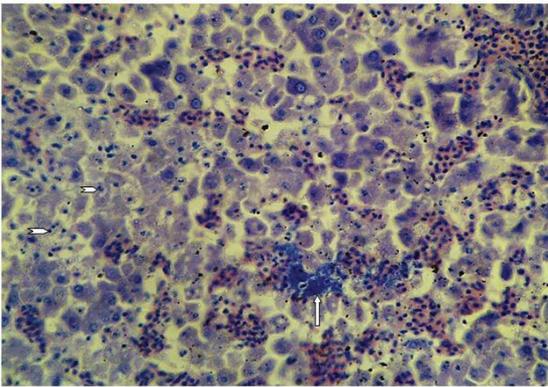
necrosis in both parietal and visceral surfaces were more common in young chicks (Figure 3). The spleens were congested and enlarged in 70% of the cases examined. There were subcapsular haemorrhages and necrosis in some cases with mottling in 40% of the cases in adult. The kidneys were congested and swollen in 80% of the cases in adult chickens and 90% in chicks. In some cases, the kidneys were swollen and pale. Gross lesions in the proventriculus were accumulation of mucus exudates in 90% of the cases, in both adult and young chickens. There were hyperemia of mucous membrane and swollen proventricular glands. In some cases, the proventricular glands were necrotic and haemorrhagic. The small intestines presented with hyperemia of serosal surfaces, distention by yellowish diarrhoic faeces with mucus exudates in 90% of the cases in adult chickens and 100% in chicks. In some cases there were hyperemia, haemorrhages and necrosis in the mucosal membrane. Gross lesions in the caeca were distention with watery diarrhoic faeces in 70% of the cases in adult chickens and 80% in chicks. There was necrosis of the caecal tonsils and mucosae and in some cases, they were haemorrhagic and contained caecal core in both adult chickens and young chicks.



**Figure 4:** Section of the lung of a 1 week-old Isa brown pullet that died from natural *Salmonella* Zega infection in Lagos State, showing necrosis of the lung parenchyma and infiltration of lymphocytes, macrophages and heterophils (arrow head); with bacterial clumps in the necrotized parenchyma (arrow) (x 400; H&E).



**Figure 5:** Section of the heart of an adult Isa brown layer chicken that died from a natural *Salmonella* Telelkebir infection in Ogun State, showing necrosis of myofibers and infiltration of *lymphocytes*, macrophages and *heterophils* in areas of the necrotized muscle fibers and inter fiber spaces (arrows) (x 400; H&E).

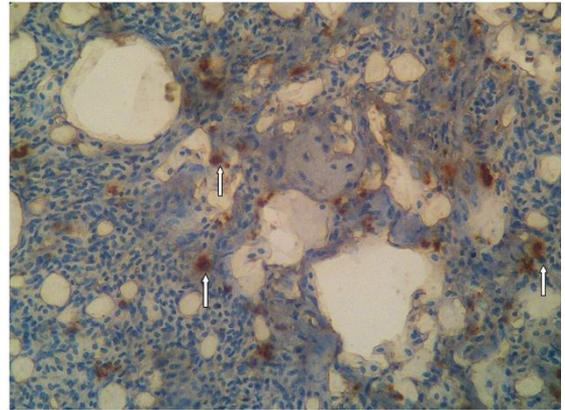


**Figure 6:** Section of the liver of an Isa brown pullet that died from natural *Salmonella* Tshiongwe infection in Ogun State showing congestion of central vein and sinusoids, coagulation necrosis and infiltration of *lymphocytes*, macrophages and *heterophils* in the necrotized parenchyma (arrow heads), with foci of bacterial clumps (arrow) (x 400; H&E).

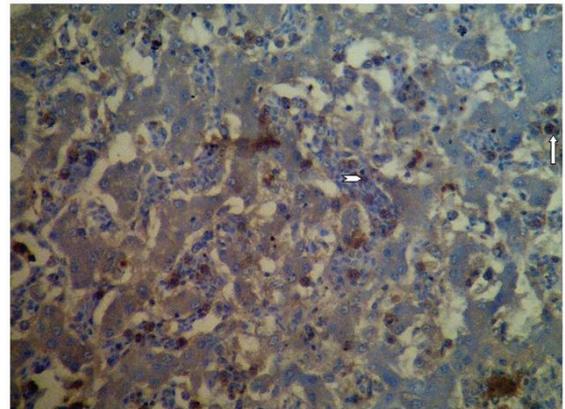
*Histopathological changes in visceral organs of chickens infected naturally with motile Salmonella serotypes:*

Microscopic changes in the lungs include severe vascular congestion and oedema in 100% of the cases in adult and 80% in young chicks. There was vasculitis, microthrombi, and haemosiderosis with infiltration of *lymphocytes*,

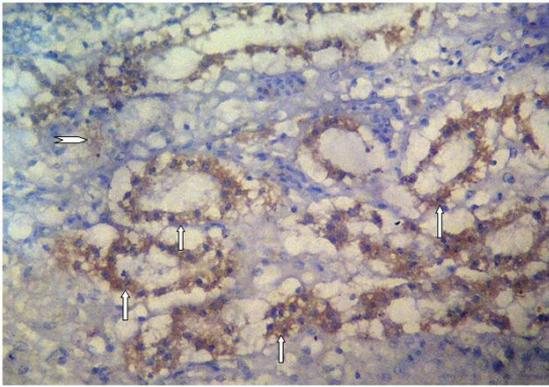
macrophages and *heterophils* in the interstitium of the alveolar septae, alveolar sacs and alveoli, and in the tertiary, secondary and primary bronchioles. Granulomatous reactions were observed in 10% of the cases in young chicks. There were also large numbers of bacterial colonies in 40 % of the cases (Figure 4). Microscopic lesions in the heart were necrosis and fragmentation of myofibers and infiltration



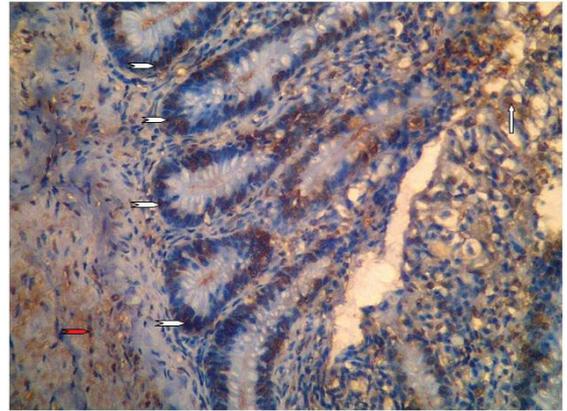
**Figure 7:** Section of the lung of an adult Isa brown layer chicken that died of natural case of *Salmonella* Zega infection in Lagos State, showing immunoreactions to *Salmonella* antigens in *pneumocytes*, interstitium of alveolar septae and in the cytoplasm of macrophages (arrows) (x 400; immunohistochemical reaction).



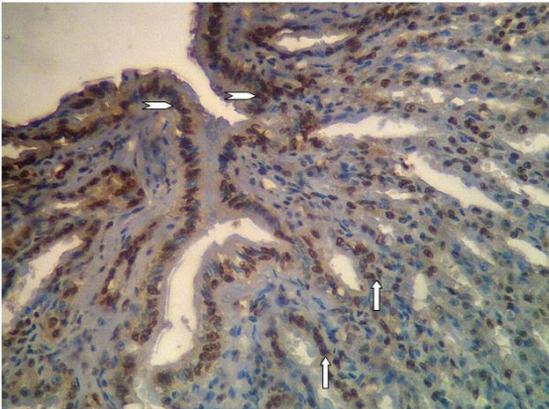
**Figure 8:** Section of the liver of an adult chicken that died of natural case of *Salmonella* Telelkebir infection in Ogun State, showing strong immunoreactions to *Salmonella* antigens in blood cells (arrow head), and in the cytoplasm of hepatocytes, *lymphocytes* and macrophages (arrow); and weak immunoreactivity in necrotic tissue debris (x 400; immunohistochemical reaction).



**Figure 9:** Section of the kidney of an adult Isa brown layer chicken that died from natural case of avian salmonellosis in Ogun State showing strong immunoreactions in tubular epithelial cells (arrows) but weak immunoreactions in necrotic debris (arrow head) (x400; immunohistochemical reaction).



**Figure 11:** Section of the intestine of an adult chicken that died from natural case of *Salmonella* Zega infection Ogun State, showing strong immunoreactions to *Salmonella* antigens in crypt (arrow heads) and mucosal epithelial (arrow) cells, lymphocytes, and macrophages; and in smooth muscles (red arrow head), but week reactions in necrotic debris (x 400; immunohistochemical reaction).



**Figure 10:** Section of the proventriculus of an adult chicken that died from natural case of *Salmonella* Tshiongwe infection in Ogun State, showing severe immunoreactions to *Salmonella* antigens in glandular (arrows) and mucosal epithelial cells (arrow heads) (x 400; immunohistochemical reaction).

of lymphocytes, macrophages and heterophils in the interfiber spaces in 70% of the cases in adult and 100% in chicks (Figure 5). In some cases large bacterial colonies were seen in the interfiber spaces. Microscopic lesions in the liver included severe congestion of the central and portal veins; and of the sinusoids, severe vacuolation and necrosis of hepatocytes in 90% of the cases examined in adult chickens and 100% in chicks. The necroses were multifocal, periportal, midzona or centrilobular with infiltration of lymphocytes, macrophages and

heterophils in 100% of the cases (Figure 6). Microscopic changes in the spleen were severe lymphoid depletion and necrosis of splenic parenchyma in 60% of the cases in adult chickens and 70% in chicks. There was hyperplasia of the lymphoid tissues in 30% of the cases. In some cases, there were infiltration of lymphocytes, macrophages, heterophils and plasma cells; and marked haemosiderosis. Microscopic changes in the kidney were severe congestion and oedema of the interstitium; and extensive necrosis of renal tubules in 100% of the cases in adult and young chickens. The glomeruli epithelial cells were necrotic and the basement membranes were thickened or disintegrated in some cases. There was infiltration of lymphocytes, macrophages and heterophil. Microscopic lesions in the proventriculus were congestion of blood vessels, hyperplasia of lymphoid tissues, moderate to severe necrosis of the glandular and mucosal epithelial cells in 85% of the cases in adult chickens and 100% in chicks. There was infiltration of lymphocytes, macrophages and heterophils in the necrotized epithelium, submucosae and peri-glandular area. Microscopic lesions in the small intestine and caecum were severe necrosis of the villi,

glandular and mucosal epithelial cells in 100% of the cases in adult and young chickens. The villi were matted and atrophied; and there were infiltrations of *lymphocytes*, macrophages and *heterophils* in the necrotized epithelium, submucosa, and muscularis mucosa.

*Immunohistochemical changes in visceral organs of chickens infected naturally with motile Salmonella serotypes:*

Immunohistochemical reactions in the lung were strong in macrophages in 90% of the cases (Figure 7), *lymphocytes*, *heterophils*, endothelial cells; and epithelial cells of the bronchi and alveolar wall (*pneumocytes*), but weak in the interstitium and necrotic debris. In the heart, immunoreactions were strong in blood cells, endothelial cells, myocytes and macrophages in 65% of the cases in adult chickens and 80% in chicks. Immunoreactions in the liver were also strong in blood cells, endothelial cells, hepatocytes, and macrophages in 100% of the cases in adult and young chickens; there was also strong immunoreactions in macrophages, but was weak in necrotic debris (Figure 8). Immunohistochemical reactivity in the spleen was strong in macrophages and *lymphocytes* in 90% of the cases in adult chickens and 100% of the cases in young chicks. Immunoreactions in the kidneys were strong in blood cells, endothelial cells; and in the epithelial cells of the renal tubules in 100% of the cases in adult and young chickens. There were also strong immunoreactions in glomeruli and macrophages (Figure 9). In the proventriculus, immunoreactions were strong in blood cells, glandular and mucosal epithelial cells in 100% of the cases in adult and young chickens; and in macrophages (Figure 10). Immunoreactions in the small intestine were strong in blood cells, glandular (crypt of Lieberkühn) and mucosal epithelial cells in 100% of the cases in adult and young chickens (Figure 11); and in smooth muscles and macrophages. The caecum showed strong immunoreactions in blood cells, epithelial cells of the crypt and mucosae in 100% of the cases in adult and young chickens; and in macrophages.

## Discussion

The clinical histories recorded in the present study have been reported in avian salmonellosis caused by different *Salmonella* serotypes (Lister and Barrow, 2008). Similar clinical signs in chickens experimentally infected with *Salmonella Gallinarum* have also been reported (de Oliveria *et al.*, 2005). However, there is no report on clinical signs of salmonellosis caused by *Salmonella* serotypes identified in the present study. The mortality of 10 to 80% recorded in the present study agreed with the 10 to 90% reported in chicken by Lister and Barrow (2008). Mortality of 40 to 88% in chickens has also been reported (Kamelia *et al.*, 2010). However, Akhtar *et al.* (2011) recorded a mortality of 10% in experimental infection of White leg horn chicks with *Salmonella* Enteritidis, while Ogunleye *et al.* (2010) recorded a mortality of 95% in pullets infected with *Salmonella* para typhi. It has been reported that mortality in avian salmonellosis can range from 0 to 100% (Lister and Barrow, 2008).

While *S. Zega* which was the most predominantly isolated serotypes in the present study has been isolated from human sources in Zaire (Fain *et al.*, 1952), *S. Kentucky*, the second most frequently isolated serotypes has been reported to infect chickens worldwide (Le Hello *et al.*, 2011). *Salmonella* Kentucky was also reported to be the most common serotype isolated from litters, dust, faeces, feed and water in Nigerian commercial chicken layer farms across the country (Fagbamila *et al.*, 2017). In other separate studies, *Salmonella* Herston has been reported to be associated with diarrheal cases in children from Niger Republic; and *S. Nima* has caused an outbreak of salmonellosis in humans through contaminated chocolates, in many countries (CMAJ, 1986). Whereas *Salmonella* Colindale was one of the most common *Salmonella* serotypes isolated from fresh lettuce in Burkina Faso (Traore and others 2015).

The gross lesions in the visceral organs of chickens naturally infected with *Salmonella* Zega, *S. Telelkebir* and *S. Tshiongwe* in the present

study were similar, although they presented differently in adult and young chicks. The lesions are also similar to the ones reported in infection with highly avian host adapted *Salmonella* serotypes, *S. Gallinarum* and *S. Pullorum* (Beyaz et al., 2010; Garcia et al., 2010). Necrosis and consolidation of the lung parenchyma with whitish nodules in young chicks observed in the present study is a common finding in pullorum disease (Shivaprasad and Barrow, 2008; Abdu, 2014). The large whitish to yellowish nodules that caused distortion in the heart has been reported in fowl typhoid (Abdu, 2014). Hepatomegaly with bronze discoloration and in some cases, whitish foci of necrosis; splenomegaly and swollen kidneys observed in the present study are consistent findings in avian salmonellosis caused by nonmotile *Salmonella Gallinarum* and *Salmonella Pullorum* in different Galiforme species (Ibrahim et al., 2003; Garcia et al., 2010; Barde et al., 2015). However, the finding of the pathological lesions caused by the present serotypes is unusual since they are not avian host adapted serotypes and report on pathological changes associated with *S. Zega*, *S. Nima*, *S. Telelkebir*, *S. Colindale* and *S. Tshiongwe* infections in chickens is rare. However, necrotic foci in the liver have been described in pullets infected with other motile *Salmonella* species, such as *Salmonella Para typhi* (Ogunleye et al., 2006). Reports of proventricular lesions associated with avian salmonellosis are rare, but enteritis and typhilitis observed in this study have also been reported in avian salmonellosis (Garcia et al., 2010; Abdu, 2014; Barde et al., 2015). The invasion of the epithelial lining of the intestine and caecum by *Salmonella* organism leads to series of pathological changes that affect intestinal fluid and electrolyte regulation resulting to diarrhea (Beyaz et al., 2010; Garcia et al., 2010).

Histopathological changes observed in the lungs in the present study have been reported in avian salmonellosis caused by the highly adaptive nonmotile *Salmonella* serotypes, *S. Gallinarum*, and *S. Pullorum* (Hossain et al., 2006; Majid et al., 2010; Beyaz et al., 2010; Barde et al., 2015). Necrosis of cells and tissues in various organs give credence to the reports of

previous workers who reported similar changes in visceral organs of different Galiforme species (Hossain et al., 2006; Shivaprasad and Barrow, 2008; Beyaz et al., 2010; Tunca et al., 2012; Nazir et al., 2012; Barde et al., 2015). *Salmonella* species are known to infect wide range of host cells where they multiply within the cells and cause necrosis (Henderson et al., 1999; Hensel, 2006; Tunca et al., 2012). Similarly, the infiltrations by *lymphocytes*, *macrophages* and *heterophils* seen in these natural cases have been reported in avian salmonellosis (Hossain et al., 2006; Beyaz et al., 2010; Nwiyi and Omadamiro, 2012; Nazir et al., 2012; Barde et al., 2015). However, report on histopathological changes in visceral organs of chickens caused by the serotypes identified in the present study is rare in the literature.

Immunoreactions in blood cells, *macrophages*, *lymphocytes*, *heterophils*, epithelial and endothelial cells of the blood vessels in visceral organs agreed with the fact that *Salmonella* species are intracellular pathogens capable of infecting a wide variety of host cells (Gorvel and Merese, 2001; Okamura et al., 2005). Antibodies binding to *Salmonella* pathogens have been reported in host cells of Galiforme species associated with salmonellosis by various workers (Henderson et al., 1999; Beyaz et al., 2010; Tunca et al., 2012). The reactions were strong in host cells but weak in interstitial spaces and necrotic debris, which suggest their ability to survive phagocytosis, multiply and subsequently kill the host cells and this give credence to other workers (Gorvel and Merese, 2001; Okamura et al., 2005; Hensel, 2006; Jones et al., 2007). The findings in this study was similar to the ones observed with *Salmonella Gallinarum*, *S. Pullorum*, *S. Typhimurium*, *S. Enteritidis* in chicken host cells (Henderson et al., 1999; Beyaz et al., 2010; Tunca et al., 2012). However, to the best of our knowledge, our study is the first to demonstrate immunoreactions to the antigens of the present *Salmonella* serotypes in the visceral organs of chickens.

In conclusion, natural infection with some motile *Salmonella* serotypes not commonly reported in chickens produced clinical signs, pathomorphological and

immunohistochemical changes in visceral organs of chickens similar to those seen in galliforme species by the nonmotile serotypes, which is unusual. To the best of our knowledge, with exception of *S. Kentucky*, these serotypes have not been reported to cause severe lesions in chickens. Therefore, in future research, including vaccine production against avian salmonellosis, attention should be focused on *Salmonella* serovars that are not known to cause serious disease in chickens.

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## PERFORMANCE OF BROILER CHICKENS INFECTED WITH HELMINTHES AND COCCIDIAN FOLLOWING THE FEEDING OF DRIED BARK OF VITEX DONIANA

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### Abstract

Study was conducted to assess performance of helminths and *Coccidia* infected broiler chickens that were fed with Bark of Vitex Doniana, ninety six day old Marshall Broiler chickens were purchased and used for the study. They were housed on deep litter pen with open sides, covered with wire mesh. They were fed with broiler starter and finisher diets from the first day of the study to 4th week and from week 5 to 10 of the study respectively. All the recommended routine broiler husbandry practices were observed. They were randomly divided into 3 groups (A= infected with helminthes and *Coccidia*, untreated, B= infected with helminthes + *Coccidia*, treated with conventional drug, C= infected with helminthes and *Coccidia*, treated with black plum tree bark powder in the feed), each group with 4 replicates of 8 birds per replicate. They were challenged orally with *Coccidial* mixed infection and the study lasted for seventy (70) days. Parameters such as feed intake, average weight gain, feed conversion ratio, mortality rate, carcass evaluation, faecal analysis, haematology and GIT histopathology were taken. The study revealed statistical significant differences in feed intake, weight gain, live and dressed weight among treatment groups but there was no statistical significant different in feed conversion ratio. Group C birds had the highest feed intake, weight gain, live and dressed weight. There were statistical significant differences between the treatment groups A and C in mortality and there was no mortality in B. *Helminthes* and *Coccidia* were cleared from birds in Groups B and C at the finisher Phase. All the haemograms in group A dropped and histopathology of groups A and B caeca show cellular necrosis with degeneration and haemorrhagic lesions respectively without lesion in group C.

**Keywords:** Broiler chicken, fed, *Coccidia*, infection, *Vitex Doniana*, medication

## PERFORMANCE DES POULETS DE CHAIR INFECTÉS PAR DES HELMINTHES ET DES COCCIDIES À LA SUITE D'UNE ALIMENTATION AUX ÉCORCES SÈCHES DE VITEX DONIANA

### Resume

Une étude a été menée dans le but d'évaluer la performance des poulets infectés par des helminthes et coccidies après une alimentation aux écorces de Vitex Doniana. Des poulets de chair Marshal âgés de quatre-vingt-seize jours ont été achetés et utilisés pour les besoins de l'étude. Ils ont été logés dans un poulailler à litière profonde aux côtés ouverts, recouvert de treillis métallique. Ils ont reçu des aliments de démarrage et de finition pour poulets de chair, respectivement du premier jour de l'étude à la quatrième semaine et de la cinquième semaine à la dixième semaine de l'étude. Toutes les pratiques d'élevage de poulets de chair recommandées ont été observées. Les poulets ont été répartis de manière aléatoire en 3 groupes (A = infectés par des helminthes et coccidies mais non traités ; B = infectés par des helminthes + coccidies et traités par des médicaments conventionnels ; C = infectés par des helminthes et coccidies et traités avec de la poudre d'écorce de prunier noir dans les aliments), chaque groupe avec 4 réplicats de 8 oiseaux par répétition. Les oiseaux ont été infectés par voie orale avec une infection coccidiale mixte, et l'étude a duré soixante-dix (70) jours. Des paramètres tels que la prise alimentaire, le gain pondéral

moyen, l'indice de consommation, le taux de mortalité, l'évaluation de carcasse, l'analyse des excréments, l'hématologie et l'histopathologie GIT ont été mesurés. L'étude a révélé des différences statistiquement significatives dans la prise alimentaire, le gain pondéral, le poids vif et paré dans les groupes de traitement, mais elle n'a relevé aucune différence statistiquement significative entre les indices de consommation. Les oiseaux du Groupe C avaient les valeurs les plus élevées pour l'indice de consommation, la prise de poids, le poids vif et le poids paré. Des différences statistiquement significatives ont été notées entre les groupes de traitement A et C au niveau des taux de mortalité, mais aucune mortalité n'a été enregistrée dans le groupe B. Les helminthes et coccidies ont été éliminées chez les oiseaux des groupes B et C à la phase de finition. Tous les hémogrammes du groupe A ont baissé, et l'histopathologie des carcasses des groupes A et B montre respectivement une nécrose cellulaire avec dégénérescence et lésions hémorragiques, mais aucune lésion dans le groupe C.

**Mots-clés :** poulet de chair, alimentés, coccidies, infection, Vitex Doniana, médicament

## Introduction

Avian coccidiosis is a disease of poultry that has accounted for an estimated economic loss of about \$800 million worldwide in the poultry industry due to mortality and performance losses (Chapman, 2009). The disease is caused by an intracellular Protozoa, *Eimeria* species, belonging to the phylum *Apicomplexa*. About nine species of *Eimeria* (*Eimeria tenella*, *E. acervulina*, *E. necatrix*, *E. maxima*, *E. praecox*, *E. mitis*, *E. hagani*, *E. mivati* and *E. brunette*) have been reported in chickens (Taylor *et al.*, 2007). The disease causes decreased feed intake, mal-absorption, inefficient feed and nutrient utilization which result in weight loss, impaired growth rate, reduce egg production and mortality (McDougald, 2003; Lillehoj *et al.*, 2004; Haq *et al.*, 2011). Birds also easily develop natural immunity to the infection but their immune systems are yet to be fully developed at the age in which broilers are generally slaughtered. To acquire natural immunity to *Coccidia*, young birds ideally need to be expose to low levels of several strains of the parasite (Williams *et al.*, 1999).

Coccidiosis damages the immune system and leave poultry more vulnerable to pathogens like clostridium, *Salmonella*, and *E. coli* (Maxey and Page, 1977). Clinical signs of the disease ranges from severe diarrhoea, decrease feed and water intake, weight loss, weakness, decreased egg production and high mortality.

Some of the predisposing factors to the disease are; deep litter system of poultry

management, high stocking rates and the environmental conditions such as high relative humidity. When birds are in direct contact with their droppings, then the risk of infection is greatly increased. Oocysts may remain in buildings for a long period of time and they may also be carried by mechanical means, like farm equipment, clothing, insects and other animals. Birds introduced to an infected building will quickly become infected. Graat *et al.* (1998) examined risk factors on 144 broiler farms and reported that poor hygiene related to personnel, feeding, and watering were important.

However, the existing indigenous technical knowledge inherited from past generations has sustained the local poultry production system (Hashemi and Davoodi, 2012; Mirzaei-Aghsaghali, 2012). This knowledge is passed on verbally and is hardly documented. Due to high cost of conventional medicines and vaccines coupled with the lack of knowledge on their use by most of the farmers, these drugs are usually out of reach of the small-scale farmers. There is, therefore, need for cheap, easy to use and sustainable local poultry disease control programs (Okitoi *et al.*, 2007; Mahima *et al.*, 2012).

Due to widespread usage of sulphanilamide, ionophorous antibiotics, amprolium or synthetic chemical compounds for the treatment of coccidiosis in poultry has resulted in emergence of drug-resistant strains and antibiotic residues in poultry meat posing serious health hazard to the consumers. Several herbs known to possess anti-*Coccidial*

properties have also been documented to increase survival rates, body weight gains of birds, reduce bloody diarrhoea and oocyst excretions from birds infected by *Eimeria tenella* (Zhang *et al.*, 2012; Dragan *et al.*, 2014). Three plants extracts namely *Tulbaghia violacea* (35 mg kg<sup>-1</sup>), *Artemisia afra* (150 mg kg<sup>-1</sup>) and *Vitis vinifera* (75 mg kg<sup>-1</sup>) showed anti-*Coccidial* action due to its antioxidant activity. *Tulbaghia violacea* significantly reduced the oocysts production in birds which can be used as prophylactic or therapeutic anti-*Coccidial* agent (Naidoo *et al.*, 2008). There is therefore need for more research into herbal products as anti-*Coccidial* drugs for treatment/or management of coccidiosis to serve as an alternative in the control of the disease in poultry production. The objective of this study was to assess the effect of black plum incorporated feed in the performance of broiler chickens infected with helminthes and *Coccidia*.

## Materials and Methods

### Study area

The study was conducted at Wukari, in Wukari Local Government Area of Taraba State, Nigeria. Wukari is located on latitude 7° 52'N and longitude 9° 46'E which lies within the Southern Guinea Savannah region of Nigeria (M.E.P., 2009).

### Experimental Animals and Housing

A total of ninety six (96) day-old Marshall Broiler chickens were used for the study. They were housed on deep litter pen with open sides, covered with wire mesh and oriented in the East-West direction. Each replication was kept in a compartment measuring 2.0m x 1.3m.

### Preparation of Test Material

The stem bark of black plum (*Vitex doniana*) trees was collected from the parent tree, about a meter from the ground level. The material was sun dried on concrete platform and then pounded into powder in a mortar. The powder was then put into sealed polyethylene bags and labelled for proper identification.

### Preparation of Feeds Incorporated with the Test Material

Proximate composition of the feed and test material were determined using the methods described by AOAC (1990). The anti-nutritional factors were determined according to Allen *et al.*, (1974) and AOAC (1990).

### Experimental Procedure

On arrival, all the chicks were given multivitamins (vitalyteR) as an anti-stress on the first day and were fed with broiler starter diet from the first day of the study to the end of the 4th week and broiler finisher diet from the beginning of week 5 to the end of the experiment (10 week). Feeds and water were given ad-libitum. All the chicks were vaccinated against infectious bursal disease (Gumboro) and Newcastle disease. All recommended routine broiler husbandry practices were observed. The unsexed broiler chickens were randomly divided into 3 groups (A B and C), each group with 4 replicates of 8 birds per replicate.

Group A, served as negative control. They were infected with helminthes and *Coccidian* but received neither coccidiostat, deworming medications nor stem bark meals.

Group B, served as positive control. They were infected with helminthes and *Coccidian*, received conventional coccidiostat [Sulphamerazine+Sulphaquinoxaline+Pyrimethamine+Furaltadone (PantacoxR)] and deworming medications [Mebendazole (ZodexR)].

Group C birds were infected and received black plum tree bark powder at 1% in the feed, and no coccidiostat or deworming medications.

The chickens in all the groups were challenged orally with *Coccidial* mixed infection from the faeces of chicken that were previously infected naturally at 4 weeks of age. Each experimental bird was challenged with approximately  $3 \times 10^3$  sporulated oocysts of *Coccidial* mixed infection. The study lasted for seventy (70) days. The Dietary and Nutrients Composition of the Broiler Starter and finisher Chickens Fed and Black plum Bark Meal are shown in tables 1 and 2 respectively.

**Table 1:** Dietary and Nutrients Composition of the Broiler Starter Chickens Fed and Black plum Bark Meal

Ingredients (%)	Treatments	
	A&B	C
Black plum bark	-	1.00
Maize	51.04	51.59
Groundnut cake	29.60	31.80
BDG	9.30	5.53
Bone ash	4.87	4.90
Fish meal	4.00	4.00
Salt	0.30	0.30
Premix*	0.25	0.25
Methionine	0.23	0.22
Lysine	0.41	0.41
Total Nutrients (%)	100.00	100.00
M.E. (kcal/kg)	2800	2800
Crude Proteins	23.00	23.00
Ether Extract	4.39	4.30
Crude Fibre	4.02	3.93
Calcium	2.00	2.00
Lysine	1.30	1.30
Phosphorus	1.00	1.00
Methionine	0.80	0.80

**Key:** Premix\*: Biomix premix supplied the following per kg of diet: Vit A, 10000 I.U.; Vit D3 2000 i.u.; Vit E, 23mg; Vit K, 2mg; Vit. B1 (thiamine) 1.8mg; Vit B2 (Riboflavin), 5.5mg; Vit B6, (Pyridoxine), 3mg; Vit. B12, 0.015mg; Pantothenic acid, 7.5mg; Folic acid, 0.75mg; Biotin, 0.06mg; Choline chloride, 300mg; Cobalt, 0.2mg; copper, 3mg; Iodine, 1mg; Iron 20mg; manganese, 40mg; Selenium 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg. M.E: Metabolizable Energy; BDG: Brewers Dried Grains

**Table 2:** Dietary and Nutrients Composition of the Broiler Finisher Chickens Fed and Black plum Bark Meal

Ingredients (%)	Treatments	
	A&B	C
Black plum bark	-	1.00
Maize	60.32	60.88
Groundnut cake	26.19	28.39
BDG	5.53	1.76
Bone ash	5.07	5.11
Fish meal	2.00	2.00
Salt	0.30	0.30
Premix*	0.28	0.26
Methionine	0.25	0.25
Lysine	0.06	0.05
Total Nutrients (%)	100.00	100.00

Ingredients (%)	Treatments	
	A&B	C
M.E. (kcal/kg)	2900	2900
Crude Proteins	20.00	20.00
Ether Extract	4.21	4.11
Crude Fibre	3.31	2.67
Calcium	2.00	2.00
Lysine	1.00	1.00
Phosphorus	0.80	0.80
Methionine	0.75	0.75

**Key:** Premix\*: Biomix premix supplied the following per kg of diet: Vit A, 10000 I.U.; Vit D3 2000 i.u.; Vit E, 23mg; Vit K, 2mg; Vit. B1 (thiamine) 1.8mg; Vit B2 (Riboflavin), 5.5mg; Vit B6, (Pyridoxine), 3mg; Vit. B12, 0.015mg; Pantothenic acid, 7.5mg; Folic acid, 0.75mg; Biotin, 0.06mg; Choline chloride, 300mg; Cobalt, 0.2mg; copper, 3mg; Iodine, 1mg; Iron 20mg; manganese, 40mg; Selenium 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg. M.E: Metabolizable Energy; BDG: Brewers Dried Grains

**Note:** The only difference between the groups is the addition of 1g of Black plum Bark Meal in group C.

### Parameters measured

The parameters measured were; feed intake, average weight gain, feed conversion ratio, mortality, carcass evaluation, faecal analysis, haematology and gastro-intestinal histopathology.

### Feed intake and average weight gain

The birds were given weighed amount of feeds daily and the left over were weighed. Daily feed intake was estimated as the difference. All birds were weighed once weekly and the weight gain was determined as the difference between the initial and final weights. Dividing this quantity by 7 and dividing again by number of birds in a replicate was used to arrive at average daily weight gain.

### Feed Conversion Ratio (FCR)

This was calculated as the ratio of feed intake to the corresponding live weight gain of the birds.

$$FCR = \text{Feed intake} / \text{Weight gain}$$

### Mortality

Mortality was calculated as the ratio of the number of dead birds to the total number of birds per replicate, expressed as percentage.

### Carcass Evaluation

At the end of the 10 weeks experiment, the birds were starved overnight to decrease faecal contamination during evisceration and weighed to obtain slaughter weight. Two birds from each replicate with average weight approximating the replicate average weight were slaughtered for carcass analysis. The birds were humanely slaughtered by severing of jugular vein and the carcasses were bled by hanging with the head downward. The carcasses were scalded at 65°C in water bath for 30 seconds before feathers were removed. The dressed chickens were then eviscerated and weighed to obtain dressed weight. Organs and abdominal fat were weighed and their proportion expressed as a percentage of live weight.

### Faecal Analysis

Faecal floatation and sedimentation techniques were used to detect helminth eggs and *Coccidial* oocysts. The modified quantitative McMaster technique was used for the examination of nematode and cestode eggs, together with *Coccidial* oocysts (MAFF, 1986). The standard qualitative sedimentation technique was used to detect trematode eggs as described by Hansen and Perry (1994)

### Haematological Analysis

Blood was collected from wing vein of 2 birds per replicate into heparinized sample bottles. The packed cell volume (PCV), haemoglobin concentration (Hb), total erythrocyte (RBC) and differential leucocytes (WBC) counts were determined according to Schalm et al. (1975).

### Gastro-Intestinal Histopathology

The intestines were dissected longitudinally and screened for the presence of helminth parasites at the end of starter and finisher phases. The intestinal contents were examined by sedimentation and floatation methods as described by Bowman and Lynn (1995) for the presence of helminthes ova and *Coccidian* oocysts.

### Data Analysis

Data obtained were subjected to Analysis of Variance (ANOVA) as outlined by Steel and Torrie (1990) using MINITAB statistical software. Significant differences in the group means were separated using the Fisher LSD methods of the MINITAB statistical software.

## Results

The results of the proximate composition and some bioactive screening of black plum stem bark used are shown in Tables 3 and 4 respectively.

There were significant differences ( $p < 0.05$ ) in the feed intake, weight gain and feed conversion ratio and there was no death recorded within the first four weeks of the experiment.

At the finisher phase, there were significant differences among the treatment group A, B and C in feed intake and weight gain, and with treatment group C having the highest feed intake and weight gain. There were no significant differences among the treatment groups A, B and C in FCR. There were significant differences between group A and C in mortalities and no mortality was recorded in group B as shown in table 7.

The result of effect of black plum

bark supplemented meal diets on carcass and visceral organ weight of the broiler chicken showed that the live weight and dressed weight were significantly different ( $p < 0.05$ ) among the treatment groups (Table 8). The birds in treatment group C were the highest in live weight and dressed weight. The visceral organ weights were not significantly different ( $p > 0.05$ ) among the treatment groups as presented in table 8.

Table 9 shows how the conventional anthelmintic-anti*Coccidian* drugs and the black plum bark reduced *Ascaris* and *Coccidian* burden from ++ to + and cleared tapeworms completely from the infected broiler chickens.

At finisher stage, the dewormer-anti*Coccidian* and Black plum were able to clear all the worms from the infected broiler chickens. The Black plum bark meal also clears the *Coccidian* infection completely but the conventional anti*Coccidian* drug reduced the *Coccidian* infection to sub-clinical level as shown in Table 10.

Photomicrograph of the gastrointestinal tract (caecum) of the experimental broiler chicken in group A (plate I), revealed cell necrosis and degeneration, group B (plate II), shows haemorrhagic lesions in caecum mucosal epithelium but no lesions was observed in group C (plate III) as normal caecal cells outline were seen.

There were no statistical significant differences ( $p > 0.05$ ) in any of the haemograms between the groups. However, group B have the lowest values of all the parameters when compare with groups A and C pre-infection as shown in Table 11.

Table 12, show the haematological parameters of the chickens after exposure to the parasites and there were statistical significant differences ( $p > 0.05$ ) between group A and the other two groups (B and C). The values of all the parameters dropped in group A at the finisher phase.

**Table 3:** Proximate composition of black plum bark

Constituents (%)	Black plum
Moisture	9.50
Crude Protein	2.42
Crude Fibre	54.88
Ether Extract	0.62
Ash	7.15
NFE	25.43
Energy ( kcal/kg )	1030.31

Each of the data was a product of average of three determinations, Energy: Gross energy and was determined using bomb calorimeter.

**Table 4:** Bioactive screening of stem bark of black plum

Bioactive Substances	Black plum
Tannin (mg/kg)	0.96
Saponin (mg/kg)	1.08
Phytic acid(mg/kg)	1.42
Oxalate(mg/kg)	3.17
Glycoside (mg/kg)	0.49
Alkaloid(mg/kg)	5.20

Each of the data was a product of average of three determinations

**Table 5:** Proximate Composition of the Experimental diets fed to Starter Broiler Chickens

Constituents	Treatments	
	A&B	C
Crude Proteins (%)	22.43	22.98
Crude Fibre (%)	5.40	4.97
Ether Extract (%)	3.92	3.34
Ash (%)	9.94	10.43
Moisture (%)	7.84	7.81
NFE (%)	50.45	50.47
*Metabolizable Energy (Kcal/kg)	2939.15	2912.49

Each of the data was a product of average of three determinations.

\*Calculated using the equation of Pauzenga (1985)

\*Metabolizable Energy (Kcal/kg) = 37 x %CP + 81 x %EE + 35.50 x NFE (Pauzenga, 1985)

NFE = Nitrogen Free Extract.

**Table 6:** Proximate Composition of the Experimental diets fed to Finisher Broiler Chickens

Constituents	Treatments	
	A&B	C
Crude Proteins (%)	19.69	19.98
Crude Fibre (%)	4.12	4.94
Ether Extract (%)	3.29	3.73
Ash (%)	11.58	9.83
Moisture (%)	7.74	8.61

Constituents	Treatments	
	A&B	C
NFE (%)	53.58	52.91
*Metabolizable Energy (Kcal/kg)	2897.11	2919.70

Each of the data was a product of average of three determinations.

\*Calculated using the equation of Pauzenga (1985)

\*Metabolizable Energy (Kcal/kg) =  $37 \times \%CP + 81 \times \%EE + 35.50 \times NFE$  (Pauzenga, 1985)

NFE = Nitrogen Free Extract.

**Table 7:** Effect of Black Plum Bark Supplements on the Overall Performance of Broiler Chickens at Finisher (Day old - 10weeks).

Parameter	A	B	C	SEM	p-Value
Feed Intake (g/bird)	5495.20 <sup>c</sup>	6059.20 <sup>b</sup>	6348.70 <sup>a</sup>	121.69	0.0034*
Weight Gain (g/bird)	2008.33 <sup>c</sup>	2184.38 <sup>b</sup>	2375.00 <sup>a</sup>	53.25	0.0036*
FCR	2.74	2.78	2.68	0.04	0.376NS
Mortality (%)	18.75 <sup>a</sup>	0.00	6.25 <sup>b</sup>	0.28	0.000*

<sup>a,b,c</sup>. Means in the same row with different superscripts are significantly different ( $p < 0.05$ ); SEM = Standard Error Means; NS = Not significant ( $p > 0.05$ ); \* = Significant difference ( $p < 0.05$ ).

**Table 8:** Effect of Experimental Diets with or without Black plum Bark Meal on Carcass and Viscera Organ Weight\*\* of the Broiler Chickens.

Parameter	A	B	C	SEM	p-Value
Live weight (g)	2187.50 <sup>c</sup>	2362.50 <sup>b</sup>	2556.25 <sup>a</sup>	52.74	0.0033*
Dressed weight(g)	1956.25 <sup>c</sup>	2111.25 <sup>b</sup>	2295.00 <sup>a</sup>	51.54	0.0026*
Viscera Weight(g)	13.01	12.98	12.95	0.11	0.055

<sup>a,b,c</sup>. Means in the same row with different superscripts are significantly different ( $p < 0.05$ )

SEM = Standard Error Means; NS = Not significant ( $p > 0.05$ ) \* = Significant difference ( $p < 0.05$ )

\*\* = Express as % of live weight

**Table 9:** Faecal analysis of Broiler Chickens Fed with Black Plum Bark Meal in Broiler Starter and Finisher Diets and Treatment for Helminthes and *Coccidia*.

Parasite types	A (Starter/Finisher)	B(Starter/Finisher)	C(Starter/Finisher)
<b>Starter Diet</b>			
Ascaris	++ /+++		+/0
Tapeworms	++/+++		0/0
Coccidial oocysts	+++ /+++		+/-

+ = 3 – 8 oocyst present per field; 0 = Absence of oocyst; A: No treatment + Feed

B: AntiCoccidial (Pantacox) + Dewormer (Zodex) + feed; C: Black plum (1%) only + Feed

**Table 10:** Faecal analysis of Broiler Chickens Fed with Black Plum Bark Meal Broiler Finisher Diet and Treatment for Helminthes and *Coccidia*

Parasite types	A (Starter/Finisher)	B(Starter/Finisher)	C(Starter/Finisher)
Ascaris	+++	0	0
Tapeworms	+++	0	0
Coccidial oocysts	+++	-	0

+ = 3 – 8 Oocyst present per field; - = 1 – 2 Oocyst present per field; 0 = Absence of oocyst

A: No treatment + Feed; B: AntiCoccidial (Pantacox) + Dewormer (Zodex) + feed; C: Black plum (1%) only + Feed

**Table 11:** Haematological Analysis of Starter Broiler Chickens Fed with Black Plum Bark Meal Diet.

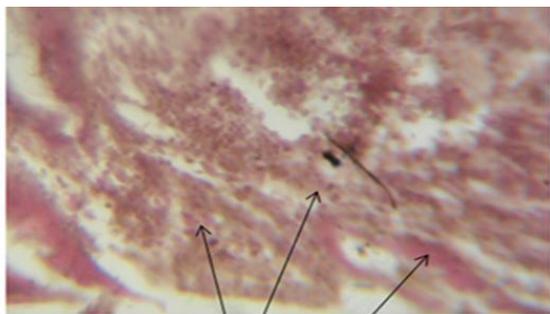
Parameter	A	B	C	SEM	p-Value
PCV (%)	30.38	25.68	29.55	2.87	0.48 <sup>NS</sup>
WBC( $\times 10^9/l$ )	1.82	1.58	1.85	0.16	0.40 <sup>NS</sup>
RBC( $\times 10^{12}/l$ )	2.20	1.80	2.19	0.21	0.46 <sup>NS</sup>
Hb(g/dl)	10.08	8.43	9.93	0.96	0.47 <sup>NS</sup>

<sup>a,b,c</sup>. Means in the same row with different superscripts are significantly different ( $p < 0.05$ ); NS = Not significant ( $p > 0.05$ ); SEM = Standard Error Means; \* = Significant difference ( $p < 0.05$ ).

**Table 12:** Haematological Analysis of Finisher Broiler Chickens Fed with Black plum Bark Meal Diet

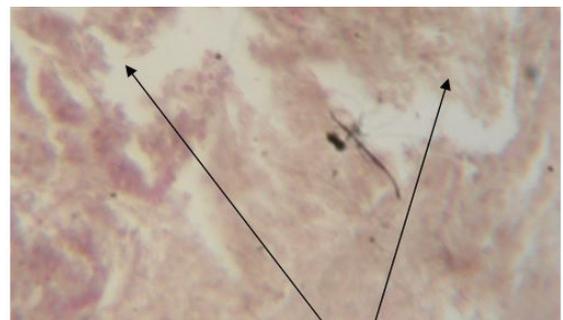
Parameter	A	B	C	SEM	p-Value
PCV (%)	24.23 <sup>c</sup>	34.35 <sup>a</sup>	33.00 <sup>b</sup>	3.09	0.14*
WBC( $\times 10^9/l$ )	1.50 <sup>c</sup>	2.13 <sup>a</sup>	2.04 <sup>b</sup>	0.17	0.23*
RBC( $\times 10^{12}/l$ )	1.84 <sup>c</sup>	2.63 <sup>a</sup>	2.47 <sup>b</sup>	0.24	0.20*
Hb(g/dl)	8.80 <sup>c</sup>	12.53 <sup>a</sup>	11.88 <sup>b</sup>	1.10	0.17*

<sup>a,b,c</sup>. Means in the same row with different superscripts are significantly different ( $p < 0.05$ ); NS = Not significant ( $p > 0.05$ ); SEM = Standard Error Means; \* = Significant difference ( $p < 0.05$ ).



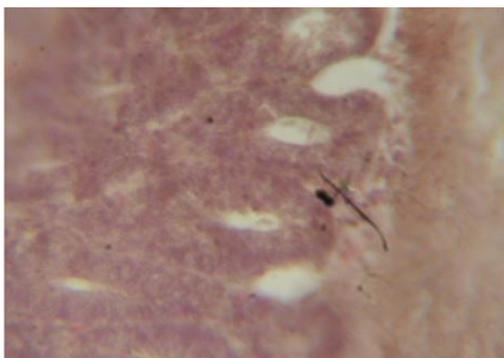
B2 x 40 N  
Cell outline not clearly visible  
Haemorrhagic lesions seen

**Plate I:** photomicrograph of group A. Broiler chicken's caecum fed with experimental diets without medication.



Caecum: Cell necrosis and degeneration observed

**Plate III:** Photomicrograph of group C. Broiler's chicken caecum fed dried bark of Vitex doniana 1% of diet.



D1 x 40 N Caecum-|No lesions observed

**Plate II:** Photomicrograph of group B. Broiler's chicken caecum fed with experimental diets and treated with proprietary drugs at the recommended dosages.

### Discussion

The results obtained revealed that there were significant differences in feed intake, weight gain and feed conversion ratio (FCR) at the starter phase (Table 7). Treatment group A had the highest feed intake but not significantly different ( $p > 0.05$ ) from feed intake of treatment group C. Treatment group A and C were significantly different from treatment group B. In weight gain, treatment group C was significantly different ( $p < 0.05$ ) from treatment group A and B. It is expected that treatment group A with highest feed intake should have the highest weight gain, but due to the effect

of *Eimeria* challenge, the feed consumed were not properly absorbed and utilized. This is in agreement with McDougald (2003) observation, that coccidiosis causes inefficient feed and nutrients utilization in chicken. The FCR were significantly different ( $p < 0.05$ ) among the treatment groups. Treatment group A was significantly different ( $p < 0.05$ ) in FCR from the treatment groups B and C. This is in agreement with Allinson et al. (2013), who reported that herbal extracts enhances the performance in poultry and increases the feed intake and weight gain ratio by significantly decreasing the bacteria and oocyst count. Mortalities were not recorded among the treatment groups during the starter phase.

The result at the finisher phase and overall performance showed that there were significant differences ( $p < 0.05$ ) in the feed intake and weight gain but no significant differences ( $p > 0.05$ ) in FCR. The feed intake and weight gain in treatment group C were the highest and were significantly different ( $p < 0.05$ ) from the feed intake and weight gain in the treatment group A and B. The test material (Black plum) in treatment group C had positive influence on performance that resulted in highest feed intake and weight gain of the birds in that group. This is in line with Wallace et al. (2010), who observed that plant extracts and various phytobiotics that originate from leaves, roots, barks, tubers, or fruits of herbs, and spices have shown to be excellent growth enhancers in poultry industry. This effect may be due to the synergistic action of various active molecules in them and the greater efficiency in the utilization of feed, resulting in enhanced growth and production (Hashemi and Davoodi, 2010). Feed conversion ratio was not significantly different ( $p > 0.05$ ) in the treatment groups. This may be due to immune response generated by the birds to the *Eimeria* challenges. This is in agreement with the report of Lee et al. (2011) that the generation of immunity improves the performance of broilers during *Eimeria* challenge, and it was evident by the significantly increased body weight gain, reduced feed conversion ratio, and in some cases, reduced mortality in post challenge broiler chickens. The highest

mortality rate that was recorded in group A, is in agreement with the observation of Tipu et al. (2002), who reported highest mortalities in birds that were infected with *Eimeria* and were not treated. Also Guha et al. (1991) reported reduced mortality rate with the use of herbal anti-Coccidial drugs as observed in this study.

The live weight and dressed weight were significantly different ( $p < 0.05$ ) among the treatment groups. The birds in treatment group C had highest live weight and dressed weight. The test material (Black plum) showed positive influence on live weight and dressed weight of the birds in treatment group C that resulted in the highest live weight and dressed weight of the birds. This is in line with Wallace et al. (2010) report that plant extracts and various phytobiotics that originate from leaves, roots, barks, tubers or fruits of herbs, and spices have shown to be excellent growth enhancers in poultry industry. This effect may be due to the synergistic action of various active molecules in them and the greater efficiency in the utilization of feed, resulting in enhanced growth and production (Hashemi and Davoodi, 2010). The organ weights were not significantly different ( $p > 0.05$ ) among the treated groups A, B, and C, indicating that the test material had no effect on the viscera organs weight of the birds. This is in line with Mandal et al. (1992) and Youn and Noh (2001) who reported that herbal anti-Coccidials are safe and does not cause tissue destruction.

At the end of starter phase, faecal analysis revealed presence of *Eimeria* oocyst despite the administration of the test material and as well as the conventional drugs. This established the occurrence of Coccidial infections during the starter phase even though the test material and the conventional drug reduced the virulence of the Coccidial parasites. The Coccidial infection was cleared completely at the finisher stage which agreed with other researchers reports (Naidoo et al., 2008; Chandrakesan et al., 2009; Arczewska-Wlasek and Swiatkiewicz, 2012), that some plants have coccidiostatic and/or coccidiocidal effects and can suppress oocysts production in birds when used as prophylactic or therapeutic anticoccidial

agent. The scanty *Coccidial* oocysts seen in the group treated with conventional anti-*Coccidial* drug confirm the occurrence of drug resistance to the conventional drugs by the parasite as earlier reported by other researchers (Boyle et al. 2007; Tacconelli, 2009). Black plum bark meal was also able to eliminate all other helminthes (*Ascaris* and Tapeworms) infestations in the chickens. This result confirm the reports of Lorimer et al. (1996), Athanasiadou and Kviriazakis, (2004); and Oliveira et al. (2009), that plants that contains bioactive compounds such as catechins, flavonoids, steroids, polyphenolics, cystein protease (bio-active enzymes), and secondary metabolites such as alkaloids, glycosides and tannins are nematocidal plants and this is true of the test material used in this study. This also is in line with the report of Fajimi et al. (2005): Fajimi and Taiwo, (2005): Assiak et al. (2001) and Jacob and Pescatore, (2011) that medicinal plants have reasonable pharmaco-therapeutic properties against intestinal worms.

The black plum was not toxic or less toxic and efficacious in the treatment of coccidial infection as shown by the histopathology result in absence of lesions in the gastrointestinal tract (caecum). This is in agreement with Molan et al. (2009) that herbal anti-*Coccidials* are safe, inhibit and destroy *Eimeria* in the challenged chickens. However, the conventional anti-*Coccidial* drug reduced the infection load but could not prevent damages to the gastrointestinal tracts of the chickens as shown in group B whereas the infected, untreated birds (group A) showed caecum degeneration.

Haematological value of the infected, untreated birds (group A), showed leucopenia which was absent in the treated groups. This is in consonance with Koinarski et al. (2001), who reported that haematological values of avian species are influenced by poultry diseases including avian coccidiosis. It is therefore concluded that *Eimeria* infection can cause leucopenia. The anaemia observed in the infected, untreated group was as a result of haemorrhages and poor feed utilization by the birds caused by the ongoing *Coccidial* infection. It can therefore be concluded that black

plum bark meal at 1% in the feed can be substituted for conventional anti-*Coccidial* and anthelmintic drugs (Pantacox and Zodex) in controlling coccidiosis and helminthiasis in broiler chicken production to reduce cost of production and to enhance productivity.

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### Conflict of interest:

The authors declare that they have no conflict of interest. The research was funded by the authors and no grant was received for the research.

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## CROSS SECTIONAL STUDY ON CALF MORBIDITY AND MORTALITY IN AND AROUND ASELLA, ARSI ZONE, ETHIOPIA

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### Abstract

A cross-sectional questionnaire survey was conducted from November 2016 to April 2017 with the objective of determining the prevalence of calf morbidity and mortality, exploring factors associated with calf morbidity and mortality in and around Asella town. A total of 384 calves (90 local and 294 cross breed) up to six months of age were selected randomly from 166 small holder dairy farms. Information on potential risk factors was collected using questionnaire survey conducted during the study period. Chi-Square statistics and comparison of proportions was used to analyze the data. Among the risk factors considered for analysis, breed of the calf, body condition of the dam, time of first colostrum feeding, housing condition, house cleanness, and awareness about importance of colostrum were significantly ( $P < 0.05$ ) associated with calf morbidity and mortality. While parity of the dam were significantly ( $p < 0.05$ ) and insignificantly ( $p = 0.791$ ) associated with calf morbidity and mortality respectively. According to the respondents diarrhea (10.4%) was found to be the most frequently observed disease syndrome followed by respiratory (*pneumonia*) disease (6.25%), tick infestation (1.56%) and navel ill (1.04%). The overall morbidity and mortality recorded were 19.3 % and 6%, respectively. In conclusion, the magnitude of calf morbidity and mortality rates found in this study area were relatively low and economically tolerable however, for better and effective productivity it is therefore, suggested that implementation of improved calf management practices were recommended.

**Keywords:** Asella, Calf, Morbidity, Mortality, Risk factors

## ETUDE TRANSVERSALE SUR LA MORBIDITE ET LA MORTALITE DE VEUX A ET AUTOUR D'ASELLA DANS LA ZONE ARSI EN ETHIOPIE

### Resume

Une enquête transversale par questionnaire a été menée de novembre 2016 à avril 2017 dans le but de déterminer la prévalence de la morbidité et de la mortalité des veaux, explorer les facteurs associés à ces phénomènes dans la ville d'Asella. Au total, 384 veaux (90 de race locale et 294 de race croisée) âgés de moins de six mois ont été sélectionnés de manière aléatoire dans 166 petites exploitations laitières. Les informations sur les facteurs de risque potentiels ont été recueillies en utilisant une enquête par questionnaire menée pendant la période d'étude. Les statistiques du chi-carré et la comparaison des proportions ont été utilisées pour analyser les données. Parmi les facteurs de risque considérés pour l'analyse, la race du veau, l'état corporel de la mère, l'heure du premier colostrum, l'état du logement, la propreté du logement et la sensibilisation à l'importance du colostrum ont été significativement ( $P < 0,05$ ) associés à la morbidité et à la mortalité des veaux, tandis que la parité de la mère a été significativement ( $p < 0,05$ ) et insignifiquement ( $p = 0,791$ ) associée respectivement à la morbidité et à la mortalité des veaux. Selon les répondants, la diarrhée (10,4%) était le syndrome de maladie le plus fréquemment observé, suivie des maladies respiratoires (pneumonie : 6,25%), de l'infestation par les tiques (1,56%) et de la maladie du nombril (1,04%). La morbidité et la mortalité globales enregistrées étaient respectivement de 19,3% et 6%. En conclusion, l'importance des taux de morbidité et de mortalité des veaux dans cette zone d'étude était relativement faible et économiquement tolérable. Cependant, pour une productivité meilleure et plus efficace, il est proposé que la mise en œuvre de meilleures pratiques de gestion des veaux soit recommandée.

**Mots-clés :** Asella, veau, morbidité, mortalité, facteurs de risque

## Introduction

Ethiopia is believed to have the largest livestock population in Africa. This livestock sector has been contributing considerable portion to the economy of the country, and still promising to play great role in the economic development of the country. There are about 56.7million cattle, 29.3million sheep, 29.1 million goats, 9.8 million equine, 1.1 million camels and 56.8 million poultry. Out of these total cattle population, the calf under six months constitute about 14.15% (CSA, 2015)

Dairy farming is a growing livestock production system in Ethiopia. Currently a number of urban and peri-urban dairy farms are the major suppliers of milk and milk products to the urban poor communities and continue to be in the future. Farmers show considerable interest in raising dairy cows and are organizing in unions to combine their efforts and money to run dairy farms. They also increase the use of exotic dairy cattle and their crosses in order to enhance milk production (Tegegne and Gebrewold, 1998).

The future of any dairy production depends among other things, on the successful program of raising calves and heifers for replacement. The productivity of cattle depends largely on their reproductive performance and the survival of calves (Amuamuta *et al.*, 2006). This means the foundation of successful dairy industry using improved breed is laid on the consistent calf production. However, since exotic cattle are less tolerant to local diseases, the dairy production is facing a great challenge due to high prevalence of diseases in dairy cows and their offspring. A successful dairy farm operation requires that a large percentage of cows wean a live healthy calf every year for replacement. Rearing healthy dairy calves to weaning time requires maximizing the calf's level of immunity against disease while minimizing its exposure to infectious agents (Lemma *et al.*, 2001; Godden, 2008).

The health of replacement calves is an important component of total dairy operation profitability as the dairy heifer calf is the foundation of the future milking herd

Furthermore, High incidence of calf morbidity and mortality incurs great economic loss to dairy producers. This arises from death loss, treatment cost, decreased lifetime productivity and survivorship. It also causes the loss of genetic material for herd improvement and decreases the number of dairy heifers available for herd replacement and expansion (Fox, 2007; Razzaque *et al.*, 2009).

Among other factors, calf diseases that cause morbidity and mortality are the major problems faced in raising replacement stock. Calf morbidity and mortality are perennial problems for dairy producers worldwide. Calf diseases that cause morbidity and mortality are the results of complex interaction of the management practices and environment, infectious agents and the calf itself. Scours in neonatal period and *pneumonia* in older calves are known to be responsible for most of calthood morbidity and mortality. Calf mortality shows wide variation ranging from 1 to 30 %. High incidence of calf morbidity and mortality incurs great economic loss to dairy producers. This arises from death loss, treatment cost, decreased lifetime productivity and survivorship. It also causes the loss of genetic material for herd improvement and decreases the number of dairy heifers available for herd replacement and expansion (Heinrichs and Radostits, 2001; Klein-Jöbstl *et al.*, 2014).

The studies, which show calf mortality in different parts of Ethiopia is ranging from 7 to 30.7% (Amuamuta *et al.*, 2006; Lemma *et al.*, 2001; Amoki, 2001; Shiferaw *et al.*, 2002; Wudu *et al.*, 2008; Bekele *et al.*, 2009 and Yeshwas *et al.*, 2014). In the highlands of Arsi zone including Asella town research has been conducted on calf morbidity and mortality by (Bulale, 2000), which is before 17 years ago. However, Nowadays, there is a change in Arsi zone regarding to husbandry system, agro ecology and knowledge of farmers on proper calf management and feeding through animal health extension and veterinary service. So it is important to quantify the extent of calf morbidity and mortality in the study area by epidemiological study. Therefore, the objectives of this study were.

Therefore, the objectives of this study were

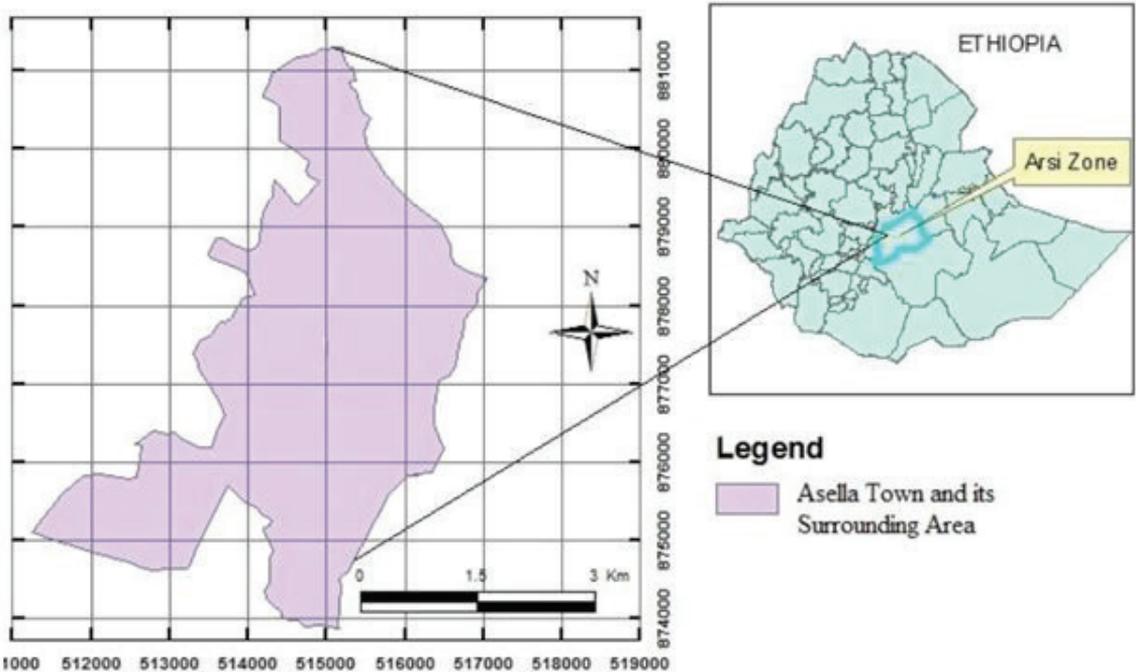
- To determine the prevalence of calf morbidity and mortality in and around Asella town dairy farms.
- To explore factors associated with calf morbidity and mortality in order to give effective advice and realistic recommendations to farmers.

## Materials and Methods

### Study Area

The research work will be carried out in and around Assela small holder dairy

farms during the period of six months from November 2016 to April 2017. Asella town, the capital of Arsi zone in the central part of the Oromia National Regional State, is located at about 175 km Southeast of Addis Ababa at 6° 59' to 8° 49' N latitudes and 38° 41' to 40° 44' E longitudes with an altitude of the area ranges from 2500 to 3000 meter above sea level. Asella town is characterized by mild sub-tropical weather with the maximum and minimum temperature ranging from 18°C and 5°C, respectively around the year. Agricultural production system of the study area is of mixed crop and livestock production.



**Figure 1:** Map showing location of the study area

### Study Population

Both local Zebu and crossbreed dairy calves of both sexes reared under small-holder dairy farms at the age of birth to 6 months were the study animals.

### Study Design

A cross-sectional study design was conducted from November, 2016 to April, 2017 to estimate the prevalence of calf morbidity and mortality.

### Sample Size Determination and sampling method

The sample size was decided by the formula of Thrusfield (2007) with assumption of 50% expected prevalence, as there were no previous study in the study area and 95 % confidence interval.

$$n = \frac{Z^2 \cdot p \cdot (1-p)}{d^2}$$

Where  $n$  = sample size,  $p$  = prevalence (50%),  $z$  = taken from the level for 95% CL (1.96) and  $d$  = the level of absolute precision (5%). Accordingly the calculated sample size was 384 calves. To achieve these sample size and to acquire necessary information about calf morbidity and mortality 166 respondents, 55 dairy farms holders located in Asella town and 111 farm holders located at the periphery of Asella town which are accessible for the main road were selected purposively. Each respondent kept one to four calves in their dairy farms.

#### Data Collection

A structured questionnaire which has been composed of various questions focused on calf management and health concerns was administered to 166 dairy farmers. Major risk factors including breed of calf, sex of calves, age of calves, body condition of the dam, parity of the dam, time of first colostrum feeding, method of colostrum feeding, housing conditions, house cleanness, ownerships of calf taker, experience of calf taker and calf taker awareness about importance of colostrum and others were the different variables covered during the interview. Data on history of calf deaths, history of illness, feeding and type of feeds, watering and health care were also recorded. Major diseases syndromes of calves were recorded during the data collection process based on owners' traditional disease description knowledge with cross referenced to scientific disease interpretation.

#### Data Management and Analysis

The data obtained from the respondents were stored, filtered in Microsoft excel spread sheet and coded and transferred to SPSS software version 20.0 for analysis. To determine the prevalence of calf morbidity and mortality the association between dependent and independent variables was tested using Chi-square statistical test. Chi-square value  $> 3.84$  and  $P$ -Value  $< 0.05$  was considered as statically significant.

## Result

#### Overall prevalence of calf morbidity and mortality

The overall morbidity and mortality recorded in this study were 19.3 %, 74/384 and 6%, 23/384 respectively. The prevalence of diarrhea in the examined dairy calves was (10.4%,  $n = 40$ ), respiratory (*pneumonia*) disease (6.25%,  $n = 24$ ), tick infestation (1.56%,  $n = 6$ ) and navel ill (1.04%,  $n = 4$ ). Of the diarrheic dairy calves, 75 % ( $n = 30$ ) were affected at the age of less than three months and 25 % ( $n = 10$ ) at the age of above three months.

#### Prevalence of Calf Morbidity and Mortality regarding with Calf related Risk Factors

Associated riskfactors (breed, sex and age) related to calf morbidity and mortality were shown in Tables 2 and 3 respectively. Calves of cross breed was significantly ( $p < 0.05$ ) linked to morbidity and mortality 22.8% and 7.5 % respectively, whereas the prevalence of calf morbidity and mortality in local breed calves was 7.8%, and 1.1%, respectively. In the present study there was no statically significantly ( $P > 0.05$ ) association between female and male animals both in morbidity and mortality of calf. The prevalence of both calf morbidity and mortality in female and male animals were 19.7%, 4.3% and 18.8%, 8% respectively.

According to the data obtained from the respondents there was no statically significant difference ( $P > 0.05$ ) between the two age groups regarding calf morbidity and mortality. The prevalence of calf morbidity and mortality in animals  $< 3$  month of age and in the age of 3 months up to 6 months were 19.6 %, 7.1% and 18.8%, 4.4% respectively.

#### Prevalence of Calf Morbidity and Mortality Related with Dam Associated Risk Factors

Factors related to dam associated (body condition and parity) with calf morbidity and mortality were shown in Table 4 and 5 respectively. According to the data obtained from the respondents in the present study there was statistically significant association ( $P < 0.05$ ) among the three categories of body condition both in calf morbidity and mortality.

**Table 2:** Calf associated risk factor related to calf morbidity

Variable (calves)	Description	No. of calves examined	No. of sick calves	Prevalence (%)	X <sup>2</sup> - value	P – value
Breed	Local	90	7	7.8	9.981	0.001
	Cross	294	67	22.8		
Sex	Male	176	33	18.8	0.057	0.897
	Female	208	41	19.7		
Age	<3 months	224	44	19.6	0.048	0.896
	≥ 3months	160	30	18.8		

**Table 3:** Calf associated risk factors related to calf mortality

Variable (calves)	Description	No. of calves examined	No. of sick calves	Prevalence (%)	X <sup>2</sup> - value	P – value
Breed	Local	90	1	1.1	4.968	0.022
	Cross	294	22	7.5		
Sex	Male	176	14	8.0	2.228	0.194
	Female	208	9	4.3		
Age	<3 months	224	16	7.1	1.270	0.285
	≥ 3months	160	7	4.4		

**Table 4:** Dam associated risk factors for calf morbidity

Variable (dam)	Description	No. of calves examined	No. of sick calves	Prevalence (%)	X <sup>2</sup> - value	P – value
Body condition of dam	Good	136	8	5.9	25.399	0.000
	medium	173	43	24.9		
	poor	75	23	30.7		
Parity	Primiparous	77	23	29.9	6.955	0.014
	multiparous	307	51	16.6		

**Table 5:** Dam associated risk factors for calf morbidity

Variable (dam)	Description	No. of calves examined	No. of sick calves	Prevalence (%)	X <sup>2</sup> - value	P – value
Body condition of dam	Good	136	2	1.5	12.157	0.002
	medium	173	11	6.4		
	poor	75	10	13.3		
Parity	Primiparous	77	5	6.5	0.043	0.791
	multiparous	307	18	5.9		

The prevalence of calf morbidity and mortality related with poor, medium and good body conditions were 5.9%, 24.9%, 30.7% and 1.5%, 6.4% 13.3% respectively.

In this study there was statistically significance difference ( $P < 0.05$ ) between calves

born from primiparous and multiparous, the prevalence of calf morbidity was higher in calves born from primiparous which was 29.9% however the prevalence of calf morbidity in calves born from multiparous was 16.6%, However there was no statistically significance

difference ( $P>0.05$ ) in the prevalence of calf mortality regarding with dam parity.

#### *Prevalence of Calf Morbidity and Mortality Related with Management Associated risk Factors*

Factors related to management (time of first colostrum feeding, method of colostrum feeding, house condition and house cleanness) associated with calf morbidity and mortality were shown in Tables 6 and 7 respectively. In this study there was statistically significant difference ( $P<0.05$ ) in calf morbidity and mortality between first colostrum feeding within 6 hours and after six hours. The prevalence of calf morbidity and mortality was higher in calves fed the first colostrum after six hours which was 64.3% and 21.4% respectively. But the prevalence of calf morbidity and mortality was lower in calves fed the first colostrum with in six hours 6.7% and 1.7% respectively.

There was no statistically significance difference ( $P>0.05$ ) in the prevalence calf morbidity and mortality between the two methods of colostrum feeding (hand feeding and suckling). The prevalence of calf morbidity and mortality in hand feeding and suckling methods were 11.1%, 3.2%, and 20.9%, 6.5%, respectively. There was statistically significant difference ( $p< 0.05$ ) in calf morbidity and mortality between calves together with their dam and separate from their dam. The

prevalence of calf morbidity and mortality was higher in calves kept together with dam which was 23.2 % and 8.7% respectively. But the prevalence of calf morbidity and mortality was lower in calves separated from their dam 14.7% and 2.8% respectively.

There was statistically significant difference ( $p< 0.05$ ) in calf morbidity and mortality between calves in unclean house and clean house. The prevalence of calf morbidity and mortality was higher in calves lived in unclean house which was 22.3% and 7.4% respectively. But the prevalence of calf morbidity and mortality was lower in calves lived in clean house 10.9% and 2% respectively.

#### *Prevalence of Calf Morbidity and Mortality Related with Attributers Associated Risk Factors*

Factors related to farm attributers (experience of ownership and awareness about importance of colostrum) associated with calf morbidity and mortality are shown in Tables 8 and 9 respectively. In this study there was no statistically significant difference ( $P>0.05$ ) in calf morbidity and mortality between the experiences of the ownerships ( $\leq 5$  years and  $> 5$  years). The prevalence of calf morbidity and mortality in  $\leq 5$  years and  $> 5$  years ownerships experience were 19.3%, 6.6%, and 19.3%, 5%, respectively. There was statistically significant difference ( $p< 0.05$ ) in calf morbidity and mortality between calves

**Table 6:** Management associated risk factors for calf morbidity

Variable (calves)	Description	No. of calves examined	No. of sick calves	Prevalence (%)	X <sup>2</sup> value	P – value
Time of colostrum feeding	within 6 hours	300	20	6.7	25.399	0.000
	after 6 hours	84	54	64.3		
Method of colostrum feeding	hand feeding	63	7	11.1	3.225	0.081
	suckling	321	67	20.9		
Housing conditions	In separate pen	177	26	14.7	4.430	0.038
	together with dam	207	48	23.2		
House cleanness	Clean	101	9	8.9	6.186	0.012
	Unclean	283	63	22.3		

**Table 7:** Management associated risk factors for calf mortality

Variable (calves)	Description	No. of calves examined	No. of sick calves	Prevalence (%)	X <sup>2</sup> value	P – value
Time of colostrum feeding	within 6 hours	300	5	1.7	9.982	0.001
	after 6 hours	84	18	21.4		
Method of colostrum feeding	hand feeding	63	2	3.2	1.061	0.396
	suckling	321	21	65.4		
Housing conditions	In separate pen	177	5	2.8	5.840	0.017
	together with dam	207	18	8.7		
House cleanness	Clean	101	2	2.0	3.912	0.041
	Unclean	283	21	7.4		

**Table 8:** Factors related to farm attributers associated with calf morbidity

Variable (calves)	Description	No. of calves examined	No. of sick calves	Prevalence (%)	X <sup>2</sup> - value	P – value
Experience of calf taker	< 5 years	244	47	19.26	0.000	1.000
	> 5 years	140	27	19.28		
Awareness about importance of colostrum	Yes	261	21	8.0	9.982	0.001
	No	123	53	43.1		

**Table 9:** Factors related to farm attributers associated with calf mortality

Variable (calves)	Description	No. of calves examined	No. of sick calves	Prevalence (%)	X <sup>2</sup> - value	P – value
Experience of calf taker	< 5 years	244	16	6.6	0.383	0.657
	> 5 years	140	7	5.0		
Awareness about importance of colostrum	Yes	261	7	2.7	4.430	0.038
	No	123	16	13.0		

reared by ownership of have not awareness about importance of colostrum and those have awareness. The prevalence of calf morbidity and mortality was higher in calves reared by the ownerships of having awareness about importance of colostrum which was 43.1% and 2.7% respectively. But the prevalence of calf morbidity and mortality was lower in calves reared by ownerships of having awareness

about colostrum for calves 8%, and 13%, respectively.

## Discussions

This study attempted to determine the prevalence of calf morbidity and mortality, identifying the importance and magnitude of the factors that put dairy calves at risk of morbidity

and mortality. The overall prevalence of calf morbidity and mortality in calves under the age of six months in and around Asella small holders dairy farms were 19.3% and 6%. This result is very close to the findings of Amuamuta *et al.*, (2006) 6.5 % from Andassa ranch and Shiferaw *et al.*, (2002) 7% from Holleta.

The prevalence of calf morbidity and mortality in this study is lower than previous findings reported 9.3% by Bekele *et al.*, (2009), 16.8% by Bangar, *et al.* (2013), 25.0 % by Sisay and Ebro (1998) and 14.2 % by Terence, (2001). This difference was might be due to management system, current better access to veterinary service in towns and their suburbs and the owner of the calves, good awareness about importance of colostrum and most of the owner was feed their calves the colostrum immediately and this difference might be due to agro-ecological difference .

The result of the present study, however, is higher than the prevalence of 3.4% previous reports by Hailemariam *et al.*, (1993a) from Abernossa Ranch. This variation is might be attributed to the differences in agro ecology, number and target group of the study animals and husbandry practices in the two study areas (2007).

The prevalence of calf diarrhea, 10.4% in the present study is comparable with the report of Endale *et al.*, (2013), 11.32% and Dersema, (2008), 13.5%. In contrary to this, the present finding is less than previous studies by Wudu *et al.*, (2008) 34%, Megersa *et al.*, (2009) 42.9% and Bekele *et al.*, (2009) 34%. The difference might be variation in ingestion of colostrums, hygienic condition of feeding utensils and condition of housing. In this study, diarrhea was the most important disease problems in the young calf and in agreement with the present findings (Lemma *et al.*, 2001; Wudu *et al.*, 2008; Trencce, 2001 and Sivula *et al.*, 1996). Among the diarrheic dairy calves occurred at the age of less than three months. This may be because newborn calves at their early age are highly susceptible to diarrhea causing agents due to their undeveloped immune system. According to McGurik and Ruegg, (<http://www.progressivedairy.com/dairy-basics/calf-and->

heifer raising /2230 -0209-pd-calf-diseases-and-prevention. referred in November 2013), the highest morbidity and mortality rates generally occur in baby calves prior to weaning.

Respiratory problem was reported with prevalence of 6.25% which is comparable with the report of Endale *et al.*, (2013) 7.5% and Wallace *et al.*, (2006) 10.6%. In contrary to this, the present finding is less than previous studies of McGurik, (2008) 25 %. This variation might be due to environmental factors, ventilation of the house and immunity development. The prevalence of tick infestation also reported 1.6% is disagrees with the report of Ykealo, (2008) who reported 12%. The difference could be attributed to the difference in the ectoparasite sprays used and degree of exposure.

In this study breed of the calf was found significantly associated with calf morbidity and mortality. Cross breed calves were found at higher risk than that of local ones these result were in agreed with many researchers (Wudu *et al.*, 2008; Debnath *et al.*, 1990 and Swai *et al.*, 2010). This is because the effect of the susceptibility of exotic genetic Bos Taurus breeds to climatic condition and disease stress in tropical environments was higher when compared to local breeds.

Regarding sex in the present study there was no statistically significant difference but more female calves were sick compared to male calves, while higher mortality occurred in male calves than in female calves. This finding agreed with the report of (Amuamuta *et al.*, 2006; Silva del *et al.*, 2007; Lombard *et al.*, 2007; Abebe *et al.*, 2008a; Bleul, 2011; Bekele *et al.*, 2012; Alemayehu *et al.*, 2013 Mellado *et al.*, 2014). This might be due to more female calves were sick probably because there were more female calves among the studied animals. More male calves died than female, this might be due to the less attention and management care given to the male calves as their role in the farms was considered not profitable and postnatal mortality for males and females had a very high genetic correlation, with direct heritability being highest for males. The other justification to this finding could be that perhaps farmers watched female calves more carefully and

diagnosed many clinical cases more effectively due to female calves deemed to be future cows and their economic importance Hansen *et al.*, (2003). According to the findings of Swai *et al.*, (2010) male animals in Tanga, Tanzania were three times more likely to die than females and also similar result was obtained by Bangar *et al.*, (2013) in India.

Poor body condition of the dam was significantly ( $p < 0.05$ ) associated with calf morbidity and calf mortality. This result was in agreement with Weaver *et al.*, (2000); lemma *et al.*, (2001) and Arthington *et al.*, (2000). This is because the poor body condition of the dam reflects its suffering from deficiency of energy, protein, vitamins and other nutrients. The prepartum diet affects colostrum quality and calves that were fed colostrum, obtained from cows that were fed restricted amounts of energy and crude protein, showed reduced absorption of immunoglobulin leading to inadequate immunity and results might be more susceptible to morbidity and mortality of calf.

In this study parity of the dam were statistically significant ( $p < 0.05$ ) associated with calf morbidity but insignificant ( $p > 0.05$ ) with calf mortality. The calves born from primiparous cows had significantly ( $p < 0.05$ ) associated with calf morbidity and non-significantly high mortality ( $p > 0.05$ ) comparing to calves born from multiparous cows. This result was in agreed with Taylor *et al.*, (1999) and Lundborg *et al.*, (2003). This is because incidence of dystocia and still birth are higher in primiparous dams than multiparous dams and immune status is better in calves from multiparous than primiparous dams; this could be due to insufficient or lower concentration of colostrum from first lactating heifers. This result was disagreed with Sargeant *et al.*, (2010) and Radostits *et al.*, (2007). This might be due to the susceptibility of cows varies considerably and new infections are most common in older cows at early lactation and when the management is poor.

In this study, calves that had their first colostrum meal after six hours of age had significantly increased mortality and morbidity. This observation was agreement

with the findings of Wudu *et al.*, (2008). On farms colostrum administration practice is the primary determinant of calf health. According to this study, some of the farm owner men/women in this study area wrongly believed that consumption of the first colostrum causes diarrhea in calves. As the result, they milk and discard the first colostrum before the newborn suckles. Hence, the newborn calves were probably allowed to consume colostrum late. Furthermore, according to Godden, (2008) colostrum immunoglobulin content is reduced with each successive milking; therefore the first milking colostrum has more immunoglobulin content than the second milking colostrum. To ensure adequate protection against disease, calves rely on the intake of an adequate amount of quality colostrum within a few hours of birth. The ability of the neonate to absorb immunoglobulin starts to decline progressively after 6 to 12 hours from birth Radostits *et al.*, (2007). Colostrum Ig concentration also decreases by 3.7% during each subsequent hour post-calving Morin *et al.*, (2010). Therefore, the late a calf consumes colostrum after birth, the lower the level of immunoglobulin absorption. As Arthington *et al.*, (2000) noted, low blood Ig concentration is directly related to calf morbidity and mortality.

Not statistically significant but increased mortality and morbidity in calves those suckling colostrum from their dam than provided by hand feeding. This result was agreed with Bringole and Stott, (1980); Gorden and Plummer (2010); Vasseur *et al.*, (2009) and Morin *et al.*, (2010). This is because passive transfer can be improved better when suckling supplemented with bottle feeding and calves take adequate colostrum to ensure adequate protection against disease. This result was disagreed with Muraguri, *et al.*, (2005). This might be due to suckling have the advantage of reducing contamination, the feeding of cold milk to the calf and incidence of *Mastitis* in the dam.

In this study, calves those together with their dam was found to be significantly associated with calf morbidity and mortality. This result was agreed with Lemma *et al.*, (2001);

Svensson *et al.*, (2006); Wudu *et al.*, (2008); Gulliksen *et al.*, (2009). This is because when the house of calf was together with their dam, the house becomes dirtier with the manure and urine of the adult animal. Under such conditions there was high chance of contamination of the house and the udder and teat of the dam with massive doses of pathogens. Thus calves might acquire these pathogens during suckling which probably resulted in diarrheal disease and death of the dairy calves. And also calves were kept in separate pen provide an opportunity for the farmer to feed, clean, and monitor them.

Based on the findings of analysis of risk factors, cleanness of the calf house was the other variable found to significantly affect calf morbidity and mortality. The higher risk of morbidity and mortality associated with dirtiness (unclean) of calf house seen in this study agrees with Bendali *et al.*, (1999); Shiferaw *et al.*, (2002); Wudu *et al.*, (2008), Phiri, (2008); Marce *et al.*, (2010); and Perez *et al.*, (1990). This is because as most of infectious agents are acquired by calves from the immediate environment, unclean houses was a full of pathogens that the calves obtain and affected. On the other hand, in this study which considered cleanness of calf house as potential risk variable on calf morbidity and mortality failed to show significant association between cleanness and calf health problems in agreed with (Wudu *et al.*, 2008 and Lance *et al.*, 1992)

In this investigation, less than five years farm work experience of herd attendants was found to be non-significantly increase calf mortality and calf morbidity. This result was in agreement with Ababu *et al.*, (2006). This was because taking care of sick calves required more work experience than caring for healthy calves.

In this study, owners of the calves those had no awareness about importance of colostrum for calves was significantly associated with calf morbidity and mortality. This result was in agreed with wudu *et al.*, (2008) and bekele *et al.*, (2012). This is because according to this study, some of the herd men/women in this study area wrongly believed that consumption of the first colostrum causes

diarrhea in calves. As the result, they milk and discard the first colostrum before the newborn suckles. Hence, the newborn calves were probably allowed to consume colostrum late. Delays in feeding of colostrum leads to lowered effectiveness of colostrum in terms of providing immunity to calves which result reduced colostrum immunoglobulin content that leads to low protections against disease.

## Conclusion and Recommendation

The health and management of replacement animals are important components of total herd profitability. The prevalence of calf morbidity and mortality found in this study area was relatively low and economically tolerable. Even if this result is low and economically tolerable it can be achieved through good management for better productivity. The association of 12 potential risk factors with dairy calf morbidity and mortality were investigated. Of these factors, cross breed of the calf, poor body condition of the dam, after six hours of colostrum feeding, calves present together with dam, unclean house, and non-awareness about of colostrum were the most important determinant factors and significantly associated with dairy calf morbidity and mortality, while parity of the dam (multiparous dam) were significantly associated with calf morbidity and insignificantly associated with calf mortality. Diarrhea was the most frequently observed disease syndrome followed by *pneumonia*, tick infestation and navel ill. Therefore,

- Even if this result is low and economically tolerable, for more reduce the calf morbidity and mortality and for better productivity in the study areas, implementation of improved calf management practices such as allowing calves to take adequate colostrums within 24 hours of birth, separation of the calf from their dam, aware of the community about colostrum importance improved health care housing and through sustainable training and optimization of appropriate crossbreeding should be warranted.

- As calf diarrhea and *pneumonia* were found to be the major diseases and of which are both etiologically complex diseases, a more comprehensive study supported by laboratory is suggested to identify the major infection causing agents.
- Further comprehensive research on the role and association of different epidemiological factors such as calf (host factor), environmental and etiological factors that might influence calf morbidity and mortality is also recommended.

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## UNDERSTANDING FISH PRESERVATION METHODS, POST HARVEST LOSSES AND KEY SOCIO-ECONOMIC CONSEQUENCES ON LAKE KARIBA

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### Abstract

Different types of preservation methods are used to prolong shelf-life of fish products. A study was undertaken to appreciate some of the traditional processing methods. Primary data were collected from 160 respondents sampled in nine selected fishing villages in Sinazongwe district using a simple two-stage cluster sampling approach. A structured questionnaire was used to capture perceptions, ideas, opinions, and thoughts. Study results revealed that besides freezing, there were three main methods of processing and preservation; smoking, salting and sun-drying. Over 67% of fish processors were women and they constituted the large numbers of small-scale informal traders, who are usually marginalized by commercialization of fish trade. Results also revealed that majority of male fish processors adopted smoking, whilst females opted to sundry their fish. On a scale of 1-4, smoking was considered to be the excellent method in preventing fish spoilage whereas salted fish was considered more prone to contamination. Fifty three percent (53%) of the respondents revealed that well processed fish had high income prospects. Most sun-dried and salted fish was meant for home consumption whilst smoked fish was mainly targeted for distant markets. As regards post-harvest losses, 50% of the respondents claimed that they lost valuable time in processing their fish, whilst 8% claimed they lost nutrition value of fish and a further 26% indicated monetary value loss. The highest post-harvest losses were experienced in the rainy season, which was compounded with poor handling, insect infestation, bad weather and bacterial contamination being some of the main causative agents for spoilage. The difference in preference of processing methods by gender was linked to the amount of input (capital, time, labour, technology) required. The conclusion drawn from the study was that different methods of processing fish had different effectiveness in reducing post-harvest losses. The study recommends improved technology, facilities and capacity.

**Key words:** shelf-life, post-harvest, small-scale, gender, processing, Kariba

## COMPRENDRE LES MÉTHODES DE CONSERVATION DU POISSON, LES PERTES POST-CAPTURE ET LES PRINCIPALES CONSÉQUENCES SOCIO-ÉCONOMIQUES SUR LE LAC KARIBA

### Résumé

Différentes méthodes de conservation sont utilisées pour prolonger la durée de conservation des produits de la pêche. Une étude a été réalisée dans le but d'évaluer certaines méthodes de traitement traditionnelles. Les données primaires ont été recueillies auprès de 160 répondants échantillonnés dans neuf villages de pêcheurs sélectionnés dans le district de Sinazongwe en utilisant une simple méthode d'échantillonnage en grappes à deux degrés. Un questionnaire structuré a été utilisé pour recueillir les points de vue, les idées, les opinions et les pensées. Les résultats de l'étude ont révélé que, outre la congélation, il existe trois principales méthodes de traitement et de conservation du poisson, à savoir le fumage, le salage et le séchage au soleil. Plus de 67% de personnes engagées dans la transformation du poisson étaient des femmes, et elles constituaient la majorité de petits commerçants informels généralement marginalisés par le secteur de commercialisation du poisson. Les résultats ont également révélé que la majorité des transformateurs de poisson de sexe masculin pratiquaient le fumage, tandis que les femmes optaient pour le séchage de leurs poissons au soleil. Sur une échelle de 1 à 4, le fumage était considéré comme une

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excellente méthode de prévention de l'altération du poisson, tandis que le salage était considéré comme étant plus sujet à la contamination. Cinquante-trois pour cent (53%) des répondants ont révélé que les poissons bien transformés avaient des perspectives de revenu élevé. La plupart des poissons séchés au soleil et salés étaient destinés à la consommation domestique, tandis que le poisson fumé était principalement destiné aux marchés éloignés. En ce qui concerne les pertes post-capture, 50% des personnes interrogées ont déclaré avoir perdu du temps précieux dans la transformation du poisson ; 8% ont déclaré avoir perdu la valeur nutritive du poisson ; et 26% ont indiqué une perte de valeur monétaire. Les pertes post-capture les plus élevées ont été enregistrées pendant la saison des pluies, aggravées par une mauvaise manipulation, les infestations d'insectes, les intempéries et la contamination bactérienne étant les principaux responsables de la détérioration. La différence au niveau des préférences entre les méthodes de traitement sur base du genre était liée à la quantité d'intrants (capital, temps, main-d'œuvre, technologie) requise. La conclusion tirée de l'étude était que les différentes méthodes de transformation du poisson avaient une efficacité différente dans la réduction des pertes post-capture. L'étude recommande donc d'améliorer la technologie, les installations et la capacité.

**Mots-clés** : durée de conservation, post-capture, artisanale, genre, traitement, Kariba

### Introduction

Fish is one of the most important sources of high quality animal protein, amino acids and absorbable dietary minerals for maintenance of good health among human beings. Despite receiving massive acceptance among consumers, fish is a highly perishable food which needs processing or storage immediately after capture. Often times, fishers catch more fish than can be consumed immediately. Surplus fish needs to be kept in good condition for later consumption or ensure an all year round supply. Notably, fish is very perishable and prone to post-harvest loss, a general term used to describe all losses that occur to fish after harvest. Maintaining good quality of fish raw material for processing is vital. However, there are generally various ways through which the quality of harvested fish can deteriorate. Getu *et al.*, (2015) and Tesfay and Teferi (2017) classified the type of losses that are encountered by fishers, fish processors and traders as either quality loss, economic loss, physical loss, market value loss, loss due to insect manifestation or loss due to processing method used. Therefore in order to prevent fish spoilage through contamination of its flesh by micro-organisms, such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Bacillus cereus*, *Shigella* spp., *Clostridium botulinum* (Obodai *et al.*, 2011), it is necessary to preserve fish if not

consumed immediately. Besides freezing, three main methods of processing and preservation include smoking, salting and sun-drying. Fish preservation is the method of extending the shelf life of fish while upholding the quality of the products. Nonetheless, processing methods vary partly because of taste and preference among consumers, and partly because of the level of patience demanded in the process, and the labour and capital investments required (Medard *et al.*, 2001).

Dried products are usually considered shelf stable due to their characteristic low water activity ( $A_w$ ) and are, therefore, often stored and distributed unrefrigerated. A water activity of 0.6 will prevent the growth of microorganisms, including pathogenic bacteria and moulds while maintaining the fish in good nutritional and organoleptic quality (Abbas *et al.*, 2009). The moisture content can be used as a pointer to the rate at which deterioration occurs in fish samples (Olagbemide, 2015). Dried fish typically has a moisture content of between 38% and 48%, depending on the product (Olaoye *et al.*, 2012). However, whilst preservation methods aim at maintaining the quality of fish for a longer period of time, losses have still remained the order of the day among the small-scale handlers as they attempt to take care of excess produce, to store and transport. Abelti (2016) reported that postharvest losses in developing countries are estimated to be up to 50% of domestic

fish production. The fish stocks of Lake Kariba are, undoubtedly, among the most important natural resources for people in the three lake riparian districts of Siavonga, Gwembe and Sinazongwe of Southern Zambia. However, considering that thousands of tons of fish are lost annually through poor handling, unhygienic treatment or inappropriate processing, a study was undertaken to investigate the traditional processing methods in-use on Lake Kariba, associated post-harvest losses and some of the key socio-economic consequences.

#### Sun-Drying

Sun-drying of fishes is simple and the oldest known method of fish preservation, common for its being least expensive yet often rudimentary and rarely hygienically practiced in rural areas (Immaculate *et al.*, 2013). Sun-drying of fish removes water which inhibits bacterial and enzymatic actions but does not add any desirable taste and odor. The drying process is a physical process where fish is exposed to air and direct sunlight. The time it takes to dry fish products depends on the nature of the product, the intensity of the sun, and the surfaces used for drying. The simplest form of drying involves exposing the fish to heat from the sun by placing products either directly on the ground, on mats placed on the ground or on racks (Immaculate *et al.*, 2013). Fish is typically sun-dried for three to ten days but drying periods of one to three days are more common. Since traditional sun-drying is weather dependent, some losses in quality also result from inadequate drying.

Drying of fishes is susceptible to many types of spoilage which can affect the quality and shelf life. Physical and organoleptic qualities of many traditional sun-dried products are unsatisfactory for human consumption (Nowsad, 2005). Damages occurring due to flies and insects are of great significance in open sun-drying and this is a serious problem in traditional drying. During rainy season, humidity levels are high, sufficient drying cannot be achieved using traditional methods; processed and stored dried fishes reabsorb moisture and become susceptible to insect attack resulting in losses.

#### Smoking

Smoking is one of the traditional fish processing methods aimed at preventing or reducing post-harvest losses. It involves application of heat to remove water which inhibits both bacterial and enzymatic actions (Kumolu-Johnson *et al.*, 2010), giving the product a desirable taste and odor and providing a longer shelf-life. Smoking is one of the preferred methods of preservation because it dries the fish, melts down some fat, and reduces microbial growth. In spite of the later mentioned benefits of smoking fish, smoked fish can be a source of microbial hazards (Salán *et al.*, 2006), this can be attributed to the growing fuel wood crisis, as local processors usually sell products that are not adequately smoked for periods long enough to reduce moisture content that acts as a determinant in microbial infestation to the recommended 10% or lower (Abolagba *et al.*, 2011). It is important to be aware that smoking requires large amounts of wood and can contribute to deforestation and pose healthy hazards to the smokers themselves.

#### Salting

Salting as a method of preserving fish has been used for centuries and in many places around the world such as Asia, Europe, and Latin America and almost throughout Africa. Salting is popular because it is a simple method of preservation, is less costly, and easily performed together with other preservation methods such as drying or smoking (Emere and Dibal (2013). Salting is a traditional method for preservation, involving salting and drying, of fish especially in rural areas (Immaculate *et al.*, 2013). The quality of salted and sun-dried fishes are adversely affected by the occurrence of microorganism (Yam *et al.*, 2015) and as such, determination of microbiological quality of such processed fish from either the market or place where the fish is processed is important in order to guarantee consumer health and hygiene.



**Figure 1:** Map of Southern Province of Zambia showing position of study site

## Materials and Methods

### Study area

Southern Province of Zambia covers an area of 85,823 km<sup>2</sup> and a projected population of 1,853,464 in 2017. The province harbors the second largest reservoir in Africa, by volume, that has made the districts that share its boundaries to be actively engaged in the fishing industry. Lake Kariba constructed in the late 1950s (277 km long; 5,364 km<sup>2</sup>; 160 km<sup>3</sup>; 29 m mean depth and 120 m max. depth) has its catchment area covering 663,817 km<sup>2</sup> extending over parts of Angola, Zambia, Namibia, Botswana and Zimbabwe. The dam wall (128 x 580 m) was completed in 1960 and the filling phase lasted from December 1958 to September 1963 when the water reached the mean operation level at 485 m above mean sea level. The lake is shared by the two riparian countries of Zambia and Zimbabwe with 45% and 55%, respectively. The area along the

lake on the Zambia side is divided into three local authorities namely; Siavonga, Gwembe and Sinazongwe. The study was conducted in Sinazongwe district (see Figure 1), originally built as a fishing and administrative center for the southern lakeshore area and currently mainly used as an outpost for Kapenta (*Limnothrissa miodon*) and bream fishing, characterized by processing of fish using various methods.

### Data collection

Nine fishing villages were selected using a systematic random sampling approach. Processors from each of the selected villages were randomly sampled proportional to zonal totals to give a sample size of 160 processors and fishers who were interviewed using a structured questionnaire to capture perceptions, ideas, opinions, and thoughts. The study adopted a simple two-stage cluster sampling approach. The field survey was conducted between March and July of 2017.

*Data analysis*

Most analyses for this study were run using SPSS and Excel computer software. Descriptive statistics were used for summarizing and presenting data from the survey and analysis for the parameters included means, frequency counts and percentages, which provided a distribution of respondents across the parameters. To investigate effectiveness of processing methods in preventing fish spoilage, freshness was assessed using sensory evaluation, a systematic assessment of the odour, flavour, appearance and texture of product.

**Results**

*Fish processing methods by gender*

Results of the study showed that smoking was widely used as a method of processing among men (at 42%) as compared to salting (23%) and sun-drying (21%), however with the females the opposite is true, smoking

was the least used among the females (at 58%) with sun-drying ranking highest (at 79%) (see Table 1). The study revealed that women are more actively involved in fish processing as compared to their male counterparts.

*Effectiveness of processing method in preventing fish spoilage*

Judgement of level of effectiveness was on the basis of sensory evaluation (odour, flavour, appearance and texture) conducted against a scale of scores from 1 to 3. This was aimed at yielding a co-operative opinion to answering questions. Results of the study revealed that smoking and sun-drying had the highest number of male and female respondents, respectively, that considered it to be excellent in terms of preventing fish spoilage. On the other hand, the three processing methods were viewed to provide 50-79% effective prevention of fish spoilage by both male and female respondents. Salting was viewed to be least effective by male respondents (see Table 2).

**Table 1:** Fish processing methods by gender

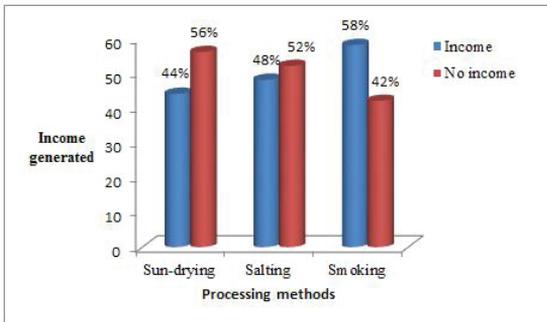
Processing method	Gender	
	Female (%)	Male (%)
Sun-drying	79	21
Salting	77	23
Smoking	58	42

**Table 2:** Majority views over effectiveness in preventing spoilage

Processing method	Average core
<b>Sun-drying</b>	
Female respondents	3
Male respondents	2
<b>Salting</b>	
Female respondents	2
Male respondents	1
<b>Smoking</b>	
Female respondents	2
Male respondents	2

*Income generation by method of processing*

Results in Figure 1 show the percent distribution of respondents that realized some form of revenue from processing fish and those that processed fish for much lower incomes. Income increased progressively from sun-drying (44%) through salting (48%) to smoking (58%). The opposite was true for those that indicated there was no income generated with figures progressive decreasing from sun-drying (56%) through salting (52%) to smoking (42%).



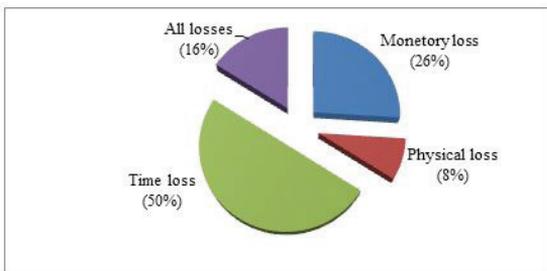
**Figure 1:** Income generation by method of processing

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*Type of losses encountered after processing the fish*

The study revealed that 26% of respondents incurred monetary losses, 8% physical losses, 50% time loss while 16% claimed a combination of losses after they processed their fish (see Figure 2). Monetary loss implies economical loss as a result of reduction in value due to quality loss. Physical loss implies loss of part or of the entire product (e.g. fragmentation during packing and transportation) whereas time loss is physical wastage of valuable time that could have been used for other things.



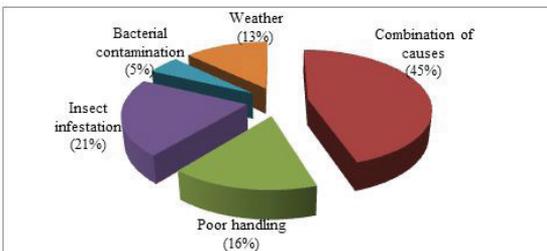
**Figure 2:** Type of losses encountered after processing the fish

*Causes of post-harvest losses*

The study revealed the following causes of fish spoilage; 21% of the respondents indicated insect infestation, 16% poor fish handling, 13% weather, 5% bacterial contamination while 45% a combination of all the aforementioned causes.

**Discussion**

The results of this study agree with findings reported by Mulenga et al. (2015) revealing that women were often responsible for post-harvest activities, such as processing and trading whereas men were more in the extractive processes. In a study conducted on Lake Victoria, Medard et al. (2001) also revealed that men preferred trading in smoked fish products while women preferred sun-dried fish products. Reasons range from cultural norms to the financial status of men and women. Women’s involvement in postharvest activities such as smoking, drying, and marketing is widespread and is regarded as an appropriate activity for women given their domestic tasks



**Figure 3:** Causes of post-harvest losses

and responsibilities. Fishing predominantly involves men and is a common practice throughout the world.

The difference in preference of processing methods between men and women was linked to the amount of input (capital, time, labour, technology, etc.) required in each type of processing method. For instance, low access to credit facilities has been one of the problems that the females face. Low access to bank loans was mentioned among women. This negatively impacts on women in the sense that they usually lack capital to buy boats or invest in more money making sub-sectors of fisheries hence left at the receiving end. Most of these factors favour men - they are physically stronger, they normally have transport (e.g. bicycles) and they have access to loan facilities. Loans from family and friends were the most common form of informal finance, an arrangement characterized by uncollateralized loans that carry little or no interest and featuring open-ended repayment arrangements with a strong focus on reciprocity. This partly explains why most women are associated with sun-dried fish products because they often require a lot of patience, require low capital investment and demand minimal labour.

From olden days, generally smoking of fish has been very common in Africa. The results of the study could be biased in that probably consumers said smoking is effective only because it is widely preferred in the respective communities. This agrees with results reported by Emere and Dibal (2013) that smoke drying is employed by remote fishing communities due to traditional preference of the local people for smoke dried fish and lack of sophisticated preservation techniques. Emere and Dibal (2013) reported that smoking deposits a coating of antimicrobial material or substance on the surface of the fish while at the same time impacting an attractive sheen and pleasant taste which is cherished by the local populace. This study revealed that spoilage causative agents like, flies, insects, molds, fungal bacterial contamination were reported lowest in smoked fish as compared to other processing methods although lack of control over drying

rate sometimes resulted in over-drying or under-drying.

Other reasons why, smoked fish was considered to be more effective in preventing spoilage could be due to the fact that most of the people involved in smoking fish had an aim of selling the fish, meaning that they put more effort in processing the fish so as to elevate its value and to prolong shelf life for transportation to markets in Lusaka and Copperbelt. On the other hand sun-drying and salting were mostly done to preserve fish for home consumption – hence less effort dedicated and resulting in characteristic short shelf life and high rates of spoilage. In all the three methods of processing, there is need to reduce the water activity in products to 0.85 or below if the product will be stored and distributed unrefrigerated in a shelf stable state. For example, salting alone will never completely stop the spoilage of fish - that is why salted fish are dried, stored at low temperatures or treated other ways.

Results revealed that most processors who smoked their fish realized some income from selling their products. This is partly explained by Salán *et al.*, (2006) who stated that smoking is not only a conservation method, but also a flavour-, aroma- and coloration-improving method and that these are attributes desired by consumers. On the other hand, sun-drying of fish was mostly done by women for home consumption.

Getu *et al.*, (2015) describe post-harvest fish loss as fish that is either discarded or sold at a relatively low price because of quality deterioration. Results of this study indicated that 50% of the respondents bemoaned the amount of time they lost in processing fish which ultimately gets spoiled – lamenting that they would have used that valuable time to venture into other enterprises. The lower percent of respondents (8%) claiming physical loss is similar to what was explained by FAO and smart fish project (2013) that poor fishing villages eat almost everything they catch in spite of low quality due to high household food insecurity in such communities. In fact, it was observed that even poor quality fish was processed and consumed locally. Similarly, a

lower percent (26%) claimed monetary losses because most products are sold locally and/or target the low class of consumers in other markets. Losses could be improved through enhanced contact with extension agents, technology adoption, provision of facilities and capacity building.

Insect infestation was observed mainly in sun-dried fish with common insects being blowflies and beetle larvae. While knowledge of bacteria was limited among most respondents, microbial spoilage perpetuated by optimal weather conditions and poor handling undoubtedly leads to huge fish losses. Fish processing was difficult during rainy season in that there is limited sun-light and intensive rainfall regimes, as well as limited access to dry wood for smoking. FAO (2010) reports that higher losses during rainy season are due to the fact that processing of fish becomes difficult and a good fraction of fish gets insect-infested and spoiled. It has also been observed that most drying, salting, and smoking platforms as well as the holding, storage and distribution facilities at village level are not hygienically clean and could harbour lots of bacteria. Huss *et al.*, (2003) observed that more than 80% of post-harvest losses are due to microbes. Post-harvest losses owing to poor infrastructure, storage facilities and transportation as well as a lack of sufficient knowledge of proper and hygienic fish handling affect most of the fish landed. As coping strategy, small-scale fishers and processors require technical assistance and their knowledge base increased in the area of fish handling procedures, storage, product diversification, value addition and packaging thus enhancing obvious economic and social benefits.

### Acknowledgements

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### Conclusion and Recommendations

Lack of affordable technology, facilities and adequate skill in fishing villages and the high rates that are charged by refrigeration service providers, fishers/traders and processors alike have opted to using traditional means of processing fish in order to deter spoilage, evidently viewed to have different degrees of effectiveness. In the lake Kariba fishery and particularly in Sinazongwe area, women are predominantly involved in fish processing and trading of fish. Women's increased involvement in fishing related activities contributes to family income, constant supply of fish/food to improve family nutrition, generation of opportunities for self-employment, uplifting overall socio-economic conditions and improved skill. With urbanization, there is high demand for fish products at urban centres. While this creates an opportunity for processed products, post-harvest losses have still remained a challenge among local level processors employing traditional methods. There is need for women empowerment if they are going to thrive in this industry – offer them credit facilities to provide start-up capital. Post-harvest losses could be improved through enhanced technology adoption, provision of processing facilities and capacity built among them, especially methods that they already using.

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## PRIORITY TRANS-BOUNDARY ANIMAL DISEASES AND ZOOSES AND THEIR PROPOSED CONTROL STRATEGIES FOR DJIBOUTI

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### Abstract

Prioritisation and categorisation of Trans-boundary Animal Diseases and Zoonoses in Djibouti was conducted a period at 21-25 February 2015 in Naivasha, Kenya and later refined in 2016 at Arusha, Tanzania, in collaboration with AU-IBAR and Phylum team. Prioritisation and categorisation tool that was developed by the OIE was used for this exercise. More than about 15 diseases were listed and analysed with various aspects of disease impact. These were: Economic impact, Impact on human health, Societal impact, Environmental impact, Feasibility and Economic impact, Societal & environmental impact. Six disease were identified as priority in Djibouti. These are: PPR and CCPP, regarding the importance of small ruminant in the country, while *Brucellosis* and TB are important zoonotic diseases in Djibouti. Likewise, CBPP and FMD are endemic and important cattle diseases in the country.

**Keywords:** Djibouti, Disease, prioritisation, categorisation, control

## MALADIES ANIMALES TRANSFRONTALIERES ET ZOOSES PRIORITAIRES ET STRATÉGIES DE LUTTE PROPOSÉES POUR DJIBOUTI

### Résumé

La priorisation et la catégorisation des maladies animales transfrontières et des zoonoses à Djibouti ont été effectuées du 21 au 25 février 2015 à Naivasha au Kenya et améliorées en 2016 à Arusha en Tanzanie, en collaboration avec l'UA-BIRA et l'équipe Phylum. L'outil de priorisation et de catégorisation développé par l'OIE a été utilisé pour cette activité. Plus de 15 maladies environ ont été répertoriées et analysées pour divers aspects de l'impact des maladies. Il s'agissait de l'impact économique, l'impact sur la santé humaine, l'impact sociétal, l'impact environnemental, la faisabilité et l'impact économique, l'impact sociétal et environnemental. Six maladies ont été identifiées comme prioritaires à Djibouti. Il s'agit de la PPR et de la PPCC compte tenu de l'importance des petits ruminants dans le pays ; et la brucellose et la TB sont des zoonoses importantes à Djibouti. De même, la péripneumonie contagieuse bovine (PPCB) et la fièvre aphteuse sont des maladies bovines endémiques et importantes dans ce pays.

**Mots-clés :** Djibouti, maladie, priorisation, catégorisation, contrôle

## Introduction

Djibouti has a significant animal populations composed of Caprines 600000, Ovines 400000, Bovines 40000 Camelines 50000, Equines 8600 (OIE, 2014). Livestock plays several roles for its products, income in society as well as being a capital and a savings for the future. The livestock-related products play an important role in food security. Livestock provides employment for about 150,000 people including pastoralists, agro-pastoral, livestock traders, butchers and related activities (DESV, 2013).

Several studies have been carried out in Djibouti on *Brucellosis* revealing 1% prevalence (Hasna.2013). Another study revealed 42.2% prevalence of toxoplasmosis and 6.5% prevalence of *Brucellosis* in humans, but RVF and Q fever were absent. Prevalence of toxoplasmosis in bovines was 2,8%, in ovines was 9.8% and in caprine was 6.4%. Likewise, the prevalence of *Echinococcus haydatosis* in bovine was 4.4%, in ovine was 12%, in caprine was 9,6 % (Chantal *et al.*, 1996). *Echinococcus granulosus* was also reported as the present disease in Djibouti in animal in slaughter houses (OIE, 2104).

First prioritisation of diseases in Djibouti was done in 2003 and indicated that: *Avian Influenza* (HPAI), PPR, RVF, and *Brucellosis* were top list. Additionally, for the exportation, the animals have to be vaccinated against the RVF during the period of the quarantine. The Prioritisation of diseases at national level has been updated which include *Brucellosis* at top list and that hinders animal production and productivity as well as the exportation of live animals as it is a zoonotic disease. PPR and CCPP are important because small ruminant dominate 80% of livestock population. In addition CBPP and FMD are endemic and also important diseases of country.

Djibouti is a small country with few animals. However, this is a strategic country for the region as the gate of the Middle East market for livestock. This is why it is important to maintain its relatively good sanitary status when a high flow of animals arriving from various

areas in the region dose cross the country to be exported. That is why this exercise of prioritisation and categoriation of diseases in collaboration with AU-IBAR and Phylum team was implemented. More than 15 diseases were selected and studied between first 21-25 February 2015 in Naivasha Kenya, and later in 2016, at Arusha Tanzania. Six diseases were identified as priority in Djibouti, these are: PPR, CCPP, *Brucellosis*, TB, CBPP and FMD.

The main objectives this study were:

- i. to select and identify the characteristics of animal diseases and zoonoses,
- ii. to prioritise diseases ,
- iii. to identify possible gaps in terms of disease knowledge.
- iv. to create awareness of the importance of the priority diseases for better planning and implementation of control measures

## Material and Methodology

### Study area

The Republic of Djibouti is located in the Horn of Africa. It is bordered by Ethiopia in the west and south, Eritrea in the north, and Somalia in the southeast. The remainder of the border is formed by the Red Sea and the Gulf of Aden in the east. It extends between latitudes 10° and 13°N, and longitudes 41° and 44°E. The total land area is about 23200km<sup>2</sup>. The population is estimated over 900,000 inhabitants. The population is growing at a rate of 3 per cent per year. Two-thirds of the population resides in Djibouti city and some other larger towns in the country. Sedentary farming associated with agriculture has developed around urban centers. The farms supply cities with milk and other animal products (DESV, 2013). French and Arabic are official languages in country. Djibouti's climate ranges from arid in the northeastern coastal regions to semiarid in the central, northern, western and southern parts of the country. Annual rainfall is less than 5 inches (131 mm); in the central highlands, precipitation is about 8 to 11 inches (200 to 300 mm). The country has two seasons' wet season (Oct- April) and dry



**Figure 1:** Map of Djibouti (source, Google, 2016)

season (May-September). The annual average maximum and minimum temperature ranges of the country are 39-42°C and 21-25°C.

#### Methodology

More than 15 diseases most important in region were listed and the Phylum tool applied to prioritisation, categorise and analyse them with regard to: Economic impact, Impact on human health, Societal impact, Environmental impact, Feasibility and Economic impact Societal & environmental impact. Score for data analysis was undertaken for final ranking.

### Result

#### Prioritisation based on each group of impact

Six diseases were identified as priority in Djibouti. These are *Brucellosis*, PPR, CCP, CBPP, TB and FMD

PPR and CCP are priority diseases due to the importance of small ruminants (80%) in the country. *Brucellosis* is zoonotic disease that hinders trade in live animal and their products; and long-term treatment may be required for human as well as there are positive cases in humans and animals. CBPP is also an important disease for the country that could justify vaccination campaigns Due to the

sensitive impact on trade, the capacity of early detection and rapid response in case of FMD should be strengthened; even the economic impact seems to be moderate and not still reported at national.

### Discussion

Nationally *Brucellosis* has a prevalence of 1% (Hasna.2013, SIS, 2013) in animals and also reported in humans. The department of livestock and veterinary service has been carrying out campaign for vaccination of *Brucellosis* in small ruminant, likewise stakeholder awareness has been done (DESV, 2008); Vaccination campaign to protect the animals and better coordination with Public Health services are needed to create of awareness especially in nomadic populations. *Tuberculosis* is also considered as an important zoonotic disease. However, the priority should be on a better coordination with Public Health Services to determine the real importance of bovine *Tuberculosis* on humans and develop a coordinated response in such case. The real prevalence should be assessed.

According by Phylum tool in Naivasha, that include study many aspect of each disease regard to the Economic impact the *Brucellosis*

had the highest score that could have been due to its categorisation because the *Brucellosis* was identified as a priority disease at national level due to impact on animal production and their productivity as well as live animal exportation and its zoonotic importance. This was followed by CBPP, TB, CCPP, PPR, respectively. Regarding human health Impact the TB had the highest score to be considered as an important zoonotic disease. However, the priority should be on better coordination with Public Health Services to determine the real importance of bovine *Tuberculosis* on humans and develop a coordinated response in such case. Its real prevalence should be. With regard to the Societal impact the order of preference was like for human health Impact. With regard to the Environmental impact the CCPP, PPR, CBPP, *Brucellosis* and TB, respectively, were ranked

in that order, because PPR and CCP are high score as a priority disease at national level due to impact on small ruminant production that is covers 80% of livestock population. Regarding to Feasibility of Control Measures the LSD, TB, PPR, CBPP and *Brucellosis* had scores as respectively. Regarding the Economic impact of Control Measures the TB, PPR, CBPP, LSD and *Brucellosis* ranked in that order respectively and regarding the Societal and Environmental Impact of Control Measures the LSD, PPR, TB, CBPP, CCPP and *Brucellosis*. All scores were used for data analysis and consequently, *Brucellosis* ranking higher highest this corroborates the results of the previous observation.

Foot and Mouth disease is an endemic but important disease, due to its impact on export trade

**Table I:** ranking of disease according to Economic impact

Impact	Rank	Diseases	Justification
Economic impact	1	<i>Brucellosis</i>	hinder trade live animal and their production, long-term treatment may be required as well as there are positive cases in human and animal
	2	CBPP	disrupt livestock/product trade high mortality Reduce output. increase production costs due to costs of disease control inhibit sustained investment in livestock production
	3	TB	Public health ,production lost
	4	PPR	disrupt livestock and their product trade, reduce production of animal (goat) high mortality
	5	CCPP	disrupt livestock and their product trade, reduce production of animal (goat) high mortality
	6	FMD	

**Table2:** Ranking of disease according to Human Health impact

Impact	Rank	Diseases	Justification
Human Health impact	1	TB	2000 -3000 cases per year according WHO reports in Djibouti
	2	<i>Brucellosis</i>	Livestock trade ,Public health , reported some cases in human
	3	PPR	Not serious disease in human
	4	CCPP	Not serious disease in human
	5	CBPP	Not serious disease in human
	6	LSD	Not serious disease in human

**Table3:** Ranking of disease according to societal impact

Impact	Rank	Diseases	Justification
Societal impact	1	TB	animal and animal production lost, transmissible from animal to human by food borne as well as air-borne disease
	2	<i>Brucellosis</i>	raw milk consumed preferably, as well as there are positive cases in human and animal in country
	3	PPR	small ruminant include 80% of livestock in country, loss huge number of goat may cause poverty of owners (stockholders)
	4	CBPP	animal and animal production lost, that cause poverty of farmers, as well as in of societal
	5	CCPP	small ruminant include 80% of livestock in country may cause poverty of owners (stackholders) high mortality in goat
	6	LSD	Loss animal production

**Table4:** Ranking of disease according to Environmental impact

Impact	Rank	Diseases	Justification
Societal impact	1	CCPP	Since small ruminant include 80% of livestock in country, high mortality may involve to contaminate in environment
	2	CBPP	dead animal secretion may contaminate in environment
	3	PPR	small ruminant include 80% of livestock in country, dead animal secretion may contaminate in environment
	4	<i>Brucellosis</i>	May contaminate the environment
	5	TB	May contaminate the environment

**Table 5:** Ranking of disease according to Feasibility of Control Measures

Impact	Rank	Diseases	Justification
Feasibility of Control Measures	1	LSD	Vaccines and pesticides are available to control the virus and the vectors
	2	TB	Affects social cohesion due to its infectiousness and long periods of treatment and
	3	PPR	vaccination and animal movement control difficult
	4	CBPP	Treatment and vaccination
	5	<i>Brucellosis</i>	Treatment and vaccination
	6	CCPP	Treatment and vaccination

**Table 6:** Ranking of disease according to Economic impact of Control Measures

Impact	Rank	Diseases	Justification
Economic impact of Control Measures	1	TB	Cost for treatment, difficult for resource financial
	2	PPR	Cost for vaccination, difficult for resource financial
	3	CBPP	Cost for treatment
	4	LSD	Cost for treatment and pesticide
	5	<i>Brucellosis</i>	Cost Treatment and vaccination
	6	FMD	

**Table 7:** Ranking of disease according to Societal and Environmental Impact of Control Measures

Impact	Rank	Diseases	Justification
Economic impact of Control Measures	1	LSD	
	2	PPR	increase production costs due to costs of disease control include vaccination
	3	TB	increase production costs due to costs of disease control include vaccination and treatment
	4	CBPP	increase production costs due to costs of disease control that including vaccination and treatment
	5	CCPP	increase production costs due to costs of disease control that including vaccination and treatment
	6	<i>Brucellosis</i>	increase production costs due to costs of disease control that including vaccination and treatment
	7	FMD	As endemic disease in neighbor countries

**Table 8:** Final proposed prioritisation

Disease	Rank	Justifications	Proposed Control strategy
<i>Brucellosis</i>	1	Hinders trade live animal and their production, long-term treatment may be required for human as well as there are positive cases in human and animal	Vaccination in animal and treatment in human and restrict animal movement Awareness in population
TB	2	according WHO in Djibouti 2000 -3000 cases per year but unknown in animal population.	Culling all animal positive , Awareness for public to raw products, vaccination for all

Disease	Rank	Justifications	Proposed Control strategy
PPR	3	Small ruminants dominate 80% of livestock in country, loss of huge number of goat & sheep may cause poverty of owners (stockholders)	Develop Vaccination program and create awareness for stockholders
CCPP	4	Disrupts livestock production and their products high mortality in goat and can cause pain and suffering to animals (Goat)	Vaccination and mass treatment campaign
CBPP	5	Increase production costs due to costs of disease control, inhibit sustained investment in livestock production.	Vaccination and mass treatment campaign
FMD	6	Is endemic in region	Vaccination

### Conclusion

This was a successful prioritisation and categorisation exercise at Naivasha and later at Arusha, using the Phylum tool, with information collected from the department of livestock and veterinary service and national public health that facilitated to rank disease in order according to all aspect of impacts.

The first prioritisation exercise of animal diseases was been done in 2003 on some diseases namely: Highly Pathogenic Avian InfluenzaA (HPAI), PPR, CBPP, CCPP, RVF, FMD, Sheep and Goat Pox, Camel pox, LSD, and *Brucellosis*. This exercise therefore updates the effort of 2003 and shows that the current the Priority diseases of Djibouti are, PPR, CBPP, CCPP, FMD, TB and *Brucellosis*.

Importance of this prioritisation of diseases is that it can lead to rational use of funds and concerned authorities will consider in designing control programmes, based on known characteristics of a disease, and create awareness in communities then to identify possible gaps in terms of disease knowledge.

National action plans should be developed based on the priority diseases and institute actions like surveillance (passive, active. ect.), control measure (vaccination and strengthen border controls, awareness in communities, harmonisation and coordination within the IGAD region.

### Conflict of interest

There was no conflict of interest identified.

### Ethical standards

The study involved review of secondary data and reports generated during routine animal and human disease surveillance. There was no direct contact with human and animal subjects. Because this was part of the routine surveillance activities which are exempted from Institutional Review Boards. The study has many benefits to the human population and to the animals by guiding policy and resource allocation for prevention and control of the diseases.

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## PRIORITISATION OF TRANSBOUNDARY ANIMAL DISEASES AND ZONOSSES FOR EFFECTIVE CONTROL IN BURUNDI

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### Abstract

Livestock plays key role in the national economy of Burundi. However, this sector has been suffering of ineffective and insufficient control of transboundary animal diseases and zoonoses. This study aimed to prioritise and categorise the principal transboundary animal diseases and zoonoses to consider in developing sustainable policies and strategies to be applied in diseases control and eradication in Burundi. A list of 23 diseases documented in national reports from the Ministry of Agriculture and Ministry of Public Health were involved in the study. The PHYLUM-OIE tool for prioritisation and categorisation of transboundary animal diseases and zoonoses was applied and critical parameters enclosed in the tool were strictly considered and analyzed. As results, two major categories of priority diseases assorted: (1) the present diseases indicated ten diseases ranked as follow: NCD, ASF, LSD, FMD, CBPP, *Porcine Cysticercosis*, BTB, *Brucellosis*, *Swine Erysipelas* and Rabies respectively; (2) the absent or uncertain diseases listed seven priority diseases ranking as follow: RVF, CSF, PRRS, S&G Pox, PPR, HPAI and CCP, respectively. This study is of great interest and will serve as good reference for the decision makers to review the investment allocated to this sector. In another hand, the results from this study would serve as baseline to the technical services to state or update the strategic documents regarding the control of transboundary animal diseases and zoonoses in Burundi.

**Key words:** prioritisation, transboundary Animal diseases, zoonoses.

## PRIORISATION DES MALADIES ANIMALES TRANSFRONTALIERES ET DES ZONOSSES POUR LEUR CONTROLE EFFICACE AU BURUNDI

### Résumé

L'élevage joue un rôle majeur dans l'économie nationale du Burundi. Cependant, ce secteur souffre de l'inefficacité et de l'insuffisance des mesures de contrôle des maladies animales transfrontières et des zoonoses. Cette étude avait pour objectif de classer par ordre de priorité et en catégories les principales maladies animales transfrontières et zoonoses à prendre en considération dans l'élaboration de politiques et stratégies durables à appliquer dans le contrôle et l'éradication des maladies au Burundi. Une liste de 23 maladies documentées dans les rapports nationaux du Ministère de l'Agriculture et du Ministère de la Santé Publique a été utilisée dans l'étude. L'outil PHYLUM-OIE de priorisation et de catégorisation des maladies animales transfrontières et des zoonoses a été utilisé et les paramètres critiques inclus dans l'outil ont été strictement pris en compte et analysés. Ainsi, deux grandes catégories de maladies prioritaires ont été identifiées : respectivement (1) dix (10) maladies actuelles, à savoir la MNC, la PPA, la DNC, la FA, la PPCB, la cysticercose porcine, la TB bovine, la brucellose, l'érysipèle porcine et la rage ; et (2) les maladies absentes ou incertaines énumérées - sept maladies prioritaires classées comme suit : la FVR, la PPC, le syndrome dysgénésique et respiratoire du porc - SDRP, la clavelée du mouton et la variole caprine, la PPR, l'IAHP et la PPCC. Cette étude est d'un grand intérêt et servira de bonne référence aux décideurs lors de l'examen des investissements alloués à ce secteur. D'un autre côté, les résultats de cette étude serviront de référence aux services techniques lors de l'élaboration ou de la mise à jour des documents stratégiques concernant le contrôle des maladies animales transfrontalières et des zoonoses au Burundi.

**Mots-clés :** priorisation, maladies animales transfrontalières, zoonoses.

## Introduction

The contribution of livestock in food security is very important in Burundi (14% of the National GDP and 29% of the Agricultural GDP, Document d'Orientation Strategique de l'Elevage (2010). The population of livestock in Burundi is mainly composed of: cattle (777786), goat (2489304), sheep (352722), pig (434204) and poultry (2705000), Direction Generale de l'Elevage (2014). However, the performance of livestock still very weak due to the persistent epizootic and zoonosis outbreaks leading to enormous economic loss at all levels. An epidemiological survey conducted in 2015 at the National Veterinary Laboratory showed high prevalence of bovine *Brucellosis* (10.2%), bovine *Tuberculosis* (9%), East Coast Fever (30.4%) and African swine fever (8.3%) for all investigated population. The competition ELISA tested a very high carriage of Foot and Mouth Disease in cattle (86.6%), while the samples tested in case of suspicions of Newcastle Disease from rural unvaccinated chickens all tested positive (National Veterinary Laboratory, Annual report (2015). This situation constitutes a barrier of increased animal production and productivity conducting to the incapability to cover public need in quantity and quality of the animal origin proteins. Consequently, the above situation negatively influenced food security and constitutes a high risk of public health.

The Ministry of Agriculture and Livestock via its Department of Veterinary Services, has however initiated animal diseases control programmes through mass vaccination and passive surveillance of principal animal diseases such as Foot and Mouth Disease (FMD), Lump Skin Disease (LSD), *Brucellosis* and Anthrax for bovine, Newcastle Disease (ND) for poultry and Rabies.

Although these efforts have been undertaken, no previous scientific studies have been performed to prove the epidemiological impact of these principal animal diseases and zoonoses. The lack of contingency plans and diseases control strategies followed by poor performances of the veterinary services are big challenges. The capacity of laboratory diagnostic

for rapid detection and diseases confirmation is very weak. From the above information, we can learn that the field of efficient diseases control is still weak for the case in Burundi. As documented from veterinary services office, the financial input into animal diseases control is largely insignificant. This is illustrated by the following observations: in 2012, 4.2% and 6.5% of cattle were vaccinated against Bovine *Brucellosis* and Lump Skin Disease, respectively; in 2013, 5.1% and 30% of cattle were vaccinated against FMD and LSD respectively, while 78.6% of rural chicken were vaccinated against Newcastle disease. In 2015, 6.1% and 14.7% of cattle were vaccinated against FMD and LSD respectively and 64.5% of rural chickens vaccinated against ND (Directorate of Animal Health, Annual reports, 2012, 2013, 2015). In contrast, in year 2014, no vaccination campaign was conducted. Therefore, from the above information, it can be seen that the coverage of mass vaccination currently ongoing, is not adequately enough to control or eradicate any of the diseases in Burundi. The objective of this study therefore, was to prioritise and categorise TADs and zoonoses in order to develop sustainable policies and strategies for better planning to enable effective and efficient diseases control and eradication in Burundi.

## Material and Methods

### *Study areas: Burundi*

Burundi is a small (27834 Km<sup>2</sup>) landlocked country located in central Africa, neighboring with Rwanda in the North, Tanzania in South-East and Democratic Republic of Congo in the West. Burundi is one of the high populated countries accounting for about 10 million people (375 inhabitants/Km<sup>2</sup>). Livestock is mainly based on subsistence system distributed in five different agro-ecologic zones: The Imbo, Mumirwa, crête Congo-Nil, Central High Lands Basin and the Depression of Kumoso and Bugesera (MOA, Strategie Agricole Nationale, SAN, 2012.) . The distribution of livestock is as follows: cattle in South-East part of the country with rich pasture; goats, sheep and poultry are distributed in the



**Figure 1:** The map of Burundi (Source, Google 2016)

whole country; and pigs in the most densely populated areas of North-Central parts of the country with limited access to land.

*Methods*

Data used in this study were documented from different reports on TDAs and zoonoses reported by the Ministry of Agriculture and Livestock and the Ministry in charge of Public Health in Burundi (MOA & MOH, 2014). Prioritisation and categorisation was conducted using a prioritisation tool for Transboundary Animal Diseases and Zoonoses developed by PHYLUM (Phylum, OIE, WORLD BANK, European Union, Kigali workshop, 2015 ). A set of criteria enclosed in the tool such as: risk of introduction, local economic impact, local public health impact, local environmental impact, local control measures feasibility, economic impact of local measure control were involved in the study . Data analysis was undertaken using scores resorted by the tool for each criterion involved and for

each criterion, diseases scoring in the range of  $\geq 3$  to 10 were considered as priority. Two principal categories of classified diseases were (1) present diseases and (2) exotic or absent diseases.

**Results**

Out of twenty three animal diseases involved in this study, seventeen had scores ranging between  $\geq 3$  to 10 forming for at least four of the seven criteria analysed. The seventeen diseases with accepted scores were divided into two groups on present and absent or uncertain diseases, respectively. Below are detailed ranking characteristics for each category regarding particular each criterion analysed.

Analysis according to the local economic impact criterion of assorted nine, the following ranking was realised in order of priority: Newcastle Diseases(ND) ranked on top and ASF, LSD, FMD, *Porcine Cysticercosis*,

*Tuberculosis, Brucellosis, Swine Erysipelas* and *Rabies* (Table 1). According to the Public Health impact analysis, four diseases were listed, among them: *Rabies* had a high score. Other diseases ranked for this according to this criterion, were: *Tuberculosis, Brucellosis* and *Porcine Cysticercosis* (Table 2).

Considering the criterion according to societal impact analysis, among the eight diseases listed: *Bovine Tuberculosis* had the highest score, followed by *Rabies, Swine Erysipelas, CBPP, ND, Brucellosis, Porcine Cysticercosis* and lastly, *LSD* (Table 3). Based on the local environmental impact criterion, the African swine fever ranked first followed by *Rabies, FMD* and *ND* (Table 4). While based on the local feasibility impact criterion, *ASF* ranked first followed by *FMD, LSD, Tuberculosis* and *Rabies* (Table 5). The *ASF* ranked first among the six diseases listed in the table taking into account the local economic impact of the

control measures. Note that *CBPP, LSD, ND, Brucellosis* and *FMD* were also included in this criterion (Table 6). Considering the criterion on Societal and environmental impact of the control measures among the six diseases listed, *NCD* had the highest score, followed by *ASF, LSD, Tuberculosis, FMD* and *Porcine Cysticercosis* (Table 7).

Ranking according to all criteria for the ten present priority diseases listed, *ND* ranked on top followed by *ASF, LSD, FMD, CBPP, Porcine Cysticercosis, Tuberculosis, Brucellosis, Swine Erysipelas* and *Rabies* (Table 8); whereas for the absent or uncertain diseases involved in the study, seven of them fulfilled the criteria and were therefore prioritised. Those are: *Rift Valley Fever* ranked on top followed by *CSF, PRRS, S&G Pox, PPR, HPAI* and *CCPP*, respectively (Table 9).

**Table 1:** Local Economic impact analysis

Ranks	Disease	Score
1	Newcastle disease (ND)	10
2	African Swine fever (ASF)	9,5
3	Lump skin disease (LSD)	8,4
4	Foot and mouth disease (FMD)	8,2
5	<i>Porcine Cysticercosis</i>	6,5
6	<i>Tuberculosis</i>	5,8
7	<i>Brucellosis</i>	5,7
8	<i>Swine Erysipelas</i>	5,5
9	<i>Rabies</i>	5,3

Local economic impact analysis listing nine priority diseases was assorted fulfilling criteria and scores: Newcastle Diseases ranked on top and *ASF, LSD, FMD, Porcine Cysticercosis, Tuberculosis, Brucellosis, Swine Erysipelas* and *Rabies*.

**Table 2:** Public health impact analysis

Ranks	Disease	Score
1	<i>Rabies</i>	10
2	<i>Tuberculosis</i>	7,5
3	<i>Brucellosis</i>	6,3
4	<i>Porcine Cysticercosis</i>	4,9

Analysis of the Public Health impact assorted four diseases, among them *Rabies* had a high score. Other diseases involved were: *Tuberculosis, Brucellosis* and *Porcine Cysticercosis*.

**Table 3:** Local societal impact analysis

Ranks	Disease	Score
1	<i>Tuberculosis</i>	8,6
2	Rabies	8,1
3	<i>Swine Erysipelas</i>	7,8
4	Contagious bovine pleuropneumonia (CBPP)	7,3
5	Newcastle disease (ND)	7,2
6	<i>Brucellosis</i>	7,0
7	<i>Porcine Cysticercosis</i>	7,0
8	Lump skin disease (LSD)	6,9

Criteria based on societal impact analysis, among the eight diseases listed Tuberculosis had a high score, followed by Rabies, Swine Erysipelas, CBPP, ND, Brucellosis, Porcine Cysticercosis and LSD.

**Table 4:** Local environmental impact analysis

Ranks	Disease	Score
1	African swine fever (ASF)	8,75
2	Rabies	6,25
3	Foot and mouth disease (FMD)	5
4	Newcastle disease (ND)	3,75

From the local environmental impact aspect, the African swine fever ranked first followed by Rabies, FMD and Newcastle disease.

**Table 5:** Local feasibility impact of control measures analysis

Ranks	Disease	Score
1	African swine fever (ASF)	10
2	Foot and mouth disease (FMD)	9,42
3	Lump skin disease (LSD)	8,83
4	<i>Tuberculosis</i>	8,54
5	Rabies	8,04

The local feasibility impact of control measures analysis resulted in ASF occupying the first place followed by FMD, LSD, Tuberculosis and Rabies.

**Table 6:** Local economic impact of the control measures analysis

Ranks	Disease	Score
1	African swine fever (ASF)	8,46
2	Contagious bovine pleuropneumonia (CBPP)	8,46
3	Lump skin disease (LSD)	7,69
4	Newcastle disease (ND)	6,9
5	<i>Brucellosis</i>	6,9
6	Foot and mouth disease (FMD)	6,15

The ASF ranked first among the six diseases listed in the table taking into account the local economic impact of the control measures, followed by CBPP, LSD, ND, Brucellosis and FMD.

**Table 7:** Societal and environmental impact of the control measures analysis

Ranks	Disease	Score
1	Newcastle disease (ND)	10
2	African swine fever (ASF)	10
3	Lump skin disease (LSD)	10
4	Tuberculosis	7,85
5	Foot and mouth disease (FMD)	7,14
6	Porcine Cysticercosis	5,71

Societal and environmental impact of the control measures analysis outlined six priority diseases among them ND had a high score, followed by ASF, LSD, BTB, FMD and Porcine Cysticercosis.

**Table 8:** Overall of present priority Transboundary Diseases and Zoonoses in Burundi

Ranks	Disease	Score
1	Newcastle disease (ND)	10
2	African swine fever (ASF)	9,5
3	Lump skin disease (LSD)	8,4
4	Foot and mouth disease (FMD)	8,2
5	Contagious bovine pleuropneumonia (CBPP)	7,3
6	Porcine Cysticercosis	6,5
7	Tuberculosis	5,8
8	Brucellosis	5,7
9	Swine Erysipelas	5,5
10	Rabies	5,3

The overall all criteria analysed listed ten present priority diseases: Newcastle Disease ranking on top and followed by African Swine Fever, Lumpy Skin Disease, Foot and Mouth Disease, Contagious Bovine Pleuropneumonia, Porcine Cysticercosis, Bovine Tuberculosis, Brucellosis, Swine Erysipelas and Rabies, respectively.

**Table 9:** Overall of absent or uncertain priority transboundary diseases and zoonosis in Burundi.

Ranks	Disease	Score
1	Rift valley fever (RVF)	10
2	Classical swine fever (CSF)	8.7
3	Porcine reproduction and respiratory syndrome (PRRS)	8.7
4	Sheep and Goat pox (S&G Pox)	7.7
5	Peste des Petits Ruminants (PPR)	6.3
6	High pathogenic Avian Influenza (HPAI)	6.2
7	Contagious caprine pleuropneumonia (CCPP)	3.75

The overall of all criteria analyzed listed seven absent priority diseases scoring in the following order: RVF was top followed by CSF, PRRS, S&G Pox, PPR, HPAI and CCPP.

## Discussion

From all criteria used by the tool, of the list of prioritised present diseases, ND ranked on top followed by ASF, LSD, FMD, CBPP, Porcine

Cysticercosis, Tuberculosis, Brucellosis, Swine Erysipelas and Rabies, respectively. According to Directorate Annual Reports (Directorate of Animal Health, Annual reports, 2012, 2013, 2015), ND ranked first probably because it is

endemic in the whole country and it's reported every year in several areas causing significant loss especially in rural smallholder poultry keepers. African Swine Fever (ASF) ranked second and this position suites the field reality as it has been reported almost every year and that about 80% of pigs' died in affected areas. Lump Skin Disease (LSD) ranked third. This is explained by the fact that outbreaks have a certain periodicity and occur at least once in two years in one or more of the regions in the country. LSD has seriously affected commercial and smallholders farms due to ineffective control measures for restricting animal movement. Foot and Mouth Disease (FMD) ranked fourth. Outbreaks are reported in one or several regions every year and hinder movement of live animals and their products to local and regional markets due quarantine restrictions. High expense of vaccines, limited access to supply of vaccines and short duration of immunity has led to unsuccessful control of FMD in Burundi. *Porcine Cysticercosis* is an important zoonosis causing serious public health problems and economic loss due to human infections and the condemnations of pig carcasses at the abattoirs, respectively. Investigations conducted in the Province of Kayanza on 98 patients, 40 tested positive and 25 of them presented a neuro *Cysticercosis* (Nzisabira *et al.*, 1992). *Cysticercosis* has been proved as risk of epilepsy in Burundi (Nsengiyumva, *et al.*, 2003). *Cysticercosis* as a major risk factor for epilepsy in Burundi, east Africa (Nsengiyumva *et al.*, 2003). In this list, *Tuberculosis* ranked at position six; the position explained by the results of a recent epidemiological survey which showed that about 10% of cattle concerned by the survey were positive ( National Veterinary Laboratory, Annual report, 2015.). These result correlate with the previous study in which one out of 46 healthy animals and 14 out of 36 *Tuberculosis* suspected animals yielded *M. bovis* isolates concluding that Bovine *Tuberculosis* remained endemic in Burundi (Rigouts *et al.*, 1996). The DNA restriction fragment typing was used in the differentiation of *Mycobacterium Tuberculosis* complex isolates from animals and humans in Burundi ( Rigouts, 1996). Bovine *Brucellosis* is

endemic in Burundi as survey conducted on *Brucellosis* in human, the aggregate prevalence of positive serology was 6.6%; such a prevalence was significantly higher in professionally at risk people (7.9%) than in people contaminated by food (3.5%), ( Laroche *et al.*, 1987). Although there is no recent objective study conducted on this disease in cattle, the routine field surveillance shows high prevalence of *Brucellosis* carriers in most of herds assessed meaning that the disease stills a priority for control as it causes both economic loss and public health threat. Although Rabies was not calamity in Burundi (Masabo and Siniremera, 2000), it remained endemic in Burundi as, thousands of people are bitten by dogs. Therefore it was recommended that use of anti-rabies vaccines (National Veterinary Laboratory, Annual report, 2015.) be scaled up because the disease causes economic loss to the Government. Swine Erysipeas has been investigated based on clinical symptoms and bacterial culture showing that the disease is present in the country and sometimes confusing the infection with African swine fever. A serological survey conducted on CBPP revealed the absence of the disease in all animals tested (National Veterinary Laboratory, Annual report, 2015.), however, the country stays at very high risk of introduction as there are regular importation of cattle from Tanzania and Uganda, where the disease has been reported.

On the other side, seven diseases were prioritised as absent or of uncertain occurrence. Of these, RVF ranked top followed by CSF, PRRS, Sheep and Goat pox, PPR, HPAI and CCPP, respectively. Although these diseases remained unknown or ignored in Burundi, there is evidence that the country is at high risk of introduction. This is due to the geographical situation of the country and the continued illegal and/or uncontrolled transboundary animal trade and movements. Currently, a significant number of cattle, goats, pigs and chicken are continually introduced from Uganda, Tanzania, Kenya and Rwanda for breeding or for slaughter (MOA, Annual report, 2014.), which could introduce some of the absent diseases . It was observed that Tanzania

is among the countries that experienced a number of RVF outbreaks (FAO/OAU/IBAR/UNDP, 2001.) and the disease re-emerged in 2006/ 2007 (Sindato *et al.*, 2011). In a study conducted on seroprevalence of PPR in Kasulu, Kibondo and Kigoma rural, three districts neighboring with Burundi and DRC proved the presence of PPR in these regions and recommended joint efforts in controlling this disease (Nkangagat, 2014). Previously, Burundi and DRC were cited among the countries in which PPR had not been reported (FAO/OMS/OIE 1985, 1986, 1987, 1988.). However, in recent years the disease has spread southwards in Africa, as far as southern Tanzania and the Democratic Republic of Congo and Angola (FAO, 2013.), showing that Burundi is at high risk of its introduction. In 2011, a Sheep and Goat pox- like disease affected more than 140 goats imported and distributed to vulnerable families in the north of Burundi. Unfortunately no confirmatory diagnostic testing was conducted before all the animals got destroyed. Although it was recommended thereafter that all imported or newly introduced goats and sheep be vaccinated against goat pox Virus, the risk of introduction still remains high due to the permeability of the borders.

### **Conclusion**

The use of PHYLUM-OIE tool for prioritisation and categorisation was capable to classify the animal diseases into two groups, namely: existing and/or at high risk of introduction diseases in Burundi. Of the twenty three diseases listed, this exercise assorted a list of seventeen priority diseases among which ten are well known or endemic diseases and seven are on high risk of introduction. The findings in this study are credible considering the situation in the field and could serve as reference for decision and policy makers to establish sustainable policy and strategies to efficiently control and eradicate these diseases in Burundi. However, more scientific based epidemiological studies on the prioritised diseases could be regularly carried out to further provide scientific proof associated

with the field situation and the current ranking reported here with.

### **Conflict of interest**

There is no conflict of interest identified.

### **Ethical standards**

The study involved review of secondary data and reports generated during routine animal and human disease surveillance. There was no direct contact with human and animal subjects. Because this was part of the routine surveillance activities which are exempted from Institutional Review Boards (IRBs) and National Council of Science and Technology (NCST) permission to conduct the study was not sought. The study has many benefits to the human population and to the animal by guiding policy and resource allocation for prevention and control of the diseases.

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## **RRIORITISATION AND CONTROL STRATEGIES OF TRANSBOUNDRY AND ZONOTIC ANIMAL DISEASES IN ERITREA**

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### **Abstract**

Disease prioritisation for Eritrea was carried out in Naivasha Kenya, from 4th to 10th July, 2015 with the objective of strengthening the ongoing disease control strategies. The method employed for the prioritisation was the PHYLUM OIE disease prioritisation tool and the main diseases prioritised were PPR, FMD, Sheep and goat pox, NCD, *Brucellosis*, *Tuberculosis* and Rabies. To sustainably updating the prioritised diseases the existing epidemiological data are found not enough. Hence, updating the surveillance systems and methods of collecting epidemiological information is required through the provision of training to the responsible staffs of the veterinary services and stakeholders.

**Key words:** Prioritisation, Transboundry, Zoonoses, Animal diseases, control strategy, Eritrea

## **PRIORISATION ET STRATEGIES DE CONTROLE DES MALADIES TRANSFRONTALIERES ET DES ZONOSSES EN ERYTHREE**

### **Résumé**

La priorisation des maladies pour l'Érythrée a été effectuée à Naivasha au Kenya du 4 au 10 juillet 2015, dans le but de renforcer les stratégies de contrôle des maladies en cours. La méthode utilisée pour la priorisation des maladies était l'outil PHYLUM-OIE, et les principales maladies définies comme prioritaires étaient la fièvre aphteuse, la variole ovine et caprine, les maladies non transmissibles, la brucellose, la tuberculose et la rage. Pour une mise à jour durable des maladies prioritaires, les données épidémiologiques existantes ne sont pas suffisantes. Par conséquent, la mise à jour des systèmes de surveillance et des méthodes de collecte d'informations épidémiologiques est nécessaire, et se ferait par la formation des membres du personnel en charge des services vétérinaires et des parties concernées.

**Mots-cles :** priorisation, transfrontalière, zoonoses, maladies animales, stratégie de contrôle, Érythrée

## Introduction

The livestock population of Eritrea are approximately; 2.2mil cattle, 4.7 mil goats, 2.5 mil Sheep, 0.5 mil camels, 0.5 mil donkeys, 0.015 mil mules and horses and 0.009 million pigs. (MOA report 2015) About 80% of Eritrea's population owns livestock and livestock sales constitute a major source of income for food purchases and it accounts for 15 % contribution to agriculture sector which accounts 20% of the GDP of the country. (IFAD Reports, 2008; OIE-PVS Evaluation – 2009).

Animal diseases are major constraints to the development of livestock productions in Eritrea, which are favoured by livestock movements through the borders and within the country. (OIE-PVS Evaluation – 2009)

In 2000 specific control strategies based on compulsory vaccinations and surveillances developed and enforced to lower the incidences of important diseases such as PPR, sheep and goat pox, FMD (only in dairy farms), NCD, IBD, *Brucellosis*, *Tuberculosis* and, LSD. These policies have succeed to reduce the incidences of some prioritised diseases, but failed to achieve successes in controlling the diseases and create stable epidemiological status of the diseases as expected. (MOA Reports 2013).

So to strengthen and improve what has been started and not achieved, the existed control strategy has revised and new country wide transboundary and zoonoses disease prioritisation has done using PHYLUM OIE disease prioritisation tools, based on the economic, societal and zoonotic potential impacts, to provide evidences the government for decision making on policies and strategy development and further supports to allocate appropriate resources to control livestock diseases with confidences and streamlining of stakeholder investment in the country's main disease priorities. The disease prioritisation at first was done together with the IGAD member states and endorsed at the national level. The objective was to update the country priority diseases and to harmonise the country disease control strategies with the regional

disease control strategies. The updated present and absent diseases of priority to Eritrea are, PPR, FMD, Sheep and Goat Pox, *Brucellosis*, *Tuberculosis* and rabies as existed and FMD exotic , CBPP, RVF and HPAI as absent diseases.

## Materials and Methods

### *The study area*

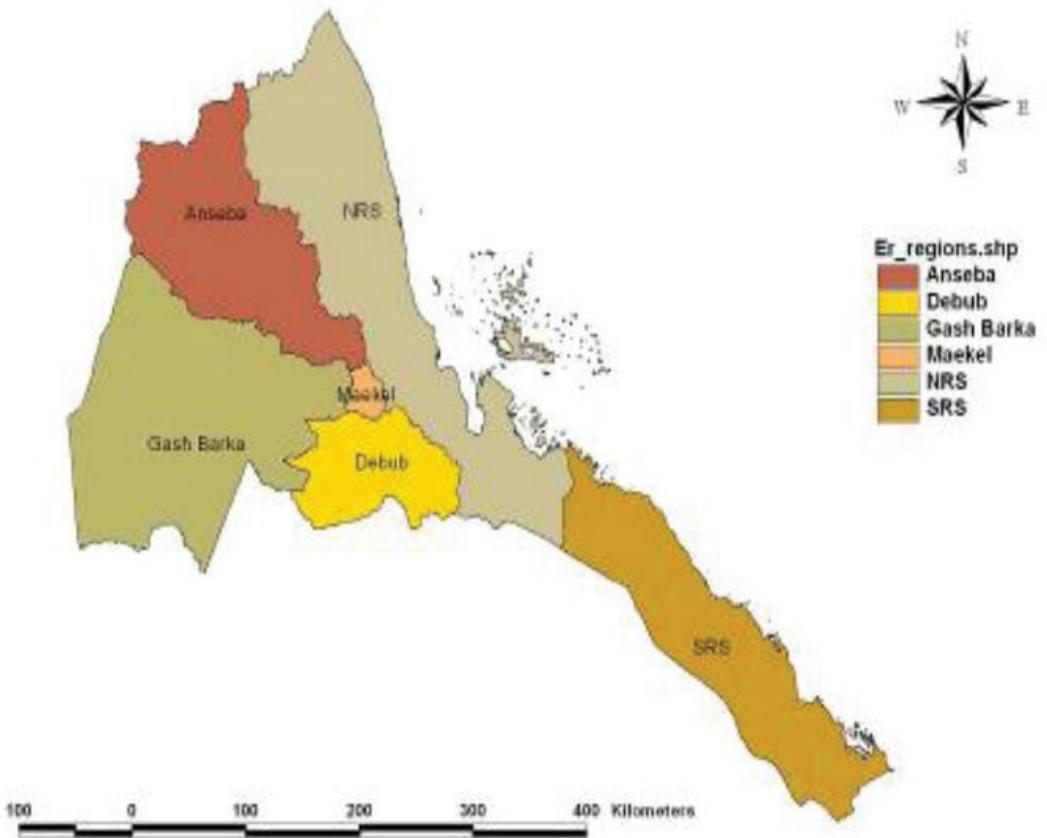
#### *Location and climate*

The state of Eritrea is located in the horn of Africa bordered with the Sudan to the west, Ethiopia to the south, Djibouti to the south-east and the Red Sea to the east. Eritrea covers an area of 121,320 Km<sup>2</sup> divided into six regions called Zobas (Figure 1), 65 sub Zobas and 2685 villages. The population of the country was estimated in 2008 to be 5,028,475 with a rural/urban ratio of 80/20. One seventh of the population lives in the capital, Asmara. Some 50% of the population is under 18 years of age (FAO, 1994 quoted by MoA, 2005).

The climate of Eritrea is influenced by the topography of the country. The coastal plain consists of semi-arid desert with mean annual temperatures varying between 26°C and 29°C. The hottest temperatures occur in the Danakil depression in the Southern Red Sea Zone, where peak temperatures of over 50°C have been recorded. The central highlands enjoy a more temperate climate with the lowest temperatures, including freezing temperatures at night, occurring during December, January and February. Rainfall is highly variable. Most of Eritrea receives rainfall from the southwest monsoon from April/May to September/October but the coastal area receives monsoon rains from the Indian Ocean. The rain is bimodal, starting with short rains in April/May, followed by a dry period before the main rainy season in July to September.

### *Agriculture and livestock*

About 80% of Eritrea's population depends on subsistence agriculture for their livelihood. Due to their limited production capacity, a large part of the population is vulnerable with limited resource base and little purchasing power. The agriculture sector



**Figure 1:** Map showing Eritrea

accounts for about 20% of the GDP, of which the livestock sub-sector accounts for 15 % (MoA, 2005).

Even though 75% of the Eritrean population depends on livestock and livestock products for their livelihood, there has been no livestock census done in Eritrea so far. The livestock population data is from a National Sample Survey of Agriculture (NSSA) conducted in 1997 as indicated in table I below.

From the livestock, cattle are by far the most important species. In the sedentary farming system in the highlands, cattle are used for draught and to a small scale for transport. In the lowlands cattle are mainly raised for milk and meat, while small ruminants and poultry are mainly kept as a readily available source of cash. (OIE-PVS Evaluation – 2009, IFAD Report, 1997)

#### *Animal disease situation in Eritrea*

Animal diseases remain one of the major constraints to the development of livestock production in Eritrea. Although the major epizootic disease the Rinderpest (RP) was eradicated in the 1990's and CBPP apparently in existent since 2001, (An outbreak of CBPP was detected in Asmara in 2001 for the last time), a number of serious transmissible diseases are still endemic or remain a serious threat favored by livestock movements through the borders and within the country.

The main trans-boundary and zoonotic diseases existed and officially reported to the OIE are the following (OIE-PVS Evaluation – 2009):

Foot and mouth disease, PPR, Sheep and Goat Pox, *Tuberculosis* and *Brucellosis*, African horse sickness, LSD, NCD, Anthrax and Infectious bursal disease (Gumboro)

The existing surveillance and reporting systems put in place by the MOA, has been allowed efficient response to reported outbreaks, and specific policies of compulsory vaccinations have been developed and now is contributing to lower the incidence of diseases such as *Brucellosis*, *Tuberculosis* and FMD (only in dairy farms), PPR, sheep pox, LSD, Rabies, NCD, IBD and ILT.

The method used to prioritize diseases stools focused on economic, human health, societal, environmental, and feasibility of control measures, economic impact of control measures, societal and environmental impact of control measures. The prioritisation based on OIE tools was a continuation of the evaluations of the existed disease prevalence and evaluations of the existed disease control strategies done locally focused mainly on PPR, Sheep and Goat CCPP, *Tuberculosis*, FMD, CBPP, rabies, NCD, infectious bursal disease, RVS and AHS.

At first of the prioritisation process a list of 16 important transboundary and zoonotic diseases were considered in Naivash kenya. These diseases were *Brucellosis*, Bovine *Tuberculosis*, Contagious Bovine and Caprine Pleuro-*pneumonia*, East Coast Fever, *Trypanosomosis*, Rift Valley Fever, Rabies, New Castle Disease, Highly Pathogenic Avian *Influenza*, Foot and Mouth Disease (present and absent serotypes), Lump skin disease, Sheep and Goat Pox, Camel Pox, African Horse Sickness, and African Swine Fever. The importance of every disease was assessed based on specific criteria including local economic impact, local public health impact, local societal, local environmental impact, and local feasibility, local economic and local societal & environmental impact of control measures. Finally new prioritised transboundary and zoonotic diseases identified and appropriate control strategies for both present and absent (or clinically absent) are designed.

#### Data Analysis

The data analysis is done using the PHYLUM OIE XL disease prioritization and identification tools on the diseases identified

as priority diseases from the list of the 16 important transboundary and zoonotic diseases. The diseases of prioritisation were identified and ranked based on the epidemiology, economic profiling, human and control strategies, risk of introduction, and the control strategies were analyzed based on Local economic impact, Local impact on human health Local societal impact and environmental impac. The priority diseases are identified and classified as present and exotic diseases with ranking to show priorities, and justifications for prioritising and proposed control strategies to control them.

## Results

The prioritised disease identified using the OIE tools focused on economic, human health, societal, environmental, and feasibility of control measures, economic impact of control measures, societal and environmental impact of control measures are presented in the tables 2 - 8 and the final prioritized diseases are presented in tables 9-10.

Based on the economic impacts and economic importance, the diseases prioritized and ranked from 1-10 are PPR, RVF, FMD Present and absent and *Tuberculosis*, NCD, Sheep pox, *Brucellosis*, CCPP, CBPP respectively. (Table, 2).

Table 3 showed, the 5 priority diseases identified based on Human Health impacts which are *Tuberculosis*, Rabies, RVF, *Brucellosis* and HPAI. All of these are prioritized based on their zoonotic nature and risks imposed on human beings.

The diseases prioritised based on societal impacts are showed in table 4. These diseases are, RVF, Rabies, AHS, TB, ECF and FMD present. They all are prioritized based on the social disturbance they create during outbreak periods.

Based on environmental impact PPR, AHS, CBPP, and CCPP are prioritized with ranks 1-4 respectively as showed in table 5.

The diseases identified based on feasibility of control measures are TB, NCD and RVF as showed in Table 6.

Table 7 showed the diseases prioritised based economic Impact of Control Measures are FMD, RVF, *Brucellosis* and HPAI attributing to the cost demanded during the control programs.

Disease priorities based on Societal and Environmental Impact of Control Measures

are showed in table 8. The priority diseases are RVF, CBPP, NCD, HPAI and TB.

At last the main diseases prioritised for Eritrea are showed in Tables 9. These diseases are, PPR, FMD present, Sheep and goat pox, NCD, *Brucellosis*, *Tuberculosis* and Rabies.

**Table 1:** Livestock Population in Eritrea

Species	Numbers of Livestock in Eritrea by Zoba						Total
	anseba	Cental/ maekel	debub	Gash- barka	Northern Red Seas	Southern Red seas	
cattle	218,923	40,505	490,093	917,344	178,532	82,060	1,927,457
Sheep	124,300	149,927	614,069	675,268	462,333	103,047	2,128,944
Goats	620,023	23,556	706,409	1,745,784	994,596	571,417	4,661,785
Camels	25,266	19,382	113,263	107,032	53,971 3	18,914	318,914
Horses		1,188	3,392	493			5,073
Donkeys	60,580	23,432	162,987	174,725	61,140	21,198	504,062
Mules	1,023	56	7,324	921			9,324
Poultry	78,247	86,425	512,776	423,898	26,867	6,052	1,134,265

Source: Survey of livestock population numbers in Eritrea, 1997

**Table 2:** Diseases Prioritized based on economic impacts

Impact	Rank	Diseases	Justification
Economic impact	1	PPR	The economic impact is great because: <ul style="list-style-type: none"> <li>The disease is highly contagious and it is killing many small ruminants (especially kids and lambs) which have great economic importance at the state and family level.</li> <li>It is greatly hindering exports of the small ruminants to the gulf countries the only existing market.</li> </ul>
	2	RVF	It is absent currently. But if newly introduced it will have great economic impacts. Because: <ul style="list-style-type: none"> <li>If the disease is newly introduced it can cause an epizootic form of outbreak that could cover large areas of a country and can cause enormous economic losses due high mortality and morbidity of the Ruminant animals.</li> <li>It can hinder export chances.</li> <li>It is a zoonotic disease which needs primary consideration to protect the human population.</li> </ul>
	3	FMD absent	<ul style="list-style-type: none"> <li>It is a highly contagious and can easily spread in the whole nation affecting all the livestock production centers and especially the high producing animals causing mortality of young animals and milk productions</li> </ul>

Impact	Rank	Diseases	Justification
			<ul style="list-style-type: none"> <li>• It can create livestock trade bans</li> <li>• High costs of prevention (costs of vaccinations including the new strains and the sanitary measures required)</li> </ul>
	4	FMD present	<ul style="list-style-type: none"> <li>• It is a highly contagious and can easily spread in the whole nation affecting all the livestock production centers and especially the high producing animals causing mortality of young animals and milk productions</li> <li>• It can create trade bans</li> <li>• High costs of prevention (costs of vaccinations and the sanitary measures required)</li> </ul>
	5	Tuberculosis	<ul style="list-style-type: none"> <li>• It is a Zoonotic and it can easily affect consumers who drink unpasteurized milk.</li> <li>• Prevention and eradication demand high cost to the government and the farmers (Surveillance, culling and compensation)</li> <li>• Costs of Public disease prevention (treatment and diagnosis) will be high</li> <li>• Establishment of pasteurizer plants and establishment of milk collection and distribution networks demand high costs</li> </ul>
	6	NCD	<ul style="list-style-type: none"> <li>• It causes high mortality and morbidity of all breed of birds (local and exotic) which creates direct and indirect losses on the poor families and investors.</li> <li>• High costs of the quarantines and movement controls <ul style="list-style-type: none"> <li>» destruction of all infected and exposed birds</li> <li>» the continuous cleaning and disinfection of the premises</li> </ul> </li> </ul>
	7	Sheep and Goat Pox	<ul style="list-style-type: none"> <li>• The risk of trade or export bans</li> <li>• The risk of mortality in young animals and high morbidity of adult animals</li> <li>• The cost vaccinations until the disease eradicated</li> </ul>
	8	Brucellosis	<ul style="list-style-type: none"> <li>• Zoonotic and it can easily affect consumers who drink unpasteurized milk.</li> <li>• Prevention and eradication demand high cost to the government and the farmers (Surveillance, culling and compensation)</li> <li>• Costs of Public disease prevention is high</li> <li>• Establishment of pasteurizer plants and establishment of milk collection and distribution networks demand high costs</li> </ul>
Impact	Rank	Diseases	Justification

9	CCPP	It is endemic in the country. However: <ul style="list-style-type: none"> <li>• it is still killing many small ruminants.</li> <li>• The therapeutic costs on the farmers is great</li> </ul>
10	CBPP	It is absent. However if newly introduced: <ul style="list-style-type: none"> <li>• The mortality and morbidity rates will be high</li> <li>• The cost of prevention and biosecurity measures inc</li> </ul>

**Table 3:** Disease priorities based on Human health impacts

Impact	Rank	Diseases	Justification
Human Health impact	1	<i>Tuberculosis</i>	It is of 1st priority regarding human health impact. Because: <ul style="list-style-type: none"> <li>• It is a Zoonotic and it can easily affect consumers who drink unpasteurized milk and contaminated meat.</li> </ul> <p>If it is not controlled at its low incidence level:</p> <ul style="list-style-type: none"> <li>• prevention and eradication will demand high cost to the government and the farmers (Surveillance, culling and compensation)</li> <li>• Costs of Public disease prevention (treatment and diagnosis) will be high</li> </ul>
	2	Rabies	<ul style="list-style-type: none"> <li>• It is zoonotic and it has high case fatality rate.</li> <li>• It demand high annual mass vaccination costs</li> <li>• The therapeutic measures in human being are high incase exposed to the infected animals</li> </ul>
	3	RVF	Currently it is absent. So the government needs to give priority for its prevention. Otherwise if the disease is newly introduced: <ul style="list-style-type: none"> <li>• It is a zoonotic disease and it demands high costs to protect the human population.</li> <li>• It can cause an epizootic form of outbreak that could cover large areas of the country and can cause enormous economic losses from high mortality and morbidity of the Ruminant animals.</li> <li>• It can hinder export chances</li> </ul>
	4	<i>Brucellosis</i>	It is a disease of priority regarding human health impact. Because: <ul style="list-style-type: none"> <li>• It is a Zoonotic and it can easily affect consumers who drink unpasteurized milk and contaminated meat.</li> </ul> <p>If it is not controlled at its low incidence level:</p> <ul style="list-style-type: none"> <li>• prevention and eradication will demand high cost to the government and the farmers (Surveillance, culling and compensation)</li> <li>• Costs of Public disease prevention (treatment and diagnosis) will be high</li> </ul>

Impact	Rank	Diseases	Justification
	5	HPAI	<p>The disease is not present in the country. But if introduced the human economic impacts will be great. Because:</p> <ul style="list-style-type: none"> <li>• It is zoonotic and can kill people</li> <li>• Therapeutic and surveillance costs will be high</li> <li>• It will create trade bans and can affect the income of the country and in return can create direct and indirect influence on human health</li> <li>• Will have negative impacts on tourism</li> </ul>

**Table 4:** Disease priorities based on societal impacts

Impact	Rank	Diseases	Justification
Societal impact	1	RVF	<p>It is absent. But if newly introduced, it will have great social impacts. Because:</p> <ul style="list-style-type: none"> <li>• It is a zoonotic and vector borne disease which can easily spread to a large area</li> <li>• It can demand high cost for surveillance and Vaccinations</li> <li>• Causes social stresses not to be contract by the disease</li> <li>• Prevention of the disease including Control of animal movements is expensive</li> <li>• Draining of stagnant water to eliminate or reduce vectors</li> <li>• Disinfestations of low depression accumulations of water where mosquitoes may reproduce</li> </ul>
	2	Rabies	<p>It is endemic. However, its social impact is great. Because:</p> <ul style="list-style-type: none"> <li>• It is a zoonotic and almost 100% fatal and this create high social stress</li> <li>• The therapeutic measure (post and pre exposures ) are expensive and it is not usually easily accessible</li> <li>• It can demand high cost for Vaccinations of all dogs</li> <li>• Causes social stresses not to be contract by the disease especially following biting by any dogs including healthy dogs</li> <li>• Prevention of the disease including culling of stray dogs and control of the canine population</li> </ul>
	3	AHS	<ul style="list-style-type: none"> <li>• It is absent. But if newly introduced</li> <li>• It is a killer disease of equines which have great social contributions as drought animals especially in the rural areas.</li> </ul>

Impact	Rank	Diseases	Justification
	4	TB	<p>It is of priority regarding social impact. Because:</p> <ul style="list-style-type: none"> <li>• It is a Zoonotic and it can easily affect consumers who drink unpasteurized milk and contaminated meat and these can create confusions on consumers</li> <li>• The raw milk and milk products consumption culture of the rural areas (usually free of <i>Tuberculosis</i>) will be disturbed</li> </ul> <p>If it is not controlled at its low incidence level:</p> <ul style="list-style-type: none"> <li>• Costs of Public disease prevention (Surveillance, treatment and diagnosis) including culling of reactor animals is high</li> </ul>
	5	ECF	<p>It is absent. But if newly introduced, it is a killer disease and unless prevented on time</p> <ul style="list-style-type: none"> <li>• The mortality and morbidity of ruminants is great. And this can create negative social impacts.</li> <li>• Tick prevention is difficult and demands high cost.</li> </ul>
	6	FMD Present	<ul style="list-style-type: none"> <li>• The disease affects directly all livestock owners and especially the farmers who keep the high producing animals causing mortality of young animals and milk productions</li> <li>• It affects indirectly the farmers <ul style="list-style-type: none"> <li>» It can create livestock trade bans</li> <li>» High costs of prevention (costs of vaccinations including the new strains and the sanitary measures required) have negative impacts on the society.</li> </ul> </li> </ul>

**Table 5:** Disease priorities based on Environmental impact

Impact	Rank	Diseases	Justification
Environmental impact	1	PPR	<ul style="list-style-type: none"> <li>• The disposal of animals</li> <li>• The contamination of the environment from infected and dead animals</li> <li>• High cost of procuring cleaning and disinfecting materials</li> <li>• Risk of contracting the endangered animals such as antelopes</li> </ul>
	2	AHS	<ul style="list-style-type: none"> <li>• The disposal of animals</li> <li>• The contamination of the environment</li> <li>• High cost of procuring cleaning and disinfecting materials</li> <li>• Risk of contracting the endangered animals such as antelopes</li> </ul>
	3	CBPP	<ul style="list-style-type: none"> <li>• The disposal of animals</li> <li>• The contamination of the environment from infected and dead animals</li> </ul>

Impact	Rank	Diseases	Justification
	4	CCPP	<ul style="list-style-type: none"> <li>• High cost of procuring cleaning and disinfecting material</li> <li>• The disposal of animals</li> <li>• The contamination of the environment from infected and dead animals</li> <li>• High cost of procuring cleaning and disinfecting materials</li> </ul>

**Table 6:** Disease priorities based on feasibility of control measures

Impact	Rank	Diseases	Justification
Feasibility of Control Measures	1	TB	<ul style="list-style-type: none"> <li>• It is a Zoonotic disease and controlling the disease has great public importance and implications</li> </ul>
	2	NCD	<ul style="list-style-type: none"> <li>• The mortality rate is high and without proper vaccinations poultry production is not sustainable hence controlling of the disease is justifiable</li> <li>• Vaccines require cold chains usually difficult in the rural areas</li> </ul>
	3	RVF	<ul style="list-style-type: none"> <li>• It is a Zoonotic disease and controlling the disease has great public importance and implications</li> </ul>

**Table 7:** Disease priorities based on Economic impact of Control Measures

Impact	Rank	Diseases	Justification
Economic impact of Control Measures	1	FMD Present	<ul style="list-style-type: none"> <li>• It is endemic and highly contagious and it causes great misery to livestock production. So control of the disease is of great economic importance</li> </ul>
	2	FMD absent	<ul style="list-style-type: none"> <li>• It is highly contagious and causes more great misery to livestock production. So control of the disease is of great economic importance</li> </ul>
	3	RVF	<ul style="list-style-type: none"> <li>• It is a Zoonotic disease and controlling the disease has great public importance and implications</li> </ul>
	4	<i>Brucellosis</i>	<ul style="list-style-type: none"> <li>• It is endemic and zoonotic causing now abortions in the livestock production with many human cases who either drink raw milk and get incontact. So control of the disease is of great economic importance</li> </ul>
	5	HPAI	<p>The disease is not present in the country. But if introduced the human economic impacts will be great. Because:</p> <ul style="list-style-type: none"> <li>• It is zoonotic and can kill people</li> <li>• Therapeutic and surveillance costs will be high</li> </ul>

Impact	Rank	Diseases	Justification
			<ul style="list-style-type: none"> <li>It will create trade bans and can affect the income of the country and in return can create direct and indirect influence on human health</li> <li>Will have negative impacts on tourism</li> </ul>

**Table 8:** Disease priorities based on Societal and Environmental Impact of Control Measures

Impact	Rank	Diseases	Justification
Societal and Environmental Impact of Control Measures	1	RVF	<ul style="list-style-type: none"> <li>It is a Zoonotic disease and controlling the disease has great public importance and implications</li> <li>It is a vector borne disease which easily entered to the country</li> </ul>
	2	CBPP	<ul style="list-style-type: none"> <li>It cause high mortality morbidity and create chronic carries</li> </ul>
	3	NCD	<ul style="list-style-type: none"> <li>It spreads due to unwise disposing of dead animals unvaccinated local birds</li> <li>Difficult to vaccinate the local birds and can affect wild birds</li> <li>If it is not controlled its social and environmental impacts is great</li> </ul>
	4	HPAI	<ul style="list-style-type: none"> <li>The disease is not present in the country. But if introduced the economic, social and environmental impacts will be great.</li> </ul>
	5	TB	<ul style="list-style-type: none"> <li>It is endemic and zoonotic and at present many human cases are diagnosed in the hospitals that mainly drink raw milk. So control of the disease has great social and environmental importance.</li> </ul>

**Table 9:** Final proposed prioritization of present diseases

Disease	Rank	Justifications	Proposed Control strategy	Current Country control strategy
PPR	1	Highly contagious and is causing high mortality	Mass vaccination for unvaccinated animals (to vaccinate each animal at least once) for 5 consecutive years with panvac certified vaccines and with identification of vaccinated animals to avoid unnecessary costs.	Mass vaccinations without identification of vaccinated animals and with not specific time frame.
FMD Present	2	Highly contagious and is causing high production causes	Biannual Vaccinations using the endemic strains and bio-security methods with sero-monitoring and bio-security measures in all dairy farms.	Biannual vaccination of dairy animals with trivalent vaccines.
Sheep and Goat pox	3	It is causing high mortality in small ruminant	Vaccination and movement control and isolation	Mass vaccinations

Disease	Rank	Justifications	Proposed Control strategy	Current Country control strategy
NCD	4	Causing high mortality in endogenous and exotic birds	Vaccinations and bio-security methods for all birds	Vaccination for exotic birds in the modern farms
<i>Brucellosis</i>	5	Zoonotic with abortions and production complications	Surveillance and culling of reactors with compensation	Surveillance and culling of reactors without compensation
TB	6	Endemic causing abortions and production complications	Surveillance and culling of reactors with compensation	Surveillance and culling of reactors without compensation
Rabies	7	Endemic causing many human cases and social complications	Annual vaccination of owned dogs and culling of stray dogs with good coverage of public awareness.	Annual vaccination of owned dogs with public awareness

**Table 10:** Final proposed prioritization of exotic diseases

Disease	Rank	Justifications	Proposed Control strategy	Current Country control strategy
FMD	1	New strains can cause more control and production complication	Biannual vaccinations with strict bio-security measures	Sero Surveillance to monitor the entrance and existing strains
CBPP	2	Trade ban and has high mortality in case introduced	Sero-surveillance, vaccination and culling of infected animals	vaccination and culling of infected animals
RVF	3	To Avoid trade bans and to protect health of human and animals	Continuous surveillance, vaccinations and bio-security measures	Sero Surveillance strict border control
HPAI	4	To Avoid trade bans and to protect human and animals health	vaccinations culling and biosecurity measures	Sero Surveillance strict border control

The absent diseases but of priority to Eritrea are explained in different tables with different stages of ranking.

### Discussions

The results of the prioritisation process using the PHYLUM OIE disease prioritisation tool are showed under different economic groups as explained in the tables 2-10.

The 10 diseases of priority based on the economic impacts are PPR, RVF, FMDP, FMDA, *Tuberculosis*, NCD, Sheep pox, *Brucellosis*, CCPP and CBPP with the ranking 1 -10, respectively. (Table, 2). These diseases are of priority according ranks noted. Because, even though, they have some variation as ranked in the table, they all cause great economic losses at the state and family level if prompt control actions are not taken. They also impose the

risk of exporting bans of ruminants to the gulf countries the only existing market gate for the country. This ranking is in agreement with current epidemiological situation of the country including the widespread PPR outbreaks currently occurring and with the ongoing disease control strategies existed since 2000. (OIE-PVS Evaluation – 2009, MOA reports 2000, 2015)

FMD is with the priority diseases considering the direct economic losses from high costs of prevention (costs of vaccinations and the sanitary measures required) and indirect economic losses expected from export bans and the cost of vaccinations. This is in agreement with the findings of the sero-surveillance studies in Eritrea (Tesfaalem Teklegiorghisa, c, Klaas Weerdmeester, Froukje van Hemert-Kluitenberg, Rob J.M. Moormann a,b and Aldo Dekker 2015) and the disease control strategies of Eritrea, since 2000. (MOA 2015)

Similarly, NCD is now causing high mortality and morbidity in all breed of birds (local and exotic) and is creating direct and indirect losses on the poor families and investors. *Tuberculosis* and *Brucellosis* are also currently considered as priority diseases taking into consideration their Zoonotic nature and the widely practiced culture of consuming unpasteurized milk and the high costs demanded the government and the farmers (Surveillance, culling and compensation) for Prevention and eradication. So generally the prioritized diseases showed in table 2 are in agreement with current diseases control strategies existed in the country.

Disease priorities based on Human health impacts are showed in Tab 3. The diseases are of priority because of their Zoonotic nature and the risks imposed on humans which are common in the country. For example the risk of *Brucellosis* and *Tuberculosis* are more in consumers who drink unpasteurized milk and livestock owners who have close contacts as indicated in the study done on prevalence of antibodies to *Brucella* spp. and risk factors related to high-risk occupational groups in Eritrea in 2002 which indicated the

risk of contaminations are relatively high in the professionals and livestock owners. (M. K. Omer, T. Asefaw, E. Skjerve, T. Teklegiorghis and Z. Woldehiwet: 2002).

Rabies is ranked a second in the priority list accounting to the zoonotic and the high costs of therapeutic measures in human being as evidenced in many parts of Eritrea. The rabies victims are also to sharply increase soon, due to the relaxation of vaccination programs, mainly due shortages of vaccine supply.

In table 4: 6 diseases are identified and ranked from 1 to 6 based on societal impacts. These diseases are RVF, Rabies, AHS, TB, ECF and FMD present and they are the diseases that have great social impacts and causes social stresses and disturbances and high costs of prevention incase outbreaks occur. However, AHS, and ECF are either not exist or are important taking in to account the current epidemiological situations of Eritrea. So the findings are only partially agreed to the disease control strategies in the country. So the prioritization should be improved based on the existing field reports and observations.

Diseases that have environmental impacts are showed in table 5. These diseases are PPR, AHS, CBPP, and CCPP. They are identified and prioritized based on environmental influences and pollutions resulted mainly from disposal of animals, contamination of the environment by infected and dead animals, and due the High cost of procuring cleaning and disinfecting materials to clean the environment. However, the findings are not in line to the epidemiological realities of the country except PPR.

Table 6 showed the diseases identified and prioritized based on Feasibility of Control Measures. These diseases are TB, NCD and RVF. The findings do not agree to the epidemiological and disease control strategies existed in the country. So updating is need to show the realities.

Table 7 showed the diseases prioritized based economic Impact of Control Measures are FMD, RVF, *Brucellosis* and HPAI attributing to the cost demanded during the control programs.

Societal and Environmental Impact of Control Measures are showed in tables 8. The diseases prioritized RVF, CBPP, NCD, HPAI, TB respectively. These diseases are prioritized taking the environmental and human well fare issues imposed the outbreaks of these diseases. The prioritized diseases except NCD and TB are not existed. So, further analysis is required.

The final prioritized diseases for Eritrea are PPR, FMD present, Sheep and goat pox, NCD, *Brucellosis*, *Tuberculosis* and Rabies as showed in tables 9. These diseases have been of priority to Eritrea and they have been under compulsory vaccination programs since 2000, although updated statistically analyzed data are lacking to analyze the achievements. (MOA, 2015, OIE-PVS Evaluation – 2009)

Different absent diseases are prioritized by the PHYNUM OIE disease prioritization tool and included in different tables with different degrees of ranking. This happened considering their zoonotic and negative economical consequences and the risks of introduction from neighboring countries. Though, this ranking does not agree with the epidemiological realities and control strategies of the country, but the finding can indicate that, contingency plans should be in place to avoid unexpected losses during emergencies.

Generally the prioritizations results using the PHYNUM OIE disease prioritization tool are in agreement with the diseases which are under compulsory vaccinations programs intended to lower the incidences and to create epidemiologically stable statuses. The strategy has so far succeed to reduce the incidences of some the prioritized diseases based on the experts' observation and disease outbreak and surveillance reports, but failed to achieve successes in controlling the diseases and create stable epidemiological status of the diseases as expected. (MOA Reports 2013). Therefore the current disease control strategies should be revised using the PHYNUM OIE disease prioritization tool combined to surveillance reports and experts' observation. For sustainable outcomes, the strategy should also be harmonized with disease control strategies of IGAD and EAC member states.

## Conclusions

The diseases identified as priority using the PHYNUM OIE disease prioritization tool are PPR, FMD , Sheep and goat pox, NCD, *Brucellosis*, *Tuberculosis* and Rabies. These identified priority diseases are in line to the ongoing disease control strategy of the country. To strengthen the ongoing disease control strategy updating the epidemiological information is required following provision of training to veterinary service staffs and stakeholders. To get appropriate and correct epidemiological information the existing surveillance systems should also be revised and updated.

The Eritrean veterinary services have little capacity to anticipate emerging problems. So general contingency plans should be put in place at least for some of the identified exotic diseases taking into consideration the epidemiological situation of the neighboring countries.

## Conflict of interest

There was no conflict of interest identified.

## Ethical standards

The study involved review of secondary data and reports generated during routine animal and human disease surveillance. There was no direct contact with human and animal subjects. Because this was part of the routine surveillance activities which are exempted from Institution Review Boards. The study has many benefits to the human population and to the animal by guiding policy and resource allocation for prevention and control of the diseases.

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## TRANSBOUNDARY ANIMALS DISEASES AND ZONOSSES PRIORITISATION AND PROPOSED INTERVENTIONS IN ETHIOPIA

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### Abstract

This study was conducted to prioritise and categorise TADs and Zoonoses, which are present or have high risk of introduction so as to facilitate veterinary authority decision making for the eventual intervention and control of the priorities. The study was done according to the OIE tool for the "Listing and categorisation of priority animal diseases including those transmissible to human" developed through a study conducted by the French Phylum Consultancy firm. The tool took into account the different aspects of the diseases especially the economic, human health, societal and environmental impacts with the feasibility of their control measures. With regards to their economic impact; PPR and LSD, societal and environment impact; Ebola and Rabies and with regards to feasibility of control measures; NCD and BTB have been identified as priorities for Ethiopia. Considering all the prioritisation criteria, the final priority diseases were classified into two categories; Diseases which are present in the country (PPR, FMD-P, LSD, NCD, Rabies, BTB and *Brucellosis*) and Diseases, which are exotic (RVF, FMD-A and HPAI). This disease prioritisation helps the veterinary services of Ethiopia to revise its annual plan and the 5 year GTP 2 Plan to accommodate the intervention and control process of some of the high prioritised diseases and revise if necessary the existing control and contingency plan of different diseases and produce such kind of plan for those not prepared. Also it helps in allocating and/or soliciting enough fund for the starting of intervention and control process as well as work closely with the AU-IBAR and IGAD regional offices and also with the member States of IGAD for information sharing and harmonizing the control of these disease along the border.

**Key words:** Control, Prioritization, TAD, Zoonoses, PHYLUM

## PRIORISATION DES MALADIES ANIMALES TRANSFRONTALIERES ET DES ZONOSSES ET INTERVENTIONS PROPOSEES EN ÉTHIOPIE

### Résumé

La présente étude a été réalisée dans le but de procéder à la priorisation et catégorisation des maladies animales transfrontières et des zoonoses présentes ou présentant un risque élevé d'introduction, afin de faciliter la prise de décision de l'autorité vétérinaire concernant l'intervention et le contrôle des maladies prioritaires. L'étude a été menée conformément à l'outil de l'OIE pour la « classification et catégorisation des maladies animales prioritaires y compris celles transmissibles à l'homme », développé à partir d'une étude réalisée par la société de conseils française Phylum Consultancy. L'outil a pris en compte les différents aspects des maladies, en particulier les aspects économique, sanitaire, sociétal et environnemental ainsi que la faisabilité des mesures de contrôle. La PPR et la dermatose nodulaire contagieuse, la fièvre Ebola et la rage, et la MNC et la tuberculose bovine ont été identifiés comme prioritaires pour l'Éthiopie, respectivement pour l'impact économique, l'impact sociétal et environnemental et la faisabilité des mesures de contrôle. Compte tenu de tous les critères de hiérarchisation, les maladies prioritaires définitives ont été classées en deux catégories : les maladies présentes dans le pays (PPR, FA-P, DNC, MNC, Rage, TBB et brucellose) et les maladies exotiques (FVR, FA-A et IAHP). Cette priorisation

des maladies aide les services vétérinaires de l'Éthiopie à réviser le plan annuel et le plan quinquennal GTP 2 du pays afin d'intégrer le processus d'intervention et de contrôle de certaines maladies hautement prioritaires, réviser si nécessaire le plan de contrôle et d'urgence existant pour différentes maladies et produire ce type de plan pour les personnes qui n'y sont pas préparées. Elle aide également les parties concernées à allouer et / ou à solliciter des fonds suffisants pour le démarrage du processus d'intervention et de contrôle et travaille en étroite collaboration avec les bureaux régionaux de l'UA-BIRA et de l'IGAD et avec les États membres de l'IGAD en vue du partage d'informations et de l'harmonisation du contrôle de ces maladies le long des frontières.

**Mots-clés :** contrôle, priorisation, MAT, zoonoses, PHYLUM

## Introduction

The economy of Ethiopia is based on agricultural sector which contributes 40 - 50% to Gross Domestic Product (GDP), over 90% to foreign exchange earnings and about 85 - 90% of employment opportunities of the country (Bureau of African Affairs, 2009). The livestock sub-sector alone contributes an estimated 12% the total and over 45% to the agricultural GDPs (National Bank of Ethiopia, 2008).

Ethiopia is believed to have the largest livestock population in Africa. However, the economic benefit derived from the livestock sector is very low compared with the overall potential. The challenge facing livestock development in Ethiopia is daunting. Inadequate feed supply and poor nutrition, high prevalence of animal diseases, poor animal genetic resource management and poor marketing are the main bottle necks for the development of the sub-sector.

The presence of a number of economically important and zoonotic diseases plays a significant role for the huge economic loss and repeated trade bans that the country is facing. The country is home to eight of the 15 former OIE List A diseases and 20 of the former 72 OIE List B diseases. Moreover, there are various diseases caused by poor animal husbandry and feeding practices. It has been estimated that mortality rates of 8 - 10% of cattle, 12 - 14% of sheep, 11 - 13% of goats, 8% of camel, 47 - 56% of Poultry due to this deadly diseases. The mortality rates are even much higher in young stock (calves, lambs and kids, 30 - 50%) (Animal Health Strategy, 2013).

Over the past several years, the

Government of Ethiopia (GoE) has made a number of concerted efforts to reduce the impact livestock diseases in the country. However, in a country such as Ethiopia where there is limited resource to address all animal health diseases, prioritising diseases of high importance in terms of economical, public health and social impact is of critical importance. Prioritisation of diseases is an important process, in that it assists the country in identifying the diseases on which it would focus its limited resources.

Based on the Livestock Sector Investment and Policy Toolkit (LSIPT) of the Livestock Development Master Plan (LDPM, 2013 - 228) of Ethiopia, 10 priority livestock diseases have been identified based on their impact on three criteria, the impact on households and their livelihoods; markets and value chains and intensification pathways in the production systems (LMP, 2015). The result showed that for cattle; FMD, CBPP, *Brucellosis*, LSD and BTB; for sheep and goats PPR, SGP and CCPP; for camels Surra and in poultry NCD have been ranked as priority diseases.

On the other hand the Veterinary Services of the country has identified 19 priority livestock diseases for the purpose of immediate notification and reporting using the cell phone based Animal Diseases Notification and Investigation System (ADNIS). Most of these identified diseases are TADs, which have a serious trade limiting and economical impact on the country (Animal Health Strategy, 2015). The Ministry of Livestock and Fisheries in close collaboration with the Ministry of Health and Ministry of Forest and Environment Protection has also identified 5 priority zoonotic diseases for cross-sectoral collaboration (Soon to be

published). The MoLF in its Livestock Master Plan (LMP) has prioritised 10 livestock diseases considering their impact on households and their livelihoods, markets and value chains and intensification pathways (LMP, 2015).

Although a number of such prioritisation exercises have been undertaken in the country at different times, most of the exercises used a limited number of criteria for a specific purpose and/or the process did not involve participation of different ministries and organisations in the livestock and health sectors.

### Objective

The objective of the study was to facilitate national veterinary authority management decision making by identifying, prioritising and categorizing TADs and zoonotic diseases of importance in a wider context of the country.

## Materials and Methods

### Description of Ethiopia

The study was conducted in Ethiopia. Ethiopia is a landlocked country found in the

horn of Africa. It is geographically located between 32° 30' - 48° 00' E and 3° 00' - 15° 00' N. It covers a land area of 1.04 million km<sup>2</sup> (CIA, 2014). The country has nine administrative regional states and two city administration. The natural vegetation and livestock distribution of the country is influenced by altitude, landform, drainage, and edaphical interaction (Tsedeki, 2004). Basically agro-ecology of Ethiopia is divided into three major categories (i.e. the highland >3000 masl, mid land 1500 - 3000 masl and lowland < 1500).

Naturally endowed with different agro-ecological zones and suitable environmental conditions, Ethiopia is home for many livestock species and suitable for livestock production. Ethiopia is believed to have the largest livestock population in Africa (CSA, 2013; Solomon et al. 2003; Tilahun and Schmidt, 2012). An estimate indicates that the country is home for about 54 million cattle, 25.5 million sheep and 24.06 million goats. From the total cattle population 98.95% are local breeds and the remaining are hybrid and exotic breeds. 99.8% of the sheep and nearly all goat population of the country are local breeds (CSA, 2013).



**Figure 1:** Administrative regions and Zones of Ethiopia

Livestock diseases are the major cause of economic losses to the peasant farmer and pastoralists in Ethiopia amounting to hundreds of millions of birr annually. Because livestock are the chief source of cash income to small holders, up to 88% in the highland livestock-cropping system, diseases are an important cause of reduced productivity of meat and milk as well as draft, hides and dung fuel. Although many of the diseases could be controlled by available vaccine technology, timely recognition of the disease followed by acquisition of the pharmaceuticals are lacking due to the remoteness of the livestock holder and the shortage of infrastructure facilities to support health services delivery. Consequently, in Ethiopia the majority of disease intervention consists of mass inoculation following outbreaks rather than preventive measures.

In Ethiopia, there are a number of diseases which occur either endemically or epidemically. Those endemic diseases have a chronic distribution with episodic flare ups. The epidemically occurring diseases manifest themselves with local peaks or regular outbreaks. As Ethiopia is a landlocked country, it shares a long stretch of land with neighboring countries. Hence, there is a considerable risk of introduction of exotic diseases such as RVF, ECF etc into the country. This is even worsened by the cross border livestock and wildlife movement, illegal trade and pastoralist movement of herds of livestock. Besides, due to the ever increasing international and regional movement of people, livestock and livestock products around the globe, there is always a notable risk of introduction of diseases absent in the country but having a devastating impact on the local livestock and on public health.

*Method: The prioritisation tool*

The prioritisation exercise used an

OIE tool for the "Listing and categorisation of priority animal diseases including those transmissible to human" developed through a study conducted by the French Phylum Consultancy firm. The exercise based its analysis on the methodological manual prepared by the French consultancy firm. This manual provided a detailed presentation of the methods of the developed tool. The tool used a qualitative and quantitative analysis of the criteria selected to prioritise and categorise the 15 - 17 TADs and Zoonoses.

The answer for each criterion was entered in the corresponding box, according to the attached definition in the tool. The "Comments" box serves to identify precisely the particular choices that had to be made in order to fill in the box. The "Lack of knowledge" box serves to identify criteria that were answered but for which additional research may be useful (due to inadequate current knowledge).

The general feature of presentation of the criteria in the tool as depicted in Table 1 has similar formats as most impact assessment modules.

The method involved two sequential steps in the analysis of prior selected 16 diseases:

- • A global characterisation of the disease, aimed at assessing inherent aspects of the disease, independently of any particular local context.
- • A local approach, aiming at refining the study of the disease within the specific context of the country or region in question.

*Design and data analysis*

Literature review (especially the FAOSTAT) and expert opinions on the characteristics of the country, prevalence

**Table 1:** Criteria presentation in the tool

Thematic Axis	Group of Criteria	Criteria 1 (Definition)	Decision rule	Answer	Comments	Lack of knowledge
		Criteria 2 (Definition)	Decision rule	Answer	Comments	Lack of knowledge
		"	"	"	"	"

rates, disease existence in the country, public health and economic importance have been used to obtain the real and/or potential impact data of the 15 - 17 diseases selected for the country prioritisation. The first step of the analysis involved qualitative profiling of the intrinsic nature of diseases in terms of their epidemiology, economic consequences and public health impacts.

Once the profiling step was made, the analysis was refined by considering the diseases in the particular context to Ethiopia. This second phase of local analysis of disease characterisation involved understanding the characteristics of the country, the epidemiology of the diseases in the country, the impact of the diseases and analysis of the possible control strategies.

The tool provided quantitative and qualitative results of the different diseases in the following three categories:

- Diseases absent;
- Diseases present and with known epidemiology;
- Disease present but with unreliable local epidemiological knowledge.

## Results

### *Priority TADs and Zoonoses according to Economic Impact*

The economical impact of the diseases in terms of local and international trade, mortality, morbidity of animals leading to production loss and costs implicated by prevention and control measures have been used by the tool to do the analysis. As a result, a list of priority diseases in terms of their economical impact has been described in the Table 2.

According to the economic impact RVF stood first for it has high impact in local and international trade if it comes in the country, and then followed by PPR with the same reason as RVF. LSD, FMD P, FMD A, and SGP are prioritised from 3rd to 6th as they have serious impact on the local and international trade. NCD and *Brucellosis* are also important diseases in poultry and dairy sector as mentioned very well in the LMP.

### **Priority TADs and Zoonoses based on public health impact**

**Table 2:** Ranking of prioritisation score of TADs and Zoonoses according to Economic impact.

No	Disease	Score	Rank	Remark
<b>Present</b>				
1	PPR	7.698458	2 <sup>nd</sup>	
2	LSD	7.692841	3 <sup>rd</sup>	
3	FMD P	6.771567	4 <sup>th</sup>	
4	FMD A	6.48050	5 <sup>th</sup>	Not present
5	SGP	6.047767	6 <sup>th</sup>	
6	<i>Brucellosis</i>	5.861455	8 <sup>th</sup>	
7	NCD	5.61124	7 <sup>th</sup>	
8	ECF	4.91895	9 <sup>th</sup>	Not present
	Absent			
1	RVF	10	1 <sup>st</sup>	Not Present
2	AHS	3.78167	10 <sup>th</sup>	
3	BTB	2.929487	11 <sup>th</sup>	
4	Rabies	2.713704	12 <sup>th</sup>	

**Table 3:** Ranking of prioritisation score of TADs and Zoonoses according to the Human health impact.

No	Disease	Score	Rank	Remark
1	Ebola	10	1st	
2	TB	9.6541	2nd	
3	RVF	8.5083	3rd	
4	<i>Brucellosis</i>	8.3166	4th	
5	Rabies	7.5041	5th	

**Table 4:** Ranking of prioritisation score of TADs and Zoonoses according to societal and environmental impacts.

No	Disease	Score	Rank	Remark
1	Ebola	7.4843	1st	
2	Rabies	7.2727	2nd	
3	FMD A	6.4616	3rd	
4	AHS	6.0113	4th	
5	ECF	4.5553	5th	

**Table 5:** Priority diseases based on feasibility of control strategies

No	Disease	Score	Rank	Remark
<b>Present Diseases</b>				
1	NCD	7.147392	1st	
2	BTB	7.024943	2nd	
3	LSD	6.602419	3rd	
4	FMD	6.034014	4th	
5	PPR	3.892668	5th	
<b>Absent diseases</b>				
6	Blue Tongue	6.327286	1st	
7	FMD-A	6.034014	2nd	
8	RVF	6.024187	3rd	
9	Ebola	4.528345	4th	

**Table 6:** Ranking of priority TADs and Zoonoses present in Ethiopia

No	Disease	Score	Rank	Remark
1	BTB	5.380189	1st	Selected
2	LSD	5.167627	2nd	Selected
3	NCD	5.131008	3rd	Selected
4	<i>Brucellosis</i>	4.590507	4rd	Selected
5	Rabies	4.527252	5th	Selected
6	FMD-P	4.48722	6th	Selected
7	PPR	3.774154	7th	Selected

**Table 7:** Ranking of TADs and Zoonoses absent in Ethiopia

No	Disease	Score	Rank	Remark
1	RVF	6.449057	1st	Selected
2	FMD-A	5.357977	2nd	Selected
3	HPAI		3rd	Latter selected

### *Priority TADs and Zoonoses based on Societal and environmental Impact*

#### *Feasibility of control measures*

Taking into account of local feasibility, subsequent associated costs, societal acceptance and environmental impact of the relevant control strategies of the diseases in the country, the following list of TADs and Zoonoses have been identified as priority diseases by the PHYLUM tool.

#### *Final Priority TADs and Zoonoses of Ethiopia*

As a result of the cumulative effect of the previous steps, the tool provided a qualitative and quantitative prioritisation score of the diseases in the country in two categories. Diseases which are present and Absent in the country. These diseases were prioritised by the tool based on their effective impact in the country on; economy, public health, societal and environmental and feasibility of associated control measures. shows the justifications of prioritisation, the current and proposed control strategies of the prioritized diseases in the country. The result of the final prioritisation exercise is depicted in Table 6 and Table 7.

However, after consultations and analysis of the final prioritisation list with experts and CVO of the country, the final ranking order of the top seven diseases present in the country have been changed as in decreasing order of importance; PPR, FMD-P, LSD, NCD, Rabies, BTB and *Brucellosis*.

Three diseases (Table 7) which are absent in the country have been given top priority based on their theoretical importance in the advent of their introduction into the country.

## **Discussion**

The final list of priority diseases identified by the prioritisation and categorisation tool were grouped in two categories. Seven of the nine diseases have endemic occurrence in the country (PPR, FMD-P, LSD, NCD, Rabies, BTB and *Brucellosis*) with a huge impact on the economy, public health and the environment. The justification behind the analysis done by the PHYLUM tool is that those diseases scored the highest as they have a significant impact on local and international trade, high morbidity and mortality rates leading to loss of production and high indirect cost associated with prevention and control.

### *PPR*

This economically important disease was one of the top diseases in the prioritisation exercise which clearly aligns with the country's top list of animal diseases. Based on reported morbidity and mortality rates and the size and structure of the small ruminant sector, PPR is considered to be one of the most economically important livestock diseases in the country (Abraham, 2005). As a result, the country has adopted and is implementing a risk based geographic approach of progressive PPR control pathway ultimately leading to eradication of the disease.

### *FMD*

Foot and Mouth Disease is equally important, if not more, to the country's local and international trade. Hence, the PHYLUM tool listed it as a priority disease of importance which is in agreement with the national priority list. FMD is endemic and known for its wider distribution in Ethiopia, although its level of prevalence may have significant variations

across the different farming systems and agro-ecological zones of the country (National FMD Control Plan, 2006). FMD is considered as one of the most important livestock diseases demanding urgent control intervention in Ethiopia. Hence, a national progressive FMD control plan has been prepared and is being implemented.

### LSD

A number of epidemiological studies have shown that Lumpy Skin Disease has a diverse distribution in the different agro-ecological and production systems of the country (Gari G, Waret-Szkuta A, Grosbois V, Jacquet P, Roger F. Risk factors associated with observed clinical lumpy skin disease in Ethiopia. *Epidemiol Infect.* 2010;138:1657–66). LSD is one of the listed notifiable animal diseases by the veterinary services of the country. Control strategies are basically based on ring vaccination around outbreak areas. However, the disease has now gained a higher national attention due to the failed efficacy and virulence of the LSD vaccine. Hence, prioritization of the LSD by the PHYLUM tool is in accordance with its importance in the country.

### Newcastle disease

Newcastle Disease is one of the most important poultry diseases found in most parts of the country that affects the poultry population. Out of the 359 disease outbreak reports of 8 transboundary diseases received in 2004-2005, NCD constitutes about 33 (9.2%) of the total report (Ministry of Livestock and Fisheries. Newcastle Disease Control Strategy, 2010). Being one of the notifiable poultry diseases in the country, the LSIPT of the LMP has prioritized NCD as one of the top ten priority livestock diseases. Therefore, the result of the prioritization study is in the interest of the country's priorities.

### Priority Zoonotic Diseases

Rabies and *Brucellosis* have been identified by the Ministry of Livestock and Fisheries and the Ministry of Health as a priority zoonotic disease. Rabies is one of

the notifiable animal disease in the country. *Brucellosis* and *Bovine Tuberculosis* are associated in the country mainly with farms and confined system with intensification. The government of Ethiopia hence, in its LMP has prioritized the two diseases as priority zoonotic diseases because of the planned intensification of dairy farms. The result of the PHYLUM tool study has hence clearly shown the country's priority in the zoonotic diseases category.

The other group of identified priority diseases (RVF, FMD-A and HPAI) are diseases which are exotic to the country but having considerable threat of introduction into the country. Besides impacting the local trade, as Ethiopia is a huge livestock and livestock products exporting country, international trade of livestock and their products would be critically hampered if such diseases are introduced into the country. Ethiopia shares a long stretch of border and pastoralist movement with neighboring and regional countries where RVF and FMD-A are endemic. Especially those foci of episodic flare up of the diseases in those countries is very much adjacent to the country's border with similar ecological niches. Epidemic and pandemic threat of *Avian Influenza* viruses such as HPAI are global priority threats let alone being the country's priority.

The results of the current prioritisation and categorisation study goes in line with existing national and regional prioritisation results and policy directions. Almost all of the nine prioritised diseases are included in the 19 national list of priority animal diseases for immediate notification through the ADNIS system (Animal health strategy, 2015). According to the LMP (2015), the LSIPT has identified 10 priority livestock diseases amongst of which are the six priority diseases of this study (PPR, LSD, FMD-P, NCD, *Brucellosis* and *Bovine Tuberculosis*). Among the five priority zoonotic diseases identified by the MoLF, MoH and MoFEP for a joint collaboration, two (Rabies and *Brucellosis*) are priority diseases in this study (MoLF and MoH Unpublished Report, 2015).

Hence, this study has clearly reflected

the interest of the country to a degree of identifying national livestock disease priorities. The proposed control measures and their feasibility results described by the study are in alignment with the control and contingency strategic plans for some of the priority diseases such as PPR, FMD, NCD, RVF and HPAI. For this reason, advocacy and alignment of priority control strategies need to be done at a national level while also preparing national control and prevention strategies for the other priority diseases. The study has also shown that some of the described diseases are identified as priority diseases by a number of countries and organisations in the region, requiring the need for regional planning and harmonisation of interventions.

### Conclusion

Despite the fact that a number of disease prioritisations have been done in the country, most of these exercises were done by a institutions and/or organisation for specific purpose using a limited number of criteria that justified the purpose. Besides, these studies usually lacked participation of varied expertise and involvement of concerned stakeholders who are intrinsic natures of any prioritisation exercises. The current study utilized an OIE endorsed PHYLUM tool of categorization and prioritisation of TADs and Zoonoses. A team of experts drawn from Ministry of Livestock and Fisheries, Ministry of Health and Ethiopian Wild Life Authority participated in the study. The method used two sequential steps in the analysis of the diseases. A global characterisation of the diseases; assessing the inherent aspects of the disease independently of any particular context and a local approach; aimed at refining the study within the specific context of the country. 7 TADs and zoonotic diseases which are present in the country in decreasing ranking order PPR, FMD-P, LSD, NCD, Rabies, BTB, *Brucellosis* and 3 TADs and Zoonoses absent in the country but which would have an enormous impact if introduced into the country RVF, FMD-A and HPAI have been prioritised.

The result also showed that with regards to their economic impact; PPR and LSD, societal and environment impact; Ebola and Rabies and with regards to feasibility of control measures; NCD and BTB to be the top priority diseases according to the respective criteria used.

A number of strategic documents have been produced and interventions on most of the prioritised diseases are being implemented in Ethiopia. Some of these documents and interventions are the progressive PPR control program, the FMD control plan, RVF contingency plan, AI contingency and preparedness plan. These government priority areas are in line with the current findings of the disease prioritisation, and can be used as control interventions for these diseases. The outcome of the prioritisation study could serve as a tool to facilitate decision making in the management of the country's veterinary authority.

In view of the findings of this study the following recommendations are forwarded:

- As is indicated in the discussion, the MoLF has to revise its annual plan and the 5 year GTP 2 plan to accommodate the intervention and control process of some of the high prioritised diseases,
- Revise if necessary the existing control and contingency plan of different diseases and produce such kind of plan for those not prepared
- Allocate and/or solicit enough fund for the starting of intervention and control process
- Work closely with the AU-IBAR, IGAD regional offices and also with the member States of IGAD for information dissemination and exchange,
- Harmonising the control of prioritised diseases along the border area with the neighbouring countries to effectively control the disease.

### Conflict of interest

There was no conflict of interest identified.

## Ethical standards

The study involved review of secondary data and reports generated during routine animal and human disease surveillance. There was no direct contact with human and animal subjects. Because this was part of the routine surveillance activities which are exempted from Institution Review Boards The study has many benefits to the human population and to the animal by guiding policy and resource allocation for prevention and control of the diseases.

## Acknowledgement.

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## **AN UPDATED REVIEW ON PREVALENCE AND MAJOR PATHOGENS OF BOVINE MASTITIS IN ETHIOPIA**

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### **Abstract**

*Mastitis* is a multifactorial disease with worldwide distribution accounting for major economic losses in the dairy cattle. *Mastitis* is one of the most costly diseases of dairy industry where affected quarters suffer 30% reduction in productivity and affected cow losses 15% of its production for the lactation. Studies in different parts of Ethiopia have showed that *Mastitis* prevalence rate is high and the prominent causative agents are *Staphylococcus aureus*, *Streptococcus agalactiae*, coagulase negatives *Staphylococcus* species, among others.

**Key words:** Causative agent, Risk factors, Mammary tissue, *Mastitis*

## **EXAMEN ACTUALISE DE LA PRÉVALENCE ET DES PRINCIPAUX AGENTS ETIOLOGIQUES DE LA MAMMITE BOVINE EN ÉTHIOPIE**

### **Résumé**

La mammite est une maladie multifactorielle répartie à travers le monde, qui représente des pertes économiques majeures chez les bovins laitiers. La mammite est l'une des maladies les plus coûteuses de l'industrie laitière où les quartiers affectés subissent une réduction de productivité de 30% et les vaches affectées perdent 15% de leur production pour la lactation. Des études menées dans différentes régions d'Éthiopie ont montré que le taux de prévalence de la mammite est élevé et que les principaux agents étiologiques sont, entre autres, les *Staphylococcus aureus*, *Streptococcus agalactiae*, les staphylocoques à coagulases négatives.

**Mots-clés :** agent étiologique, facteurs de risque, tissu mammaire, mammite

## Introduction

Diary production is a biologically efficient system that converts feed and roughages to milk (Yohannes, 2003). Despite large number of dairy cows in Ethiopia, milk production does not satisfy the nations' demand. *Mastitis* (inflammation of mammary gland) is among important health problems in dairy cattle and has been considered one of the most important threats affecting the dairy industry throughout the country.

The term "*Mastitis*" comes from the Greek derived word elements mast referring to the mammary gland and itis meaning inflammation (Blood and Studdert, 1999). Although *Mastitis* could technically be used to describe any udder injury that may result in inflammation, it is generally accepted that the causative agents for the inflammatory reaction are microorganisms that have gained entry into the teat canal and mammary tissue.

*Mastitis* is an inflammation of the mammary gland and udder tissue, and is a major endemic disease of dairy cattle that follows a number of factors (Radostits *et al.*, 2007) and risk factors depends on three components i.e. exposure to the microbes, cow defense mechanism, and environment and management factors (Suriyasathaporn *et al.*, 2000).

The causes of *Mastitis* are almost entirely infectious and bacteria though, rarely other pathogens and chemical or physical agents can be involved (Wellenberg *et al.*, 2002). They are broadly classified into contagious and environmental agents (Quinn *et al.*, 2002).

*Mastitis* is the common and costly disease causing loss in milk yield, treatment cost for dairy farmers and culling of animals at unacceptable age (Vaarst and Envoldsen, 1997).

*Mastitis* is considered as the most complex disease because of its multi factorial causation (Nibret *et al.*, 2011) with worldwide distribution accounting for major economic losses in dairy cattle (DeGrave and Fetrow, 1993). Several scholars agree that *Mastitis* is one of the most costly diseases of dairy industry worldwide. It is estimated that on average an affected quarter suffers 30% reduction in

productivity and an affected cow loses 15% of its production for the lactation (Radostits *et al.*, 2007; Bartlet *et al.*, 1991).

The economic implication of bovine *Mastitis* is derived from the high costs of treatment and diagnosis, loss of milk production, early culling, and cost of the control program in clinical and subclinical cases. A large proportion of *Mastitis* is not detectable only by clinical examination of the udder and milk that poses economic loss from decreased production, which makes it difficult to estimate the economic losses (Brinda *et al.*, 2009). In addition, the bacterial contamination of milk from affected cows may render it unsuitable for human consumption due to zoonosis, food poisoning, and antibiotic residue in the milk following *Mastitis* (Radostits *et al.*, 2007).

So far, few studies on bovine *Mastitis* have been conducted in Ethiopia in the past five years. The objective of this review paper is to examine those studies conducted at different parts of the country and consolidate their findings.

### Prevalence of *Mastitis*

An effort has been made by different authors to assess the occurrence of *Mastitis* among dairy herds at different parts of Ethiopia. In the last few years studies have been conducted on the prevalence of bovine *Mastitis* and reports has showed that the prevalence rate is high (19.5 to 75.8%) and location specific prevalence rates were 41.7 % in Ambio district of West Shewa zone (Sarba and Tola, 2017), 75.8% in urban, peri urban and rural areas of Sidamo zone (Yibrah and Tsega, 2016), 63.02 % in and around Bahir Dar town (Gashaw, 2016), 32.92% in and around Sodo town (Endale *et al.*, 2016), 38% in Shashemene and Kofale of West Arsi zone (Umer *et al.*, 2015), 19.5% in eighty two small holder urban and pri-urban dairy farms of Tigray (Haftom *et al.*, 2015), and 63.11% in Hawassa and Wendo-genet towns (Fentaye *et al.*, 2014).

These variations between different research findings could indicate that *Mastitis* is a complex disease and the interaction of different factors like management system

of the farms, breeds of the study population, awareness of the owners toward the disease, geographical locations of the study areas and variation in veterinary service delivery and coverage, among others, could play a major role.

Even though the disease has been reported with varying prevalence in different parts of Ethiopia, incidence of *Mastitis* has not been studied like that of prevalence. Besides, the spreading of *Mastitis* among cows was not determined in the country.

#### Major causative agents

*Mastitis* is an important factor that limits dairy production due to its heavy financial losses involved and the existence of latent infections characteristics. The causes of *Mastitis* are almost entirely infectious and bacteria though, rarely other pathogens and chemical or physical agents can be involved (Wellenberg *et al.*, 2002). The majority of microorganisms that are responsible for *Mastitis* could be *Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis*, *Mycoplasma* species, *Streptococcus uberis*, coliforms (*Escherichia coli*, *Klebsiella* species and *Enterobacter aerogenes*), *Serratia*, *Pseudomonas*, *Proteus* species, environmental *Streptococci*, *Enterobacter* species (Quinn *et al.*, 2002).

The reports of Umer *et al.*, (2015), Abera *et al.*, (2010) and Bitew *et al.*, (2010) has revealed that the predominant bacterial pathogens species causing *Mastitis* in varied areas of Ethiopia is *Staphylococcus aureus*, *Streptococcus agalactiae* and coagulase negatives *Staphylococcus* species. The high prevalence of this organism may be associated with its frequent colonization of teats, its abscesses in the udder and hence resistant to antibiotic treatment (MacDonald 1997). The bacteria usually establish chronic, subclinical infections and are shed in the milk, which serves as a source of infection for other healthy cows during the milking process (Radostits *et al.*, 1994) and / or attributed to the wide distribution of the bacteria on the skin of teats and udder (Mac Donald, 1997).

#### Risk factors associated with Mastitis

Prevalence of *Mastitis* related to specific risk factors is determined as the proportion of affected cows out of the total examined. *Mastitis* risk factors depend on three components; exposure to microbes, cow defense mechanism, environmental and management factors (Quinn *et al.*, 2002).

Based on different studies the major exposing factors that lead *Mastitis* could be breed, lactation stage, management problems, age and parity, among others.

According to Yibrah and Tsega (2016) the major exposing factors that lead *Mastitis* were breed, lactation stage, management problems, age and parity. Sarba and Tola (2017) have acknowledged that the prevalence of *Mastitis* was significantly higher in crossbred than local cows, as well older cows than young adult cows.

As it has been explained by Radostits *et al.*, (2007/1994), the higher susceptibility of higher-yielding cows to *Mastitis* could be attributed to the anatomy of teat and udder and certain physiological characteristics such as fewer phagocytic cells in higher yielding cows associated to dilution. It can be also assumed that as the parity of cow advances and the age increases cows become prone to *Mastitis*.

A wide range of farm and animal level management factors can also influence husbandry conditions. This was obvious in most studies and attributed to the variation in hygienic dairy environment and milking conditions which favors the proliferation and transmission of *Mastitis* causing organisms.

Most of the studies revealed that *Mastitis* was associated with herd management system, drainage, floor types and level, milking procures, manure removal schedule, hygienic of the utensils used for milking practice, milking order and proper housing. Under Ethiopian condition most dairy farmers do not exercise management practices such as pre-milking udder cleaning, teat dipping, milking with gloves and machine milking. Therefore, hygienic milking practice, milking order, hygienic of the utensils used for milking practice, proper manure removal and housing and management

improvements are some of the important procedures which need attention while the prevention of the disease.

### Concluding remarks

*Mastitis* is one of the complex diseases of dairy cows which involve an interaction between management practice and infectious agent(s). Studies clearly showed that, it is a major health problem to small holder dairy farms. Therefore, capacity building through continuous hands-on training as well as *Mastitis* control and prevention strategies should be designed and implemented. To shape the existing control and prevention strategies, detailed studies on epidemiological, microbiological, and economic analysis at national level are suggested

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## MOLECULAR IDENTIFICATION OF CRYPTOSPORIDIUM SPECIES IN GRASSCUTTER (THRYONOMYS SWINDERIANUS)

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### Abstract

There is currently no information on the occurrence of *Cryptosporidium* infection in the grasscutter or cane rat (*Thryonomys swinderianus*), a rodent found in tropical sub-Saharan Africa and consumed as meat. In this study, we examined faecal samples from 74 grasscutters at a rearing facility in Ibadan, Nigeria for the presence of *Cryptosporidium* species using polymerase chain reaction (PCR) amplification of the 18S rRNA gene. The overall infection rate was 12.2% (9/74) and *Cryptosporidium* infection had no significant association ( $P > 0.05$ ) with sex, age and management practices. Sequencing of the small-subunit rRNA gene identified *Cryptosporidium muris* as the only genotype infecting the grasscutters. These findings suggest that grasscutters may harbour *Cryptosporidium* species with potential public health consequences since *C. muris* has been implicated in human infections. It is therefore recommended that precautions be taken while processing the rodent meat for human consumption to avoid zoonotic infection. This is the first report on molecular identification of *Cryptosporidium* species in the grasscutter.

**Keywords:** *Cryptosporidium muris*; Cryptosporidiosis; Genotyping; Grasscutter; Rodent; Nigeria

## IDENTIFICATION MOLECULAIRE DES ESPECES CRYPTOSPORIDIUM CHEZ L'AULACODE (THRYONOMYS SWINDERIANUS)

### Résumé

Il n'existe actuellement aucune information sur la présence de l'infection à *Cryptosporidium* chez l'aulacode (*Thryonomys swinderianus*), un rongeur trouvé en Afrique sub-saharienne tropicale et consommé comme viande. Dans cette étude, nous avons examiné des échantillons d'excréments de 74 aulacodes dans une structure d'élevage à Ibadan, au Nigeria, pour rechercher la présence de *Cryptosporidium* en utilisant l'amplification en chaîne par polymérase (PCR) du gène de l'ARNr 18S. Le taux global d'infection était de 12,2% (9/74) et l'infection par *Cryptosporidium* n'avait pas d'association significative ( $P > 0,05$ ) avec le sexe, l'âge et les pratiques de gestion. Le séquençage du gène de l'ARNr a identifié *Cryptosporidium muris* comme le seul génotype infectant les aulacodes. Ces résultats portent à croire que les aulacodes peuvent abriter *Cryptosporidium* avec des conséquences potentielles sur la santé publique puisque *C. muris* a été impliqué dans des infections humaines. Il est donc recommandé de prendre des précautions lors du traitement de la viande de rongeur destinée à la consommation humaine afin d'éviter les infections zoonotiques. Cette étude est le premier rapport de l'identification moléculaire des espèces *Cryptosporidium* chez l'aulacode.

**Mots-clés :** *Cryptosporidium muris*; cryptosporidiose ; génotypage ; aulacode ; rongeur ; Nigeria

## Introduction

*Cryptosporidiosis* is an important parasitic zoonosis caused by *Cryptosporidium*, an Apicomplexan protozoon parasite. The infection is most often characterised by diarrhoea in animals and humans, especially in immunocompromised individuals (Fayer, 2004). *Cryptosporidium* infection can be acquired through the ingestion of contaminated food and water or via direct contact with infected animals, humans or fomites (Casemore, 1990). Several *Cryptosporidium* spp. have been identified as pathogens of humans and a wide range of mammals, including rodents (Fayer, 2004).

*Cryptosporidium* infection has been reported in over 40 different rodent species (Lv et al., 2009) and the infecting species have been isolated from several rodents. Some host-adapted species like *Cryptosporidium* mouse genotype (I - II), deer mouse genotype (I - IV), shrew genotype, vole genotype, muskrat genotype (I - II), squirrel genotype, chipmunk genotype (I-II) and beaver genotype, respectively have been previously described (Feng, 2008; Danisova et al., 2017). *Cryptosporidium* species known to infect other mammals have also been isolated in rodents (Lv et al., 2009; Danisova et al., 2017). The detection of zoonotic *C. parvum*, *C. hominis*, *C. meleagridis* and *C. muris* in some rodents suggest that these animals may play a potential role in the epidemiology of human *Cryptosporidiosis* (Lv et al., 2009; Quy et al., 1999; Morgan et al., 2000; Palmer et al., 2003; Danisova et al., 2017).

Although *Cryptosporidium* infection has been reported in several rodents worldwide, there is currently no information on the infection in grasscutters (also known as greater cane rats) found in sub-Saharan Africa. This rodent can grow to nearly 0.6 metre in length and weigh up to 8 kg (Akinloye, 2005). It is usually reared commercially for meat in West Africa where it is regarded as a delicacy in meals. This study was therefore aimed at investigating the occurrence of *Cryptosporidium* infection in the grasscutter (*Thryonomys swinderianus*) and to characterize the species infecting this

rodent.

## Materials And Methods

### Sample collection

Faecal samples were obtained from 74 grasscutters housed in a rearing facility in Ibadan, Nigeria between February and October, 2015. The samples were kept at 4°C until processed for molecular analysis. Information on gender and age of the animal, and management practices under which they were reared were obtained during the collection of samples.

### DNA extraction and genotyping

Faecal DNA was extracted from samples using the Ultra-pure® DNA Kit (Roche, Indianapolis, USA). *Cryptosporidium* species were detected by nested polymerase chain reaction (PCR) amplification of a ~590 bp fragment of the 18S rRNA gene using 18SiCF2 (5'-GACATATCATTCAAGTTTCTGACC-3') and 18SiCR2 (5'-CTGAAGGAGTAAGGAACAACC-3') primers, followed by a nested amplification using primers 18SiCF1 (5'-CCTATCAGCTTTAGACGGTAGG-3') and 18SiCR1 (5'-TCTAAGAATTTACCTCTGACTG-3'), as previously described (Ryan et al., 2003). The cycling conditions for both primary and secondary amplification were as follows: 94°C for 5 min (initial denaturation), followed by 45 cycles of 94 °C for 30 s (denaturation), 58 °C for 30 s (annealing) and 72 °C for 30 s (extension), with a final extension of 72 °C for 10 min. *Cryptosporidium parvum* from mice (TUI14) and ultra-pure PCR water were used as positive control and negative controls, respectively. All PCR amplicons were visualised by electrophoresis in 1.5% agarose after ethidium bromide staining. Visible PCR amplicons were purified using Mini elute kit (Roche, indianapolis, USA) and sequenced at the Tuft University sequencing facilities (tuft.org). Sequence alignments were performed using MEGA software, version 5.2.2 (www.megasoftware.net). The obtained sequences were compared with *Cryptosporidium* sequences

found in the GenBank (<http://www.ncbi.nlm.nih.gov/>) using BLAST. Phylogenetic trees to visualise the similarities between obtained nucleotide sequences and selected reference sequences were inferred using the neighbour joining method (Saitou *et al.*, 1987) with model that best fits the alignment using MEGA 6 (Tamura *et al.*, 2013). The identical nucleotide sequences obtained from the isolates were deposited in the GenBank database with accession number KX811729.

## Results

Nested PCR amplification of the 18S rRNA gene revealed that 12.2% (9/74) of the tested faecal samples were positive for *Cryptosporidium* species. Sequencing of the secondary products of PCR amplification identified *C. muris* as the only species infecting the grasscutters. The infection rate of *Cryptosporidium* was comparable among sexes and there was no significant association ( $P>0.05$ ) with age of rodents and management practices. Phylogenetic analysis of the 18S gene sequences showed that the *C. muris* obtained from the grasscutters had 100% sequence

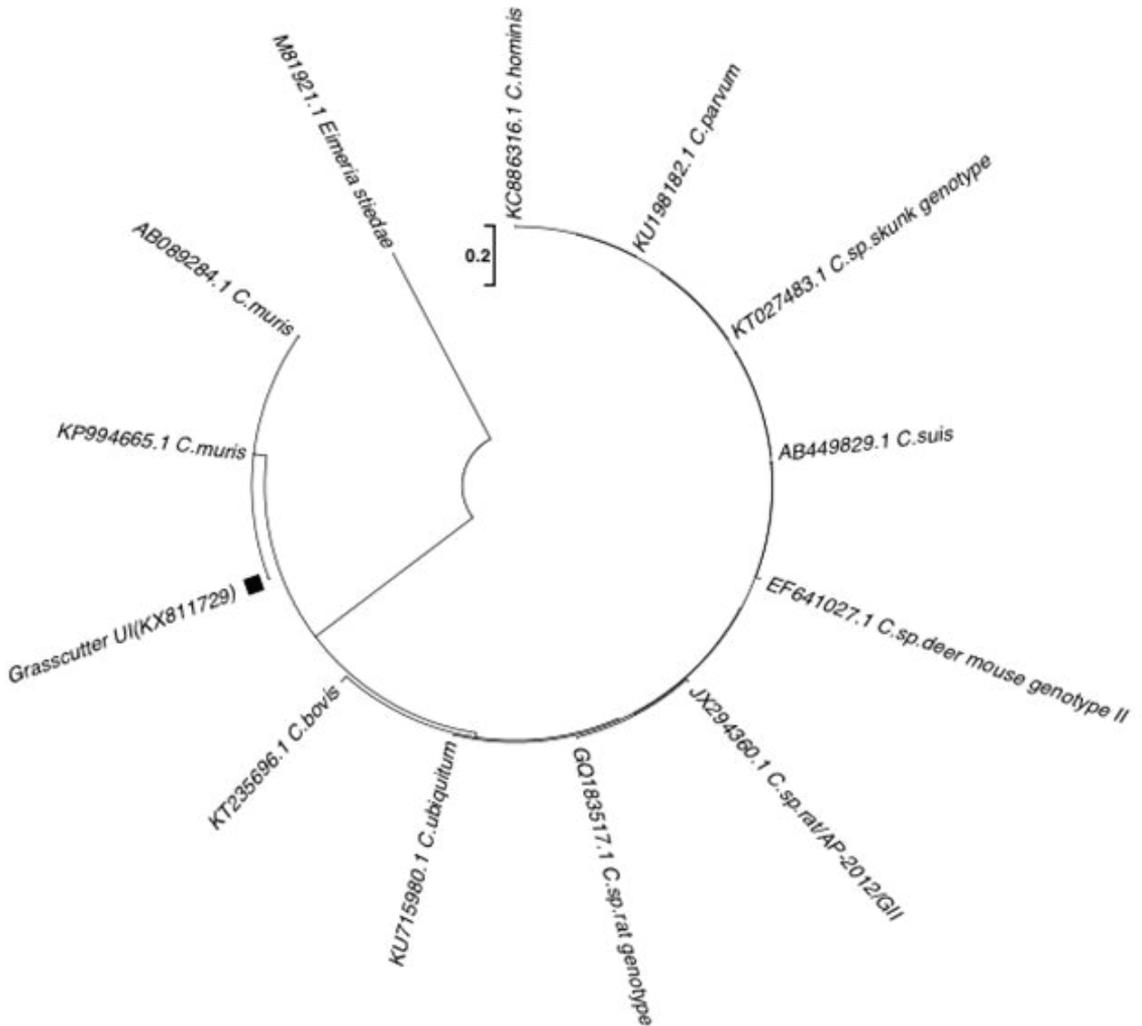
similarity with GenBank reference sequence KP994665.1 obtained from an orangutan (*Pongo* spp.) in Indonesia.

## Discussion

In the present study, we investigated the prevalence of *Cryptosporidium* infection in the grasscutter (*Thryonomys swinderianus*), a rodent domestically raised and sold as meat for human consumption in West Africa. The overall prevalence of *Cryptosporidium* infection (12.2%) obtained in the sampled grasscutters is similar to the range (10 -16%) previously reported in some rodents in other geographical areas like China (Lv *et al.*, 2009), United Kingdom (Chalmers *et al.*, 1997) and Brazil (Bomfim *et al.*, 1998). This low prevalence may be attributed to the good management practices observed in the rearing facilities. The reared grasscutters were fed mainly with concentrates and carefully selected shrubs, grasses, fruits and legumes, thereby reducing exposure to feeds contaminated with *Cryptosporidium* oocysts shed by other infected hosts.

**Table 1:** Occurrence of *Cryptosporidium* oocysts by sex, age, management type and location in domesticated *Thryonomys swinderianus* (grasscutter) in Ibadan, Nigeria

Characteristics	Test	Postive (%)	CI	P value
Total	Grasscutter	74	9(12.2)	
Sex	Male	48	8 (16.7)	0.5894 – 42.42
	Female	26	1 (3.8)	
Age (yrs)	1-3	52	7 (13.5)	0.297 -8.160
	3-5	22	2 (9.1)	
Feeding & watering	Well & Concentrates, Corn husk	44	3 (6.8)	0.067- 1.279
	Tap water & Cane grass	30	6 (20)	
Location	A	44	3 (7.0)	0.067- 1.279
	B	30	6 (20)	



**Figure 1:** Phylogenetic relationships inferred by neighbour-joining analysis of the SSU rRNA gene among *Cryptosporidium* species from grasscutter and other reference sequences from rodents in the GenBank.

This study revealed *C. muris* as the only *Cryptosporidium* species infecting the investigated grasscutters. *Cryptosporidium muris* had been previously reported in various rodents worldwide (Lv *et al.*, 2009; Morgan *et al.*, 2000; Bomfim *et al.*, 1998, Danisova *et al.*, 2017) and also in diverse hosts including dogs, pigs, cats, monkeys, birds, giraffe and ruminants (Feng, 2008; Palmer *et al.*, 2003; Lupo *et al.*, 2008; Kvac *et al.*, 2009). In addition, *C. muris* was recovered from humans in Peru (Palmer *et al.*, 2003), India (Muthusamy *et al.*, 2006) and Kenya (Gatei *et al.*, 2006). The identification of

*Cryptosporidium* species circulating in rodents is essential to better understand their role in the epidemiology of human *Cryptosporidiosis*.

In the present study, we report for the first time the occurrence of *C. muris* in domesticated grasscutters. The grasscutter is increasingly consumed by humans as a delicacy (bush meat) in Nigeria and other West African countries. The findings of this study might be of public health significance, since *C. muris* has been implicated in human infection as a potential zoonoses, particularly in immunocompromised individuals (Slapeta, 1987; Palmer *et al.*, 2003).

However, more studies are needed to clarify whether *C. muris* infection is a “true” zoonoses. It will also be valuable to investigate whether its occurrence in the grasscutter is adaptational or coincidental. Previous investigations have reported an array of zoonotic gastrointestinal parasites (*Schistosoma haematobium*, *Hymenolepis* sp. and *Strongyloides* sp.) that occur in grasscutters (Adu *et al.*, 1999; Awah-Ndukum *et al.*, 2001; Opara & Fagbemi, 2008). These findings further suggest that grasscutters may play a potential role in zoonotic infections.

In conclusion, the present study which to the best of our knowledge is the first to report *Cryptosporidium* infection in the grasscutter, suggests that the rodent might play a critical reservoir role for a pathogen of potential public health importance. It is therefore recommended that appropriate precautions that eliminate faecal contamination should be taken during rearing and consumption of the rodent to avoid human infection.

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## PRIORITISATION OF TRANSBOUNDARY ANIMAL DISEASES AND ZOOSES TO STRENGTHEN CONTROL MEASURES IN KENYA

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### Abstract

The prevention and control of Trans-boundary animal diseases (TADs) and zoonoses involves dealing with numerous pathogens and diseases, each of which poses a specific threat to human and animal health. Disease prioritisation is therefore motivated by the need to ensure that limited resources are targeted at the most important problems to achieve the greatest benefit. The objective of this work was therefore to prioritise the TADS and zoonoses in order to rationally allocate the limited resources for research, surveillance and control. We used the OIE prioritisation tool in which a panel of experts identified diseases to be subjected to the tool. The tool was divided into seven themes; each had at least two parameters with criteria against which the scoring was conducted. Average thematic scores were standardised and compared to produce a disease ranking, separately for present and absent diseases. The ranking from the tool was subjected to stakeholder review before developing the final list of prioritisation. Fifteen diseases were subjected to the prioritisation process. The ranked list from the highest were: Rift Valley fever (RVF), African Swine Fever (ASF), Circulating Foot and Mouth Disease (FMD), *Peste des Petits Ruminants* (PPR), *Contagious Bovine Pleuro-Pneumonia* (CBPP), *Contagious Caprine Pleuro-Pneumonia* (CCPP), Rabies, *Brucellosis*, Newcastle Disease (ND), *Tuberculosis* (TB), Lumpy Skin Disease (LSD), East Coast Fever (ECF) and Sheep & Goat Pox. Following stakeholder consultation, the final prioritised list from the highest was as follows; Circulating FMD, PPR, *Brucellosis*, CBPP, CCPP, RVF, ND, ASF, TB, Rabies, ECF, Sheep & Goat Pox, and LSD. From the tool, most of the diseases which ranked high were episodic, occurring in epidemics and not widespread in the country. The stakeholder meeting gave more emphasis to the endemic diseases which contribute to a bigger burden. There is need to consider and make appropriate provisions for seasonal and endemic diseases when using the tool. The tool is an opportunity for countries and regions with limited data to have a rational basis for allocation of scarce resources.

**Key words:** Trans-Boundary Animal Diseases, Zoonoses, Prioritisation, Kenya,

## PRIORISATION DES MALADIES ANIMALES TRANSFRONTALIERES ET DES ZOOSES POUR RENFORCER LES MESURES DE CONTROLE AU KENYA

### Résumé

La prévention et le contrôle des maladies animales transfrontières (MAT) et des zoonoses font appel à la lutte contre de nombreux agents pathogènes et maladies, dont chacun constitue une menace spécifique pour la santé humaine et animale. La priorisation des maladies est donc motivée par la nécessité de veiller à ce que les ressources limitées soient ciblées sur les problèmes les plus importants pour en tirer le meilleur parti. L'objectif de ce travail était donc de prioriser les MAT et les zoonoses afin d'allouer rationnellement les ressources (limitées) pour la recherche, la surveillance et le contrôle. Nous avons utilisé l'outil de priorisation de l'OIE, et un panel d'experts a identifié les maladies à soumettre à l'outil. L'outil était divisé en sept thèmes, chacun ayant au moins deux paramètres avec des critères sur la base desquels la notation a été effectuée.

Les scores thématiques moyens ont été normalisés et comparés pour produire un classement des maladies, séparément pour les maladies présentes et absentes. Le classement basé sur l'outil a été soumis à un examen par les parties prenantes avant l'établissement de la liste définitive des priorités. Quinze maladies ont été soumises au processus de priorisation. Les maladies classées en tête sur la liste étaient la fièvre de la Vallée du Rift (FVR), la peste porcine africaine (PPA), la fièvre aphteuse circulante, la peste des petits ruminants (PPR), la pleuropneumonie contagieuse bovine (PPCB), la pleuropneumonie contagieuse caprine (PPCB), la rage, la brucellose, la maladie de Newcastle (MNC), la tuberculose (TB), la dermatose nodulaire contagieuse (DNC), la fièvre de la Côte orientale (FCO) et la variole caprine et clavelée du mouton. Après la consultation des intervenants, la liste des grandes priorités dans l'ordre décroissant se présentait de la manière suivante : la fièvre aphteuse circulante, la PPR, la brucellose, la péripneumonie contagieuse bovine, la PPCC, la FVR, la MNC, la peste porcine africaine, la tuberculose, la rage, la variole caprine et clavelée du mouton et la DNC. Il ressort de l'outil que la plupart des maladies classées en tête de liste étaient épisodiques, survenant dans les périodes épidémiques, et n'étaient pas répandues dans le pays. La réunion des parties prenantes a mis davantage l'accent sur les maladies endémiques qui contribuent à une charge plus lourde des maladies. Il est nécessaire d'envisager et de prendre des dispositions appropriées pour les maladies saisonnières et endémiques lors de l'utilisation de l'outil. Cet outil donne une occasion aux pays et régions ayant peu de données d'avoir une base rationnelle d'allocation de leurs maigres ressources.

**Mots-clés :** maladies animales transfrontalières, zoonoses, priorisation, Kenya

### Introduction

Livestock contributes about 26% of the total national Agricultural GDP. Promotion of commercially oriented agriculture is identified as one of the pillars of the development blueprint, vision 2030. Kenya is a member of three Regional economic communities namely: Common Market for Eastern and Southern Africa (COMESA), East African Community (EAC) and Intergovernmental Authority on Development (IGAD). This region experiences significant cross-border movement of animals.

Kenya's animal resource base comprises of 17.5 million cattle, 27.7 million goats, 17 million sheep, 3 million camels, 31.8 million poultry, 1.8 million donkeys, 415,000 pigs (KNBS, 2009). Animal resources provide livelihoods and wealth for Kenyans, and significantly contribute to the national economy. In totality, livestock, contribute about 12% of the Gross Domestic Product. In addition, the livestock sub-sector employs 40% of the agricultural labour-force of whom 60-80% are women.

The livestock industry makes a critical contribution to the national economy, food

security, household income and employment creation. The Contribution of livestock to the National GDP in 2011 was estimated at 917 million USD (Economic Survey, 2012). Services from agricultural and animal husbandry services contributed 36 million USD making up 7.4% of the national GDP.

The dominant methods of livestock production are traditional pastoral systems for the beef and small sector while small holder production prevails in the dairy sector. Kenya is endowed with a high population of wildlife which poses a challenge in disease control as some of them harbour important livestock and zoonotic diseases while others act as reservoirs to some of these diseases.

Past studies carried out on livestock keeping have shown that there are several challenges affecting the livestock industry. Among these are endemic livestock diseases, weak disease surveillance systems in the country, inadequate resources with laboratory capacity being poorly equipped and timeliness of data collection and reporting. There is poor infrastructure especially in the Arid and Semi-Arid Lands where the bulk of the country's livestock is found e.g. inaccessible roads and

inadequate quarantine facilities for animals. At the ports of entry and border posts, the quarantine facilities for both animals and humans are limited. (Matheka *et al.*, 2013). The country has expansive porous borders where uncontrolled livestock movement in search of water and pastures and to some extent cross-border livestock trade hinders effective disease control measures.

Trans-boundary Animal Diseases (TADs) cause devastating economic losses to livestock through morbidities, mortalities and direct losses to production. Outbreaks of TADs limit market access locally, regionally and internationally. Transboundary animal diseases are of significant economic, trade and/or food security importance. They can easily spread within the country and its neighbours reaching epidemic proportions with heavy losses. For example the RVF outbreak in 2006/2007 in Kenya caused losses amounting to more than 9.2 million US dollars in the livestock sector (Murithi *et al.*, 2010) and also resulted in 404 human cases with 118 deaths (CDC report, 2007).

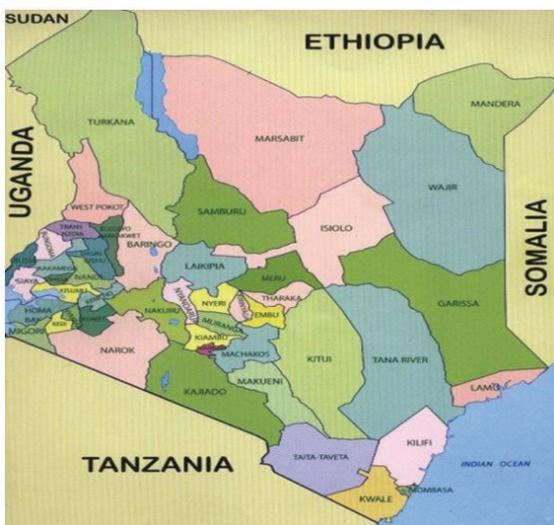
The prevention and control strategies require cooperation between several Counties and Countries. Several TADs are known to be endemic in Kenya and considering the limited resources, the control approach needs to be

effective and economically feasible. Among the TADs identified in Kenya, some are of public health (Zoonotic diseases) importance. These include Rift Valley Fever (RVF), *Brucellosis*, *Trypanosomosis*, *Bovine Tuberculosis*, Anthrax and Rabies. To control zoonotic diseases, Kenya has adopted the One Health Approach and established a Zoonotic Disease Unit in 2011, which brings together the Directorate of Veterinary Services and the Ministry of Health. There have been several attempts to identify and prioritise animal diseases in Kenya. The Zoonotic Disease Unit identified priority zoonotic diseases in the country among them being viral hemorrhagic fevers (CCHF, Dengue, RVF, Yellow Fever, Ebola, and Marburg), *Avian Influenza* and other pandemic influenza viruses, *Brucellosis*, Leishmaniasis, Leptospirosis, Anthrax and Rabies. The Smallholder Dairy Commercialisation Programme in its development of a control strategy listed priority diseases in the dairy sector, among them being ECF, *Trypanosomosis* and Anthrax. Therefore the objective of this work was to prioritise the TADS and zoonoses in order to rationally allocate the limited resources for research, surveillance and control.

## Materials and Methods

Kenya has an area of approximately 584,000 km<sup>2</sup> and borders Tanzania to the South, Uganda to the West, Ethiopia to the North, South Sudan to the North-West, Somalia to the East, and the Indian Ocean to the South-East. Only about one third of the total land area of Kenya is arable, which includes the Kenyan highlands, coastal plains and the lake region. The other two thirds of the land area is Arid Semi-Arid characterised by low, unreliable and poorly distributed rainfall. These areas are mainly used for pastoral farming with 70% of livestock in Kenya found here (FAO STAT, 2005).

A panel of multidisciplinary multisectoral experts from animal and human health was selected to undertake the prioritisation process. The team identified diseases for prioritisation based on expert



**Figure 1:** Map of Kenya showing the counties and the neighbouring countries (Source, Google 2016)

opinion and on the impact they cause in the country. The data were sourced from the directorate of Veterinary Services, the Kenya National Bureau of Statistics and FAO Statistics. Disease which are absent in the country but would have a significant consequence if introduced were also taken into account during the prioritisation.

In this study, the process of prioritisation was undertaken using the OIE tool (Phylum, 2015) by listing and categorising priority TADs including those transmissible to humans. The tool looked at the impact of the diseases and the control measures. Other aspects that were analysed included the production systems, the importance of animal production and the respective importance of the different major categories of animals and animal products for the local economy (Table 1). The analysis of a disease consisted of two sequential steps; 1. A global characterisation of the disease, aimed at assessing the epidemiology, economic consequences and human health impacts within the region, independent of any particular local context; 2. A local approach, aimed at refining the study of the disease within the country. In the local disease characterisation, the Economic impact, on human health, societal impact of the disease and environmental impact were considered.

In analysing the control measures of each disease, the following factors were considered: Feasibility of control, economic impact of control, societal and environmental impact of the control measures. The tool used for the prioritisation proposed a series of numerical indicators for each of the main types of impacts of the disease and of the corresponding control measures in the country. Depending on the selected criteria, the characteristics of the studied diseases and local contingencies, the scores may not be homogenous between the different modules applied. Thus, in order to propose relevant elements of interpretation, a mathematical correction was done to standardise these results. The presentation of the summary table of results, which provides elements to adapt the weighting of each type of criteria and to

obtain general interpretation clues for the disease prioritisation, is shown in Tables 1 and 2.

The diseases were ranked using the results from the prioritisation tool, expert opinion and the country's control strategy and status. These results were then subjected to discussions by stakeholders in the country who provided their inputs and a final list of prioritisation was arrived at as shown in Table 5.

## Results

The country priority list of TADs and zoonotic diseases as generated by the OIE ranking tool were Rift Valley Fever (RVF), Circulating Foot and Mouth Disease (FMD) strains, Lumpy skin disease (LSD), East Coast Fever (ECF), Newcastle disease (ND), African Swine Fever (ASF), contagious bovine pleuropneumonia (CBPP), *Peste des Petits Ruminants* (PPR), contagious caprine pleuropneumonia (CCPP), Rabies, Bovine Tuberculosis (BTB), sheep & Goat pox, *Brucellosis*, Exotic FMD and highly pathogenic *Avian Influenza* (HPAI). In the analysis of production by species in the livestock subsector, cattle had the majority of the share of animal production at 84% followed by chicken and goats at 5% and 4% respectively. Chicken constituted the majority of national share of exports of animal and animal products at 91% followed by pigs at 3% as shown in Table 3.

The summary of the weighted values of the prioritised diseases by theme, for diseases present in the country, RVF, circulating FMD strains, *Brucellosis* and ASF /ND, had the highest weighted value on economic impact, human health, societal impact and environmental impact, respectively. Under control measures TB had the highest weighted value while ASF had the highest weighted value on economic impact and societal and environmental impact as shown in Table 2.

From the results shown in Table 4, the diseases present in the country were provisionally ranked by the tool in the following order of priority: RVF, ASF, Circulating FMD, PPR, CBPP, CCPP, Rabies, *Brucellosis*, ND, BTB, LSD,

**Table 1:** Weighted values by disease and themes

	<b>RVF</b>	<b>FMD circulating</b>	<b>FMD exotic</b>	<b>CBPP</b>	<b>CCPP</b>	<b>PPR</b>	<b>Rabies</b>	<b>S&amp;G pox</b>	<b>ASF</b>	<b>LSD</b>	<b>ND</b>	<b>HPAI</b>	<b>Brucellosis</b>	<b>TB</b>	<b>ECF</b>
Economic impact	10.0	5.2	4.0	5.9	3.3	4.6	1.1	2.2	2.7	1.2	3.8	5.2	2.4	0.0	1.9
Impact on human health	7.9	1.5	0.9	1.5	1.5	2.9	8.2	0.0	0.9	0.9	1.7	8.8	10.0	9.8	0.9
Societal impact	7.0	7.1	10.0	3.1	3.1	4.8	4.9	1.4	4.3	3.8	2.8	4.9	2.9	0.6	0.0
Environmental impact	0.0	5.0	10.0	0.0	2.5	3.8	0.0	0.0	7.5	0.0	7.5	10.0	0.0	0.0	3.8

RVF- Rift Valley fever, ASF- African Swine Fever, FMD-Circulating Foot and Mouth Disease, PPR- Peste des Petits Ruminants, CBPP-Contagious Bovine Pleuro-Pneumonia, CCPP-Contagious Caprine Pleuro-Pneumonia, ND-Newcastle Disease, TB-Tuberculosis, LSD- Lumpy Skin Disease, ECF-East Coast Fever and S&G-Sheep & Goat Pox.

**Table 2:** weighted values by disease and themes on control measures

	<b>RVF</b>	<b>FMD circulating</b>	<b>FMD exotic</b>	<b>CBPP</b>	<b>CCPP</b>	<b>PPR</b>	<b>Rabies</b>	<b>S&amp;G pox</b>	<b>ASF</b>	<b>LSD</b>	<b>ND</b>	<b>HPAI</b>	<b>Brucellosis</b>	<b>TB</b>	<b>ECF</b>
Economic impact	2.2	1.8	7.6	2.5	3.6	0.1	1.4	1.3	8.6	1.0	0.0	4.8	5.5	10.0	0.9
Impact on human health	6.0	8.0	9.0	4.0	4.0	4.0	1.0	4.0	9.0	5.0	3.0	10.0	0.0	2.0	0.0
Societal impact	6.0	5.0	10.0	7.0	7.0	5.0	6.0	0.0	10.0	0.0	0.0	7.0	4.0	3.0	2.0
Environmental impact	0.0	5.0	10.0	0.0	2.5	3.8	0.0	0.0	7.5	0.0	7.5	10.0	0.0	0.0	3.8

RVF -Rift Valley fever, ASF- African Swine Fever, FMD-Circulating Foot and Mouth Disease, PPR- Peste des Petits Ruminants, CBPP-Contagious Bovine Pleuro-Pneumonia, CCPP-Contagious Caprine Pleuro-Pneumonia, ND-Newcastle Disease, TB-Tuberculosis, LSD- Lumpy Skin Disease, ECF-East Coast Fever and S&G-Sheep & Goat Pox.

**Table 3:** Analysis of production sectors

Species and production		Share of national animal production	Share of national exports of animal and animal products
Cattle	Live animals	-	1.07%
	Meat	28.29%	1.89%
	Milk	55.75%	--
	Hides	0.01%	--
	Total Cattle	84.06%	2.96%
Camels	Live animals	--	---
	Meat	1.05%	--
	Milk	-	-
	Total Camels	1.05%	
Goats	Live animals	--	--
	Meat	4.43%	1.95%
	Milk	--	--
	Skins	0.00%	--
	Total Goats	4.43%	1.95%
Sheep	Live animals	-	--
	Meat	3.95%	0.32%
	Milk	--	--
	Wool and skins	--	--
	Total Sheep	3.95%	0.32%
Pigs	Live animals	--	--
	Meat	1.12%	3.43%
	Skins	--	--
	Total Pigs	1.12%	3.43%
Chickens	Live animals	-	-
	Meat	2.47%	91.34%
	Eggs	2.90%	-
	Total Chickens	5.37%	91.34%
Bees (hives)	Live animals (hives)	-	-
	Honey	0.01%	-
	Total Bees	0.01%	-

**Table 4:** Standardised results of the Indicative prioritisation score

	<b>RVF</b>	<b>ASF</b>	<b>FMD circulating</b>	<b>PPR</b>	<b>CBPP</b>	<b>CCPP</b>	<b>Rabies</b>	<i>Brucellosis</i>	<b>ND</b>	<b>TB</b>	<b>LSD</b>	<b>ECF</b>	<b>S&amp;G pox</b>	<b>FMD exotic</b>	<b>HPAI</b>
Present diseases	6.14	5.72	5.30	4.18	3.57	3.57	3.52	3.22	3.12	2.57	1.80	1.43	1.27	-	-
Risk of introduction	-	-	-	-	-	-	-	-	-	-	-	-	-	High	High
Absent diseases	-	-	-	-	-	-	-	-	-	-	-	-	-	7.35	7.25

RVF - Rift Valley fever, ASF- African Swine Fever, FMD- Circulating Foot and Mouth Disease , PPR- Peste des Petits Ruminants, CBPP- Contagious Bovine Pleuro-Pneumonia , CCPP- Contagious Caprine Pleuro-Pneumonia , , ND- Newcastle Disease , TB- Tuberculosis, LSD- Lumpy Skin Disease , ECF- East Coast Fever and S&G- Sheep & Goat Pox

**Table 5:** Final list of disease prioritisation for diseases present in the country and control strategies

<b>Disease</b>	<b>Rank</b>	<b>Justifications</b>	<b>Control strategy</b>
Foot & Mouth Disease Sero Types O,A, C, Sat 1 and Sat 2	1	Disease present almost the whole country High impact on production Vaccines available locally Trade sensitive disease	Mass vaccination, movement control, surveillance and zoning. Zonal cleaning of the country from the disease beginning from the Dairy Zone Intensification of animal movement control
<i>Peste des Petits Ruminants</i>	2	Small ruminants play a major role in food security & livelihood in the ASAL region of Kenya Vaccine locally available	Mass vaccination for three years and detection and immunisation, sterilization, movement control, risk based surveillance and zoning.
<i>Brucellosis</i>	3	Disease present almost the whole country Heavy impact on human health Trade sensitive disease	Public health education, surveillance, selective vaccination in high value dairy and selective culling
<i>Contagious Bovine Pleuropneumonia</i>	4	Zonal presence of disease Trade sensitive Vaccine locally available	Risk based surveillance and zoning Massive vaccination in infected zones Movement control Selective culling in uninfected zones
<i>Contagious Caprine Pleuropneumonia</i>	5	Small ruminants play a major role in food security & livelihood in the ASAL region of Kenya Vaccine locally available	Mass vaccination, movement control, risk based surveillance and zoning.
Rift Valley Fever	6	The economic impact is high when the disease occurs It occurs sporadically and infrequently (5-15 years) High impact on food security and human health	Surveillance, Public health education, Mass routine vaccination in high risk areas. Forecasting and climate models for the occurrence of disease, mass vaccination in medium risk areas
New Castle Disease	7	Poultry is kept by majority of the households. The disease occurs countrywide with high mortalities in case of an outbreak	Vaccine manufactured and available locally. Involve all stakeholders in immunisation of especially the free range
African Swine Fever	8	The disease is a big threat to the pork industry – rapid spreading in case of an outbreak. High mortalities	No vaccine no treatment. Surveillance and disease outbreak investigations. Licensing of pig producers. Livestock movement control
<i>Tuberculosis</i>	9	Disease difficult to diagnose clinically. High impact on human health	No vaccine No treatment Surveillance with modified stamping out.

Disease	Rank	Justifications	Control strategy
Rabies	10	Public health importance. Available animal vaccine locally	Mass vaccinations in dogs and cats. Managing dog population Strategic vaccination of livestock
East Coast Fever	11	High impact on Dairy production Available control measures for the vector Available chemotherapeutic drugs and vaccine	Control of ticks. Strategic vaccination
Sheep & Goat Pox	12	Small ruminants play a major role in food security & livelihood in the ASAL region of Kenya Vaccine locally available	Strategic vaccination Movement control
Lumpy Skin Disease	13	Seasonal occurrence in most parts of the country	Strategic vaccination Movement control

**Table 6:** Final list of disease prioritisation for exotic diseases and control strategies

Disease	Rank	Justifications	Control strategy
Foot & Mouth Disease Sero Types Sat 3 & Asia 1	1	High impact on production Vaccines not available locally Trade sensitive disease Sero-type Sat 3 present within the region	Surveillance and stamping out in index herd
Highly Pathogenic Avian Influenza	3	Naïve livestock population High impact on production and trade Impact on tourism	Surveillance in wild bird and in live bird markets

ECF and S & G Pox; while for the diseases that are absent in the country, the Exotic strains of FMD was ranked higher than HPAI. Zoonotic diseases ranked as follows: RVE, *Brucellosis*, BTB and Rabies.

The stakeholders subjected the prioritised list from the tool to other considerations such as prevalence, distribution and seasonality of disease, the presence of disease control strategies and impact on production and trade that generated a new ranking as shown in Table 5 and 6 where FMD circulating, PPR and *Brucellosis* were ranked highest.

### Discussion

Prioritisation of diseases has acquired major interest within the past few years, especially from a prevention point of view

and in the sector of public and animal health (Humblet *et al.*, 2012). Such a method is important within the context of emerging diseases due to limited knowledge on how severe socioeconomic consequences would be in case of an outbreak. Our study included TADs and zoonoses and as compared to Rushdy and O’Mahony (1998 as cited by Rist *et al.*, 2014) it is noted that historically recognised methods for prioritisation have been adapted by health officials to identify infectious diseases, of both public and animal health importance, for national surveillance and risk-assessment. In general, after determining the diseases to be prioritised, the ranking processes have employed a hybrid of methods to 1) select the criteria used to define the importance of diseases, 2) apply weights to individual criteria, and 3) to score the diseases within each criterion.

As the main purpose of our work was to guide surveillance and disease control activities in Kenya, most of our categories relate strongly to the impact and feasibility of control measures. Similar to the cited prioritisation efforts, our approach makes the scoring more transparent and reproducible by explicitly defining each score. Although complete standardisation is not possible, clear definitions of the scores allowed exaggerated scoring to be identified, discussed and corrected. This ensured all the experts had a common understanding of the scoring criteria, which is another reason for the importance of clear definitions. The number and content of the categories in other studies varies considerably depending on the main purpose of the prioritisation. Some studies used categories including incidence, burden of disease and the opportunity for public-health interventions. Other criteria reflect the demands of specific groups, such as veterinary-health factors being used to prioritize zoonoses, or international aspects for prioritisation efforts involving regional collaboration.

Our findings indicated that cattle contribute 84 % of the national production of livestock and livestock products which is lower than 93% reported by FAO (FAO livestock sector brief, 2005). The difference could be attributed to the increase in promotion and consumption of white meat. From the results obtained using the phylum tool, for diseases present in the country, RVF had the highest economic impact compared to the other endemic diseases and this could be attributed to the fact that this disease had very high disease-related impact on local trade and movements, and disease-related loss in affected production sectors. *Brucellosis* had the highest impact on human health compared to other diseases under consideration which could be due to the fact that there is high prevalence of this disease in Kenya ranging from 2-14% in selected counties (Ogola *et al.*, 2014). With regard to the societal impact circulating serotypes of FMD had the highest score that could have been due to its categorisation as to have a high bioterrorism potential. ASF and ND had

the highest environmental impact compared to other diseases which could be attributed to lack of regulation on the disposal of dead animals as these diseases cause high mortalities hence contamination of the environment. In addition, lack of capacity to monitor wildlife health contributed to the high environmental impact. The feasibility of applying control measures for the various diseases was determined by considering diagnosis and surveillance, culling and compensation, trade and movement measures, vaccination, medical treatment and biosecurity. *Tuberculosis* and ASF had the highest weighted score for feasibility of control measures. This could be attributed to weak surveillance system, uncontrolled livestock movement, lack of treatment and vaccine for these diseases. The control measures for ASF, FMD and RVF had the highest economic impact which could be due to the weak surveillance systems in the country, the endemicity of the diseases and hindrance to trade. The societal and environmental impact of the control measures was also assessed by the tool and this was high for ASF, CBPP, CAPP, RVF, Rabies, PPR and FMD due to restrictions in movement, confinement of animals, uncontrolled culling, isolations and quarantines and the various control measures especially for Rabies cause pain, injury and death. The results from the tool ranked zoonotic diseases as follows; RVF, Rabies, *Brucellosis* and BTB. This ranking differs significantly from the ranking done by the Zoonotic Diseases Unit which ranked Rabies highest on the priority list followed by *Brucellosis* and Rift valley fever. However, there was agreement in that the four diseases are ranked in the top priority zoonoses in the country.

The final list of TADs and zoonoses prioritisation was revised from the results of the phylum tool after stakeholder consultation and expert opinion. The factors considered by the experts were the prevalence, distribution and seasonality of the disease in the country, impacts on production, vaccine availability, impact on trade, role of livestock in food security and as a source of livelihood and zoonotic nature of the diseases. For instance RVF is seasonal and

has inter-epidemic period of about 5-15 years which the tool did not consider. This disease is also restricted to known endemic areas and this explains why RVF was ranked lower than it was in the phylum tool output. Outbreaks of ASF are sporadic in the country and the pig population is not high compared to other livestock and hence the reason for ranking the disease lower than it was ranked by the phylum tool. The risk of introduction of the HPAI and exotic serotypes of FMD into the country is high and this can be attributed to the fact that there is trade in animal and animal products and that the disease is present in neighboring countries.

### Conclusion and Recommendations

The tool provides a fairly unbiased process of comparing and prioritising diseases across several criteria. It offers an opportunity for countries and regions with limited data to have a basis for allocation of scarce resources. The final prioritised list from the highest was as follows: Circulating FMD, PPR, *Brucellosis*, CBPP, CCPP, RVF, ND, ASF, TB, Rabies, ECF, Sheep & Goat Pox, and LSD. However, there is room for improvement by taking into consideration and making appropriate provisions for seasonal and endemic diseases. We recommend that the ranking of the diseases be incorporated in the national planning and budgeting as it will be an important justification for resource mobilisation for the livestock sector which in the past has received low funding. It will be important to carry out a similar prioritisation exercise at sub-national level as the relative importance of diseases will vary. For meaningful regional economic gains, there is need for a regional approach to the control and management of the TADs and zoonoses and regularly review the list as the epidemiology, socio-economic of disease and other factors change.

### Conflict of interest

There is no conflict of interest identified.

### Ethical standards

The study involved review of secondary data and reports generated during routine animal and human disease surveillance. There was no direct contact with human and animal subjects. Because this was part of the routine surveillance activities which are exempted from Institution Review Boards (IRBs) and National Council of Science and Technology (NCST) permission to conduct the study was not sought. The study has many benefits to the human population and to the animal by guiding policy and resource allocation for prevention and control of the diseases.

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## HAEMATOLOGICAL AND SERUM INDICES OF FINISHING BROILER CHICKENS FED GRADED LEVELS OF GUINEA HEN WEED (*PETIVERIA ALLIACEA*) PARTS

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### Abstract

A 56-day feeding trial was carried out to evaluate the effect of feeding guinea hen weed (*Petiveria alliacea*) leaf and root meals as phytobiotics on growth, carcass, haematological and serum indices of finishing broiler birds using 192 day old chicks. Eight treatment groups were arranged in a 2 × 4 factorial arrangements of 2 plant parts; *Petiveria* leaf meal (PLM) and *Petiveria* root meal (PRM) at 4 levels (0mg/kg, 500mg/kg, 1000mg/kg and 1500mg/kg). Each group was replicated three times with 8 birds per replicate. Haematological results revealed that most of the parameters measured were significantly affected ( $p < 0.05$ ) by *Petiveria* plant parts. Higher values were observed for PCV, Hb, RBC, and WBC count in birds fed diet containing PRM compared to birds on PLM. A reverse trend was, however observed in lymphocytes, eosinophil, MCV and MCH counts with birds on PLM diet showing higher ( $P < 0.05$ ) values for these parameters. Varying levels of inclusion of PLM and PRM revealed significant ( $p < 0.05$ ) effects on RBC, heterophil, lymphocyte, eosinophil, MCV, MCH and H/L counts. Lymphocyte counts values increased in 0-1000 ppm supplementation but declined at 1500 ppm inclusion level. Serum total protein, albumin, globulin and glucose of birds fed diet containing PRM showed ( $p < 0.05$ ) higher values compared to those on PLM however, increased serum urea concentration was observed for PLM fed birds. Graded levels of *Petiveria* plant parts had ( $p < 0.05$ ) influence on serum glucose, urea and HDL. Highest ( $p < 0.05$ ) urea concentration was observed in birds fed diets containing 1000 ppm of *Petiveria* plant parts while HDL was elevated ( $p < 0.05$ ) when *Petiveria* plant parts were included to diet at 500 ppm. It could be concluded that varying inclusion of PLM and PRM could be used as immune booster in finishing broiler chickens.

**Keywords:** *Petiveria alliacea*, finishing broiler, serum, haematology

## INDICES HEMATOLOGIQUES ET SÉRIQUES DE POULETS DE CHAIR EN STADE DE FINITION RECEVANT DES NIVEAUX GRADUELS DE PARTIES D'HERBE DE PINTADE (*Petiveria alliacea*)

### Résumé

Un essai alimentaire de 56 jours a été réalisé dans le but d'évaluer l'effet de l'alimentation aux feuilles et racines d'herbe de pintade comme (*Petiveria alliacea*) comme phytobiotiques sur la croissance, la carcasse, les indices hématologiques et sériques des poulets de chair en stade de finition en utilisant des poussins de 192 jours. Huit groupes de traitement ont été conçus dans des schémas factoriels 2 × 4 de 2 parties de plantes - farine de feuilles de *Petiveria* (PLM) et farine de racine de *Petiveria* (PRM) à 4 niveaux (0mg / kg, 500mg / kg, 1000mg / kg et 1500mg / kg). Chaque groupe a été répliqué trois fois avec 8 oiseaux par réplicat. Les résultats hématologiques ont révélé que la plupart des paramètres mesurés étaient significativement affectés ( $p < 0,05$ ) par les parties de la plante *Petiveria*. Des valeurs plus élevées ont été observées pour les numérations d'hématocrite, d'hémoglobine, d'érythrocytes et de leucocytes chez les oiseaux nourris à PRM par rapport aux oiseaux soumis à PLM. Une tendance inverse a toutefois été notée au niveau des numérations de lymphocytes, d'éosinophiles, de VGM et HCM, les oiseaux soumis à PLM montrant des valeurs plus élevées ( $P < 0,05$ ) pour ces paramètres. Des niveaux variables d'inclusion de PLM et de PRM ont révélé des effets significatifs ( $p < 0,05$ ) sur les numérations d'érythrocytes, d'hétérophiles, de lymphocytes, d'éosinophiles, de VGM, de HCM et du rapport H / L. La numération lymphocytaire a

augmenté en supplémentation 0 - 1 000 ppm, mais a diminué au niveau d'inclusion de 1 500 ppm. Les protéines sériques totales, l'albumine, la globuline et le glucose des oiseaux soumis à PRM ont montré des valeurs plus élevées ( $p < 0,05$ ) par rapport à ceux recevant PLM, cependant, une augmentation de la concentration sérique d'urée a été observée chez les oiseaux soumis à PLM. Les concentrations de parties de *Petiveria* ont eu une influence ( $p < 0,05$ ) sur le glucose sérique, l'urée et la HDL. La plus forte concentration d'urée ( $p < 0,05$ ) a été observée chez les oiseaux recevant 1000 ppm de parties de *Petiveria* tandis que la HDL était élevée ( $p < 0,05$ ) lorsque des parties de *Petiveria* étaient incluses dans le régime alimentaire à 500 ppm. On pourrait conclure que l'inclusion variable de PLM et de PRM peut être utilisée comme stimulant immunitaire chez les poulets de chair en stade de finition.

**Mots-clés :** *Petiveria alliacea*, poulet de chair en stade de finition, sérum, hématologie

## Introduction

Contemporary biosecurity threats arising from the increasing resistance of pathogenic microbes to antibiotics and the accumulation of antibiotics in the edible products (Simon, 2005 and Abo-Omar, 2102) have elicited a ban of antimicrobial growth promoters. The use of herbs and plant extracts (phytobiotics), as alternatives to antibiotic feed additives in diets for monogastric animals have been explored (Bedford, 2000; Wenk, 2003). Phytobiotics are plant-derived compounds and natural bioactive compounds that can be incorporated into diets in order to enhance the performance and well-being of animals. The large variety of plant compounds used as phyto-genic feed additives (PFA) are assembled according to their origin and the active substances in these products can vary greatly depending on what part of the plant that is used, the harvesting season and geographical location (Kamel, 2001). The beneficial multifunction aspects of most phytobiotics are derived from their specific bio-active components. Phytobiotics contain many pharmacologically active components which play a major role in the defence system of the plant. Wald (2003) and Máthe (2009) reported that phytobiotics are generally assumed as Generally Recognized as Safe (GRAS). Previous studies have investigated the efficacy of nutritive and non-nutritive feed additives in the diets of livestock animals with resultant improved growth and enhanced nutrient utilization (Aderemi, 2003; Amad, 2011). Phyto-genic effects have been proven in poultry for feed palatability and quality, growth promotion, gut function and

nutrient digestibility, gut microflora, immune function and carcass meat safety and quality (Mountzouris *et al.*, 2009). Guinea hen weed (*Petiveria alliacea*) is a perennial shrub from the order- Caryophyllales and family- Phytolaccaceae. Globally, its use in livestock production has not been properly harnessed except for a few cases. The present study was therefore designed to investigate the effect of root and leaf meals of the plant on serum and haematological parameters of finishing broiler chickens.

## Materials and Methods

### *Experimental site and test ingredient*

This experiment was carried out at the Teaching and Research Farm, Federal University of Agriculture, Abeokuta, Ogun State. The area lies on latitude 7°10'N and longitude 3°2'E. It is 76m above sea level. The climate is tropical humid with a mean annual rainfall of 1037mm, 34.70C temperature and relative humidity of 82%. (Google earth, 2014). The plant was obtained within the Federal University of Agriculture, Abeokuta (FUNAAB). It was uprooted completely; the leaves and the roots were cut off from the stalk separately, chopped into bits, washed to remove debris. The leaves were spread separately on polyethene bags and air dried under a shade ( $29 \pm 2$ o C) without altering the greenish colour of the leaf and the roots were sundried ( $\leq 90\%$  DM) for 14 days until they become crispy and easy to break. Both were milled (1mm sieve) into powdered form using a laboratory mill and stored separately in air tight containers at room temperature till the time of use: as *Petiveria* leaf meal (PLM) and



Ingredient	PLM (ppm)				PRM (ppm)			
	0	500	1000	1500	0	500	1000	1500
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
*Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
PLM	-	+	++	+++	-	-	-	-
PRM	-	-	-	-	-	+	++	+++
Total	100	100	100	100	100	100	100	100
<b>Calculated Analysis</b>								
ME (Kcal/Kg)	2933.20	2933.20	2933.20	2933.20	2933.20	2933.20	2933.20	2933.20
Crude Protein%	20.74	20.74	20.74	20.74	20.74	20.74	20.74	20.74
Crude Fibre%	3.41	3.41	3.41	3.41	3.41	3.41	4.41	4.41
Fat%	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Calcium%	1.52	1.52	1.52	1.52	1.52	1.52	1.52	1.52
Phosphorus	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

\*Vitamin Mineral premix provided (per kg of diet): Vit A 11500IU, Vit D3 1600IU, Riboflavin 9.9mg, Biotin 0.25mg, Pantothenic acid 11.0mg, Vitamin K 3.0mg, Vit B2 2.5mg, Vit B6 0.3mg, Vit B12 8.0mg, Nicotinic acid 8.0mg, Iron 5.0mg, Manganese 10.0mg, Zinc 4.5mg, Cobalt 0.02mg, Selenium 0.01mg

*Petiveria* root meal (PRM).

#### Experimental birds and dietary treatments

A total of 192 day-old broiler chicks of commercial strain (ANAK 2000) were purchased from a reputable commercial hatchery in Abeokuta. The poultry house and equipments were thoroughly washed and disinfected before the arrival of chicks. The birds were raised on deep litter system for 56-days. The birds were allotted to eight treatment groups of 24 birds each. Each treatment group was further divided into three replicate groups of eight birds each in a 2 × 4 factorial arrangements of; 2 plant parts (leaf and root) and 4 inclusion levels of PLM and PRM (0 ppm, 500 ppm, 1000 ppm and 1500 ppm). Eight experimental diets containing varying levels of *Petiveria* leaf meal (PLM) and *Petiveria* root meal (PRM) were formulated. Diets 1 - 4 contained *Petiveria* leaf meal (PLM) at 0 mg/kg, 500 mg/kg, 1000 mg/kg, and 1500 mg/kg while Diets 5 - 8 contained *Petiveria* root meal (PRM) at 0 mg/kg, 500 mg/kg, 1000 mg/kg, and 1500 mg/kg respectively. The gross composition of starter and finisher diets are as shown on Tables 1 and 2 respectively.

#### Data Collection

At 56th day of the study, blood samples were drawn from the wing (bronchial vein) of the birds. About 2ml of blood each were collected from two birds per replicate into a tube containing Ethylene diamine tetra acetate (EDTA) as anti-coagulant to determine haematological indices according to Jain (1996). Another 2ml blood each was collected into sterile bottles without anti-coagulant for serum biochemical components. The blood samples were assayed for serum total protein, albumin, globulin, serum enzymes, cholesterol and uric acid according to the standard procedures described by Davice and Lewis (1991).

#### Statistical Analysis

Data obtained in this experiment were laid out in a 2 × 4 factorial arrangement, subjected to Analysis of Variance (Steel and Torrie, 1980) and analysed using SAS (2000) to determine main and interaction effects. Level of probability was expressed at 5% and significant means separated using Duncan multiple range test (Duncan, 1955). Polynomial contrast (linear and quadratic) was applied to determine the effect of inclusion levels of *Petiveria* leaf and root meals (PLM; PRM).

**Table 3:** Main effect of *Petiveria* plant parts and level of inclusion on the haematological parameters of finishing broiler chickens

Measurements	Plant parts			Levels of inclusion (ppm)					P-Value		
	PLM	PRM	SEM	P-Value	0	500	1000	1500	SEM	L	Q
	PCV (%)	28.36 <sup>b</sup>	28.50 <sup>a</sup>	0.961	0.007	33.30	29.00	30.80	30.80	1.360	0.358
Hb (%)	9.01 <sup>b</sup>	9.08 <sup>a</sup>	0.283	0.003	10.53	8.95	9.90	9.95	0.400	0.651	0.053
RBC ( $\times 10^{12}/l$ )	2.30 <sup>b</sup>	2.33 <sup>a</sup>	0.094	0.002	2.90 <sup>a</sup>	2.40 <sup>b</sup>	2.40 <sup>b</sup>	2.80 <sup>ab</sup>	0.132	0.484	0.004
WBC ( $\times 10^9/l$ )	11.04 <sup>b</sup>	12.50 <sup>a</sup>	0.227	0.016	11.70	12.03	11.93	11.43	0.321	0.528	0.217
HET (%)	27.63 <sup>b</sup>	32.63 <sup>a</sup>	0.718	0.000	32.00 <sup>a</sup>	26.50 <sup>b</sup>	28.75 <sup>b</sup>	33.30 <sup>a</sup>	1.016	0.205	0.000
LYM (%)	70.88 <sup>a</sup>	66.30 <sup>b</sup>	0.588	0.000	67.50 <sup>b</sup>	71.50 <sup>a</sup>	69.75 <sup>a</sup>	66.00 <sup>b</sup>	0.718	0.159	0.000
EOS (%)	0.30 <sup>a</sup>	0.13 <sup>b</sup>	0.051	0.000	0.00 <sup>c</sup>	0.50 <sup>a</sup>	0.30 <sup>b</sup>	0.00 <sup>c</sup>	0.072	0.450	0.000
BAS (%)	0.50	0.13	0.135	0.137	0.00	0.50	0.30	0.50	0.191	0.163	0.522
MONO (%)	0.75	0.88	0.184	0.107	1.00	1.00	1.00	0.30	0.260	0.071	0.169
MCV (fL)	123.30 <sup>a</sup>	122.32 <sup>b</sup>	2.150	0.001	114.83 <sup>b</sup>	120.83 <sup>a</sup>	128.33 <sup>a</sup>	110.00 <sup>b</sup>	3.041	0.735	0.000
MCH (Pg)	39.20 <sup>a</sup>	38.97 <sup>b</sup>	0.952	0.027	36.31 <sup>b</sup>	37.30 <sup>b</sup>	41.30 <sup>a</sup>	35.54 <sup>b</sup>	1.346	0.657	0.007
MCHC (%)	31.77	31.85	0.322	0.140	31.62	30.86	32.14	32.31	0.456	0.090	0.408
H/L	0.41 <sup>b</sup>	0.53 <sup>a</sup>	0.017	0.002	0.50 <sup>ab</sup>	0.40 <sup>b</sup>	0.50 <sup>ab</sup>	0.53 <sup>a</sup>	0.024	0.260	0.002

<sup>ab</sup> Means on the same row with different superscripts are significantly different ( $p < 0.05$ )

**Table 4:** Interaction effects of *Petiveria* plant parts and levels of inclusion (ppm) on the haematological parameters of finishing broiler chickens

Measurements	PLM					PRM					SEM	P-Value
	0	500	1000	1500	0	500	1000	1500				
PCV (%)	33.50 <sup>ab</sup>	28.00 <sup>c</sup>	25.50 <sup>c</sup>	26.50 <sup>c</sup>	33.00 <sup>ab</sup>	30.00 <sup>b</sup>	36.00 <sup>a</sup>	35.00 <sup>a</sup>	0.963	0.007		
Hb (%)	10.65 <sup>ab</sup>	8.50 <sup>c</sup>	8.40 <sup>c</sup>	8.60 <sup>c</sup>	10.40 <sup>ab</sup>	9.40 <sup>b</sup>	11.35 <sup>a</sup>	11.35 <sup>a</sup>	0.299	0.003		
RBC ( $\times 10^{12}/l$ )	2.90 <sup>ab</sup>	2.00 <sup>c</sup>	2.00 <sup>c</sup>	2.00 <sup>c</sup>	2.90 <sup>ab</sup>	2.80 <sup>b</sup>	2.80 <sup>b</sup>	3.20 <sup>a</sup>	0.103	0.002		
WBC ( $\times 10^9/l$ )	12.00 <sup>b</sup>	11.20 <sup>bc</sup>	11.30 <sup>bc</sup>	10.40 <sup>c</sup>	12.10 <sup>b</sup>	12.90 <sup>a</sup>	12.60 <sup>ab</sup>	12.50 <sup>ab</sup>	0.215	0.016		
HET (%)	34.52 <sup>a</sup>	23.50 <sup>c</sup>	29.00 <sup>ab</sup>	28.50 <sup>b</sup>	34.50 <sup>a</sup>	29.50 <sup>ab</sup>	28.50 <sup>b</sup>	38.00 <sup>a</sup>	0.948	0.000		
LYM (%)	64.50 <sup>bc</sup>	73.50 <sup>a</sup>	70.00 <sup>ab</sup>	70.50 <sup>ab</sup>	64.50 <sup>bc</sup>	69.50 <sup>b</sup>	69.50 <sup>b</sup>	60.50 <sup>c</sup>	0.793	0.000		
EOS (%)	0.00 <sup>c</sup>	1.00 <sup>a</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.50 <sup>b</sup>	0.00 <sup>c</sup>	0.078	0.000		
BAS (%)	0.00	0.50	0.50	1.00	0.00	0.50	0.00	0.00	0.107	0.137		
MONO (%)	1.00	1.50	0.50	0.00	1.00	0.50	1.50	0.50	0.149	0.107		
MCV (U3)	115.52 <sup>b</sup>	140.00 <sup>a</sup>	127.50 <sup>ab</sup>	132.50 <sup>a</sup>	113.79 <sup>b</sup>	107.14 <sup>c</sup>	128.57 <sup>ab</sup>	109.40 <sup>c</sup>	2.537	0.001		
MCH (Pg)	36.72 <sup>b</sup>	42.50 <sup>a</sup>	42.00 <sup>a</sup>	43.00 <sup>a</sup>	35.86 <sup>b</sup>	33.57 <sup>b</sup>	40.54 <sup>ab</sup>	35.50 <sup>b</sup>	0.865	0.027		
MCHC (%)	31.79	30.40	32.94	32.50	31.51	31.33	31.53	32.43	0.256	0.140		
H/L	0.55 <sup>b</sup>	0.30 <sup>c</sup>	0.55 <sup>b</sup>	0.40 <sup>bc</sup>	0.55 <sup>b</sup>	0.60 <sup>ab</sup>	0.30 <sup>c</sup>	0.70 <sup>a</sup>	0.020	0.000		

<sup>abc</sup> Means on the same row with different superscripts are significantly different ( $p < 0.05$ )

**Table 5:** Main effect of *Petiveria* plant parts and level of inclusion on the serum biochemical parameters of finishing broiler chickens

Measurements	Plant parts			Levels of inclusion (ppm)					P-Value		
	PLM	PRM	SEM	P-Value	0	500	1000	1500	SEM	L	Q
Total protein (g/dl)	4.50 <sup>b</sup>	5.05 <sup>a</sup>	0.082	0.000	4.80	4.60	4.95	4.95	0.12	0.071	0.148
Albumin (g/dl)	2.78 <sup>b</sup>	2.86 <sup>a</sup>	0.076	0.018	2.98	2.82	2.67	2.83	0.22	0.644	0.148
Globulin (g/dl)	1.72 <sup>b</sup>	2.19 <sup>a</sup>	0.060	0.001	1.82	1.78	2.28	2.12	0.20	0.059	0.473
Glucose (g/dl)	119.12 <sup>b</sup>	128.25 <sup>a</sup>	1.406	0.000	132.25 <sup>a</sup>	112.25 <sup>c</sup>	123.25 <sup>ab</sup>	127.00 <sup>ab</sup>	3.48	0.601	0.000
Urea (mg/dl)	2.71 <sup>a</sup>	2.50 <sup>b</sup>	0.121	0.005	2.78 <sup>a</sup>	2.60 <sup>b</sup>	2.80 <sup>a</sup>	2.22 <sup>c</sup>	0.21	0.654	0.033
AST ( $\mu$ /l)	78.58	75.42	2.039	0.079	83.00	77.00	78.50	69.50	2.76	0.081	0.498
ALT ( $\mu$ /l)	21.83	20.75	0.983	0.820	23.33	24.33	18.67	18.83	2.30	0.905	0.196
Cholesterol (mg/dl)	95.10	89.75	2.268	0.492	94.17	96.83	88.00	90.67	3.10	0.125	0.969
Triglyceride (mg/dl)	83.30	79.50	2.666	0.384	79.67	79.33	80.00	86.50	2.88	0.158	0.263
HDL (mg/dl)	58.42	57.30	2.241	0.221	59.17 <sup>b</sup>	64.00 <sup>a</sup>	53.50 <sup>b</sup>	54.67 <sup>b</sup>	3.82	0.032	0.846
LDL (mg/dl)	12.35	10.95	0.387	0.055	19.07	19.95	18.50	18.70	0.94	1.000	0.057
VLDL (mg/dl)	16.65	15.91	0.533	0.384	15.93	15.88	16.00	17.30	0.96	0.158	0.754

<sup>abc</sup> Means on the same row with different superscripts are significantly different ( $p < 0.05$ )

**Table 6:** Interaction effect of *Petiveria* plant parts and levels of inclusion of PLM and PRM on the serum parameters of finishing broiler chickens

Measurements	PLM				PRM				SEM	P-Value
	0	500	1000	1500	0	500	1000	1500		
Total protein (g/dl)	5.35 <sup>ab</sup>	4.70 <sup>c</sup>	4.30 <sup>d</sup>	5.10 <sup>b</sup>	5.40 <sup>ab</sup>	4.40 <sup>d</sup>	5.60 <sup>a</sup>	4.90 <sup>bc</sup>	0.111	0.000
Albumin (g/dl)	2.70 <sup>b</sup>	2.60 <sup>bc</sup>	2.60 <sup>bc</sup>	2.70 <sup>b</sup>	2.70 <sup>b</sup>	2.40 <sup>c</sup>	3.20 <sup>a</sup>	2.95 <sup>ab</sup>	0.070	0.002
Globulin (g/dl)	2.65 <sup>a</sup>	2.10 <sup>b</sup>	1.70 <sup>bc</sup>	2.40 <sup>ab</sup>	2.70 <sup>a</sup>	2.00 <sup>c</sup>	2.40 <sup>ab</sup>	1.95 <sup>bc</sup>	0.069	0.018
Glucose (g/dl)	135.00 <sup>a</sup>	106.00 <sup>c</sup>	117.50 <sup>bc</sup>	124.00 <sup>b</sup>	135.50 <sup>a</sup>	118.50 <sup>bc</sup>	129.00 <sup>ab</sup>	130.00 <sup>ab</sup>	2.006	0.000
AST ( $\mu$ /l)	68.50	77.50	76.50	67.00	81.00	79.00	63.50	72.00	1.722	0.069
ALT ( $\mu$ /l)	20.00	21.50	20.00	18.00	17.50	19.50	21.00	19.00	0.638	0.729
Urea (mg/dl)	2.40	2.70	2.40	1.80	1.70	2.10	2.80	2.50	1.543	0.055
Cholesterol (mg/dl)	94.50	93.50	82.00	88.50	88.00	92.00	87.00	84.00	1.593	0.492
Triglyceride (mg/dl)	96.30	85.80	99.80	96.30	94.50	94.80	95.50	106.30	1.926	0.384
HDL (mg/dl)	63.50	63.00	51.00	55.50	57.50	61.00	49.50	51.00	1.711	0.221
LDL (mg/dl)	12.60 <sup>a</sup>	11.40 <sup>b</sup>	10.60 <sup>c</sup>	11.00 <sup>bc</sup>	12.60 <sup>a</sup>	12.10 <sup>ab</sup>	8.40 <sup>d</sup>	11.80 <sup>ab</sup>	0.395	0.006
VLDL (mg/dl)	19.30	17.20	19.95	19.30	18.90	18.95	19.10	21.30	0.385	0.384

<sup>abc</sup> Means on the same row with different superscripts are significantly different ( $p < 0.05$ )

## Results

Table 3 shows the main effect of *Petiveria* plant parts and levels of inclusion on the haematological parameters of finishing broiler chickens. The result revealed that most of the parameters measured were significantly affected ( $p < 0.05$ ) by *Petiveria* plant parts. Higher values were observed for PCV, Hb, RBC, and WBC count in birds fed diet containing PRM compared to birds on PLM. A reverse trend was, however, observed in *lymphocytes*, *eosinophil*, MCV and MCH counts with birds on PLM diet showing higher values for these parameters. Varying levels of inclusion of PLM and PRM revealed significant ( $p < 0.001$ ) effects on RBC, *heterophil*, *lymphocyte*, *eosinophil*, MCV, MCH and H/L counts. Similar values were observed in RBC count across the dietary treatments with control birds having higher value compared to birds on PLM and PRM. With white blood cell differential, *heterophil* concentration values were similar in birds fed control diet and those on 1500 mg/kg. *Lymphocyte* counts values increased in 0-1000 mg/kg supplementation but declined at 1500 mg/kg inclusion level. Meanwhile, MCV and MCH values were elevated at 500 and 1000 mg/kg inclusion level with 1500mg/kg recording the lowest values. The interaction effects of *Petiveria* plant parts and levels of inclusion on the haematological parameters in finishing broiler chickens presented in Table 4. Dietary treatments did exert significant ( $p < 0.05$ ) interaction on the parameters measured. PCV, Hb and RBC counts were found to decrease as the level of PLM increased compared with their counterparts fed PRM. White blood cell (WBC), *heterophil* and *lymphocyte* counts were statistically similar. MCV and MCH values increased as the level of supplementation of PLM increased. Broiler chickens fed 500 mg/kg PLM recorded ( $p < 0.05$ ) highest MCV and MCH values.

Effects of main and interactive of *Petiveria* plant parts and levels of inclusion on the serum bio-chemicals are presented in Tables 5 and 6. Serum total protein, albumin, globulin and glucose of birds fed diet containing PRM showed ( $p < 0.05$ ) higher values compared

to those on PLM. Increased serum urea concentration was, however, observed for PLM fed birds. Graded levels of inclusion of *Petiveria* plant parts had ( $p < 0.05$ ) influence on serum glucose, urea and HDL. Glucose value reduced in birds fed varying levels of plant parts. Levels of inclusion at 500 mg/kg had better serum glucose in broiler chickens when compared to other inclusion levels. Highest urea concentration was observed in birds fed diets containing *Petiveria* 1000 mg/kg plant parts while HDL was ( $p < 0.05$ ) elevated when *Petiveria* plant parts were included the diet at 500 mg/kg. The result revealed that the dietary treatments significantly ( $p < 0.05$ ) affected the serum total protein, albumin, globulin, glucose and LDL concentration. Serum protein, albumin, globulin and glucose concentrations were statistically similar despite different plant parts fed. Birds fed 1000 mg/kg PRM gave the highest total protein when compared with other supplementation levels and plant part. Broilers fed 0 mg/kg plant parts had improved LDL value.

## Discussion

Haematological characteristics of livestock have been observed as one of the factors that determine the response of livestock to the diet fed (Khan and Zafar, 2005; Madubuike and Ekenyen, 2006). Graded levels of inclusion of *Petiveria* plant parts did exert significant effect on haematological parameters (RBC, HET, *Lymphocyte*, *eosinophil*, MCV, MCH and H/L) of finishing broiler chickens. The higher value observed for RBC count for birds on control diet may be due to stress condition. Slight increases in the peripheral RBC mass may occur in the excited or stressed avian species (Fudge, 2000). The RBC values in this report fell within the range  $3.7 - 7.5 \times 10^6 \mu\text{l}$  reported by Hewitt et al. (1989). And also lies within the reports of Ali et al. (2004) and Islam et al. (2004). Increased *heterophil* counts recorded for birds in control group and those in 1500 ppm; increased *lymphocyte* at 500 and 100 ppm and *eosinophil* counts at 500 ppm by dietary treatments could be attributed to

the synergetic effect of the active ingredients in the diet to combat foreign bodies. These WBC differentials play protective roles and associated with the production of antibodies and recognition of foreign substance such as bacteria and viruses (Oso, 2007). High values above normal values could be implicated in infectious inflammatory conditions (Oso, 2007). Also the observation could be as a result of stress. Stressors, including food or water deprivation, temperature extremes, constant light, and exposure to novel social situations elevate the number of *heterophils* and depress the number of *lymphocytes* (Gross and Siegel, 1983). Meanwhile, these observations were synonymous to the reports of (Obikaonu et al., 2014) when broiler finishers' diets were supplemented with neem leaf meal. However, PLM and PRM at 500 and 1000 ppm inclusion appreciated MCV concentration and this could indicate traces of anaemia. According to Adebisi (2007) and Post et al. (2007) increase in MCV and MCHC an indication of microcytic anaemia. Moreover the relevance of MCV, MCH and MCHC measurements lies in their use in the diagnosis of anaemia an index of the capacity of bone marrow to produce RBC (Aletor and Egberongbe, 1992); the result of this study is in harmony with the reports of Oso et al. (2014) when broiler diet was supplemented with bitter leaf extract. Furthermore, the occurrence of variations in H/L as well as elevation in its concentration may be due to stressors (unfavourable environmental conditions) as reported earlier. The interaction effect showed that dietary treatment significantly influenced haematological parameters (Hb, PCV, RBC, WBC, *lymphocyte*, *eosinophil*, MCV, MCH and H/L) of broiler chickens. The low haemoglobin, packed cell volume, red blood cell observed in birds fed graded levels of diet containing PLM could be an indication of anaemia. Anaemia is an indication of reduced concentration of haemoglobin circulation in the blood (Allison, 1999). Coles (1986) reported that PCV is the quickest indirect way of assessing values of red blood cells in circulation and is often used as a simple screening test for anaemia. High WBC count was recorded for birds on 500 ppm

inclusion of PRM compared to those on other levels of dietary treatments nevertheless the value observed was normal and fell within 5.0 - 15.00x 10<sup>9</sup>/l reported by McDonald (1996). It also fell within the range reported by Ali triacylglycerols (2004) and Islam et al., (2004). The difference observed in the mean WBC values of birds could be due to differences in the intrinsic body defence system (Ganong 1991). However, the result was in line with the reports of Ayodeji et al. (2005) who fed laying hens Siam weed (*Chromolaena odorata*) leaf meal. The increase in *heterophil* count at 1500 ppm inclusion PRM; increase in *lymphocyte* count and *eosinophil* count at 500 ppm inclusion of PLM respectively may be due to the synergetic effect of these WBC differentials and the diet at this levels to combat foreign bodies as stated earlier by (Oso, 2007). The increase in *eosinophil* in birds fed diet containing 500 ppm PLM was an indication that the birds were exposed to foreign bodies. These WBC differentials act as agent against foreign substances (Oso, 2007). High values above normal values could be implicated in infectious inflammatory conditions, stress condition or conditions induced by steroids (Oso, 2007). However, values obtain for MCV of broiler chickens fell within the reference range 90 to 140 fl for healthy birds as reported by Mitruka and Rawnsley (1977).

At finishing phase, birds on PRM obtained a more appreciative value for serum total protein, albumin, globulin and glucose compared to those in PLM. These findings did not agree with the findings of Al-Homidan (2005) and Ademola et al. (2009) who found a significant decrease in blood serum glucose, total protein and cholesterol when feeding chicks up to 6% ginger root meal and also with the results of Zomrawi et al. (2013). Tewe and Maner (1980) reported that serum protein was related to the availability of protein in the diets. The values obtained for urea concentration for birds on PLM, showed indication of better protein utilisation in the diet (Eggum, 1970; 1976). The quadratic increase in glucose concentration observed in broiler chickens fed control diet could be linked to inflammation

of the liver. High serum glucose is an indication of pancreatic or liver inflammation (Cheesebrough, 1999). Urea concentration was poor in broilers fed diet containing 1500 ppm inclusion of dietary treatments compared to other levels of inclusion; this indicated that at this level protein was better utilized. Blood urea is inversely related to the quality of dietary protein (Eggum, 1970) and high serum urea indicates poor dietary protein utilization (Awosanya *et al.*, 1999). Values obtained for serum urea in this study higher than (1.80-2.16 mg/dl) reported by Sogunle *et al.* (2005). Meanwhile, HDL was influenced by *Petiveria* plant parts and 500 ppm inclusion recorded the highest value. This increase could be as a result of harmonious gut environment suitable for the release and assimilation of digestive nutrients necessary for growths (Elangovan *et al.*, 2000) and it is also in harmony with the reports of Prasad *et al.*, (2009) who recorded decreased total cholesterol, *triacylglycerols*, LDL and VLDL while HDL was significantly increased by garlic supplementation in chicken up to 8 weeks of age in comparison to control. In the present study, the interaction effect showed that dietary treatments had a positive influence on the serum total protein, albumin, globulin, glucose of finishing broiler chickens. The values for serum total protein varied significantly across the group but a more appreciative value was obtained for birds on diet containing PRM at 1000 ppm. However the values were within the range of 3.25g/dl to 7.61g/dl observed by Rajurker *et al.*, (2009). It has been shown that phytochemicals contain a high level of plant proteolytic enzyme (Thompson *et al.*, 1973; Ziauddin *et al.*, 1995; Naveena *et al.*, 2004) that could help birds digest dietary protein upon ingestion. The result in this study fell in line with the observations of Zhang *et al.*, (2009) who had similar results when broiler chickens were fed Ginger root meal. In contrast, Farinu *et al.*, (2004) reported that supplementation of ginger at the rate of 5, 10, or 15 g/kg did not affect total protein and albumin in the serum of broilers. Values obtained for albumin and globulin was within reference range for healthy bird, albumin was a bit higher than the range

of 1.25g/dl to 2.20g/dl observed by Akinmutimi and Onen (2008). These values obtained in this study were similar to those observed by Obikaonu *et al.*, (2014) who fed broiler finisher diet containing neem leaf meal. The increase in globulin concentration in birds fed control diet could be indicative of their immune system combating foreign bodies. Babatunde and Oluyemi (2000) opined that, the higher the value of serum globulin the better the ability to fight against disease. Serum glucose of broiler chickens was also higher in birds fed control diet and this could also be linked to the report earlier stated. Meanwhile dietary treatments led to a decrease in LDL concentration at 1000 ppm inclusion. This was in consonance with the reports of Prasad *et al.*, 2009 whereas total cholesterol, triglycerides, LDL and VLDL were significantly decreased by *Allium sativum* and also the reports of Heinrich *et al.*, (2004) and Forster (2008) in which animals were treated with garlic extract.

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## EFFECTS OF WONDERFUL KOLA (*BUCHHOLZIA CORIACEA*) SEED MEAL ON NUTRIENT DIGESTIBILITY AND CARCASS YIELD OF GROWER RABBITS

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### Abstract

The experiment was conducted to investigate the effect of wonderful kola (*Buchholzia coriacea*) seed meal (BSM) on nutrient digestibility and carcass yield of grower rabbits. A total of 24 rabbits were randomly allotted into four dietary groups of six rabbits with three replicate of two rabbits. The diets contained BSM at four levels of inclusion (0, 250, 500 and 750 mg/kg). The experiment was arranged in a completely randomized design (CRD). The proximate analysis of *Buchholzia coriacea* seeds revealed 12.77% crude protein, 2.00% crude fibre, 4.65% ash, 78.38% nitrogen free extract and 2.20% ether extract. Furthermore, phytochemical screening showed that *Buchholzia coriacea* seeds contained 3.27 mg/g of alkaloid, 0.29 of mg/g tannin, 0.56 of mg/g flavonoid and 1.35 of mg/g of saponin. There were significant ( $P < 0.05$ ) effect of BSM at varying levels on digestibility study. Dry matter and crude protein digestibilities were increased ( $P < 0.05$ ) in rabbits fed diets containing 250 mg/kg and 500 mg/kg of BSM than those on control diets and 750 mg/kg. When compared to rabbits fed control diets and 250 mg/kg of BSM, crude fiber and ash digestibilities were better ( $P < 0.05$ ) at 500 mg/kg and 750 mg/kg. Shoulder in rabbits fed 500 mg/kg of BSM was reduced ( $P < 0.05$ ) compared to other varying inclusion levels of BSM. Incorporation of 0, 250 and 750 mg/kg had heavier ( $P < 0.05$ ) neck than that of 500 mg/kg of BSM. There was an increase ( $P < 0.05$ ) in kidney weight of rabbits as levels of inclusion increases, however it was vice-versa in lungs weight. It could be concluded that addition of 500 mg/kg of BSM resulted in effective utilization of nutrients for grower rabbits.

**Keywords:** *Buchholzia coriacea*, Rabbits, Proximate, Digestibility, Carcass

## EFFETS DE LA FARINE DE GRAINE DU WONDERFUL KOLA (*Buchholzia coriacea*) SUR LA DIGESTIBILITÉ DES NUTRIMENTS ET LE RENDEMENT BOUCHER DES LAPINS EN CROISSANCE

### Résumé

L'expérience a été menée dans le but d'étudier l'effet de la farine de graines (BSM) du wonderful kola (*Buchholzia coriacea*) sur la digestibilité des nutriments et le rendement boucher de lapins en croissance. Au total, 24 lapins ont été répartis de manière aléatoire en quatre groupes alimentaires de six lapins avec trois répétitions de deux lapins. Les régimes contenaient de la BSM à quatre niveaux d'inclusion (0, 250, 500 et 750 mg / kg). L'expérience a été organisée selon un schéma complètement randomisé. L'analyse immédiate des graines de *Buchholzia coriacea* a révélé 12,77% de protéines brutes, 2,00% de fibres brutes, 4,65% de cendres, 78,38% d'extrait exempt d'azote et 2,20% d'extrait étheré. En outre, l'examen phytochimique a montré que les graines de *Buchholzia coriacea* contenaient 3,27 mg / g d'alkaloïde, 0,29 mg / g de tanin, 0,56 mg / g de flavonoïde et 1,35 mg / g de saponine. On a noté un effet significatif ( $P < 0,05$ ) de la BSM à des niveaux variables sur l'étude de digestibilité. La digestibilité des matières sèches et des protéines brutes a augmenté ( $P < 0,05$ ) chez les lapins soumis aux régimes contenant 250 mg / kg et 500 mg / kg de BSM par rapport aux régimes témoins et à 750 mg / kg. Quant à la comparaison avec les lapins soumis aux régimes témoins et à 250mg/ kg de BSM, les digestibilités des fibres brutes et des cendres étaient meilleures ( $P < 0,05$ ) à 500 mg / kg et à 750 mg / kg). L'épaule des lapins recevant 500 mg / kg de BSM a été réduite ( $P < 0,05$ ) en dimension par rapport à d'autres niveaux

d'inclusion variables de BSM. L'incorporation de 0, 250 et 750 mg / kg a conduit à un cou plus lourd ( $P < 0,05$ ) que celle de 500 mg / kg de BSM. On a noté une augmentation ( $P < 0,05$ ) du poids des reins chez les lapins parallèlement à l'augmentation des niveaux d'inclusion ; cependant, la situation était inverse pour le poids des poumons. On pourrait conclure que l'ajout de 500 mg / kg de BSM a entraîné une utilisation efficace des nutriments chez les lapins en croissance.

**Mots-clés :** Buchholzia coriacea, lapins, immédiat, digestibilité, carcasse

## Introduction

In Nigeria just like in other developing countries of the world, the food supply is inadequate and protein most especially is in short supply (Nwakpu *et al.*, 2000). To alleviate this protein malnutrition in this part of the world (Africa), rabbit production is one of the cheapest and reliable ways of achieving this (Mohammed, 2003). Rabbit production serves as a means of utilizing small rural holdings in a profitable manner and it is a more efficient means of converting low quality feed ingredients into meat for human consumption. Besides, rabbit production can also be a family hobby for semi-rural and urban families and at the same time could give the families a supply of very nutritious meat with all amino acids required for the human body.

In addition, the meat is low in cholesterol and high in omega 3 fatty acids (McCroskey, 2000). There are pockets of information online that support the beneficial effects of antibiotics as additive, when antibiotics is added into the feed of animal, it ensures more efficient conversion of nutrient elements to tissues elements. The meat obtained from animals supplemented with antibiotic as feed additives is always better in terms of quality and the amount of protein is always high with low fat content when compared to that obtained from animals not supplemented with antibiotics (Hughes and Heritage, 2002). Gustafson and Bowen, (1997) reported that the use of tetracycline and penicillin in chicken feed led to a significant improvement in the production of eggs and hatchability besides feed efficiency. However, there are problems associated with usage of antibiotics such as drugs toxicity, residual effects and development of microbial resistance. The negative impact on consumers of meat due to residual effects has also raised

some concern. Antibiotic-resistant strains have always been having a positive correlation with use of antibiotics, resistance is detected even in bacteria obtained from places which are not only uninhabited but also, thinly populated (Chattopadhyay and Grossart, 2010).

Judging from this backdrop, the possibility that its presence in the animal feed may contribute to the crisis is high and has brought forth a serious controversy over the years. This has consequently led to the ban on the use of antibiotics as growth promoters since 2006 by the European Union. Since prehistoric times, plants and their extracts have been used for their healing properties. Due to the nutritional content like, protein, carbohydrates, fats and oils, minerals, vitamins, and water, plants are responsible for growth and development in man and animals. In addition to vitamins and pro-vitamins in fruits and vegetables, the presence of bioactive plant components often called phytochemicals has been considered of crucial nutritional importance in the prevention of chronic diseases such as cancer, cardiovascular disease and diabetics (Aruona, 2003). Therefore, the study was undertaken to check the effect of wonderful kola (*buchholzia coriacea*) seed meal on nutrient digestibility and carcass yield of grower rabbits.

## Materials and methods

### Study Area

The experiment was carried out at the Teaching and Research Farm of Federal University of Agriculture, Alabata, Abeokuta, Ogun State, Nigeria. Alabata is located in the south-west Nigeria lying between Latitude 6.2 N and 7.8 N and longitude 3 E and 5 E and altitude of 76 meters above the sea level (Google earth, 2014).

#### Source and preparation of test ingredient

*Buchholzia coriacea* seeds were purchased from a local market in Ibadan, Oyo State, Nigeria. The seeds were washed to remove the adhering particles, chopped into pieces, sun-dried for 1 week (< 90% DM) and pulverised into powder using laboratory mill (1 mm sieve) and stored separately in air tight containers at room temperature till the time of use.

#### Management of experimental animals and diets

Twenty four (24) grower rabbits were purchased from a reputable farm in Abeokuta, Ogun State Nigeria. They were allotted to four (4) treatment groups, each treatment consisted of (six) 6 rabbits and replicated three (3) times with two (2) rabbits per replicate. The rabbits were housed individually in all-wire metabolic cages, which were designed for easy collection of faeces and urine. They were provided with feed with feeding trough and water ad-libitum throughout the whole experiment. Diet one served as the control diet (without BSM), diet 2, 3, 4 contained 250 mg/kg, 500 mg/kg and 750 mg/kg of BSM respectively.

#### Data Collection

##### Proximate analysis of *Buchholzia coriacea*

The test ingredient, *Buchholzia coriacea* was subjected to proximate analysis (AOAC 2000).

##### Phytochemical screening of *Buchholzia coriacea*

Alkaloid determination was done using Harbone (1973) method; Flavonoid determination was done through the method described by Boham and Kocipai-Abyazan (1974); Saponin was determined by method described by Trease and Evans (1978) while the polyphenols (tannin acid) was determined in 0.05, 0.2 or 0.5ml aliquot using Folinocalteu (Sigma) and standard tannic acid (0.5mg/ml) as described by (Makkar *et al.*, 1996).

#### Digestibility Study

At the end of the experiment, one rabbit per replicate was taken at random and was housed in an individual cage for 7 days.

Three days of acclimatization was allowed prior to the collection of faeces. Excreta collected per replicate per day was oven dried (60 °C) and used for analysis. Proximate composition of feed and dried excreta samples were analyzed for dry matter crude fibre, ether extract, ash and crude protein using standard methods of (AOAC 2000).

#### Carcass evaluation

At the end of the experiment (eight weeks), the experimental animals were starved for 12 hours to empty their gastro-intestinal tract (GIT) for a very easy and cleaner evisceration. The selected rabbits were slaughtered and carcasses were drained of blood for 2 minutes and were skinned as described by Omojola and Adesehinwa (2000). Evisceration was accomplished by removing the gastro-intestinal tract (GIT). The carcasses were dissected into promal cuts of loin, shoulder, hind-limb, fore-limb, legs, neck, head, tail, and the organs such as GIT, liver, kidney and lungs weight were recorded according to Aduku and Olukosi (1990).

#### Statistical Analyses

All data collected were subjected to one way analysis of variance (SAS, 2008). Differences between the treatment means were separated using Duncan's New Multiple Range Test (1955) at 5% level of significant.

## Results

Table 2 shows the proximate composition of *Buchholzia coriacea*. The results revealed that *Buchholzia coriacea* seeds contained 12.77% crude protein, 2.00% crude fibre, 4.65% ash, 78.38% nitrogen free extract and 2.20% ether extract.

The Phytochemical composition of *Buchholzia coriacea* is presented in Table 3. The result showed that *Buchholzia coriacea* seeds contained some anti-nutritional factors like, alkaloid (3.27 %), tannin (0.29 %), flavonoid (0.56 %) and saponin (1.35 %).

Table 4 shows the apparent nutrient digestibilities of rabbits fed diets containing BSM.

**Table 1:** Gross composition of the experimental diets

Ingredients	BSM (mg/kg)			
	0	250	500	750
Maize	35.00	35.00	35.00	35.00
Soybean	8.00	8.00	8.00	8.00
Groundnut	6.30	6.30	6.30	6.30
Palm kernel cake	15.00	15.00	15.00	15.00
Wheat offal	30.00	30.00	30.00	30.00
Bone	3.00	3.00	3.00	3.00
Oyster shell	2.00	2.00	2.00	2.00
Grower premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10
Wonderful kola	-	+	++	+++
Total	100.00	100.00	100.00	100.00
<b>Calculated chemical analysis (%)</b>				
Metabolizable energy (kcal/kg)	2365.15	2365.15	2365.15	2365.15
Crude protein (%)	16.59	16.59	16.59	16.59
Fat (%)	3.97	3.97	3.97	3.97
Crude fibre (%)	6.00	6.00	6.00	6.00

**Table 2:** Proximate Analysis of *Buchholzia coriacea* Seed meal

Parameters	Composition (%)
Dry matter	90.30
Crude protein	12.77
Crude fiber	2.00
Ether extract	2.20
Ash	4.65
NFE	78.38

**Table 3:** Phytochemical composition of *Buchholzia coriacea*

Anti-nutritional factors	Quantity (%)
Tannin	90.30
Alkaloid	0.29
3.27	2.00
Flavonoid	0.56
Saponnin	1.35

**Table 4:** Apparent nutrient digestibility of grower rabbits fed diets containing BSM

Parameters	BSM (mg/kg)				SEM
	Control	250	500	750	
DM (%)	57.90 <sup>b</sup>	64.14 <sup>a</sup>	63.82 <sup>a</sup>	60.16 <sup>b</sup>	1.18
CP (%)	77.40 <sup>b</sup>	80.14 a	82.36 <sup>a</sup>	75.72 <sup>b</sup>	1.03
CF (%)	49.42 <sup>b</sup>	50.17 <sup>b</sup>	53.71 <sup>a</sup>	55.12 <sup>a</sup>	0.94
NFE (%)	56.24	57.81	59.42	58.21	0.82
Ash (%)	62.51 <sup>b</sup>	63.12 <sup>b</sup>	67.43 <sup>a</sup>	68.14 <sup>a</sup>	1.01
EE (%)	64.69	65.82	65.31	65.92	0.87

<sup>ab</sup>means on the same row having different superscript were significantly different ( $P<0.05$ ). SEM = Standard Error of Means

There were significant ( $P<0.05$ ) differences in dry matter, crude protein, crude fibre and ash digestibilities. Dry matter and crude protein digestibilities were higher ( $P<0.05$ ) in rabbits fed diets containing 250 mg/kg and 500 mg/kg of BSM than those on control diets and 750 mg/kg. When compared to the rabbits fed control diets and 250 mg/kg of BSM, crude fiber and ash digestibilities were elevated ( $P<0.05$ ) at 500 mg/kg and 750 mg/kg.

Carcass characteristics and organ weight of rabbit is presented in Table 5. The inclusion of BSM reflected significant ( $P<0.05$ ) effect on shoulder, tail, neck, gastro intestinal tract, kidney and lungs. Rabbits fed diets added with 0 and 250 mg/kg of BSM had better ( $P<0.05$ ) tail when compared with 500 and 750 mg/kg of BSM. Shoulder in rabbits fed 500 mg/kg of BSM was reduced ( $P<0.05$ )

**Table 5:** Carcass characteristics and organ weight of rabbit fed experimental diet

Parameters	BSM (mg/kg)				SEM
	0	250	500	750	
Final live-weight (g)	1516.70	1600.00	1566.70	1546.70	155.75
Carcass weight (g)	1000.00	1033.33	1008.33	1041.67	58.94
Dressing percentage	65.93	64.58	64.36	67.35	3.78
Cut off parts					
Head Weight (%)	7.80	6.93	7.52	7.48	0.74
Fore-limb Weight (%)	7.61	7.18	7.61	7.21	0.74
Hind-limb Weight (%)	16.85	17.40	16.96	18.76	1.75
Shoulder (%)	8.06 <sup>ab</sup>	8.03 <sup>ab</sup>	6.83 <sup>b</sup>	8.44 <sup>a</sup>	0.78
Loin (%)	12.91	15.45	14.55	14.13	1.53
Tail (%)	0.41 <sup>a</sup>	0.37 <sup>a</sup>	0.22 <sup>b</sup>	0.23 <sup>b</sup>	0.03
Neck (%)	1.71 <sup>a</sup>	1.86 <sup>a</sup>	1.29 <sup>b</sup>	1.98 <sup>a</sup>	0.17
GIT (%)	0.84 <sup>ab</sup>	0.93 <sup>a</sup>	0.96 <sup>a</sup>	0.71 <sup>b</sup>	0.09
<b>Organs weight</b>					
Liver (%)	0.16	0.15	0.15	0.15	0.02
Kidney (%)	0.03 <sup>b</sup>	0.04 <sup>a</sup>	0.04 <sup>a</sup>	0.04 <sup>a</sup>	0.00
Lungs (%)	0.04 <sup>a</sup>	0.02 <sup>a</sup>	0.02 <sup>b</sup>	0.02 <sup>b</sup>	0.00

<sup>ab</sup>means on the same row having different superscript were significantly different ( $P<0.05$ ). SEM = Standard Error of Means

compared to other varying inclusion levels of BSM. When compared with 0 and 750 mg/kg of BSM, rabbits fed diets included with 250 and 500 mg/kg of BSM had poorer ( $P < 0.05$ ) neck weight. Decreased ( $P < 0.05$ ) GIT was obtained in rabbits fed diets containing 750 mg/kg of BSM when compared to other inclusion levels. There was a decrease ( $P < 0.05$ ) in kidney weight of rabbits fed diets incorporated with 0 mg/kg of BSM. Lungs of rabbit fed control diets was heavier ( $P < 0.05$ ) when compared with 250, 500 and 750 mg/kg of BSM.

### Discussion

The crude protein value (12.77%) observed for *Buchholzia coriacea* seeds in this study was slightly lower when compared to 13.28% reported by Ameachi, (2009). Nevertheless higher than 8.9% crude protein for kolanut, as reported by Jaiyeola, (2001), 3.95 % crude protein for bitter kola has revealed by (Eleyinmi *et al.*, 2006). The crude fibre (2.0%) obtained in this study was better than 1.7% obtained by Ameachi (2009) but lower than what was recorded in bitter kola 11.4% by Eleyinmi *et al.* (2006) and 7.3% as observed by (Jaiyeoba, 2001). Ash content reflects the mineral elements reserved in the seeds. The ash content (4.65%) of the seeds obtained in this work was slightly higher than 4.33% obtained by Ameachi (2009). The crude fat of 2.20% was bit lower when compared with 2.50% recorded by Ameachi, (2009), but higher than the value of 0.92% observed for kolanut by (Jaiyeola, 2001). The differences could result from type and part of plant used and their physical properties, time of harvest, preparation method of phytogenic additive and compatibility with other food components as reported by (Yang *et al.*, 2009).

The presence of bioactive compounds in *Buchholzia coriacea* like alkaloid (3.27%) has been found to have microbiocidal effects (Trease and Evans, 1978). The tannin (0.29%) present in the seeds could be partly responsible for the hot taste of *Buchholzia coriacea* seeds and it has been found to be toxic to the filamentous fungi, yeast and bacterial (Jones *et al.*, 1994). The biological function of flavonoid

(0.56%) includes protection against microbes, allergies, inflammation, platelets (Ibrahim *et al.*, 2012). The seeds contain saponnin (1.35%), which inhibits  $\text{Na}^+$  concentration in the cells, activating a  $\text{Na}^+$ - $\text{Ca}^{2+}$  anti-porter in cardiac muscle (Ibrahim *et al.*, 2012).

Nutrient utilization is the degree to which an ingested nutrient from a particular source is absorbed in a form that can be utilized in metabolism by the animal (Ammerman *et al.*, 1995). Results obtained from this study on apparent nutrient digestibility showed that supplementing diets of rabbit with BSM meal significantly affected the crude fibre digestion. Decreased digestibility of crude protein observed in rabbits fed 750 mg/kg of BSM may be attributed to the high quantity of tannins present at this level. Jansman, (1993) reported that tannins depress nutrient digestibility by forming indigestible complexes with nutrients and inhibiting the activities of digestive enzymes. The significant increase recorded in shoulder and neck at 250 and 750 mg/kg of BSM might be due to the improvement in the digestibility of feed ingredient by the inclusion of herbal dietary supplements (Jamroz *et al.*, 2003). This finding was in line with the concept that plant extracts improved the carcass yield of broiler chicken as reported by Alcicek *et al.* (2004). On the contrary, Adama and Haruna (2002), Adama and Danwake (1999) and Sankhyan *et al.* (1991) reported insignificant effects of varying inclusion of BSM on most of the carcass weights observed. Furthermore, Abdullah and Al-Kuhla, 2010 reported no significant differences in any carcass traits found with a supplementation of 5% or 10% *Nigella sativa* seeds

Significant differences achieved on lungs could be related to the biological function of this additive which enhances immune response (El-Ghamry, 2004). The decreased in lungs' weight of the rabbit fed diets containing BSM could mean that phytochemical present in BSM is not toxic to the health status of the rabbit. This corroborates the report of Ahamefule *et al.* (2006) who reported that increased metabolic rate of the organs in their attempts to reduce the anti-nutritional factors in livestock feeds to non-toxic metabolites may as well cause

increase in their weights. In contrast, Raeesi et al. (2010) reported that garlic powdered meal had no significant effects on digestive organs among different treatments.

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## FERTILITY, HATCHABILITY AND CHICK QUALITY IN ANDASSA POULTRY MULTIPLICATION CENTER, BAHIR-DAR, ETHIOPIA

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### Abstract

The present study was conducted at Andassa Poultry Multiplication Center (APMC) from October, 2014 to May, 2015 to assess the infertility rate, embryonic death, hatchability and chick quality. In this survey, a structured questionnaire was prepared to assess factors affecting the quality of hatching eggs and other related management practices in the breeder farm. Questionnaire based survey, observational survey during the hatching process and retrospective data and data during the study period was collected for assessing the infertility rate, embryonic death, hatchability and chick quality in the center. The poultry breeds found at APMC include Lohmann White, Bovan White, Bovan Rhode Island Red (Bovan RIR), ISA Brown and Hubbard Classic. The result of this study showed that the fertility, hatchability and chick quality varies from breed to breed. The infertility rate for Lohman white, Bovan White, Bovan RIR, ISA Brown and Hubbard classic at APMC varies from 16.8%-66.6%, 5.2% - 8.8%, 22.5% - 37.1%, 16.7% -82.7% and 58.7% - 69.7%, respectively. In general from the five breeds in the center Bovan White had the lowest infertility rate (highest fertility, 91.2 – 94.8%). The average hatchability rate at the center was also highest for Bovan White breed (91.0%), whereas the lowest hatchability rate was recorded for Bovan RIR (52.2%). Chick quality was fairly good for all the breeds, however, it was highest for Hubbard Classic (97.7%-100%) followed by Bovans White (92.8%-99.7%). The fertility rate recorded in the center indicates that the issue of infertility is a big challenge to the multiplication center. Therefore, Andassa Poultry Multiplication Center, in order to be profitable and sustainable in the business sector, it must assess and solve the environmental problems that significantly affect the fertility and hatchability.

**Keywords:** Chick Quality, Fertility, Hatchability, Humidity, Incubation, Temperature

## Introduction

Poultry production in Ethiopia is an important part of the mixed crop-livestock farming system practiced by most households where it makes a vital function through the provision of meat and eggs for home consumption and for the generation of cash income through market exchange. According to the official report of the Central Statistical Authority of Ethiopia (CSA, 2012), the total poultry population at country level is estimated to be about 45 million. With regard to breed, 96.5% and 3.5% of the total poultry were reported to be local village level chicks and hybrid/commercial, respectively. The total egg produced in 2011/12 is estimated about 95 million, where 79 and 16 million eggs were produced from local and commercial poultry, respectively. In the country, there is huge demand of poultry meat and eggs which has led to an emerging establishment and expansion of modern and organized poultry farms in the entire country particularly in peri-urban and urban areas (Tadelle *et al.*, 2003; Dana *et al.*, 2010). Recently, greater efforts are being made to transform the production system into a more commercialized and intensive large-scale system so as to improve the livelihood and nutrition of poor farmers and further to contribute to the national economy at large (Aklilu *et al.*, 2007; Dana *et al.*, 2010).

The numbers of large scale poultry farms in the country are limited and currently there are about five large scale poultry farms. There is no specific data on the number of medium and small scale poultry farms in the country, even though there are large numbers of small scale poultry farms. These small scale poultry farms are highly concentrated in and around Addis Ababa. There are a number of emerging small and medium scale poultry farms in and around regional capitals and zonal administrations. The small scale poultry farmers are solely dependent on large and medium scale poultry farms and government based hatcheries for supply of day old chicks (DOCs), feed and veterinary inputs (drugs, vaccines, etc) (HAPP, 2012). In total there are

around 30 hatcheries in the country, with a total setting capacity of 1.3 million eggs. About half of these hatcheries are government owned or have recently been privatized. Currently, several small scale hatcheries are emerging in the country. The government owned hatcheries are established with the objective of multiplying and distributing exotic and cross-bred poultry breeds to small and medium scale poultry farmers to enhance the poultry productivity and optimize the contribution of chicks to the national economy. However, still farmers are suffering from high loss of chicken and low production status of the sector. Among other factors, this could be associated with the breeder farm management, quality of hatching eggs and the management process in the hatchery.

The measure of success of any hatchery is the number of first-quality chicks produced. This number expressed as a percentage of all eggs set for incubation is normally termed hatchability. Optimum hatchability and chick quality can only be achieved when the egg is held under optimum conditions between laying and setting in the incubator (Elibol *et al.*, 2002; Dymond and Vinyard, 2013). The hatching egg is not “just like any egg”. It contains a living embryo which has all the genetic potential. In order to enable the embryo to express this potential during incubation and in later life as a pullet, laying hen or broiler, good hatching egg quality and good management during the hatching process are crucial. There are several factors that affect the fertility, hatchability and chick quality beginning from the parent stock farm to the hatchery (Gonzalez *et al.*, 1999; Fassenko *et al.*, 2001; Ruiz and Lunam, 2002; González-Redondo, 2006; Labaque *et al.*, 2004; Reijrink *et al.*, 2010; Gucbilmez *et al.*, 2013). While storing hatching eggs the temperature, relative humidity and position of the egg are most important conditions to be given a serious attention (PTC+, 2012).

Hatching egg quality defects lower the grade, storage/shelf life, hatchability, increase egg breakage and embryonic mortality, which generally leads to poor hatchery output and lower the profitability of hatcheries (Ruiz &

Lunam, 2002; Reijrink *et al.*, 2010). Setting a good quality hatching egg by itself can't guarantee the success of hatching process; the disinfectant used, the temperature set, the relative humidity, ventilation, turning of eggs and hygiene in the hatchery are most important factors to be considered by hatchery managers (COBB, 2008; King'ori, 2011).

Andassa Poultry Multiplication Center is one of the government owned breeder farm and hatchery that supplies DOCs of commercial breeds and feed to small and medium scale poultry farmers in Amhara National Regional State and neighboring regions. However, reports indicate that in the government owned poultry multiplication centers there is a problem of fertility (i.e. the infertility rate is high), high embryonic death, poor hatchability rate and chick quality. In addition, the factors associated with problem of fertility, embryonic death, hatchability and chick quality are not well investigated and known. In view of the above facts, the present study was undertaken to assess the rates of infertility, embryonic mortality, hatchability and chick quality. Moreover, the study determined the major factors at the breeder farm and hatchery levels that affect the hatching process.

## Materials and Methods

### Study Area

The study was conducted in Andasa Poultry Multiplication Center (APMC) from October 2014 to May 2015. APMC is located 22 km Southeast of Bahir-Dar, capital city of Amhara National Regional State. It is located 11029'N latitude and 37029' E longitude with an altitude of 1730 m.a.s.l. The minimum and maximum temperature is 13.1°C and 27.9 °C, respectively (ANRSAB, 1999). APMC is a government based hatchery and poultry farm that supplies DOCs and poultry feed to the small scale farms in the region and neighboring regions. Currently, the farm have five breeds namely: Lohmann white, Bovin white, Bovan RIR, ISA brown and Hubbard classic. The breeder farm has about 14,000 poultry with a floor system and the male to female ratio in

the farm is 1:10. The hatchery has 2 Setters (Victoria, Italy) each with a setting capacity of 15,000 eggs and 1 Hatcher (Victoria, Italy) with a capacity of 12,000 eggs. Each setter has two compartments.

### Study Subjects and Study Design

The breeder farm and hatchery of the poultry multiplication center were the target groups of the present study where the hatching eggs supplied to the hatchery, the hatching process and DOCs were considered as the major target areas of the study. Longitudinal study was conducted with regular visit to the farm and hatchery. In additional, personal observations were made during the farming activity and process of hatching.

### Questionnaire Survey:

A structured questionnaire was designed and administered to obtain information from hatchery and breeder farm at APMC. The questionnaire for hatchery focused on points like grading eggs up on arrival, egg storage condition, hatchery management (temperature and relative humidity control, setting eggs, candling), hatchability recording, etc. While the questionnaire for breeder farm mainly investigated health status of the parent stock; age of the parent stock; feed and water quality; type of housing; climate; percentage and quality of males; type and cleanness of nest boxes; house temperature; collection of hatching eggs; storage and handling of the hatching eggs; disinfection of the hatching eggs; transpiration of hatching eggs, etc.

### Fertility, Embryonic Death, Hatchability and Chick Quality Assessment

The strategy implemented at APMC to assess the fertility rate and embryonic death was the candling technique. The rate of infertility was calculated by dividing the infertile eggs to the total number of hatching eggs set at day zero. Embryonic death was calculated by dividing the number of dead embryos to the total number of hatching eggs set at day zero. The hatchability rate was determined by the number of hatched eggs at day 21 plus hours.

Hatchability was calculated by dividing the total number of chicks hatched to the total number of fertile eggs. Hatch% was calculated by dividing the total number of hatched eggs to the total number of hatching eggs placed in the setter at day zero (King'ori, 2011).

Chick quality was assessed following the Pasgar©Score quality determination protocol. In this chick quality scoring method, vitality (reflex), naval, legs, beak and belly were taken as downgrading criteria for the hatched chicks. For each chick all the five parameters were considered. The scores were given for each individual chick against the five parameters (0 = good condition, 1 = bad condition). 30-50 DOCs were randomly sampled and a score was given individually for the randomly selected chicks for the five parameters and recoded. The Pasgar©Score for each chick was calculated separately by subtracting each score from 10. Finally, the mean Pasgar©Score for all chicks in one sample was calculated. An average Pasgar©Score of 9 was considered good hatch (Hatchery Management Guide, 2012). The result for the primary/first grade chicks was calculated by dividing the primary DOCs to the total number of hatched chicks (primary and secondary DOCs).

#### *Data Management and Statistical Analysis*

The generated primary and secondary data was entered and managed in Excel Microsoft Spread sheet, and analyzed using STATA version 12.1 (StataCorp, 2011).

## **Result**

### *Management at Breeder Farm Level*

At APMC dirty eggs are cleaned by disinfectant like potassium permanganate and formaldehde. Eggs are stored in the breeder farm for 5-7 days before transferring them to the hatchery. However, the average transfer time of eggs to the hatchery is 3-4 days of laying. If they are stored longer in the breeder farm, they are stored with maintained temperature and relative humidity (Table 1). If eggs are stored for more than 7 days, the sharp end will be kept down. At the time of the study period the age of the parent flock was greater than 40 wks. The average % of first class eggs collected in the farm during the study period was 85%. Transportation of eggs to the hatchery is done manually and the temperature is not controlled while transporting eggs as it is nearby.

### *Management during the Hatching Process*

The types of eggs incubated in the hatchery at APMC are both layers and broilers. The age of the flock is recorded up on receiving eggs and grading of eggs is done up on arrival to the hatchery. The average weight of hatching eggs received in the hatchery varies from 56-65g. On average, eggs are stored in the hatchery before incubation for 3-4 days (Table 2). "Sweating" of eggs is not a problem in the hatchery. Prior to putting eggs in the setter/incubator, pre-heating is conducted at room temperature for 8 hours. Disinfection

**Table 1:** Storage temperature and humidity level to store eggs at the breeder farm

<b>Parameter</b>	<b>1-4 days</b>	<b>5-7 days</b>	<b>8-10 days</b>	<b>&gt;10 days</b>
Temperature (°C)	20	19	18	16
Humidity (%)	70	70	70	70

**Table 2:** Storage temperature and humidity level to store eggs

<b>Parameter</b>	<b>1-2 days</b>	<b>3-4 days</b>	<b>5-7 days</b>	<b>&gt;7 days</b>
Temperature (°C)	18-21	15-17	10-12	8-12
Humidity (%)	75	75	80-88	80-88

**Table 3:** Temperature, humidity and ventilation in the setter and Hatcher at APMC

	Setter	Hatcher
Temperature (°C)	37-38	37
Humidity (%)	60	85
Ventilation	15-30	25-30

of hatching eggs prior to setting is done using formaldehyde and potassium permanganate. Regular turning of eggs takes place automatically every hour. Setted eggs are candled at 18th day. Eggs hatch on average at 21 days and 6 hrs. The average weight of chicks at hatching is about 40-45g. The temperature and relative humidity in the hatchery is controlled (Table 3).

#### *Infertility Rate and Embryonic Death*

The infertility rate and embryonic death for Lohmann White varies from 16.4%-66.6% and 8.8%-40%, respectively. The average infertility rate and embryonic death of this breed is 29.7% and 25.5%, respectively. The infertility rate of Bovan White, Bovan RIR, ISA Brown and Hubbard Classic at APMC varies from 5.2% - 8.8%, 22.6% - 41.9%, 16.8% - 82.8% and 58.7% - 75%, respectively and their average infertility rate was found to be 7.1%, 30.1%, 39% and 68%, respectively. In general from the five breeds in the center Bovan White had the lowest infertility rate (i.e. highest fertility, 92.9%) and Hubbard Classic had the highest infertility (Fig 1, Fig 5).

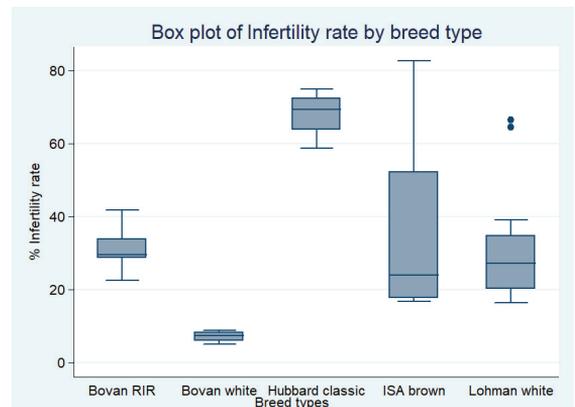
The embryonic death of Bovan White, Bovan RIR, ISA Brown and Hubbard Classic varies from 5% - 12.8%, 22.9% - 54.8%, 9% - 22.6% and 5% - 11.3%, respectively and their average embryonic death was found to be 8.4%, 34.9%, 17.8% and 7.7%, respectively. In general, Lohmann White had the highest embryonic death and Hubbard Classic had the lowest embryonic death during the hatching process at APMC (Fig 2, Fig 5).

#### *Hatchability Rate and Chick Quality*

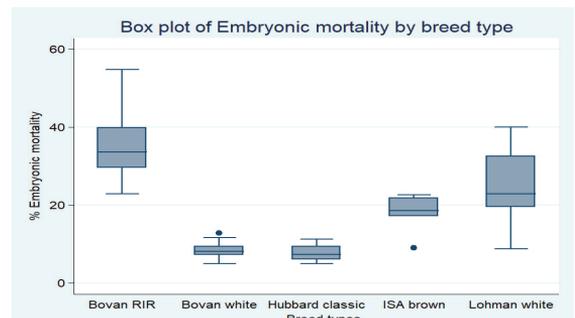
The hatchability for Lohmann White, Bovan White, Bovan RIR, ISA Brown and Hubbard Classic ranged from 38.7%-83.0%, 86.1%-94.7%, 36.6%-66.7%, 17.3%-81.4%

and 71.8%-83.7%, respectively. The average hatchability of these breeds with the same order as above was 63.9%, 91.1%, 52.2%, 63.0% and 75.6%, respectively. In general, Bovan White had the highest hatchability rate and Hubbard Classic had the lowest (Fig 3, Fig 5).

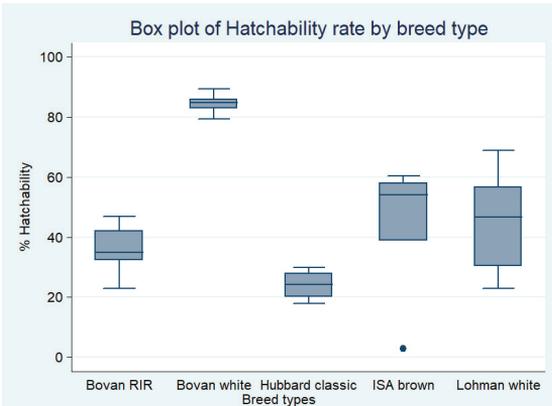
Hubbard Classic had the highest chick quality (98.9%) followed by ISA brown (96.9%). The lowest chick quality was observed for Lohmann White compared to other breeds (84.8%). The average chick quality of Lohmann White, Bovan White, Bovan RIR, ISA Brown and Hubbard Classic ranged from 70%-99.6%, 92.8%-99.7%, 77.5%-99.1%, 94.9%-98.8% and 97.7%-100%, respectively (Fig 4, Fig 5).



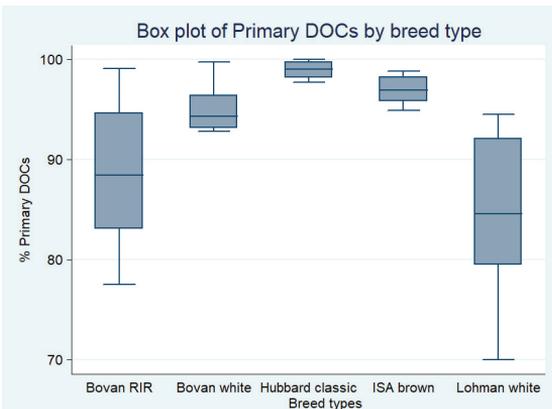
**Figure 1:** Infertility rate at Adanssa Poultry Multiplication center by breed type



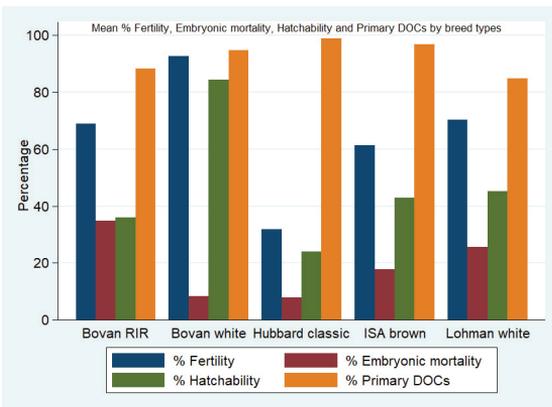
**Figure 2:** Embryonic mortality at Adanssa Poultry Multiplication center by breed type



**Figure 3:** Hatchability rate at Adanssa Poultry Multiplication center by breed type



**Figure 4:** Primary DOCs at Andassa Poultry Multiplication center by breed type



**Figure 5:** Mean % of Fertility, Embryonic mortality, Hatchability and Primary DOCs by breed types

## Discussion

The management of hatching eggs at the breeder farms, age of the parent stock, breed of the parent stock, storage condition of eggs and male to female ration are among the major factors that influence the fertility rate and hatchability rate of hatching eggs. Usually, storage of the eggs prior to incubation is unavoidable. The storage time and, above all, the temperature and relative humidity at which the eggs are stored are very important for the hatching result (Hatchery Management Guide, 2012; Horrox, 2015). The infertility rate, embryonic death and hatchability at Andassa Poultry Multiplication Center observed in the present study were far below standards. The average infertility rate was high for all breeds at APMC (29.7% for Lohmann white, 30.1% for Bovan RIR, 39% for ISA brown and 68% for Hubbard classic), except for Bovan white where the average infertility rate was 7.1%. Similarly the embryonic death was also higher for the rest of the breeds at the center except Hubbard classic (7.7%) and Bovan white (8.4%). The high infertility rate coupled with the high embryonic death at the center resulted in poor hatchability for all the breeds at the center except Bovan white where the average hatchability of this breed during the present study was 91.0%. It has been documented that at peak production for COBB breeds, it is possible to achieve up to 96.7% fertility and 93.5% hatch of fertile (COBB, 2008), however, there is some variation between breeds. But in general it has been reported that a hatchability of 80-90% for the exotic breeder birds under Kenyan condition (MOALD, 1993). Fertility and hatch percent standards are set according to the age of the breeders.

To get good fertility rate male to female ratio should be maintained to the standard, good nutrition for males, males must be active for mating and there should be replacing of inactive males at least every 20-25 weeks (COBB, 2008; ISA Brown Management Guide, 2010; PTC+, 2012). It is recommended to start at 1 day old between 10% and maximum 12% of cocks, with no special selection until production. When

moved to laying house, cocks must be reduced to a maximum of 10%, excluding immature and deformed cocks and selecting those within the expected average weight range and with good balance: the biggest and smallest will then be eliminated (ISA Brown Management Guide, 2010). However, at APMC the males are not replaced throughout the production time; this could be the probable reason for the poor fertility rate (high infertility) at the center in addition to the other factors.

Fertility and hatchability are major parameters of reproductive performance in poultry breeding programs which are most sensitive to environmental and genetic influences (King'ori, 2011). Heritability estimates for fertility and hatchability in chickens range from 0.06-0.13 (Sapp *et al.*, 2004); that means compared to the genetic factors, the non-genetic ones have a higher influence on these traits. A number of other factors including egg age (length of storage) and storage condition (Labaque *et al.*, 2004; Kozuszek *et al.*, 2009; Dymond and Vinyard, 2013), age of flock (Roque and Soares, 1994; Elibol *et al.*, 2002), system of husbandry and rearing technology (King'ori 2011), incubation temperature, relative humidity and eggs turning angle (Elibol *et al.*, 2002; COBB, 2008; ISA Brown Management Guide, 2010; Hatchery Management Guide, 2012; King'ori, 2011) have been shown to influence the hatchability of poultry eggs.

At APMC dirty eggs are cleaned by disinfectant like potassium permanganate and formaldehyde. The formaldehyde used in the breeder farm to clean dirty eggs, and hatchery for fumigation could have an impact on the life of the growing embryo and cause significant mortality (Yildirim *et al.*, 2003; Cadirci, 2009; Roger, 2013). On average, eggs are stored 5-7 days before putting them in to the setters in the study poultry multiplication center. As the length of storage increases, the hatchability rate decreases and also the hatch time increases. For every one day increase in storage after the second day, the hatchability rate will decrease by 0.7% and the hatch time increases by 1 hour (Labaque *et al.*, 2004; González-Redondo, 2006;

PTC+, 2012). In general, if eggs are stored for more than 7 days, it is recommended to put them with the sharp end down.

One of the major factors that affect the fertility, embryonic death and hatchability is the age of the parent stock. Hen age has an influence on the fertility of eggs and there is a general tendency of fertility to decline with age (Elibol *et al.*, 2002; King'ori, 2011). At the time of the study period the age of the parent flock was greater than 50 wks and this could be one of the reasons for the less hatchability rate at APMC for that specific period.

At APMC hatchery, pre-warming of eggs is not exercised. However, scientific evidences recommend pre-warming of eggs before setting them into the hatchery because uneven pre-warming increases variation in hatch time – precisely the opposite of the desired effect of pre-warming. Even with good air circulation, it will take 8 hours for eggs on a buggy to reach 78 °F (25 °C), irrespective of their initial temperature. With poor air circulation, it may take twice as long. So the recommendation is to: Provide good air circulation around the eggs and allow 6 to 12 hours for pre-warming (Reijrink *et al.*, 2010; Gucbilmez *et al.*, 2013).

The embryonic death of the different poultry breeds at APMC recorded during the study period was higher ranging from 8.8%-40% for Lohmann White (Av. 25.5%), 5 % - 12.8% for Bovan white (Av. 8.4%), 22.9% - 54.8 % for Bovan RIR (Av. 34.9%), 9% – 22.6% for ISA Brown (Av. 17.8%) and 5% - 11.3% for Hubbard Classic (Av. 7.7%). Embryonic death was also found to be one of the major problems at this poultry multiplication center. Among the causes of embryonic death several studies have indicated evidence of embryonic mortality during incubation due to excessive disinfection with formaldehyde, improper temperature and relative humidity and problems in turning of eggs (Yildirim *et al.*, 2003; Cadirci, 2009; King'ori, 2011).

After approximately 17.5-18.5 days of incubation, eggs must be transferred to the hatcher. Often, before transferring the eggs to the hatcher cabinets, eggs are candled to remove infertile eggs and eggs containing

dead embryos. At APMC candling of eggs and transferring to the hatcher takes place at Day 18. Transferring too early or too late will result in embryos being subjected to sub-optimal conditions causing lower hatchability. This should be considered in any decision to vary the transfer time. Transfer times will differ according to the different types of setters (18 to 19 days are usually the norm) (ISA Brown Management Guide, 2010; Hatchery Management Guide, 2012).

Fertility and hatchability are the major determinants of profitability in the hatchery enterprise and therefore must be given appropriate attention in breeding programs for commercial layers and broilers. According to the present finding the profitability and sustainability of Andassa Poultry Multiplication Center is doubtful, if it is going to continue with the current performance. Hatchability comprises of several sub-traits which are susceptible to genetic and environmental factors arising from various sources (Wondmeneh *et al.*, 2011).

### Conclusion

Andasa poultry multiplication center had five species of poultry and they had different percentage of fertility, hatchability and chick quality. The chick quality for all breeds is fair enough. However, the fertility and hatchability of all the breeds at the center is far below the standards except for Bovan white. The fertility rate recorded in the center indicates that the issue of infertility is a big challenge to the multiplication center. The government is currently either transferring the government owned hatcheries to private entrepreneurs or shifting them into enterprises. Therefore, Andassa Poultry Multiplication Center, in order to be profitable and sustainable in the business sector, it must assess and solve the environmental problems that significantly affect the fertility and hatchability. Moreover, selection should be made on the poultry breed that suits best to the tropical condition.

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## EFFECT OF DIETARY INCLUSION OF CASHEW NUT SHELL LIQUID ON THE PERFORMANCE, NUTRIENT DIGESTIBILITY AND BLOOD CONSTITUENTS OF WEST AFRICAN DWARF GOATS

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### Abstract

This study aimed to evaluate the effects of dietary inclusion of cashew nut shell liquid (CNSL) on performance, nutrient digestibility and blood constituents of West African dwarf (WAD) goats. Twenty-four WAD goats were divided into four groups to attain comparable group mean weights. Animals were fed diets of *Panicum maximum* supplemented with concentrate pellets. Four dietary treatments were tested, the control (CNSL0, pellets without CNSL), CNSL5 [pellets with CNSL at 5 ml kg<sup>-1</sup> dry matter (DM)], CNSL10 (pellet with CNSL at 10 ml kg<sup>-1</sup> DM) and CNSL15 (pellet with CNSL at 15 ml kg<sup>-1</sup> DM). Each animal group was randomly assigned to one of the four treatments during a 98-day trial in a completely randomized design. Goats fed CNSL5, CNSL10 and CNSL15 had reduced dry matter intake and increased ( $P < 0.05$ ) daily weight gain. Crude protein (CP) digestibility was higher ( $P < 0.05$ ) with CNSL10 and CNSL15 while organic matter digestibility (OMD) increased ( $P < 0.05$ ) with CNSL15. Animals fed CNSL had lower ( $P < 0.05$ ) feed conversion and higher ( $P < 0.05$ ) protein efficiency ratio. Blood constituents were unaltered with CNSL. Inclusion of CNSL as feed additive up to 15 ml/kg in supplemental pellets for goats could be recommended for efficient feed to weight gain conversion and nutrient utilization with no deleterious effect on animal health.

**Keywords:** Cashew nut shell liquid, feed additives, goats, performance, nutrient digestibility, Blood.

## EFFET DE L'INCLUSION ALIMENTAIRE D'UN LIQUIDE DE COQUE DE NOIX DE CAJOU SUR LA PERFORMANCE, LA DIGESTIBILITÉ DES NUTRIMENTS ET LES CONSTITUANTS SANGUINS DE CHERVRES NAINES D'AFRIQUE DE L'OUEST

### Résumé

Cette étude visait à évaluer les effets de l'inclusion alimentaire du liquide de coque de noix de cajou (CNSL) sur la performance, la digestibilité des nutriments et les constituants sanguins des chèvres naines d'Afrique de l'Ouest (WAD). Vingt-quatre chèvres WAD ont été réparties en quatre groupes pour atteindre des poids moyens de groupe comparables. Les animaux ont reçu des régimes de *Panicum maximum* complétés avec des pastilles de concentré. Quatre traitements diététiques ont été testés, le régime témoin (CNSL0, pastilles sans CNSL), CNSL5 [pastille avec CNSL à 5 ml kg<sup>-1</sup> matière sèche (DM)], CNSL10 (pastille avec CNSL à 10 ml kg<sup>-1</sup> MS) et CNSL15 (pastille avec CNSL à 15 ml kg<sup>-1</sup> DM). Chaque groupe d'animaux a été affecté de manière aléatoire à l'un des quatre traitements au cours d'un essai de 98 jours conçu selon un schéma complètement randomisé. Les chèvres nourries au CNSL5, au CNSL10 et au CNSL15 ont réduit leur consommation de matière sèche et ont augmenté ( $P < 0,05$ ) leur gain pondéral quotidien. La digestibilité de la protéine brute (CP) était plus élevée ( $P < 0,05$ ) pour CNSL10 et CNSL15, tandis que la digestibilité de la matière organique (OMD) a augmenté ( $P < 0,05$ ) avec le CNSL15. Les animaux nourris au CNSL avaient un taux de conversion alimentaire inférieur ( $P < 0,05$ ) et un rapport

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d'efficacité protéique plus élevée ( $P < 0,05$ ). Les composants sanguins n'ont pas été modifiés par le CNSL. L'inclusion de CNSL comme additif alimentaire jusqu'à 15 ml / kg dans des pastilles / suppléments pour chèvres pourrait être recommandée pour une conversion efficace aliments - gain pondéral et l'utilisation des nutriments sans effet délétère sur la santé des animaux.

**Mots-clés :** Liquide de coquille de noix de cajou, additifs alimentaires, chèvres, performance, digestibilité des nutriments, sang

## Introduction

Since discovery in 1940s, the use of antibiotics at low doses as feed additives to stimulate growth in animals had been a common practice and the benefits associated with their use include reductions in feed cost, efficient conversion of feed to animal product and improvement in animal growth rate up to 10% (Chattopadhyay, 2014). However, their use as growth promoters is being restricted or banned in most countries due to risk of development of resistant bacteria strains, appearance of antibiotic residue in meat and milk products and concerns for human health (Yang *et al.*, 2005; Calsamiglia *et al.*, 2007; Chattopadhyay, 2014; Beyene, 2016). Recent research has therefore, focused on the use of natural additives emerging from plants and plant extracts as potential feed additives to promote growth. Cashew nut shell liquid (CNSL) is an extract from cashew nut shell, and based on the mode of extraction from the shell it is classified as solvent-extracted immature CNSL or technical CNSL (Lopez *et al.*, 2012). Studies in vitro to evaluate the potency of CNSL as a feed additive for ruminants have shown that it inhibits the growth of some rumen microbes leading to reduction in methane production and enhanced propionate production (Watanabe *et al.*, 2010; Oh *et al.*, 2017). Studies of Mitsumori *et al.* (2014) confirmed the effect of CNSL in increasing propionate production in cows in vivo. The reported effect of CNSL on methane and propionate production could influence animal performance but few studies have evaluated its effect on animal responses in vivo. The few studies available are on cattle with limited reports on small ruminants. The specific dose in feed for improving animal performance is not also established. Shinkai *et al.* (2012) have proposed that in order to apply CNSL to

animal feed industry, in vivo feeding trials are required. This study therefore, investigates the effect of varying levels of cashew nut shell liquid inclusion in supplemental pellet diets for West African dwarf goats on growth performance, total tract nutrient digestibility and blood parameters.

## Materials and methods

### Location of study area

The study was conducted at the small ruminant farm of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. All experimental procedures and animal handling were carried out in accordance with the principles of the small ruminant technical committee of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta with prior approval and were in line with international standard.

### Experimental diets

The experimental diets used in this study consisted of *Panicum maximum* as basal diet and a supplemental concentrate diet in pellet form (Table 1). The concentrate supplement was compounded using wheat offal, rice bran, groundnut cake, brewers' dried grain, cassava flour, bone meal and salt as ingredients. The ingredients were properly mixed to ensure homogeneity. Thereafter, the complete concentrate mixture was incorporated with CNSL at varying inclusion levels of 0 ml kg<sup>-1</sup> DM (CNSL0), 5 ml kg<sup>-1</sup> DM (CNSL5), 10 ml kg<sup>-1</sup> DM (CNSL10) and 15 ml kg<sup>-1</sup> DM (CNSL15). Solvent-extracted CNSL with a density of 0.95 was used in this study. The concentrate mixture according to the different levels of CNSL inclusion was thoroughly mixed and then processed into pellets (9 mm

diameter sized-pellets) to give four different dietary treatments.

#### *Animals and management*

Twenty-four growing West African dwarf goats ( $8 \pm 2$  kg initial body weight, below 12 months of age), were used for this study. The animals were purchased from small ruminant farmers within surrounding villages. On arrival at the farm, the animals were given routine prophylactic treatments and allowed free movement in an enclosed paddock for a stabilization period of one month during which they were maintained on grass and supplemental concentrate diet with water supplied ad libitum. At the end of the stabilization period, the initial weights of the animals prior to data collection were taken. Thereafter, the animals were allocated into four groups of six animals each on initial weight basis with each group having comparable average weights. The animals were housed and fed individually in well-ventilated pens. Each group of animals were randomly allotted to one of four experimental diets consisting of basal diets of *Panicum maximum* supplemented with the pellets containing CNSL at varying levels as described above in a completely randomized design. Animals were fed at 50 g kg<sup>-1</sup> of body weight with considerations for changes in weekly weight. The basal diet was offered at 70% of the animals' daily feed requirement while the pellets made up the remaining 30%. The diets were offered from 8.00 am daily to animals in separate feeding troughs. Animals were allowed an adaptation period of seven days to adjust to the diet. Aliquot samples of the feeds offered were collected and oven-dried at 65°C until constant weight to determine dry matter content. Oven-dried samples were then used for further chemical analysis.

#### *Feed intake and weight gain measurements*

To evaluate animal performance on the dietary treatments, quantities of *Panicum* and pellets offered to individual animals and quantities of the feed refusals the following morning were measured daily during 84 days of feeding trial to compute daily feed intake.

After the initial individual animal weights were measured at the commencement of the experiment, weights were subsequently measured weekly to determine weight changes. Daily weight changes were calculated by dividing the weekly weight changes by the number of days per week. Feed conversion ratio was calculated by dividing the feed consumed (in grams per day) by animals in each group by their corresponding weight gain (in grams per day). Protein efficiency was calculated by dividing the weight gain (in grams per day) in animals by the protein intake (in grams per day).

#### *Digestibility measurement*

At the end of the feeding trial, animals were transferred into metabolic crates with provision for separate collection for faeces and urine. Total faeces voided from individual animals were collected and weighed daily during a 7-day collection period preceded by a 5-day adjustment period. About 5% of total faeces voided per day from each animal was subsampled and oven-dried at 65°C until constant weight. The subsamples for the seven days were then pooled by animal and stored for subsequent analysis. Digestibility of dry matter and nutrients were calculated using the formula for total collection technique (Khan *et al.*, 2003).

#### *Blood collection*

Blood samples were collected from each animal via the jugular vein puncture using hypodermic syringes at the beginning and at the end of the feeding trial. Blood collection was done before morning feeding. About 10 ml of blood was collected from each animal at each collection period. Five milliliters of the blood sample collected were immediately drawn into anticoagulant test tubes for haematological studies. The remaining 5 ml of blood samples were drawn into plain test tubes and then allowed to stand for about 6 hours until complete coagulation. The samples were then centrifuged for 10,000 × g for 15 minutes to harvest serum for the determination of some serum biochemical indices.

### Chemical analysis

Oven-dried samples of the feeds offered and faeces voided were milled to pass through a 1 mm mesh for the determination of proximate composition according to the official methods of AOAC (2000). The ash contents in samples were determined by igniting in a muffle furnace overnight at 550°C. Ether extract (EE) was determined by soxhlet extraction using petroleum ether (laboratory grade with boiling point 40 – 60°C) as solvent. The nitrogen (N) content was determined by Kjeldahl method and crude protein (CP) calculated as  $N \times 6.25$ . Organic matter (OM) content and non-structural carbohydrate content were calculated by difference as  $(100 - \% \text{ ash content})$  and  $[100 - (\% \text{NDF} + \% \text{CP} + \% \text{Fat} + \% \text{Ash})]$ , respectively. The metabolizable energy value of the diets was calculated according to Ponzenga (1985). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were determined by the methods of Van Soest *et al.*, (1991). The blood plasma samples were used for the determination of packed cell volume, haemoglobin concentration, red blood cell and white blood cell. The packed cell volume (PCV) of blood was determined by the microhaematocrit method. Haemoglobin (Hb) concentration was determined by Sahli's (acid haematin) method (Benjamin, 1978). Red blood cell was determined using an automated haemocytometer and the number of erythrocytes in a circumscribed volume was counted microscopically. Mean corpuscular volume (MCV) was calculated from the value of PCV and Red blood cell count, mean corpuscular haemoglobin (MCH) was calculated from haemoglobin and red blood cell values while mean corpuscular haemoglobin concentration (MCHC) was calculated by expressing the value of Hb in relation to PCV values as reported by Sarma (1990). White blood cell was also determined using an automated haemocytometer and counting done microscopically. From the serum samples, serum glucose was determined by the glucose oxidase method as described by Bauer *et al.* (1974). Serum protein was determined using the biuret method while globulin was estimated

as the difference between total serum protein and albumin content. The albumin:globulin ratio was calculated by dividing the albumin fraction value by the globulin fraction value of each sample. Serum urea-nitrogen was determined by the method of Marsh *et al.*, (1965).

### Statistical analysis

Data were subjected to analysis of variance in a completely randomized design using the one-way analysis of variance procedure of SAS (1999). Linear model of the study is as follows:

$$Y_{ij} = \mu + \alpha_i + \sum_{ij}$$

Where,  $Y_{ij}$  is the observation,  $\mu$  is the overall population mean,  $Y_i$  is the effect of CNSL inclusion in the diet ( $i = 1 - 4$ ) and  $\sum_{ij}$  is the residual error. Where significant differences occurred, treatment means were separated using LSD (SAS, 1999) with significant differences accepted if  $P \leq 0.05$ .

## Results

### *Effect of cashew nut shell liquid on animal weight and dry matter intake*

The data presented on Table 2 shows the effect of CNSL on average weights and dry matter intake in goats. Animals has similar ( $P > 0.05$ ) initial weight gain confirming the distribution of animals to attain comparable weight among groups at the beginning of the experiment. There was an increase ( $P < 0.05$ ) in average daily weight gain with CNSL. Goats fed CNSL5, CNSL10 and CNSL15 had higher ( $P < 0.05$ ) average daily weight gain ranging between 29.2 and 31.0 g/d relative to 23.4 g/d obtained in animals on CNSL0.

The inclusion of CNSL5, CNSL10 and CNSL15 in supplemental concentrate pellets for goats caused a reduction ( $P < 0.05$ ) in the basal, supplemental, total and metabolic dry matter intake. The total dry matter intake was reduced ( $P < 0.05$ ) by about 10.7, 18.3 and 21.1% in goats fed CNSL5, CNSL10 and CNSL15, respectively. Goats supplemented with pellets containing CNSL5, CNSL10 and

**Table 1:** Composition (in grams per kilograms) of experimental diets

Ingredient composition	Pellet with varying levels of cashew nut shell liquid				Panicum maximum
	0 ml kg <sup>-1</sup>	5 ml kg <sup>-1</sup>	10 ml kg <sup>-1</sup>	15 ml kg <sup>-1</sup>	
Wheat offal	400	400	400	400	
Rice bran	140	140	140	140	
Groundnut cake	50	50	50	50	
Brewers' dried grain	180	180	180	180	
Cassava flour	200	200	200	200	
Bone meal	20	20	20	20	
Salt	10	10	10	10	
Cashew nut shell liquid	-	+	++	+++	
Total	1000 g	1000 g	1000 g	1000 g	
<b>Nutrient composition<sup>2</sup></b>					
Dry matter (as-fed basis)	958.0	965.2	967.0	963.4	407.0
Crude protein	140.2	141.1	140.0	142.1	90.0
Ether extract	83.8	106.2	111.5	115.2	32.5
Ash	107.0	105.5	106.0	105.0	71.2
Organic matter	893.0	894.5	894.0	895.0	928.8
Non-structural carbohydrate	198.0	178.4	177.1	173.6	320.6
Neutral detergent fibre	471.0	468.8	465.3	464.1	485.6
Metabolizable energy <sup>3</sup> (Kcal kg <sup>-1</sup> )	3194	3296	3318	3344	2330
Metabolizable energy (MJ kg <sup>-1</sup> ) <sup>4</sup>	13.37	13.80	13.89	14.00	9.75

<sup>1</sup>Cashew nut shell liquid was included as feed additive to a complete (1000 g) concentrate pellet at 0 ml kg<sup>-1</sup> (-), 5 ml kg<sup>-1</sup> (+), 10 ml kg<sup>-1</sup> (++) and 15 ml kg<sup>-1</sup> (+++); <sup>2</sup>nutrient composition in g/kg DM derived from means of three values (df=2); <sup>3</sup>SME (Kcal kg<sup>-1</sup>) = 37 x % CP + 81 x % EE + 35 x % NFE (Pauzenga, 1985); <sup>4</sup>1 MJ kg<sup>-1</sup> is equivalent to 238.903 Kcal kg<sup>-1</sup>.

CNSL15 had lower (P < 0.05) feed conversion ratio and higher (P < 0.05) protein efficiency ratio relative to the group fed CNSL0.

*Effect of cashew nut shell liquid on nutrient intake and digestibility*

The effect of CNSL on nutrient intake and digestibility in goats is presented in Table 3. Intake of nutrients except for ether extract were lower (P < 0.05) in animals fed CNSL5, CNSL10 and CNSL15 relative to animals fed CNSL0. The digestibility of crude protein was higher (P < 0.05) in goats fed pellets containing CNSL10 and CNSL15 while organic matter digestibility was higher (P < 0.05) in goats fed

CNSL15. Digestibility of dry matter, ash and neutral detergent fibre were not significantly different (P>0.05) with inclusion of CNSL in the diet.

*Effect of cashew nut shell liquid on blood parameters in goats*

Table 4 shows the haematological parameters measured in goats in response to CNSL. Haematological parameters in goats were similar (P > 0.05) at the start of the experiment and these were not affected (P>0.05) by dietary inclusion of CNSL at the end of the feeding period. Similarly, serum values (Table 5) in goats fed CNSL5 CNSL10

**Table 2:** Performance and digestibility in goats fed cashew nut shell liquid

Parameters	Levels of cashew nut shell liquid in pellets				SEM ( $\pm$ )
	0 ml kg <sup>-1</sup>	5 ml kg <sup>-1</sup>	10 ml kg <sup>-1</sup>	15 ml kg <sup>-1</sup>	
<b>Dry matter intake and weight gain:</b>					
Average initial live weight (kg)	7.3	7.2	7.3	7.2	0.31
Average daily weight gain (g)	23.4 <sup>c</sup>	29.2 <sup>b</sup>	30.1 <sup>ab</sup>	31.0 <sup>a</sup>	0.62
Basal diet DMI (g day <sup>-1</sup> )	385 <sup>a</sup>	351 <sup>b</sup>	302 <sup>c</sup>	295 <sup>d</sup>	1.04
Supplemental diet DMI (g day <sup>-1</sup> )	193 <sup>a</sup>	166 <sup>b</sup>	170 <sup>b</sup>	161 <sup>b</sup>	1.89
Average total DMI (g day <sup>-1</sup> )	578 <sup>a</sup>	516 <sup>b</sup>	472 <sup>c</sup>	456 <sup>d</sup>	2.30
Feed conversion ratio	24.7 <sup>a</sup>	17.0 <sup>b</sup>	15.7 <sup>c</sup>	14.8 <sup>c</sup>	0.89
Protein efficiency ratio	0.38 <sup>c</sup>	0.53 <sup>b</sup>	0.60 <sup>bc</sup>	0.64 <sup>a</sup>	0.24
<b>Nutrient intake (g day<sup>-1</sup>):</b>					
Crude protein	61.67 <sup>a</sup>	54.95 <sup>b</sup>	51.00 <sup>c</sup>	49.42 <sup>d</sup>	0.30
Ether extract	28.67	29.01	28.80	27.96	0.20
Organic matter	529.58 <sup>a</sup>	473.95 <sup>b</sup>	432.50 <sup>c</sup>	418.05 <sup>d</sup>	2.07
Neutral Detergent Fibre	277.67 <sup>a</sup>	247.98 <sup>b</sup>	225.76 <sup>c</sup>	218.00 <sup>d</sup>	1.09
<b>Nutrient digestibility (%):</b>					
Crude protein	67.38 <sup>b</sup>	68.76 <sup>ab</sup>	69.39 <sup>a</sup>	70.12 <sup>a</sup>	0.33
Ether extract	67.02	70.37	70.38	70.64	0.47
Organic matter	66.47 <sup>b</sup>	68.11 <sup>ab</sup>	68.16 <sup>ab</sup>	68.39 <sup>a</sup>	0.31
Neutral detergent fibre	75.56	76.57	76.62	76.47	0.23

<sup>a,b</sup>Means along the same row with different superscript letters are significantly different ( $P < 0.05$ );

SEM: Standard error of mean

and CNSL15 did not vary ( $P > 0.05$ ) from values measured in goats fed CNSL0. Although the mean values of blood parameters (Table 6) showed positive mean differences while few others were negative, the inclusion of CNSL in the diet had no influence ( $P > 0.05$ ) on the mean differences observed.

## Discussion

The observed increase in daily weight gain with cashew nut shell liquid suggests its potential to enhance the rate of animal growth. Studies of Valero et al. (2016) reported better average daily weight gain and final weight in bulls supplemented with 3 g per animal per day of a mixture of essential oils containing anacardic acid which according to Morias et al. (2017), is the major active ingredient derived from CNSL. Although the biological mode of action by which CNSL caused an increase in

daily weight gain is not clearly understood in this study, it is presumed to be related to the chemical constituents in CNSL which possibly caused certain modifications in animal's metabolic processes in terms of digestion, absorption and utilization of feed nutrients. In the regulation of metabolic processes, cashew nut shell liquid has been reported to exhibit selective activity against rumen microbes, leading to fermentation that results in decreased methane and enhanced propionate production (Watanabe et al., 2010; Shinkai et al., 2012). The increased weight gain in this study, could be a likely reflection of lowered methane and increased propionate production in the rumen.

The lower dry matter intake in goats with increasing levels of CNSL in the diet was thought to be a possible reflection of reduced diet palatability. Since cashew nut shell liquid contains anacardic acid, a polyphenol, as its

**Table 3:** Haematology and serum profile of goats fed cashew nut shell liquid

Parameters	Reference ranges <sup>1</sup>	Start of experiment					End of experiment				
		Levels of cashew nut shell liquid in pellets, ml kg <sup>-1</sup>					Levels of cashew nut shell liquid in pellets, ml kg <sup>-1</sup>				
		0	5	10	15	SEM (±)	0	5	10	15	SEM (±)
Packed cell volume (%)	22-38	30.00	28.67	29.00	32.67	0.74	30.67	31.33	30.67	34.67	0.86
Hb concentration (g dL <sup>-1</sup> )	8-12	9.50	9.33	9.10	10.33	0.31	10.17	10.43	10.13	11.67	0.32
Red blood cells (x10 <sup>6</sup> µL <sup>-1</sup> )	8-18	8.57	8.72	8.51	8.93	0.11	11.93	12.40	11.97	13.00	0.34
White blood cell (x10 <sup>3</sup> µL <sup>-1</sup> )	4-13	7.87	8.03	7.867	8.13	0.30	8.63	9.07	8.93	9.10	0.36
MCV (fL)	16-25	34.96	32.88	33.99	36.55	0.58	30.17	30.03	30.32	29.75	0.24
MCH (pg)	8-12	11.07	10.65	10.65	11.57	0.26	8.52	8.42	8.47	8.98	0.07
MCHC (g dL <sup>-1</sup> )	31-34	31.67	32.44	31.31	31.69	0.67	33.15	33.30	32.99	33.64	0.18
Serum glucose (mg dL <sup>-1</sup> )	50-75	68.73	69.23	70.07	70.33	1.26	73.67	74.87	76.67	77.40	1.06
Serum protein (g dL <sup>-1</sup> )	6.2-7.9	5.63	5.80	5.76	5.73	0.16	7.27	7.47	7.73	8.13	0.18
Albumin (g dL <sup>-1</sup> )	2.9-4.3	2.90	2.50	2.67	2.67	0.14	3.20	3.37	3.43	3.83	0.13
Globulin (g/dL)	3.3-3.6	2.73	3.30	3.10	3.07	0.22	4.07	4.03	4.30	4.30	0.13
Albumin: Globulin	0.88-1.19	1.07	0.81	1.00	0.88	0.08	0.81	0.84	0.81	0.89	0.04

<sup>a,b</sup>Means along the same row with different superscript letters are significantly different (P < 0.05)

<sup>1</sup>Jackson and Cockcroft (2002); SEM: Standard error of mean

**Table 4:** Mean differences in blood parameters of goats fed pellets containing cashew nut shell liquid

Parameters	Levels of cashew nut shell liquid in pellets				SEM ( $\pm$ )
	0 ml kg <sup>-1</sup>	5 ml kg <sup>-1</sup>	10 ml kg <sup>-1</sup>	15 ml kg <sup>-1</sup>	
Packed cell volume (%)	0.67	2.67	1.67	2.00	0.43
Haemoglobin (g dL <sup>-1</sup> )	0.67	1.10	1.03	1.33	0.14
Red blood cell (x10 <sup>12</sup> L <sup>-1</sup> )	3.36	3.68	3.46	4.06	0.24
White blood cell (x10 <sup>9</sup> L <sup>-1</sup> )	0.77	1.03	1.07	0.97	0.09
Mean corpuscular volume (fL)	-9.26	-7.60	-8.32	-9.84	0.58
Mean corpuscular haemoglobin (pg)	-2.56	-2.24	-2.18	-2.59	0.25
Mean corpuscular haemoglobin concentration (%)	1.48	0.86	1.68	1.95	0.69
Serum glucose (mg dL <sup>-1</sup> )	4.93	5.63	6.60	7.07	0.70
Total serum protein (g dL <sup>-1</sup> )	1.63	1.60	1.97	2.40	0.19
Serum albumin (g dL <sup>-1</sup> )	0.30	0.87	0.77	1.17	0.14
Serum globulin (g/dL)	1.33	0.73	1.20	1.23	0.22
Albumin: Globulin ratio	-0.26	0.03	-0.18	0.02	0.08

<sup>a,b</sup>Means along the same row with different superscript letters are significantly different ( $P < 0.05$ );

SEM: Standard error of mean

main constituent (Philip *et al.*, 2008; Subbarao *et al.*, 2011; Morias *et al.*, 2017), the presence of this polyphenol could cause an astringent taste thereby inhibiting intake by animals. The high constituent of plant secondary metabolites have been linked to cause reduction in feed intake due to low palatability (Lamy *et al.*, 2011). The reduced dry matter intake in goats fed CNSL could also be attributed to higher levels of metabolizable energy content of the diet arising from increased levels of oil contributed by CNSL. Feeds with oil content have been reported to have higher metabolizable energy levels (Silva *et al.*, 2012). In ruminants, feed intake is regulated by dietary energy density and the dry matter intake in growing goats had been reported to decrease when pelleted diets containing increasing levels of metabolizable energy concentrations were fed (Lu and Potchoiba, 1990). Contrary to our study, Olagoke *et al.* (2015) in a similar study using same levels and mode of inclusion of CNSL reported no variation in feed intake in West African dwarf goats fed for a 21-d period. Osmari *et al.*, 2017, Coutinho *et al.* (2014) and Branco *et al.* (2015) in studies conducted with cows reported no effect of CNSL on feed

intake when cows consumed up to 1200 mg per kg dry matter, 7 g day<sup>-1</sup> and 30 g per cow per day of CNSL, respectively. Variations in animal feed intake response to CNSL feeding could be due to animal differences, the type of CNSL used, the level of inclusion in the diet, or length of feeding CNSL in the diet.

The better feed conversion and protein efficiency ratio in animals fed cashew nut shell liquid implied that less feed was required to produce a unit change in weight gain with an efficient utilization of dietary protein particularly at inclusion level of 15 ml kg<sup>-1</sup>. Several plant-based oils have been reported to stimulate better feed utilization efficiency when included in ruminant diet. Tassoul and Shaver (2009) reported greater feed efficiency in terms of kilogram of milk per kilogram of dry matter intake in lactating cows fed mixtures of essential oils.

The reduction in intake of dietary crude protein, ash, non-structural carbohydrate, organic matter and neutral detergent fibre with feeding of CNSL pellets is presumed as a direct effect of reduced dry matter intake. However, ether extract intake which was unaltered was

probably due to the higher contributions of ether extract in the diet with increasing levels of cashew nut shell liquid. The higher crude protein and organic matter total tract digestibility at 15 ml kg<sup>-1</sup> of CNSL feeding implied that the inclusion of cashew nut shell liquid at these levels in the diet had no adverse effect on goat nutrition in spite of the reductions in dry matter and nutrients intake. Studies of Branco *et al.*, (2015) reported higher neutral detergent fibre digestibility while the apparent total tract digestibility of other nutrients were not affected in cows supplemented with 30 g per cow per day of technical cashew nut shell liquid. When lactating dairy cows were supplemented with 0%, 0.012%, 0.024% and 0.036% of cashew nut shell liquid in the diet, dry matter intake, nutrients intake and apparent digestibility of nutrients were not affected by the inclusion of CNSL in the ration (Coutinho *et al.*, 2014).

The similarity in blood parameters and mean differences of goats fed with and without CNSL implied that CNSL did not alter the blood constituents, physiological functions and health of goats throughout the feeding period. According to Shrivastav and Singh (2012), haematological and biochemical studies are important tools in evaluating and interpreting the health status in terms of physiological functions of various organs. In this present study, haematological and serum biochemical values in all animal groups were within normal reference range values for goats as given by Jackson and Cockcroft (2002) implying that feeding CNSL at a dose rate of 5 – 15 ml kg<sup>-1</sup> of supplemental pellet diet did not impose any adverse effect on the health of the animals. It could therefore, be considered safe for inclusion in the diet. Similar to our result, plasma urea and glucose concentrations were reported not to be affected in cows supplemented with or without TCNSL (Branco *et al.*, 2015).

### Conclusion

The inclusion of CNSL as feed additive in supplemental concentrate pellet for goats up to 15 ml kg<sup>-1</sup> improved animal performance and nutrient utilization with no deleterious

effect on the haematological and biochemical status of West African dwarf goats. Cashew nut shell liquid therefore, could be safely included as feed additive in supplemental pellets for goats up to 15 ml kg<sup>-1</sup> to enhance feed and nutrient utilization without imposing any adverse effect on the health status of the animals. Animal responses to cashew nut shell liquid in the diet are however, not conclusive and could be dependent on level and mode of inclusion and length of feeding in the diet.

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### Conflict of interest

The authors whose names appear in this manuscript certify that they have no conflict of interest.

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## REPRODUCTIVE PERFORMANCE, EGG QUALITY AND WELFARE OF EGG-TYPE CHICKENS AS INFLUENCED BY LITTER MATERIALS

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### Abstract

There has been an unprecedented hike in the cost of wood shavings, a conventional litter material in Nigeria recently owing to competition on its use, thereby considerably adding to the cost of production. This study was designed to evaluate the reproductive performance, egg quality and welfare of egg-type chicken as affected by litter materials. 120 Nera black pullets were assigned to three different litter materials viz. chopped dry *Panicum maximum* (guinea grass), crushed dry corn cobs and wood shavings having four replicates of 10 birds each in a Completely Randomized Design (CRD). At weeks 21 and 36, two birds in each replicate were slaughtered for the assessment of reproductive apparatus. Five eggs were randomly selected weekly to assess the external and internal egg characteristics. Blood samples were taken from 2 hens per replicate at week 21 and 36 for the determination of *heterophil/lymphocyte* ratio (H/L). The results revealed that the different litter materials used did not have significant effect on body weights, liver weights and small yellow follicles at week 21. On the contrary, the ovary weight of the birds reared on wood shavings and *Panicum maximum* was higher than those raised on maize cobs litter material. A similar trend was observed on the oviduct weights and lengths of the birds. The large yellow follicles of the birds reared on *Panicum maximum* was similar to those of wood shavings and maize cobs but the birds on wood shaving litter material had a higher large yellow follicles than those in Maize cobs. Most of the external and internal egg characteristics were similar across the treatment groups. Moreover, the different litter materials used did not result into any significant difference on the HL of the birds. It was concluded that chopped *Panicum maximum* may be used as litter materials to replace wood shavings without adverse effect on reproductive performance, welfare and egg quality characteristics of laying chickens.

**Key words:** Hen, reproduction, egg, litter materials

## PERFORMANCE REPRODUCTIVE, QUALITÉ DES OEUFS ET BIEN-ÊTRE DES POULETS DE TYPE PONDEUSES, TEL QU'INFLUENCÉES PAR LES MATIÈRES DE LITIÈRE

### Résumé

Une hausse sans précédent a été enregistrée récemment en ce qui concerne le coût des copeaux de bois, une matière de litière conventionnelle au Nigeria en raison de la concurrence relative à son utilisation, augmentant ainsi considérablement les coûts de production. Cette étude a été conçue pour évaluer les performances reproductives, la qualité des œufs et le bien-être des poulets de type pondeuses affectés par les matières de litière. 120 poulettes noires Nera ont été affectées à trois matières de litière différentes, à savoir *Panicum maximum* (herbe de pintade), épis de maïs secs concassés, et copeaux de bois ayant quatre réplicats de 10 oiseaux chacun dans un schéma entièrement randomisé. Aux semaines 21 et 36, deux oiseaux dans chaque réplicat ont été abattus pour évaluation de l'appareil reproductif. Cinq œufs ont été sélectionnés de manière aléatoire chaque semaine pour évaluation des caractéristiques des œufs externes et internes. Des échantillons sanguins ont été prélevés sur 2 poules par réplicat aux 21<sup>ème</sup> et 36<sup>ème</sup> semaines en vue de la détermination du rapport hétérophile / lymphocyte (H / L). Les résultats ont révélé que les gros follicules jaunes des oiseaux élevés sur du *Panicum maximum* étaient similaires à celles recevant des copeaux de bois et épis de maïs, mais les oiseaux sur litière de copeaux de bois avaient des follicules jaunes plus gros que ceux logeant sur des épis de maïs. Il a été conclu que *Panicum maximum* haché peut être utilisé comme matière de litière en remplacement de copeaux de bois sans effet négatif sur les performances de reproduction, le bien-être et la qualité des œufs des poules pondeuses.

**Mots-clés :** Poule, reproduction, oeuf, matériaux de litière

## Introduction

The poultry litter has been considered as one of the most important and integral elements in providing the proper environment inside the building to achieve efficient productive and reproductive performance of poultry (Carr *et al.*, 1990). In spite of wide expansion of the poultry industry, the supply of the litter materials are not always available to meet the demand in Nigeria. This has forced the poultry industry to search for other alternative litter materials (Al-Homidan and Robertson, 2007 and Sharnam *et al.*, 2008).

Wood shavings is the commonest litter materials used in poultry production in Nigeria (Dafwang, 1990). However, wood-based litter materials are being diverted into the manufacturing of wood products (Charles, 2005). This implies that the use of wood shavings as litter materials may not be favoured and the cost magnified in poultry production (Charles, 2005) making it difficult for farmers to secure adequate quality materials for poultry production (Adene, 1989). Moreover, the increasing number of poultry producers has necessitated the search for alternative litter materials other than wood shavings.

Donham (2000) found that the presence of moisture, in conjunction with high temperature promoted the bacterial growth, causing the decomposition of the litter organic materials and producing the ammonia, which is responsible for a large number of health-and density-related welfare problems in poultry production. Also, they added that, exposing the pullets to several increasing levels of ammonia from 7 to 10 weeks of age reduced their feed intake and body weight and egg production during the laying season. Grimes *et al.* (2002) stated that wood shaving, rice hulls and straw shops were considered as satisfactory litter materials as they are available, cheap and didn't pack down easily and however, they were not good water absorbent.

Egg quality and animal welfare can be affected seriously by production systems. Poultry products and their quality are affected significantly by genetic and ecological conditions

such as the indoor environment of the poultry house (Bell and Weaver, 2002; Ahmed and Singh, 2007).

Previous researchers reported significantly different performances for laying hens in caged houses because of varying housing systems and indoor environmental conditions of the poultry house (Duncan, 2001; De Boer and Cornelissen, 2002; Awoniyi, 2003).

External and internal quality of eggs are dependent on genotype and environmental factors such as housing system, oviposition time, age, ambient temperature and nutrition, as well as their interactions (Charvátová & Tůmová, 2010). External egg quality, namely egg weight, poorer and variable eggshell quality, and shape index, and internal egg quality, namely proportion of yolk, albumen and lipid, depend on the age of laying hens (Rizzi and Chiericato, 2005; Tůmová and Ledvinka, 2009; Charvátová and Tůmová, 2010). Egg weight, eggshell content, albumen height and albumen pH are affected by interactions between the age of laying hens, oviposition time and housing system (Van den Brand *et al.*, 2004; Tůmová *et al.*, 2011).

The aim of the present study was to determine the feasibility of utilizing maize cobs and *Panicum maximum* as alternative litter materials for raising laying hens under the prevailing environmental conditions in Nigeria, in an attempt to ensure satisfactory and cost-effective bedding supplies. This study therefore sought to evaluate the reproductive performance and egg quality of egg-type chickens as influenced by litter materials using maize cobs, *Panicum maximum* and wood shavings.

### Experimental Location

The Experiment was carried out at the poultry unit of the Teaching and Research Farms of the Federal University of Agriculture Abeokuta. It is located within the rainforest zone of south western Nigeria at latitude 70 13', 490 46'N, longitude 30 26', 110 98'E and altitude 76mm above sea. The climate is humid with a mean annual rainfall of 1037mm. The annual mean temperature and humidity are usually 34°C and 82 %, respectively.

### Experimental Birds, Materials and Management

A total number of 120 Nera black pullets were obtained from a reputable commercial Farm. Birds were reared on 3 different litter materials viz. chopped dry *Panicum maximum* (guinea grass), crushed dry corn cobs and wood shavings. Birds were individually weighed at the beginning of the experiment and randomly assigned into three experimental groups. Four replicates of 10 birds (a total of 40 birds per treatment) were used for each of the experimental groups. Wood shaving was obtained at the sawmills around, while guinea grass (*Panicum maximum*) was harvested, dried and cut into pieces of approximately 3 cm, and corn cobs was sourced for from maize farmers and then crushed. All materials were air-dried before being spread as litter materials.

About 8-10 cm depth of litter materials was used as bedding. Routine and occasional management practices in poultry production was carried out. Feeders and drinkers was cleaned, and litter was changed as at when due. Sanitation of the environment was maintained throughout the experimental period. The experimental diet is shown in Table 1.

**Table 1:** Percentage Composition of Layer Diet used in the experiment

Ingredient	Percentage
Maize	48
Soybeans	11
Groundnut cake	8.75
Fishmeal	1.5
Palm kernel cake	5
Wheat offal	14.00
Bone meal	2.5
Limestone	8.5
*Premix	0.25
Salt	0.25
Lysine	0.1
Methionine	0.15
Total	100

Ingredient	Percentage
<b>Calculated feed Analysis</b>	
Me (Kcal/kg)	2,489.05
CP (%)	17.13
CF (%)	4.30
Ether extract (%)	4.30
Calcium (%)	4.11
Phosphorus (%)	0.92
Lysine (%)	0.88
Methionine (%)	0.44

\*Supplied per kg diet: Biotin = 40gm; Zn = 58mg; Fe = 5800mg; Vit A = 1,000,000 i.u Folic acid = 500mg; Se = 120mg; I = 60mg; Nicotinic acid = 2800mg; Cu = 700mg; Mn =4800mg; Vit k = 1,500mg; Riboflavin = 500gm; Co = 300g

### Data Collection

#### Reproductive Traits

Two birds in each replicate group at weeks 21 and 36 were deprived feed and water overnight prior to processing to facilitate gut emptying. Birds were killed by cervical dislocation and then dissected for determination of the weight of liver and oviduct. Ovaries were removed for assessment of ovarian morphology (ovary weight, weight and the number of small [5–10 mm diameter] follicles and large [>10 mm diameter] yellow hierarchical follicles). Oviduct length was also measured.

#### Egg Quality Characteristics

Five eggs were selected at random from each replicate once a week and were weighed and the following quality parameters of eggs were determined:

- Egg weight (g):** The Egg Weight (EW) was measured with an electronic balance to the nearest 0.01 g.

Where YI = Yolk index  
YH = Yolk height  
YW = Yolk width

**Shape Index:** The shape index was calculated using the following formula (Anderson et al., 2004):

$$SI = \frac{W}{L} \times 100$$

Where : W = Width of egg  
L = Length of egg

**Eggshell thickness;** Thickness was measured after removing the internal membranes of the eggshell. A precision micrometer was used to the nearest 0.01mm (Mitutoyo Dial Thickness Gage). Measurements were taken at the three regions of the shell and the means were calculated.

#### *Egg specific gravity determination*

The assessment of the eggs' specific gravity was based on Archimedes' principle. The eggs were weighed in air on a Mettler scale. The weight of the water (at 22°C) displaced by the eggs was determined by submerging the eggs in a beaker water on the same tared scale (Valkonen et al., 2008). Egg specific gravity was then determined using the equation:

*Specific gravity* = Egg weight in air/displaced water weight

**Haugh Units :** Individual Haugh Unit (HU) score was calculated using the egg weight and albumen height (Haugh, 1937). Albumen height was measured using P6085 spherometer (tripod micrometer) with 0.01mm accuracy in a flat dish

The Haugh Unit values was calculated for individual eggs using the following formula:

$$HU = 100 \log_{10} (H + 7.5 - 1.7W^{0.37})$$

Where: H = Observed height of the albumen in mm

W = Weight of egg in grams

#### *Welfare Measurement*

##### *Heterophil/Lymphocyte Ratios (H:L)*

Blood samples were taken from 2 hens from each replicate and 2 blood smears per hen were made immediately after drawing blood using the 2-slide wedge method (Houwen, 2000). After air drying, the slides were fixed in methanol and stained with Diff Quik stain (Dade Behring Inc., Deerfield, IL). One hundred leucocytes, including granular (heterophils, basophils) and nongranular (lymphocytes, monocytes), were counted once on each slide using oil immersion microscopy at 100x magnification using the method of Davis et al., (2004).

## **Results and Discussion**

#### *Reproductive Apparatus*

Table 2 shows the effects of litter materials on the reproductive traits of the egg-type chickens. At week 21, litter materials did not have effect on body weights, liver weights and small yellow follicles. However, the ovary weight of the birds reared on wood shavings and *Panicum maximum* was higher than those raised on maize cobs litter material. The oviduct weights and lengths followed a similar trend. The large yellow follicles of the birds raised on *Panicum maximum* was similar to those of wood shavings and maize cobs but the birds on wood shaving litter had a higher large yellow follicles than those in Maize cobs.

At 36 weeks, apart from large yellow follicles, none of the other reproductive apparatus was affected by litter materials. The large yellow follicles of the birds reared on *Panicum maximum* and wood shavings were higher than those reared on maize cobs.

#### *Egg quality characteristics*

Table 3 shows the effects of litter materials systems on the Internal and external egg characteristics. Litter materials did not affect egg weights, yolk, albumen, shell weights, shell thickness, egg specific gravity, albumen heights, Haugh units and egg shape index. However, the egg length of the birds reared on *Panicum maximum* was similar to those

**Table 2:** Effects of litter materials on the reproductive traits of the egg-type chicken

Parameters	Litter type			SEM
	<i>Panicum maximum</i>	Maize cobs	Wood shavings	
<b>(Early production: 21 weeks old)</b>				
Body weight (g)	1383.25	1343.50	1455.75	37.588
Ovary weight(g)	29.0 <sup>a</sup>	18.25 <sup>b</sup>	29.75 <sup>a</sup>	2.287
Oviduct weight (g)	50.50 <sup>a</sup>	35.75 <sup>b</sup>	49.75 <sup>a</sup>	2.740
Oviduct length (cm)	62.0 <sup>a</sup>	50.25 <sup>b</sup>	62.75 <sup>a</sup>	2.460
Liver weight (g)	34.0	31.50	32.00	1.505
LYF	3.75 <sup>ab</sup>	2.75 <sup>b</sup>	4.50 <sup>a</sup>	0.310
SYF	13.75	13.25	10.25	1.672
<b>(Peak production: 36 weeks old)</b>				
Body weight (g)	1525.0	1485.75	1506.25	17.607
Ovary weight(g)	21.5	30.75	31.75	2.273
Oviduct weight (g)	38.25	54.50	46.50	3.583
Oviduct length (cm)	49.0	57.75	61.0	2.732
Liver weight (g)	28.25	27.0	29.5	1.538
LYF	5.75 <sup>a</sup>	3.50 <sup>b</sup>	5.75 <sup>a</sup>	0.369
SYF	11.75	8.75	15.0	1.953

<sup>abc</sup> Means within rows with different superscripts are significantly different ( $P < 0.05$ )

Legends: LYF: Large yellow follicles; SYF: Small yellow follicles

on wood shavings but significantly higher than those reared on maize cobs. The width of the eggs from the hen on *Panicum maximum* was higher than those laid in maize cobs but similar to those in wood shavings. The width of the eggs laid by the hen on wood shavings was however similar to those of the hen on maize cobs.

Effects of litter materials systems on the Internal and external egg characteristics is shown in Table 4. Across the treatment groups, there was no difference in the HL of the birds.

The findings in the present study has shown that litter materials (wood shavings, maize cobs and *Panicum maximum*) did not significantly affect the body weight of laying chickens. This is in agreement with other studies. Anisuzzaman and Chowdhury (1996) reported that different litter materials including sawdust, paddy straw, sand and rice husk had no significant effects on weight gain and feed conversion ratio of broiler chickens. Moreover, Abdul Hafeez *et al.*, (2009) comparing sawdust,

sand and wheat straw as litter materials in broiler chickens indicated that there was no difference in the weight gain.

Ovarian morphology influences egg production and laying sequence (Hocking *et al.*, 1987). It has been reported that excess of large yellow follicles (LYF) in the ovary increases the potential for cull eggs and erratic laying (Yu *et al.*, 1992). The similarity of the large and small yellow follicles, ovary and oviduct weight of the birds raised on chopped *Panicum maximum* and wood shavings can be regarded as a beneficial index for possible replacement of wood shavings with chopped *Panicum maximum*. In agreement with the report of Enaiat *et al.*, (2009) that large yellow follicles and small white follicles may be related to the rate of laying, the rate of laying of the birds on *Panicum maximum* was higher than those reared on maize cobs litter. Oke *et al.*, (2016) also reported a positive relationship between yellow follicles and egg production. The improved reproductive apparatus of the hens reared on chopped *Panicum maximum*

**Table 3:** Effects of litter materials systems on the Internal and external egg characteristics

Qualities	Rearing systems			SEM
	<i>Panicum maximum</i>	Maize cobs	Wood shavings	
Egg weight (g)	56.32	54.64	58.99	1.348
Egg length (cm)	5.25 <sup>a</sup>	4.73 <sup>b</sup>	5.07 <sup>a</sup>	0.067
Egg width (cm)	3.92 <sup>a</sup>	3.47 <sup>b</sup>	3.87 <sup>ab</sup>	0.090
Yolk weight (g)	13.30	13.94	14.26	0.304
Shell weight (g)	5.83	6.33	6.00	0.235
Albumen height (mm)	6.11	5.69	6.11	0.192
Albumen weight (g)	37.19	34.37	38.73	1.209
Shell thickness (mm)	0.91	0.94	0.90	0.012
Egg shape index	74.54	73.26	76.54	1.569
Specific gravity	1.32	1.37	1.13	0.008
Haugh unit	77.52	75.70	77.09	1.412

<sup>ab</sup> Means within rows with different superscripts are significantly different ( $P < 0.05$ )

**Table 4:** Effects of litter materials systems on the Internal and external egg characteristics

Parameters	Rearing systems				P value
	Wood shaving	<i>Panicum maximum</i>	Maize cobs	SEM	
HL at week 21	0.58	0.55	0.65	0.02	0.1278
HL at week 36	0.64	0.61	0.56	0.02	0.5877

HL: Heterophil/lymphocyte

and wood shavings litter types in the present study translated to a better egg production performance than that of maize cobs. Grimes *et al.*, (2002) reported that chopped straw was a more effective litter material than whole straw. The absence of caking in all the litter materials in the present study is an indication of their ability to absorb moisture showing their potential as good litter materials.

*Heterophil/lymphocyte* ratio is a sensitive indicator of stress indicating the activity of the HPA (Mitchell and Kettlewell, 1998). 0.2, 0.5 and 0.8 characterize low, optimum and high levels of stress, respectively (Gross, and Siegel, 1983). The results obtained in the present study revealed that there was no difference in the H/L ratios of the birds across the treatment groups. The data obtained were within the range of the optimum heterophil/lymphocyte of birds (Gross, and Siegel, 1983).

## Conclusion

Based on the findings of the present study, it could be concluded that the use of chopped *Panicum maximum* has beneficial effects and can be used as litter materials to replace wood shavings without adverse effect on reproductive performance and egg quality characteristics of laying chickens.

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## PRIORITISATION OF TRANSBOUNDARY ANIMAL DISEASES AND ZONOSSES TO STRENGTHEN CONTROL IN UGANDA

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### Abstract

Prioritisation and categorisation of transboundary animal diseases and zoonoses was done to guide efficient allocation and utilisation of resources in Uganda. Information was obtained from secondary data collected during routine disease surveillance in animals and humans. Data was also collected using a structured questionnaire developed by the Ministries of Health and Agriculture Animal Industry and Fisheries. The data was analysed using an OIE Phylum Tool. The study showed that the major priority diseases in order of importance were Foot and Mouth Disease Circulating, Contagious Bovine Pleuropneumonia, New Castle Disease, African Swine Fever, Lumpy Skin Disease, Peste Petit Ruminants, RFV, Sheep and Goat Pox, *Trypanosomosis*, East Coast Fever, *Brucellosis*, *Tuberculosis* and Rabies. With regards to the new and emerging diseases, Highly Pathogenic Avian Influenza ranked highest followed by Foot and Mouth Disease for strains absent (SAT 3, ASIA, Type A and Type C). The output of the study will be linked to the the Vision 2040; the National Development Plan, ; the Agriculture Sector Strategic Development programme; and the Health Sector Investment Plan 2020, to guide investment in the Agriculture and health asectors. The study recommends that a more comprehensive undertaking be done to incorporate more diseases such as the other emerging viral haemorrhagic fevers in the prioritisation process.

**Key words:** transboundary, zoonoses, Uganda, animal, human, disease, prioritisation, categorisation

## PRIORISATION DES MALADIES ANIMALES TRANSFRONTIERES ET DES ZONOSSES POUR RENFORCER LEUR CONTROLE EN OUGANDA

### Résumé

La priorisation et la catégorisation des maladies animales transfrontières et des zoonoses ont été réalisées dans le but de guider de manière efficace l'allocation et l'utilisation des ressources en Ouganda. Les informations ont été obtenues à partir des données secondaires collectées pendant la surveillance de routine des maladies animales et humaines. Les données ont également été recueillies à l'aide d'un questionnaire structuré élaboré par les ministères de la Santé et de l'Agriculture, de l'Elevage et des Pêches. Ces données ont été analysées à l'aide de l'outil Phylum - OIE. L'étude a révélé que les principales maladies prioritaires par ordre d'importance étaient la fièvre aphteuse circulante, la pleuropneumonie contagieuse bovine, la maladie de NewCastle, la peste porcine africaine, la dermatose nodulaire contagieuse, la peste des petits ruminants, la FVR, la trypanosomiase, la fièvre de la côte orientale, la brucellose, la tuberculose et la rage. En ce qui concerne les maladies nouvelles et émergentes, l'influenza aviaire hautement pathogène est la plus fréquente, suivie de la fièvre aphteuse pour les souches absentes (SAT 3, ASIA, type A et type C). Le résultat de l'étude sera relié à la Vision 2040, au plan de développement national, au Programme de développement stratégique du secteur agricole et au Plan d'investissement du secteur de la santé 2020, pour orienter l'investissement dans les secteurs de l'agriculture et de la santé. L'étude recommande qu'une étude plus globale soit menée pour incorporer plus de maladies telles que les autres fièvres hémorragiques virales émergentes dans le processus de priorisation.

**Mots-clés :** transfrontalière, zoonoses, Ouganda, animal, humain, maladie, priorisation, catégorisation

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## Introduction

Livestock production is a major component of the Uganda's economy and it greatly contributes to the livelihoods of many farming households (Benson and Mugarura, 2013). Livestock is an important subsector of agricultural production contributing 8% to the gross domestic product (GDP) and 21% to the agricultural GDP (UBOS, 2014). The subsector plays an important role in the socio-economic development of the country (Perry *et al.*, 2011). According to the Uganda National Bureau of Statistics (UBOS), more than 60% of the rural households in Uganda derived their livelihoods from livestock in 2014.

The Livestock sector has been recognised globally as a major opportunity and a vital tool for contributing to rural poverty alleviation. The demand for livestock products regionally and globally is projected to double in the next 30 years from 2000. In Uganda, the demand for livestock and livestock products has been increasing in the recent years. This could be due to the growing human population, increasing urbanisation and opening of regional markets in South Sudan, Democratic Republic of Congo, Burundi and Somalia.

Previous attempts have been done to prioritise in the world. The World Animal health organisation had earlier classified animal diseases as list A, B and C based on the impact of the disease. Due to the potential impact of each of these diseases if they occurred, OIE changed the list to include all listed diseases. At regional level, the African Union Inter-African Bureau of Animal Resources (AU-IBAR) under Standard Methods and Procedures Project (SMP) in 2012 prioritised trade sensitive diseases in the Great Horn of Africa. The priority diseases include; Foot and Mouth Disease (FMD), Contagious Bovine PleuroPneumonia (CBPP), Rift Valley Fever (RVF), Pestes de Petits Ruminants (PPR), Contagious Caprine PleuroPneumonia (CCPP), *Brucellosis*, camel pox, sheep and goat pox, Rinderpest, Lumpy Skin Disease (LSD) (AU-IBAR, 2014). The East African community in 2011 also prioritised diseases for purposes of harmonising reporting within the region. The

diseases prioritised were; pathogenic *Avian Influenza* (HPAI), RVF, FMD, CBPP, New Castle Disease (NCD), *Trypanosomosis*, PPR, CCPP, LSD, rabies and African Swine Fever (ASF) (EAC, 2011).

In Uganda prioritisation has also been done in the past using several methods such as ranking using participatory epidemiology methods and also use of intuition. In this respect the Ministry of Agriculture, Animal Industry and Fisheries selected four diseases including FMD, CBPP, Rabies and Rinderpest as a priority for state control (MAAIF, 2002). A study done in Uganda by Nantima *et al* (2015) prioritised livestock diseases according to economic importance as *Trypanosomosis*, FMD, CBPP Worms and East Coast Fever (ECF). Another study also carried out in Uganda by Nantima *et al* (2009) prioritised poultry diseases in order of importance as New Castle Disease, Fowl Typhoid, Coccidiosis, Chronic Respiratory Disease (CRD) and Fowl Pox.

Recent studies have indicated that there is an upsurge of transboundary animal diseases and zoonoses especially emerging and re-emerging zoonotic diseases resulting in serious socio-economic disruption, ill-health and sometimes deaths. Notable among re-emerging diseases are viral haemorrhagic fevers namely: Ebola (MacNeil *et al.*, 2012; Mbonye *et al.*, 2012), Marburg (Green, 2012), Rift Valley Fever and Yellow Fever; (Wamala *et al.*, 2012) and bacterial infections like Anthrax (Nabukenya *et al.*, 2014). Some of the diseases such as rabies, *Brucellosis* and bovine *Tuberculosis* are endemic (Makita *et al.*, 2008; Muwonge *et al.*, 2012; Nyakarahuka & Tweheyo, 2012). The first outbreak of Rift Valley Fever in Uganda has recently been confirmed in Kabale district, South Western Uganda resulting into serious socioeconomic disruptions and deaths of animals and people. (MAAIF Reports, 2016; MOH Reports, 2016). Recent reports have also indicated rampant outbreaks of major animal diseases such as FMD, (Baluka, 2014) ASF, (Nantima *et al* 2015, Muhangi, 2014) and PPR, (Ruhweza *et al*, 2010, Mulindwa *et al*, 2011.). These disease outbreaks have resulted in serious socioeconomic and public health impacts on the population and

livestock of Uganda.

Several efforts are being made to control the transboundary animal diseases and zoonoses in Uganda using a multispectral approach. The current strategies include; strengthening disease surveillance, monitoring, vaccination, vector control, treatments among others. However, despite these measures, the diseases have continued to reoccur implying that more efforts are required to address their control. Given the importance of the diseases on the livestock resource and public health in Uganda and considering their high prevalence and socio- economic impacts, the high cost of control, it was found necessary to undertake prioritisation and categorisation of the diseases to ensure efficient and rational allocation of resources to address these challenges.

The aim of this study, therefore, was to prioritise and categorise transboundary animal disease and zoonoses in order to guide efficient resource allocation and utilisation in Uganda. The output of this study is expected to contribute to inform policy on prevention, control and eradication of transboundary animal diseases and zoonosis in Uganda.

**Materials and Methods**

*Study area*

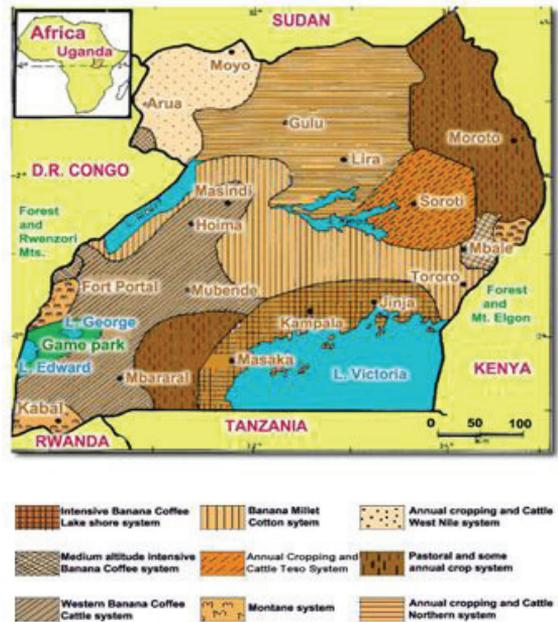
The study was carried out in Uganda covering all the 112 districts. The country covers approximately 241,000 km<sup>2</sup>, with a human population of about 34 million (UBOs, 2015). The country is subdivided into seven agro-ecological zones as indicated in Figure 1. These include; banana/coffee system, Banana/Millet/Cotton system, Montane system Teso systems, Northern systems, Pastoral systems and West Nile system. Uganda borders Kenya, Tanzania, Rwanda, Democratic Republic of Congo and South Sudan. There is free movement of animals and people across the borders which predispose the country to cross border animal and human diseases. In addition, the people on either side of the borders have similar cultural and social practices and are inter-dependent. Furthermore Uganda is located in the Congo-Albertine ecosystem which predisposes to

outbreaks of dangerous diseases such as Ebola and Marburg. The majority of the livestock in Uganda are kept in the cattle corridor using pastoral and agro pastoral farming systems (Figure 2) with poor husbandry practices. The estimated livestock population is 14 million cattle, 16.59 million goats, 4.3 million sheep, 4.04 million pigs and 61.66 million chickens (UBOS, 2014).

*Study design*

The study involved use of national data and secondary data collected during routine disease surveillance in animals and humans. The study design was a cross sectional study which employed quantitative techniques. Data was collected using a structured questionnaire developed by the Ministries of Health and Ministry of Agriculture. The variables captured in the questionnaire included; epidemiologic parameters, national data on livestock population, demographic data and socioeconomic indicators among others.

*Data management and analysis*



**Figure 1:** Map showing the agro-ecological zones of Uganda

Data was collected coded, cleaned and stored in spread-sheet. Data was analysed using the OIE Phylum Tool (Cros *et al.*, , 2010) to generate figures and scores. The Phylum Tool uses criteria such as, economic impact, human health impact, environmental impact, local feasibility and societal impact to rank diseases.

The Phylum tool consists of two sequential steps in the analysis of diseases. Global characteristics of the disease aimed at assessing inherent aspects of the disease, independently of any particular local context. A local approach aiming at refining the study of the disease within the specific context of the country. This global characterisation takes into account the following steps: The nature of the disease, animal or zoonosis and possible impact on human health. The possibility of vector-borne transmission of the pathogen in relationship to the epidemiology (transmissibility and persistence in animals) and human health (transmissibility in humans) is important. This is useful on the assessment of possible control measures and any disease-related societal impact. It also considers the absence or presence of the disease in a given country.

## Results



Figure 2: The Cattle corridor of Uganda

### Categorisation of priority diseases

Results of categorisation revealed that the animal diseases present in Uganda were; FMD circulating, CBPP, CCPP, PPR, Sheep and Goat Pox, ASF, LSD, NCD, ECF, Tuberculosis, Rabies, Brucellosis, Rabies, Trypanosomosis and Viral Haemorrhagic Fever including Ebola, Marburg, yellow fever, Congo haemorrhagic Fever and Rift valley.

The diseases that were categorised as absent were Highly Pathogenic Avian Influenza and FMD exotic strains including SAT 3, ASIA, Type A and Type C.

### Prioritisation of transboundary animal diseases according to local economic importance

The priority diseases according to the above criteria were; RVF FMD absent PPR HPAI ASF NCD Brucellosis, FMD circulating CCPP CBPP as shown in Table 2.

### Prioritisation of transboundary animal diseases according to local impact on human health

The priority diseases according to the above criteria were; RVF Rabies Brucellosis TB HPAI FMD absent Brucellosis as shown in Table 2.

### Prioritisation of transboundary animal diseases according to local society impact

The priority diseases according to the above criteria were; RVF, HPAI, TB FMD circulating FMD absent CCPP Trypanosomosis as shown in Table 2.

### Prioritisation of transboundary animal diseases according to Local Environmental Impact

The priority diseases according to the above criteria were; Trypanosomosis, ASF HPAI FMD circulating FMD absent ECF Brucellosis as shown in Table 2.

### Comparative analysis of transboundary animal diseases according to Local Environmental Impact

The priority diseases according to all the above criteria were; Foot and Mouth Disease Circulating, Contagious Bovine Pleuropneumonia, New Castle Disease, African Swine Fever, Lumpy Skin Disease, Peste Petit

Ruminants, Viral Haemorrhagic Fever, Sheep and Goat Pox, *Trypanosomosis*, East Coast Fever, *Brucellosis*, *Tuberculosis* and Rabies. With regards to the new and emerging diseases, Highly Pathogenic *Avian Influenza* ranked highest

followed by Foot and Mouth Disease strains absent.

**Table 1:** Categorisation of priority diseases. Diseases were categorised as present or absent

	Tryps	Rabies	TB	HPAI	Brucellosis	ECF	NID	LSD	ASF	S& G Pox	PPR	CCPP	CBPP	FMD exotic	FMD Circulating	RVF
Epidemiological status of the disease in the country	P	P	P	A	P	P	P	P	P	P	P	P	P	A	P	P

**Table 2:** Scores of priority TADs and Zoonoses

	RVF	FMD Circulating	FMD exotic	CBPP	CCPP	PPR	S& G Pox	ASF	LSD	NID	ECF	Brucellosis	HPAI	TB	Rabies	Tryps
Local Economic	3.9	2.6	3.7	2.3	2.5	3.6	2.1	3.1	1.4	3.1	2.0	3.1	3.4	2.4	0.6	2.06
Local on Human	5.9	1.2	2.0	1.2	1.2	1.2	1.2	0.8	0.3	0.8	0.3	5.2	4.7	4.9	5.2	3.20
Local Societal Impact	7.0	5.7	5.3	5.2	3.5	5.1	2.7	4.2	3.7	4.3	4.3	5.6	6.9	6.4	4.9	4.87
Local Environmental Impact	1.3	2.5	1.9	1.3	0.6	1.3	0.6	3.8	0.6	0.6	1.3	1.3	3.1	1.9	1.9	8.1
Local Economic Impact on	7.3	6.1	6.2	4.8	5.3	5.9	5.5	7.3	5.8	5.5	4.4	6.5	5.7	7.2	5.2	2.6
Local Environmental mpact	1.3	2.5	1.9	1.3	0.6	1.3	0.6	3.8	0.6	0.6	1.3	1.3	3.1	1.9	1.9	8.1

**Discussion**

The importance of categorisation and prioritisation has been recognised earlier by several experts and countries. It is argued that given the ever diminishing resources that are available for animal disease control, it is prudent to create a list of diseases according to priority for efficient resource utilisation. The OIE had also earlier classified animal diseases

according to epidemic nature impact on trade, zoonotic potential and production systems. This compelled member states to report to the OIE on only those priority diseases on a regular basis. However considering the global threat of each of the diseases, OIE changed the prioritisation to include all reportable diseases countries. Prioritisation of trade sensitisation diseases was also considered by AU-IBAR under Standard Methods and Procedures

Project to enhance impact of the project by focusing on a few diseases (AU-IBAR, 2012). Similarly, the East African Community member states also prioritised diseases to aid countries in the East African Region to use a harmonised list of diseases for reporting (EAC, 2011). However, all these efforts to categorise diseases has been influenced by socioeconomic impact of the disease, impact on trade, reporting requirements and the tendency for donors to tag aid on recipient countries accepting certain conditions. In Uganda previous efforts to categorise livestock diseases and zoonoses were also mainly donor driven and carried out under various projects and programmes. For instance, during the Global threat of Highly Pathogenic Avian Influenza, the most important poultry diseases in Uganda were ranked according to priority (Nantima et al., 2015). Similarly during the Global eradication of Rinderpest, all livestock diseases were ranked according to economic importance.

Recent advances by the OIE which developed the Phylum Tool has enabled countries to consider all aspects such as economic impact human health impact, societal impact environmental impact and impact of control measures. This tool has an edge over earlier efforts as it takes into consideration all the important factors associated with disease epidemiology, control and impact. This tool is timely as it will enable countries to make priority decisions on legislation, surveillance, on farm biosecurity, control and monitoring of animal diseases, public/private partnerships, awareness campaigns and new research programs. The Phylum tool which uses criteria such as, economic impact, human health impact, environmental impact, local feasibility and societal impact to categorise and rank diseases tends to weight human impact of a disease more than economic impact.

The Phylum tool puts all the issues into consideration while weighting them to come up with a holistic prioritisation and categorisation. This is in contrast with previous attempts where categorisation was oriented to the existing issues at the point in time.

In Uganda, the study ranked Viral Haemorrhagic Fever highest priority while FMD and CBPP which are the more plausible were ranked lower than VHF. This finding is not supported by the current disease prevalence and socioeconomic impacts of these diseases in the country. However, combining the outputs of the tool and actual surveillance and economic data at national level helps to arrive at a more realistic and rational decision for efficient resource allocation and utilisation. Therefore in this study, Foot and Mouth Disease was ranked as the most priority disease in Uganda.

Previous studies carried out in Uganda also highlighted the importance of Foot and Mouth Disease as a most priority disease to livestock due to its socioeconomic impact (Ademun 2012, Baluka, 2014; Ayebazibwe et al., 2010; Rutagwenda, 2007). The government and development partners have also recognised that FMD is an important disease. OIE has put in place a stepwise approach to control FMD globally through the Progressive Control Pathway (PCP). All countries are required to adopt this pathway. At the national level, a risk based Control strategy for FMD has been put in place and this is which is in line with the OIE/FAO Progressive Control of Foot and Mouth Disease has been developed for implementation

Results of this study also that Contagious Bovine Pleuropneumonia was ranked second. This is backed by the fact that the disease is wide spread in the country and treatment is very difficult. It affects major specie and it is a trade sensitive disease and therefore it is associated with trade bans

New Castle Disease was the third most important poultry disease. It is an important disease in Uganda because poultry is an important livelihood activity. Almost all households keep poultry and vaccines are available to control the disease and are cheap. The study by Nantima et al (2009) carried out in Uganda highlighted the importance of New Castle disease as it was ranked most important among the poultry diseases. However the importance of NCD as a priority disease is not a priority and its control is not catered for in the National Development programmes.



**Table 4:** Strategies for the control of priority transboundary animal diseases

<b>Disease</b>	<b>Rank</b>	<b>Justifications</b>	<b>Control strategy</b>
FMD	1	<p>Disease of high economic impact</p> <p>Outbreaks have been occurring in many districts in the country affecting multiple species including wild animals</p> <p>It is a hindrance to trade in livestock and livestock products</p> <p>Disease results in loss of production</p> <p>It is fairly easy to control</p>	<p>Mass vaccination for three years followed by strategic outbreak vaccinations</p> <p>Creation of export zones that could be cleaned regularly</p> <p>Regional and national FMD control strategies using PCP</p> <p>Framework vaccination contracts</p> <p>Draft FMD control strategy and policy</p>
PPR	2	<p>Disease affects goats and sheep which are a major livelihood activity</p> <p>It is relatively new with many naïve hosts and its associated with high mortalities</p> <p>Uncontrolled cross border movement control</p> <p>Disease has a fairly high economic and environmental impact</p> <p>Disrupts livelihoods of millions of people in Uganda</p> <p>Goats are a priority specie in the restocking programmes of government</p> <p>Shoats have a high reproductive rate and are easily convertible into financial returns</p>	<p>Establish the epidemiology of the disease in the country and zone according to risk</p> <p>Mass vaccination for three years where the prevalence is high and follow it up with</p> <p>risk based surveillance and strong monitoring</p> <p>PPR control strategy not available</p>
ASF	3	<p>Affects pigs which are a major livelihood activity in Uganda.</p> <p>Pigs have a high fecundity</p> <p>Pigs are a priority specie in the restocking programmes of government</p> <p>Difficult disease to control, no vaccine and no treatment</p>	<p>Establish epidemiological status</p> <p>Zone the country according to productivity</p> <p>Need to develop strategy for its control</p>
NCD	4	<p>Poultry is an important livelihood activity</p> <p>Almost all households keep pigs</p> <p>Vaccines available and are cheap</p>	<p>Mass vaccination in the commercial sector</p> <p>Encourage vaccination in the backyard</p>
<i>Brucellosis</i>	5	<p>The country has a large dairy sector which is very important</p> <p>Has high impact on human health (zoonosis)</p> <p>Feasible to control at low cost</p>	<p>Establish epidemiological status ; zone the country according to productivity;</p> <p>establish rapid diagnosis near the farmers (at milk collecting centers);</p> <p>Undertake a national study to establish the economic cost of controlling <i>Brucellosis</i>.</p>
CBPP	6	<p>Disease affects a major livestock species; trade sensitive and therefore associated with trade bans; it is wide spread in the country; treatment is very difficult because it leads to lungers</p>	<p>Mass vaccination in high risk zones and movement control;</p> <p>Post-mortem inspection to determine chronic carrier status</p>

<b>Disease</b>	<b>Rank</b>	<b>Justifications</b>	<b>Control strategy</b>
Rabies	7	Zoonosis with high case fatality rate in humans and animals Has a fairly high impact on society	Mass vaccination of dogs for 3 years Develop a rabies communication strategy Promotion of exposure prophylaxis Develop education programme targeting children and incorporate it in the curriculum
CCPP	8	Disease affects small ruminants which play a major role in food security	Screening and mass vaccination in high risk zones
Trypanosomosis	9	The disease widely spread in the country Has high impact on human health (zoonosis) Availability of therapeutic and prophylactic treatment	Mass rearing of sterile males ongoing in the country Treatment of sic animals and people
ECF	10	Disease associated with very high mortalities in cattle, especially calves; acaricide resistance on the increase; Drugs and vaccines are available for its control	Strategic vaccination, formulate and enforce a National Tickr Control Policy.

**Table 5:** Strategies to controlling exotic diseases

<b>Disease</b>	<b>Rank</b>	<b>Justifications</b>	<b>Control strategy</b>
Highly Pathogenic Avian Influenza	1	It is zoonosis; has high economic and societal impact; hindrance to international trade; and has high environment impact if it occurs	Review emergency, preparedness and response/contingency plan; institute Border Controls; procure Vaccine Framework contracts; and formulate and enforce a national Slaughter and Compensation Policy.
Rift Valley Fever	2	New emerging zoonotic disease and therefore mortality would be high leading to enormous economic and public health impacts; Affects multiple species; vector borne disease that makes it difficult to control; Associated with risk of bans to international trade in livestock and livestock products; and Disrupts movement of humans and the tourism industry.	Develop contingency plans; institute Border Controls; procure Vaccine Framework contracts; and formulate and enforce a National Vector Control Policy.
Exotic FMD (Strains Absent-SAT3, ASIA, Type A and Type C)	3	High economic impacts because it is a new disease in a naïve population, may lead to failure to export; High cost of controlling the disease; and Difficult ies in timely getting of appropriate vaccines incorporating the new strains.	Develop contingency plans; institute border Controls; procure Vaccine Framework contracts; and formulate and enforce a National slaughter and Compensation Policy.

As such there are no national programmes for controlling NCD because it is considered as a private good and farmers are required to privately control the disease.

African Swine Fever was ranked the fourth most important disease. The disease is endemic in Uganda and outbreaks are rampant throughout the country resulting in serious socioeconomic impacts and loss of livelihoods. The disease affects pigs which are priority specie in the restocking programmes of government for wealth creation. Control of this disease is difficult because of lack of a known vaccine and treatment coupled with inadequate control strategies and policies. The importance of ASF was highlighted by previous studies carried out in Uganda (Nantima *et al.*, 2015; Muhangi, 2014).

Peste Petit Ruminatum was also selected as a priority disease because of its high economic and environmental impact on livelihoods. It is relatively new disease and when it was first confirmed in Uganda in 2007, it killed more than a half million goat and sheep in Karamoja within one year. Therefore it has many naïve hosts associated with high mortalities in small ruminants. The disease affects goats which are priority specie in the restocking programmes of government for wealth creation. The disease has been prioritised by international and regional partners for eradication and as such strategies to control PPR have been developed at national, regional and Global levels.

Although earlier studies carried out in Mbarara district of Uganda among the agro pastoral and pastoral communities revealed that *trypanosomosis* was the most important disease in Uganda according to livestock farmers' perceptions (Nantima *et al.*, 2015), this study ranked the diseases as number nine. Whereas the Phylum tool uses all the indicators including economic impact, human health impact, societal impact,, environmental impact and impact of control, the study carried out in Uganda only considered economic impact and impact of control.

Viral Haemorrhagic Fevers (VHFs) were listed as the most important zoonotic

disease in Uganda according to the tool. This is justifiable because VHFs are zoonoses and some of them like RVF are vector-borne. They constitute over 70% of the global emerging and re-emerging disease burden. Uganda has experienced eight VHF outbreaks in the past five years (Nabukenya *et al.*, 2013), resulting into disruption of economic activities including tourism and hampering local and international trade. Haemorrhagic fevers are very infectious and need specialised isolation facilities for their prevention and control. Implementation of control measures is often very costly. The diseases disrupt social services, trade and tourism because of trade embargos thus leading to enormous economic and public impacts. Similar findings were reported in studies carried in the region (Himeidan *et al.*, 2014).

*Brucellosis* was also ranked second highest among the zoonotic diseases. This is because the disease affects the dairy sector which is a very important in Uganda. It is a debilitating disease in humans and in regions where the infection persists in domestic animals, consequent, transmission to the human population frequently occurs. It also affects a wide range of wildlife including wild pigs, bison and antelopes (OIE, 2009). In contrary to the current perception of the communities and the impact of the disease, other zoonotic diseases rank highest compared to *Brucellosis*. Ministry of Health and World Health Organisation has ranked *Brucellosis* lowest compared to other diseases (MOH 2015; WHO, 2014).

Rabies was ranked least in order of priority although available data indicate that the disease is very important because of the case fatality rate associated with lack of treatment of the affected people.

With regards to new and emerging diseases, Highly Pathogenic Avian Influenza ranked highest because it is zoonosis, has high economic and societal impact, high hindrance to international trade, has high environmental impact through culling if it occurs. Lastly, Foot and Mouth Disease new strain such as SAT3, ASIA, Type A and Type C were ranked second highest because of its potential economic impact

if the strains are introduced leading to failure to export livestock and livestock products. In order to mitigate the high consequences of outbreaks from the non-existing FMD strains, Uganda needs to put in place contingency plans, policies, border controls that can identify the new strains and establish framework contracts with vaccine manufacturers. In case of an outbreak from such emerging diseases Uganda will identify the affected herds or areas for slaughter and compensation.

The study was limited by combining criteria that are of human nature. The tool overweight's human health. These various social networks due to cross border movement of people, animals and the social cultural practices poses a great challenge in controlling transboundary animal diseases and, harmonisation with neighbouring countries to undertake joint transboundary and zoonotic diseases.

### Conclusion and Recommendation

In conclusion this study was able to comprehensively prioritise and categorise transboundary animal disease and zoonoses to guide efficient resource allocation and utilisation in Uganda. The output of this study will contribute to developing strategies and policies for prevention, control and eradication of transboundary animal diseases and zoonosis in Uganda. The study results should be used as tool to advocate for resources by policy makers in Uganda and East African Community during planning and implementation of National and continental Developmental plans. A more comprehensive study should be done to incorporate more diseases such as viral haemorrhagic fevers in the prioritisation process. There is urgent need to put in place contingency plan for emerging diseases to enhance control when there are introduced into the country.

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### Conflict of interest

There is no conflict of interest identified.

### Ethical standards

The study involved review of secondary data and reports generated during routine animal and human disease surveillance. There was no direct contact with human and animal subjects. Because this was part of the routine surveillance activities which are exempted from Institution Review Boards (IRBs) and National Council of Science and Technology (NCST) permission to conduct the study was not sought. The study has many benefits to the human population and to the animal by guiding policy and resource allocation for prevention and control of the diseases.

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## **PRIORITISATION OF TRANS-BOUNDARY ANIMAL DISEASES (TTADS) AND ZONOOSES FOR THE DEVELOPMENT OF AN EFFECTIVE DISEASE CONTROL STRATEGY IN SOUTH SUDAN**

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### **Abstract**

The Ministry of livestock and Fisheries Industry (MLFI), South Sudan in collaboration with AU-IBAR carried out a disease prioritisation exercise on TADs and zoonotic diseases in South Sudan so as to guide the ministry in planning the available limited resources for the control of livestock diseases and development of an informed control strategy for TADS and zoonotic diseases. Based on the prevalence and the equal distribution of livestock disease in the country, the study covered the ten states of South Sudan from 2013 to 2015.

Three staff from South Sudan, ministry of livestock and Fisheries industry and ministry of health were trained on the use of OIE disease ranking PHYLUM tool in Entebbe 2013, followed by the exercise of disease prioritisation on July 2015 in Naivasha, Kenya, by inserting animal health data and information into OIE -PHYLUM tool for ranking of diseases, the team concluded a list of twelve present priority diseases, FMD, PPR, RVF, *Brucellosis*, CBPP, HPAI, CCP, NCD, ECF, Rabies, Shoats pox, BTB, and Anthrax respectively and three absent exotic diseases (HPAI, Ebola, Camel Pox) to be the most current priority diseases in South Sudan. These results coincided with the previous studies on disease prioritisation and the actual animal health information in the country. Henceforward the government will use these results in updating and formulating new strategies for the control of Trans-boundary Animal Disease and Zoonotic Diseases

**Keywords:** Prioritization, TAD, Zoonoses, Control, South Sudan, PHYLUM tool

## Introduction

Livestock population and its distribution in South Sudan is based on estimates as it is difficult to quantify actual figures because of seasonal frequent livestock movements, displacement of communities due to insecurity, raiding or theft and a cultural practice against counting livestock. Livestock farming is a key livelihood vocation for over 80% of the total population in South Sudan. It is estimated that the cattle population is 10 million, 13 million sheep, 12 million goats, 8,000 pigs, 671,200 donkeys, 4500 camels, 6 million chickens and wildlife in the national parks and game reserves (MLFI, 2014). More than 90% of the populations in South Sudan live in rural areas practicing traditional agriculture, animal husbandry and fisheries as a source of their livelihood. It is estimated that 80% of the South Sudanese keep livestock, small ruminants, mainly sheep and goats, play an important role in the livelihoods and food security of poor families. Goats and sheep are ranked number two and three respectively after cattle by the agro-pastoralist communities of South Sudan (South Sudan PPR strategy 2015). In South Sudan, the contribution of livestock to the national gross domestic product (GDP) stands at 8.894 billion SSPs or 3.015 billion USD (IGAD 2015).

In spite of the enormous available resources, the country finds itself importing some animal and animal products from the neighboring countries and beyond. South Sudan has a wide cross section of livestock diseases of socio-economic and public health importance although, little information about their presence, distribution and importance exist.

According to previous disease prioritisation and consultative discussion with pastoralists and veterinary professionals and socio-economic studies carried out in the country, the total farm gate value and national value loss associated with 13 priority animal diseases is US \$ 436,434,336 and US \$ 264,029,603.00, respectively (T.M. Kimani and S.W. Njue 2007). Based on these values CBPP emerged the most economically important

disease followed in order of decreasing importance by rinderpest, rift valley fever (RVF), east coast fever (ECF), hemorrhagic septicemia (HS), foot and mouth disease (FMD), pest des petits ruminants (PPR), black quarter (BQ) and anthrax, contagious caprine pleuropneumonia (CCPP) and lastly highly pathogenic *Avian Influenza* (HPAI) and this was also supported by the report of the directorate of veterinary services (Anonymous 2009), which indicated a high prevalence of livestock diseases in the country such as transboundary, vector borne, zoonoses and emerging diseases that often present a big challenge to the development of the livestock industry. That report listed the following disease as priority diseases; foot-and-mouth disease (FMD), contagious bovine pleuropneumonia (CBPP), contagious caprine pleuropneumonia (CCPP), Newcastle disease, anthrax, trypanosomiasis, rabies, bovine *Tuberculosis* (TB), *Brucellosis*, tick-borne diseases (TBDs), bovine theileriosis or east coast fever (ECF), anaplasmosis, bovine babesiosis, heart-water, poultry diseases (Newcastle disease, coccidiosis, fowl pox, fowl typhoid, infectious coryza, infectious bursal disease (gumboro)). As indicated by the cross sectional study conducted in five states in south Sudan, bovine theileriosis, anaplasmosis and babesiosis are endemic and existent in an unstable state (Kivaria 2010).

Further studies by Dr. Maximillion P.O Baumann in August 2010 reconfirmed the aboved outcome on priority diseases in South Sudan, in which Anthrax, *Brucellosis*, Rabies, Rift Valley Fever, Bovine *Tuberculosis* and Foot and Mouth Disease were identified as priority disease for multispecies. However, in cattle, shoats and poultry the following diseases were ranked as priority diseases CBPP, FMD, LSD,, PPR, CCPP, Sheep and Goats Pox and NCD.

In an effort by the directorate of veterinary services to control the prevalent of many Livestock diseases and their devastating socioeconomic impact on the livelihood of the pastoralists, an annual vaccination calendar for livestock disease control was developed and implemented, whereby a number of animals from different species were vaccinated against

several diseases, in which 703,000, 571,500, 924,205, 313,45,000, 154,400,, 20,341, 437,900, 6,200 ,18,400 animals were immunised against Anthrax, PPR, *Haemorrhagic Septicaemia*, CCPP, LSD, Sheep and Goat Pox, CBPP, BQ , Rabies and NCD respectivel ( Anonymous DVS Report 2009).

Because of this high negative impact of livestock diseases on livelihood of pastoralists community compounded with high cost of disease control and demographic changes caused by the current conflict in the country, the ministry of livestock and fisheries industry in collaboration with AU-IBAR decided to carry out more prioritisation studies on TADs and zoonotic diseases in South Sudan so as to guide the ministry in planning and utilisation of resources for the control of livestock diseases and to inform the development of control strategies for TADS and zoonotic diseases.

### Material and Methods:

#### Study Site

The study site was selected based on the prevalence and distribution of livestock disease in the country, many previous studies on the prioritisation and distribution of TADs and other zoonotic diseases in South Sudan suggested that livestock diseases are equally distributed all over the country, based on this, the disease prioritisation exercise covered the ten states of South Sudan from 2013 to 2015.

The Republic of South Sudan covers an area of 644,329 km<sup>2</sup>, with a population of 8.26 million, it is bordered by six countries namely; Sudan to the North, Ethiopia to the east, Kenya to the Southeast, Uganda to the South, Democratic Republic of Congo to the Southwest, and Central African Republic to the West. The country has three distinct agro ecological zones namely, the flat savannah and flood prone plains along the river Nile and its tributaries; the rocky semi-arid region of the southeast; and the rain forest of the hilly ironstone plateau of the west and south west. The climate varies from extremely hot and dry to humid and rainy season.



Figure 1: The of Southern Sudan showing locations of ten states of the study

#### Materials

Inventory of animal health and production information from Directorate Veterinary Services and Animal production of Ministry of livestock and Fisheries Industry (Passive and active disease reports, out break investigation , disease surveillance , animal production reports), agencies , NGOs reports and published scientific papers on TADs and Zoonotic Disease in South Sudan were the sources of data used in this study.

#### Methodology

The approach for disease prioritisation study started with training of three staff from South Sudan, two from the ministry of livestock and fisheries industry and one from the ministry of health on the use of OIE disease ranking PHYLUM tool in Entebbe 2013.

In July 2015 the trained staff at the country level came to Naivasha-Kenya and conducted disease prioritisation exercise using PHYLUM disease ranking tool in which the country team filled 15 excel spread sheets for 15 TADs and Zoonotic diseases (present/ absent) and then scores of these 15 diseases were copied and paste into the comparative table and based on the parameters in the comparative table, the team concluded the list of the thirteen priority diseases in South Sudan. The study was based on diseases ranking by applying comparative table and scoring system, which was designed by PHYLUM considering

the following parameters; Epidemiological (Prevalence, mortality, morbidity rates ), Economical (Cost of control measures, export, livelihood, livestock production), Human health impact (Public health), Socioeconomic impact Socio-environmental and Risk analysis parameters .

To validate the results of the exercise many consultations with stakeholders at the national level were carried out.

#### *Data Analysis*

The data on diseases prioritisation were collected and analyzed using comparative tables of the Oie -PHYLUM tool for scoring and ranking of disease .

### **Results**

#### *Disease prioritisation based on the economic impact*

From table 4.1 the diseases were prioritised based on their economic impact and the results were as follow; *Brucellosis* was rank number one followed by Contagious caprine pleuropneumonia, Newcastle, Contagious bovine pleuropneumonia, PPR and Foot-and mouth disease, respectively.

#### *Disease prioritisation based on the human health impact*

From the table 4.2 the diseases were prioritised based on their impact on human health and the results were as follow: *Brucellosis*, Rift valley fever, Rabies and Bovine *Tuberculosis* respectively

#### *Disease prioritisation based on the societal impact*

From Table 4.3 the results of the scoring were as follow: *Brucellosis*, Rabies, PPR, contagious caprine pleuropneumonia, Bovine *Tuberculosis* and contagious bovine pleuropneumonia, respectively.

#### *Disease prioritisation based on the Environmental impact*

The below table 4.4 shows that Contagious caprine pleuropneumonia was leading followed by Newcastle, Foot-and-mouth

disease, Contagious bovine pleuropneumonia, Lymph skin disease and lastly PPR

#### *Disease prioritisation based on the Feasibility of Control Measures*

Table 4.5 Diseases were prioritised based on their feasibility of control measures in which Rift valley Fever was on the top of the list ,followed by Foot and Mouth Disease, Lumpy skin disease, Bovine *Tuberculosis* , PPR, and Newcastle Disease respectively.

#### *Disease prioritisation based on the Economic impact of Control Measures*

Based on the economic impact of control measures PPR was ranked number one on the list followed by sheep & goat pox, FMD, CCPP, CBPP and Newcastle Disease was the lastly in list (Table 4.6)

#### *Disease prioritisation based on the Societal and Environmental Impact of Control Measures*

From table 4.7 Bovine *Tuberculosis* was the leading disease followed by Sheep & goat pox, Rift valley Fever, Lumpy skin disease, Newcastle Disease and lastly PPR Final Priority Diseases

#### *Present diseases*

FMD: As shown by the final priory list of livestock disease in South Sudan Food and Mouth Disease (FMD) was ranked on the top of the disease priory list due to its known sever impact on the livestock trade , livestock productions , livestock movements as well as being an endemic disease in the whole country with multiple hosts margin and high mortality rate in the naïve and young populations, as indicated by the an overview report on FMD epidemiology in Unity and lake state ( Erneo. B Ochi et al 2014 ) the overall prevalence was 56% in unity and 25% in lake state respectively and 50% moratlity rate in Young animal and 20% in adult cattle , sheep and goat.

PPR: Ranked the second on the disease priority list because of its high mortality rate, negative impact on the livestock trade, livelihood, cause of conflict between pastoralists and for its potential used as a Bioterrorism tool.

**Table 1:** Diseases ranking based on economic impact

Impact	Ranking	Disease	
Economic impact	1	<i>Brucellosis</i>	Endemic in the whole country, zoonotic, abortion in major species, foodborne
	2	CCPP	Endemic, high mortality, impact on international trade, severe impact food industry, risk of ban live animal, sever impact on the livelihood of the pastoralists communities
	3	NCD	Endemic in the whole country, high mortality
	4	CBPP	Endemic in the whole country, loss in production of milk & meat, seasonal, chronic
	5	PPR	Endemic, high mortality, effect on productivity and livelihood of the pastoralists communities
	6	FMD	Endemic, whole country, impact on production and livestock trade

**Table 2:** Diseases ranking based on human health impact

Impact	Ranking	Disease	
Human health impact	1	<i>Brucellosis</i>	Endemic in the whole country, zoonotic, foodborne, high number of human cases, highly transmittable
	2	RVF	Zoonosis, endemic, fatal, wide range of hosts, vector borne
	3	RABIES	Zoonosis, endemic, stray dogs, high case fatality, low transmissibility than the above ones
	4	BOVINE TB	Zoonosis, cost of treatment is high, foodborne, airborne, direct/indirect, disabling disease, curable

**Table 3:** Diseases ranking based on societal impact

Impact	Ranking	Disease	
Societal impact	1	BRUCELOSIS	Public health, occupational, impact on labour, Orchitis/infertility , potential bioterrorism
	2	Rabies	Public health, occupational, isolation of patient, Bioterrorism potential
	3	PPR	Inter-communal Conflict , high mortality, effect on livelihood , Bioterrorism potential
	4	CCPP	High fatal which causes poverty (effect on pastoralist welfare, movement restriction, Bioterrorism potential (USDA),
	5	Bovine TB	Public health, occupational, impact on labor, disability, high transmissible disease, stigma
	6	CBPP	Isolation of sick animal, backyard slaughtering, chronic and disabling

**Table 4:** Diseases ranking based on Environmental impact

Impact	Ranking	Disease	
Environmental impact	1	CCPP	High mortality , threat to endangered species of wildlife , using of chemicals for disinfection , treatment
	2	NCD	High mortality , threat to wild birds, scontaminated manure , using of disinfectants
	3	FMD	Wide range species including, cattle, shoats, pig and wildlife
	4	CBPP	Medical treatment , contaminated manure, contiguous
	5	LSD	Use of pesticide in control of vectors
	6	PPR	High mortality , wildlife,

**Table 5:** Diseases ranking based on Feasibility of Control Measures I impact

Impact	Ranking	Disease	
Feasibility of Control Measures	1	RVF	Confined, floods, availability of vaccine, use of pesticide for control of vectors
	2	FMD	Mass vaccination, affect multiple species
	3	LSD	Sporadic cases, isolation of sick animals
	4	Bovine TB	Testing and culling , Sporadic,
	5	PPR	Mass vaccination , narrow range of affected species
	6	NCD	Mass vaccination, affordability of the vaccine

**Table 6:** Diseases ranking based on Economic impact of Control Measures

Impact	Ranking	Disease	
Economic impact of Control Measures	1	PPR	Mass vaccination , control of livestock movement
	2	Sheep & goat pox	Vaccination , contiguous
	3	FMD	6 Serotype, mass vaccination , affect trade , control of livestock movement
	4	CCPP	Medical treatment , mass vaccination , movement control , high mortality
	5	CBPP	Medical treatment , vaccination , chronic , decrease of milk , meat production
	6	NCD	Mass vaccination , high mortality ,disposal and disinfection of dead birds , control movement t of birds

RVF: Rift Valley Fever was ranked third in the priority list because of its Public health concern , impact on the livestock trade, restriction of livestock movement , being fatal and affect multiple species including human being ,and for the previous history of its presence in the country.

*Brucellosis*: From the result of the analysis in table 4.1, table 4.2 and table 4.3 *Brucellosis* was ranked on the top of all livestock diseases due to its endemicity in the whole country, negative economic impact and being a zoonotic disease with high number of human cases and relapses , causing a lot of abortions in major species. This was supported by a *Brucellosis*

study report conducted in Melut, upper Nile state ( Sixl W et al.,1988) which shows 9.2% of investigated cattle and 16.2% of the green long tail monkeys, 3.7% of school children, 5.2 % of hospital patients, 2.2% of village children and adult positive for *Brucella abortus* and 12% of sheep and goats, 8.7% cattle, 2.2% school children, 5.2% hospital patient, 3.6% of district children and adult were positives for *Brucella Melitensis*. Also it was reveal by another study in Terekeka County of central Equatoris state ( Lado et al., 2012 ) that a total of 58 of human cases tasted positive for *Brucellosis* using rapid antigen test. These studies is supporting the result of the ranking and confirming the public health concern, the economic impact of the disease and the wide spread of it in both human and animal in South Sudan

**CBPP:** CBPP was ranked in the fifth position due to it Impairment to livestock trade, endemic in the whole country, its chronicity, and sever lost of livestock production.

**CCPP:** was included in the priority list because of its high economic cost due to high mortality rates, mass vaccination and treatment costs.

**NCD:** Newcastle Disease is the only poultry disease in the priority list due to its high mortality rates among the local native chicken.

*The proposed control measures are; Annual mass vaccination.*

ECF, Rabies, shoats fox, Bovine Tuberculosis and Anthrax were perceived to be of the least priorities and ranked at the end of the priority list of disease.

*Exotic diseases*

**HPAI:** was ranked in the first position due to it pubic health concern, Pandemic, previous history in the country 2006, endemic in some of the countries of the continent , fatal , highly pathogenic

**Ebola:** Pandemic diseases with high mortality rate, previous history of the disease in South Sudan ( WHO, CDC, KMRI 2004, 1976)

**Camel pox :**Present of high population of camels in the neighboring, countries and the present of camel in South Sudan, free movement of livestock

**Table 7:** Diseases ranking based on Societal and Environmental Impact of Control Measures

Impact	Ranking	Disease	
Societal and Environmental Impact of Control Measus	1	Bovine TB	Persistent of the pathogen in the environment, Public health concern, occupational, impact on labour, disability, high transmissible disease, stigma.
	2	Sheep& goat pox	Disposal and disinfectant of infected carcasses, un control use of pharmaceuticals, contiguous
	3	RVF	Use of pesticide for control of the vector, zoonosis, control and banning of slaughter activities, high numbers of aborted fetus, phobia , control of livestock movement
	4	LSD	Use of pesticide for control of the vector, reduction of animal production (milk, meat , hide and skin), contaminated semen
	5	Newcastle	Contaminated manure, high mortality, disposal and disinfection of dead birds, control movement of birds, people and vehicle
	6	PPR	High mortality, wildlife, affect trade, movement control , disposal and disinfection of carcasses

**Table 8:** Present diseases

<b>Disease</b>	<b>Rank</b>	<b>Justifications</b>	<b>Proposed Control strategy</b>	<b>Current Country control strategy</b>
FMD (FMD ASIA I)	1	Livestock trade, decrease in livestock production, endemic in the whole country, effect on livestock movement and trade , multiple hosts High mortality rate in naïve population	Partial vaccination (Ring vaccination) , massive vaccination, livestock movement control, serotype identification, epidemiological studies, Zonation, LITs	Passive and active surveillance, sample collection, outbreak investigations
PPR	2	High mortality , livestock trade , source of conflict , affect livelihood , Inter-communal Conflict , Bioterrorism potential	More scientific Studies and Mapping, Mass vaccination, Livestock movement control , biosecurity , epidemiological studies	Response to outbreaks, partial vaccination, outbreak investigation
RVF	3	Public health and livestock trade, history of the present of the disease , fatal , affect multiple species	Vaccination during outbreaks, control of livestock movement, surveillance, sentinel animals	Passive and active surveillance, sample collection
<i>Brucellosis</i>	4	Endemic in the whole country, zoonotic , abortion in major species, foodborne , high number of human cases	Vaccination of young animals, pasteurization of milk, awareness raising , bio-security , coordination and information sharing with stakeholders	No strategy, un control use of pharmaceuticals
CBPP	5	Impair livestock trade, endemic in the whole country , disabling , chronic , lost of livestock production	Treatment, bio-security measures, Partial and massive vaccination, livestock movement control	Treatment, out breaks investigation, spot vaccination
CCPP	6	High mortality	Mass vaccination and treatment	Response to out breaks, partial vaccination and treatment
NCD	7	High mortality	Annual mass vaccination	Response to out breaks
ECF	8	High mortality	Tick control, vaccination & treatment	Tick control
RABIES	9	zoonotic	Vaccination	
SHOAT POX	10	Affects export trade	Vaccination	
BTB	11	Zoonotic	No vaccine; No treatment	
ANTHRAX	12	Zoonotic	Vaccination	

**Table 9:** Exotic diseases

Disease	Rank	Justifications	Proposed Control strategy	Current Country control strategy
HPAI	1	Public health, Pandemic, previous history, endemic in Egypt, fatal	Culling, surveillance	Passive surveillance
EBOLA	2	Public health, Pandemic, fatal	Bio-security measures, awareness raising	Screening of passengers at the international entry points
CAMEL POX	3	Present of high population of camels in the neighbouring countries	Bio-security measures, surveillance	Nothing

## Discussion

The disease prioritisation exercise results were in a sequence such that to established diseases prioritisation list based on their economic impact, human health impact, societal impact, environmental impact, feasibility of control measures and environmental impact of control measures.

Food and Mouth Disease (FMD) was on the top of the disease priority list due to its known sever impact on the livestock trade , livestock production, livestock movement as well as being an endemic disease in the whole country with multiple hosts margin and high mortality rate in the naïve and young populations, As indicated by an overview report on FMD epidemiology in Unity and lakes states ( Erneo. et al 2014 ), the overall prevalence is 56% and 25% respectively, 50% moratlity rate in Young animals, 20% in adult cattle, sheep, and goats.

Last studies also prevail the present of 6 Foot-and-Mouth Disease Virus serotype specific antibodies (O, A, C, SAT 1 ,SAT2 and SAT 3). To deal with the disease the ministry is planning to conduct partial vaccination (ring vaccination), and in some cases mass vaccination, livestock movement control, conduct more epidemiological studies, zonation and Livestock Identification and Traceability (LITs)

PPR was identified as one of the most important priority diseases of small ruminants because of its high mortality rate, negative

impact on the livestock trade, livelihood, cause of conflict between pastoralists and for its potential used as a Bioterrorism tool which is in line with the previous disease prioritization ( Boumann 2010), the positive results of PPRV Lineage 4 circulating in Warab state ( OIE/ FAO Refrence Laboratory in Vienna , Austrai 2011 report ) and the regular annual reports of disease outbreak in the country. In efforts to contained the disease, the ministry of livestock and Fisheries Industry developed an strategic plan for the control and eradication of PPR (Mass vaccination, Livestock movement control, biosecurity, epidemiological studies)

Rift Valley Fever because of its Public health concern, its impact on the livestock trade, livestock movement restriction, high fatality, being affecting multiple species including human beings ,and for the previous history of it's detection in human and animal sera in the country (DVS Report 2007 ) and for the fears of it spreading from the neighboring countries as in the report of CDC volume 19 2013) (Global Alert and Response. Geneva: WHO; 2007). As part of the contingency plan the Ministry developed a draft strategic plan for the prevention and control of RVF disease which include preparation for ring vaccination during outbreaks, control of livestock movement, surveillance sentinel animals.

*Brucellosis* was also identified as one of the important public health and economic livestock disease due to its endemicity in the whole country, its negative economic impact,

being a zoonotic disease with high number of human cases and relapses, and causing annual abortions in major species. This was supported by a *Brucellosis* study report conducted in Melut, Upper Nile state (Sixl *et al.*, 1988) which shows 9.2% of investigated cattle and 16.2% of the green long tail monkeys, 3.7% of school children, 5.2 % of hospital patients, 2.2% of village children and adult positive for *brucella abortus* and 12% of sheep and goats, 8.7% cattle, 2.2% school children, 5.2% hospital patient, 3.6% of district children and adult were positives for *brucella Melitensis*. Also it was reveal by another study in Terekeka County of central Equatoris state (lado *et al.*, 2012 ) that a total of 58 of human cases tasted positive for *Brucellosis* using rapid antigen test. These studies is supporting the result of the ranking and confirming the public health concern, the economic impact of the disease and the wide spread of it in both human and animal in South Sudan. Strategy for the control of the diseases would be through vaccination of young animals, pasteurisation of milk, awareness raising, bio-security, coordination and information sharing with stakeholders.

N.B Due to lack of laboratory confirmation of cases, scarcity of data on the disease, absent of livestock export , negative perceptions of the pastoralists on the important of disease and comparing the result of the analysis tool with the previous studies , *Brucellosis* was ranked number four in the final list of priority diseases .

CBPP, CCPP, Bovine *Tuberculosis*, NCD, Sheep and goats pox, Lumpy Skin Disease were among the top priority diseases that followed in the list in South Sudan, these diseases are endemic in the whole country and their outbreaks are reported through out the year from almost the ten states of South Sudan, as confirmed by earlier studies on disease prioritization in the country ((Maximillion P.O Baumann in August 2010), (Kivaria 2010), (Kimani and Njue, 2007) these diseases have a devastating impact on the livelihood of the pastoralists, economy of the country , environment as well as 80% of the whole South Sudan societies, therefore the government

conduct an annual regular and emergency vaccination and treatment against these diseases, bio-security isolation and livestock movement control as a policy to contain and control their spread ( ML&FI Reports 2007,2008, 2009, 2010, 2011,2012)

HPAI, Ebola and Camel pox were identified as the most feared exotic diseases with the potential of entering in to the country from the neighboring states of the region, based on this fears and the previous experience of Ebola and HPAI outbreaks in 2004, 1976 and 2006 (Reports WHO, KMRI, CDC, FAO) respectively, the Ministry of livestock and Fisheries and Ministry of Health has put in place contingency plans to prevent the reentering of this pandemic diseases.

## Conclusion

From the results of the above disease prioritisation exercise combined with the previous studies on diseases prioritisation in South Sudan it has became crystal clear that FMD, PPR, RVF, *Brucellosis*, CBPP, HPAI, CCPP, NCD, ECF, Rabies, Shoats pox, BTB, and Anthrax respectively are the most present priority diseases in the country.

The results of this exercise will guide the Ministry of Livestock and Fisheries Industry, Ministry of health and other stakeholders in the development of comprehensive control strategies for TADS and zoonotic diseases and the allocation of resources for their control. Recognising the un control movement of livestock between the neighboring countries of the regional and the important of harmonised Livestock diseases control strategies with the neighboring regional countries, IGAD, EAC, AU/IBAR and other regional and global disease control strategies and pathways would be consider in developing South Sudan Disease control strategies .e.g. Global PRR Control and Eradication Strategy, FMD Global Progressive Control pathway etc.

Initiation and implementation of a memorandum of understanding (MOU) with neighboring countries for development of joined project to control TADs and Zoonotic

diseases in the region.

Sharing the outcome of this exercise with relevant stakeholders including, ministries, private sector, pastoralists communities, Non-Governmental organization, and agencies operating in animal health sector is a must, to allow them to add inputs in planning the final control strategy.

Updated action plan and the annual vaccination calendar for control of TADs and Zoonotic diseases livestock disease in South Sudan would be done.

### **Conflict of interest**

There was no conflict of interest identified.

### **Ethical standards**

The study involved review of secondary data and reports generated during routine animal and human disease surveillance. There was no direct contact with human and animal subjects. Because this was part of the routine surveillance activities which are exempted from Institution Review Boards (IRBs) and National Council of Science and Technology (NCST) permission to conduct the study was not sought. The study has many benefits to the human population and to the animal by guiding policy and resource allocation for prevention and control of the diseases.

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## EFFET DU NIVEAU DE FERTILISATION A LA FIENTE DE POULE SUR LA PRODUCTION DU ZOOPLANCTON EN BAC BÉTONNÉ

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### Resume

Entre Mai et Octobre 2014, l'effet du niveau de fertilisation à la fiente de poule sur la production zooplanctonique a été étudiés à la station de l' institut de recherche agricole pour la développement (IRAD) de Foumban, Ouest Cameroun (LN : 5° 21' à 5° 58' et LE : 10° 17' à 11° 02') (LN : 5° 21' à 5° 58' et LE : 10° 17' à 11° 02'). Pour cela, quatre niveaux de fiente de poule correspondant aux traitements: D0 (0 g/m<sup>3</sup>); D450 (450 g/m<sup>3</sup>); D600 (600 g/m<sup>3</sup>) et D750 (750 g/m<sup>3</sup>), ont été distribué de manière aléatoire à raison de trois répétitions par traitement dans 12 bacs bétonnés identiques. Ces bacs ont été rempli chacun à 500 litres d'eau. Le zooplancton a été ensemencé dans chaque bac six jours après fertilisation à raison de 7 individus par litre (ind/l). Les résultats montrent qu'indépendamment de la dose de fiente, la production du zooplancton quel que soit le groupe, a été optimale entre les 6 et 10ème jours. La production des rotifères, des cladocères et des copépodes a été optimale respectivement avec D600 (3428 ind/l), D450 (521 ind/l) et D750 (325 ind/l). La densité, la production journalière et le taux d'accroissement de la biomasse des zooplanctons les plus élevé ont été obtenus dans le traitement D750 soit 3592; 597 et 1 ind/l respectivement, tandis que les valeurs les plus faible sont été obtenues avec D0 (1581; 262 et 0.86 ind/l respectivement). L'indice d'abondance relative la plus élevée a été obtenue avec D600, les rotifères ayant été les plus abondants (90%) contrairement aux cladocères (8%).

**Mots clés :** fiente de poule; niveau; zooplancton; production.

### Abstract

Between May and October 2014, the effect of the level of fertilization using poultry dung on the production of zooplankton was conducted at the Koupa-Matapit IRAD fishculture station, Western Cameroon (LN: 5° 21' to 5° 58' and LE: 10° 17' to 11° 02'). Four levels of poultry dung corresponding to treatments D0 (0 g/m<sup>3</sup>); D450 (450 g/m<sup>3</sup>); D600 (600 g/m<sup>3</sup>) and D750 (750 g/m<sup>3</sup>), were randomly divided into 3 replicates in 12 identical concrete tanks (1 x 0.75 x 1.2m: height x width x length) containing 500 liters of water. The zooplankton was seeded in each tank 6 days after fertilization at the rate of 7 individuals per liters. Results showed that independently of the level of poultry dung, the zooplankton production was best between the 6th and 10th day. The production of rotifers, cladoceras and copepods was highest with D600 (3428 ind/l), D450 (521 ind/l) and D750 (325 ind/l) respectively. The highest value of the density, daily production and growth rate of the biomass of zooplankton were obtained in the treatment D750 (3592; 592 ind/l respectively) while the lowest values were with D0 (1518; 262 and 0.86 ind/l respectively). The highest values of relative abundance index were obtained with D600, rotifers being more abundant (90%) contrary to cladoceras (8%).

**Key words:** poultry dung; level; zooplankton; production.

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## Introduction

L'importance du poisson de nos jours dans la nutrition des populations Africaine et mondial n'est plus à démontrer. En Afrique subsaharienne et plus spécifiquement au Cameroun, 46% des 20g de protéines animales consommées par jour par habitant sont couvert par le poisson, l'essentiel de ce poisson provenant des pêches et des importations et seulement 1% de l'aquaculture (FAO, 2008). Cependant, la baisse des stocks de pêches survenue ces dernières années du fait de la Surpêche, la destruction des zones humides et des changements climatiques font de l'aquaculture la seule alternative pour lutter contre l'insatisfaction des besoins en poisson des populations (Tacon, 2004 ; Marra, 2005). Or ce secteur fait face depuis 50ans à cinq grandes contraintes (Big five) dont la principale est le manque d'alevins de qualité (Moehl *et al.*, 2006). Cette rareté d'alevins est lié aux faibles taux de survie larvaires dans les éclosiers ce qui serait imputable à plusieurs raisons dont la majeure est la non maîtrise de la production qualitative et quantitative de proies vivantes adaptées tels que les zooplanctons (aliments naturels des larves) (Awaiss, 1992). A cet effet, plusieurs recherches à l'instar de ceux de Dossou (2008); Agadjihouèdé *et al.* (2011a, 2011b) ont montré que l'usage des déchets agricoles et principalement la fiente de poule permet une meilleure production de ces zooplanctons. Les travaux de Agadjihouèdé *et al.* (2011b) proposent 600 g/m<sup>3</sup> de fiente de poule comme étant le meilleur niveau de fertilisation par rapport à 300 et 1200 g/m<sup>3</sup>. L'écart entre ces niveaux nécessite une détermination plus précise du niveau optimale en vue de son économie et de la protection de l'environnement. Ce travail a pour objectif de contribuer à une meilleure connaissance sur la production des alevins de qualité à travers une meilleure production du zooplancton. Plus spécifiquement, il s'agit d'évaluer l'effet du niveau de fertilisation à la fiente de poules sur la densité, la biomasse, la production journalière et l'indice d'abondance spécifique des zooplanctons en bacs bétonnés.

## Matériels et Methodes

### Zone de l'étude

L'étude s'est déroulée de Mai à Juillet 2014 à la station IRAD de Foumban, plus spécifiquement au sein de sa ferme d'expérimentation piscicole de Koupa Matapit. Koupa Matapit situé à une dizaine de kilomètres de la ville de Foumban dans la Région de l'Ouest Cameroun. Selon un relevé GPS, la localisation exacte de cette station est 5°45,826' de latitude Nord et 10°48,516' longitude Est, à une altitude de 1147 m. Le climat est de type Soudano-guinéen et comprend une saison de pluie (Mars – Octobre) et une saison sèche (Novembre – Février). La température et la pluviométrie sont respectivement de 22° C et de 1800 mm/an en moyenne (Mikolasek *et al.*, 2006).

### Préparation des bacs et fertilisant

L'essai a été réalisé dans 12 bacs bétonnés identiques de 1,2 m de long, 0,75 m de large et 1 m de hauteur chacun, sur lesquels une serre en matière plastique a été construite en vue de réguler la température. Deux semaines avant le démarrage de l'essai, les bacs ont été lavés et désinfectés à l'eau de javelle (proportions 1g/200litres) puis mis en assec pendant 3 jours au terme desquels une mise en eau (eau de forage) d'un volume de 400 l a été faite, suivie du chaulage avec de la chaux vive à raison de 21g pour chaque bac en vue de stimuler la productivité naturelle du milieu et relever le pH.

Les fientes sans litière de copeau ont été collectées dans une ferme d'élevage en cages des poules pondeuses de la localité, puis conditionnées à l'ombre à température ambiante (22° C en moyenne). Avant utilisation, les fientes ont été préalablement tamisées en vue de les débarrasser de toute grosse particule puis un échantillon a été analysé, lequel a révélé les caractéristiques en pourcentage de matière sèche ci-après: 19,24; 0,87 et 0,61% respectivement de cellulose brute d'azote et de phosphore.

### Matériel biologique

Le matériel biologique était constitué

d'un échantillon concentré de zooplanctons qui a été récolté entre 6-7 heures du matin dans un étang fertilisé de la station à l'aide d'un chalut à plancton de 40 µm de maille tiré d'un bord à l'autre de l'étang. Cet échantillon de zooplancton a été concentré et reparti de manière homogène dans 13 tubes à essai de 25 ml chacun.

#### Conduite de l'essai

Une semaine après le chaulage, chaque bac a été fertilisé de manière aléatoire et en trois répétitions à D0, D450, D600 et D750 correspondant respectivement à 0; 450; 600 et 750g /m<sup>3</sup> de fientes de poule. Trois jours après cette fertilisation les bacs ont été ensemencés en phytoplanctons (aliment du futur zooplancton). Pour cet ensemencement, 100 litres d'eau d'un étang de stockage de tilapia ont été prélevés, filtrés à 50µm de mailles afin de ne laisser passer que le phytoplancton, puis ajoutées dans chaque bac. Trois jours plus tard, un échantillon de zooplanctons a été récolté entre 6-7 heures du matin dans un étang à Tilapia de la station. Comme décrit plus haut, cet échantillon de zooplancton a été concentré et reparti de manière homogène dans 13 tubes à essai de 25 ml chacun. Les 12 bacs ont reçu chacun le contenu d'un tube. Le contenu du 13ème tube a été fixé au formol à 5% puis acheminé immédiatement au laboratoire d'aquaculture de l'IRAD de Foumban pour un inventaire qualitatif et quantitatif au microscope optique type MOTIC à l'objectif 10X des organismes ensemencés suivant la méthode préconisée par Agadjouédé et al (2011a). Il en est ressorti que le zooplancton a été inséminé dans les bacs à une densité de 7 ind/l (soit 1; 2 et 4 ind /l respectivement de rotifères, cladocères et copépodes). Les caractéristiques physico-chimiques de l'eau ont été mesurées de manière hebdomadaire entre 6-8 heures.

Les caractéristiques physico-chimiques de l'eau ont été mesurées au début de l'essai puis hebdomadairement entre 6 et 8 h à l'aide d'un pH-mètre, d'un Oxy-thermomètre, d'un conductimètre d'un disque de sécchi et d'un bambou gradué respectivement pour les mesures du PH, oxygène dissout, température,

conductivité, transparence et profondeur de l'eau. Aux mêmes dates, un échantillon d'eau était collecté en vu du dosage au Laboratoire d'Ichtyologie et d'Hydrobiologie Appliqué de l'université de Dschang des sels nutritifs à savoir: nitrates, nitrites, azote ammoniacal et phosphates. Ce dosage s'est fait à l'aide d'un spectrophotomètre de marque HACH respectivement d'après les méthodes de nitra ver III, nitri ver III, Nessler et de phos ver V recommandée par Zébazé (2000). La concentration en ammoniac non ionisée s'est déduite de celle de l'azote ammoniacal selon la formule de Pihan et Landragin (1985) ci-dessous:

$$N-NH_3 = \frac{N-NH_{4+}}{1 + 10^{10-pH-0,03T}}$$

Avec N-NH<sub>3</sub> et N-NH<sub>4+</sub> en mg.L<sup>-1</sup> ; T Température en °C.

Ces caractéristiques physicochimiques de l'eau sont présentées dans le tableau 1

#### Collecte des données et paramètres étudiés

La production du zooplancton a été suivie de manière bi-journalière pendant 30 jours suivant le protocole décrit par Needham (1962) et repris par Agadjouédé et al. (2011b). Pour cela, 20 litres d'eau étaient prélevés de chaque bac tous les deux jours entre 6 et 8 h à l'aide d'un récipient d'un litre (donc 15 points de prélèvement sur les bords et 05 points au milieu du bac), puis filtrés à travers un tamis à zooplanctons de mailles 50µm en vue de retenir le zooplancton qui était ensuite concentré dans un tube à essai de 25ml puis fixé au formol à 5% et acheminé au laboratoire d'aquaculture de l'IRAD de Foumban. Trois sous-échantillons de 1ml de chaque tube à essai étaient prélevés au moyen d'une pipette pasteur en plastique et transmis sur une boîte de pétri quadrillée pour identification et comptage systématique au microscope décrit si haut.

A l'issu de cette démarche, les données ont été calculées tel que indiqué dans le tableau 2:

**Tableau 1:** Variation des caractéristiques physicochimique de l'eau en fonction du niveau de fiente de poules.

Caractéristiques physicochimique	Traitements			
	D0	D450	D600	D750
Température	26,89 ± 0,15	27,09 ± 0,73	27,01 ± 0,26	27,05 ± 0,38
pH	10,78 ± 0,23	10,75 ± 0,22	10,44 ± 0,28	10,16 ± 0,11
Oxygène dissout	9,59 ± 0,42	8,98 ± 0,69	7,94 ± 0,40	7,21 ± 0,35
Conductivité	105,56 ± 4,19	195,00 ± 13,23	200,56 ± 12,62	242,78 ± 9,77
Transparence	70,10 ± 1,15	70,83 ± 1,04	70,43 ± 0,81	69,73 ± 1,20
Profondeur	70,10 ± 1,15	70,83 ± 1,04	70,43 ± 0,81	69,73 ± 1,20
NH4+	0,47 ± 0,08	0,46 ± 0,08	0,87 ± 0,29	0,79 ± 0,07
NH3	0,45 ± 0,07	0,45 ± 0,08	0,85 ± 0,31	0,76 ± 0,06
NO3-	0,12 ± 0,08	0,16 ± 0,11	0,29 ± 0,14	0,23 ± 0,26
NO2-	0,25 ± 0,05	0,04 ± 0,01	0,07 ± 0,01	0,14 ± 0,01
PO42-	0,48 ± 0,05	1,34 ± 0,09	2,17 ± 0,11	1,97 ± 0,31

**Tableau 2:** Estimation de la production du zooplancton

Caractéristiques zooplanctonique	Formules	références
Densité (D)	$D = n / v_1 \times v_2 / v_3$	(Cacot, 2007)
Biomasse(B)	$B = mi \times D$	(Legendre et al., 1987)
Production journalière (P)	$P = (N_f - N_0) / t$	Agadjihouede et al., 2010
Taux d'accroissement de la biomasse (Kr)	$K_r = (\ln BmT - \ln Bm_0) / t$	
Indice d'abondance relative (IAR)	$IAR = NG / NT \times 100$	Agadjihouédé et al. (2011b)

Avec : n = nombre d'individus compté ;  $v_1$  = volume du sous échantillon prélevé ;  $v_2$  = volume de l'échantillon concentré ;  $v_3$  = volume total d'eau filtré ;  $mi$  = masse individuelle, NG = Nombre d'individus d'un groupe ; NT = Nombre total d'individus ;  $N_f$  = Nombre final par ml de filtrat ;  $N_0$  = Nombre initial/ml de filtrat et t = Durée de colonisation des espèces zooplanctoniques (en jours) ;  $BmT$  = Biomasse final / l de filtrat ;  $Bm_0$  = Biomasse initial/l de filtrat ; t = Durée de colonisation des espèces zooplanctoniques (en jours).

Notons qu'en absence d'une microbalance, la biomasse (masses moyennes individuelles sèches des zooplanctons) en microgrammes a été calculée en utilisant les données de la littérature selon la méthode développée par Gras et Saint-Jean (1981); Legendre et al. (1987) à savoir 0,07 µg/ind pour les rotifères, 0,08 µg/ind pour les copépodes au stade nauplius et 0,47 µg/ind pour les mélanges de copépodes adultes et enfin 3,5 ; 2,7 ; 0,2 µg/ind (poids sec) pour les femelles adultes, les juvéniles et les néonates de cladocères respectivement. En définitive la biomasse de chaque groupe zooplanctonique correspondait à sa densité multipliée par ces masses individuelles préalablement défini.

#### Analyses statistiques

Les données collectées ont été soumises à l'analyse de la variance à un facteur (ANOVA 1). Lorsqu'il existait de différences significatives entre traitements, le test de Duncan au seuil de 5% a été utilisé pour séparer les moyennes. Le test de corrélation a été utilisé pour déterminer la relation entre les caractéristiques zooplanctonique et les caractéristiques de l'eau. Le logiciel statistique SPSS Version 20.0 a été utilisé pour ces analyses.

## Résultats et discussion

### Résultats

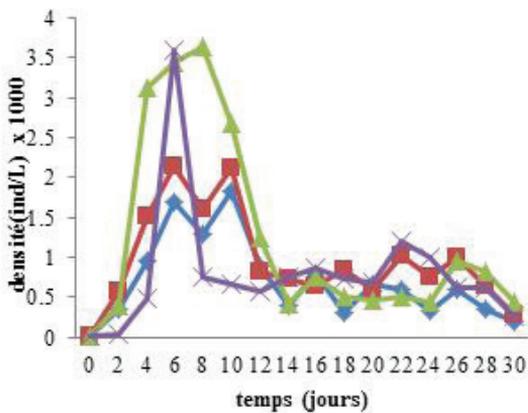
Effet du niveau de fertilisation à la fiente de poule sur l'évolution des groupes zooplanctoniques.

L'évolution des densités moyennes des groupes de zooplanctons en fonction du niveau de fientes de poules est illustrée par la figure 1A pour le total de zooplanctons, 1B pour les rotifères, 1C les copépodes et 1D les cladocères.

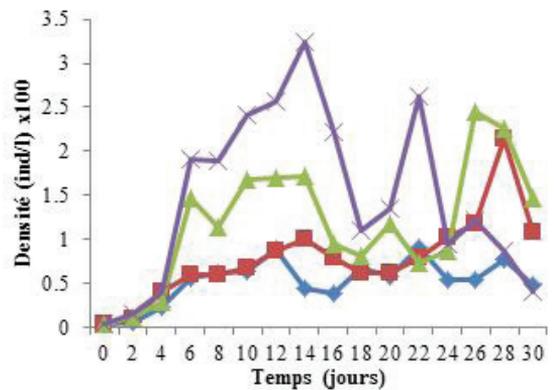
Il ressort de la figure 1 que indépendamment du niveau de fiente et du groupe, l'évolution de la

Les courbes de l'évolution de la densité zooplanctonique en fonction des doses de fiente utilisées pour la fertilisation présentent

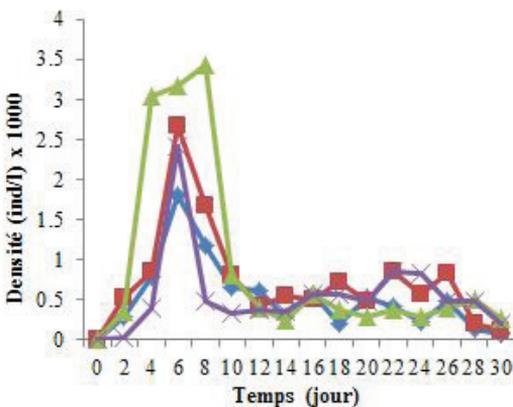
un profil, une allure et tendance identique, avec une phase de colonisation entre le jour zéro et le 6-10ème jour après ensemencement des bacs aux zooplanctons et une phase de décolonisation qui s'étend entre les jours 6 - 10 jusqu'au 12-14ème jour. De plus, à l'exception des copépodes dont les pics sont plutôt atteints entre les jours 14 et 28, tous les groupes ont atteint leur pic de production entre le 6ème et le 8ème jour (rotifères) et entre le 6ème et le 10ème jour (cladocères). Par ailleurs, le pic le plus haut a été obtenu au 10ème jour et cela avec les lots ayant reçu une fertilisation de 600g/m<sup>3</sup>.



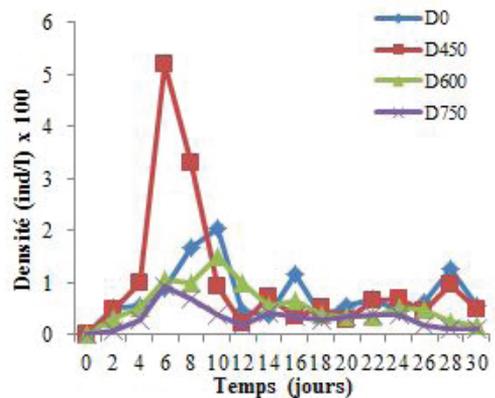
A : zooplanctons



C : copépodes



B : rotifères



D : cladocères

D0; D450; D600 et D750: bacs fertilisés respectivement à 0, 450, 600 et 750g/l de fiente de poule

**Figure 1:** Evolution de la densité zooplanctonique par niveau de fiente en fonction du temps

*Effet du niveau de fertilisation à la fiente de poules sur la densité du zooplancton*

L'analyse de la variance a montré que le niveau de fiente a eu un effet significatif sur la densité de tous les groupes zooplanctonique comme illustré dans le tableau 3.

Il découle du tableau 3 que, la densité des espèces de rotifères et de copépodes a augmenté significativement ( $p < 0,05$ ) avec le niveau croissant de fertilisation. Ainsi, les valeurs les plus faibles ont été obtenues avec les lots non fertilisés et ceux ayant reçus le plus faible niveau de fiente. Pour ce qui est des cladocères, les densités ont été significativement plus élevées avec les lots ayant reçus 450 g/m<sup>3</sup> de fiente de poules. Il est à remarquer qu'une corrélation significative ( $P < 0,05$ ) et positive a été observée entre la densité des zooplanctons, la conductivité et l'ortho-phosphate à D600.

*Effet du niveau de fertilisation à la fiente de poules sur la biomasse des groupes zooplanctonique*

L'analyse de la variance a montré que le niveau de fiente a eu un effet significatif sur la biomasse de tous les groupes zooplanctonique. Ainsi, le tableau 4 résume l'effet du niveau de fiente de poule sur la biomasse zooplanctonique.

Il ressort du tableau 4 que, la biomasse des espèces de rotifères et de copépodes a augmenté significativement ( $p < 0,05$ ) avec

le niveau croissant de fertilisation. Ainsi, les valeurs les plus faibles ont été obtenues avec les lots non fertilisés et ceux ayant reçus 450g de fiente de poule. Pour ce qui est des cladocères, la biomasse a été significativement plus élevée avec les lots fertilisés au plus faible niveau de fiente.

*Effet du niveau de fertilisation à la fiente de poules sur la production journalière du zooplancton*

L'analyse de la variance a montré que le niveau de fertilisation à la fiente de poule a eu un effet significatif sur la production journalière de tous les groupes zooplanctonique. Ainsi, l'influence du niveau de fiente de poule sur la production journalière du zooplancton est résumée dans le tableau 5

Il découle du tableau 4 que, la production journalière de toutes les espèces de rotifères et des copépodes a augmenté significativement ( $p < 0,05$ ) de manière générale d'un niveau de fertilisation à l'autre. Ainsi, les valeurs les plus élevées ont été obtenues avec les niveaux les plus élevés. Toutefois, la production journalière des cladocères a été significativement plus élevée dans les bacs ayant reçus 450g/m<sup>3</sup>. En ce qui concerne le total de zooplanctons, les valeurs de la production journalière ont été significativement plus élevées dans les plus hauts niveaux.

**Tableau 3:** Densité (ind/L) des différents groupes zooplanctonique en fonction des niveaux .de fiente de poule

Groupes zooplanctoniques	espèces	Traitements			
		D0	D450	D600	D750
Rotifères	<i>B calyciflorus</i>	684,72 ± 42 <sup>a</sup>	668,75 ± 10 <sup>a</sup>	2090,55 ± 18 <sup>b</sup>	2096,25 ± 3 <sup>b</sup>
	<i>keratellasp</i>	408,33 ± 62 <sup>a</sup>	434,16 ± 47 <sup>a</sup>	635,55 ± 17 <sup>b</sup>	629,27 ± 2 <sup>b</sup>
	<i>A fissa</i>	283,33 ± 27 <sup>a</sup>	275,00 ± 28 <sup>a</sup>	448,61 ± 19 <sup>b</sup>	459,37 ± 40 <sup>b</sup>
	Total Rotifères	1376,38 ± 12 <sup>a</sup>	1377,91 ± 13 <sup>a</sup>	3174,72 ± 17 <sup>b</sup>	3184,89 ± 3 <sup>b</sup>
Cladocères	<i>Daphniasp</i>	87,50 ± 11 <sup>a</sup>	520,83 ± 19 <sup>a</sup>	104,44 ± 14 <sup>a</sup>	92,50 ± 10 <sup>a</sup>
Copépodes	<i>Nauplius</i>	66,66 ± 3 <sup>a</sup>	58,05 ± 50 <sup>a</sup>	113,61 ± 70 <sup>b</sup>	175,55 ± 20 <sup>c</sup>
	<i>Cyclops sp</i>	26,94 ± 30 <sup>a</sup>	31,66 ± 20 <sup>b</sup>	33,33 ± 40 <sup>b</sup>	90,27 ± 20 <sup>c</sup>
	Total copépode	93,61 ± 30 <sup>a</sup>	89,72 ± 6 <sup>a</sup>	146,94 ± 10 <sup>b</sup>	265,83 ± 20 <sup>c</sup>
Total	zooplancton	1581,66 ± 12 <sup>a</sup>	2032,91 ± 15 <sup>b</sup>	3441,38 ± 20 <sup>c</sup>	3592,67 ± 26 <sup>c</sup>

D0; D450; D600 et D750: bacs fertilisés respectivement à 0, 450, 600 et 750g/m<sup>3</sup> de fiente de poule

<sup>a, b, c, d</sup> les moyennes portant des lettres identiques sur la même lignes ne présentent aucune différence significative ( $P > 0,05$ ).

**Tableau 4:** Biomasse ( $\mu\text{g}$ ) des différents groupes zooplanctoniques en fonction des niveaux de fiente

Groupes zooplanctoniques	espèces	Traitements			
		D0	D450	D600	D750
Rotifères	<i>B calyciflorus</i>	47,93 $\pm$ 2,99 <sup>a</sup>	46,81 $\pm$ 7,17 <sup>a</sup>	146,34 $\pm$ 1,33 <sup>b</sup>	146,74 $\pm$ 0,20 <sup>b</sup>
	<i>keratellasp</i>	28,58 $\pm$ 4,39 <sup>a</sup>	30,39 $\pm$ 3,29 <sup>a</sup>	44,49 $\pm$ 1,20 <sup>b</sup>	44,05 $\pm$ 0,15 <sup>b</sup>
	<i>A fissa</i>	19,83 $\pm$ 1,93 <sup>a</sup>	19,25 $\pm$ 1,99 <sup>a</sup>	31,40 $\pm$ 1,32 <sup>b</sup>	32,16 $\pm$ 0,25 <sup>b</sup>
	Total Rotifères	96,35 $\pm$ 8,19 <sup>a</sup>	96,45 $\pm$ 9,19 <sup>a</sup>	222,23 $\pm$ 1,19 <sup>b</sup>	222,94 $\pm$ 0,20 <sup>b</sup>
Cladocères	<i>Daphniasp</i>	236,25 $\pm$ 28,73 <sup>a</sup>	1406,3 $\pm$ 50,71 <sup>b</sup>	282,00 $\pm$ 38,60 <sup>a</sup>	249,8 $\pm$ 27,28 <sup>a</sup>
Copépodes	<i>Nauplius</i>	5,33 $\pm$ 0,20 <sup>a</sup>	4,64 $\pm$ 0,37 <sup>a</sup>	9,09 $\pm$ 0,56 <sup>b</sup>	14,04 $\pm$ 1,58 <sup>b</sup>
	<i>Cyclops sp</i>	12,66 $\pm$ 1,20 <sup>a</sup>	14,88 $\pm$ 0,68 <sup>b</sup>	15,67 $\pm$ 1,71 <sup>b</sup>	42,43 $\pm$ 0,60 <sup>c</sup>
	Total copépode	17,99 $\pm$ 1,15 <sup>a</sup>	19,53 $\pm$ 1,01 <sup>a</sup>	24,76 $\pm$ 2,16 <sup>b</sup>	56,48 $\pm$ 1,63 <sup>c</sup>
Total	zooplancton	426,2 $\pm$ 30,4 <sup>a</sup>	1592,4 $\pm$ 54,8 <sup>d</sup>	650,2 $\pm$ 47,7 <sup>b</sup>	738,5 $\pm$ 33,4 <sup>c</sup>

D0; D450; D600 et D750: bacs fertilisés respectivement à 0, 450, 600 et 750g/l de fiente de poule

<sup>a,b,c,d</sup>les moyennes portant des lettres identiques sur la même ligne ne sont pas significativement ( $P>0,05$ ) différents.

**Tableau 5:** Production journalière (P) (ind/L) des différents groupes de zooplanctons en fonction des niveaux de fiente de poule

Groupes zooplanctoniques	espèces	Traitements			
		D0	D450	D600	D750
Rotifères	<i>B. calyciflorus</i>	114,02 $\pm$ 7,13 <sup>a</sup>	111,36 $\pm$ 17,06 <sup>a</sup>	348,33 $\pm$ 3,16 <sup>b</sup>	349,28 $\pm$ 0,49 <sup>b</sup>
	<i>keratellasp</i>	68,02 $\pm$ 10,46 <sup>a</sup>	72,32 $\pm$ 7,84 <sup>a</sup>	105,89 $\pm$ 2,85 <sup>b</sup>	104,84 $\pm$ 0,36 <sup>b</sup>
	<i>A. fissa</i>	47,18 $\pm$ 4,58 <sup>a</sup>	45,79 $\pm$ 4,73 <sup>a</sup>	74,72 $\pm$ 3,15 <sup>b</sup>	76,52 $\pm$ 0,59 <sup>b</sup>
	Total Rotifères	229,23 $\pm$ 19,50 <sup>a</sup>	229,48 $\pm$ 21 <sup>a</sup>	528,95 $\pm$ 2,83 <sup>b</sup>	530,65 $\pm$ 0,46 <sup>b</sup>
Cladocères	<i>Daphniasp</i>	14,25 $\pm$ 1,77 <sup>a</sup>	86,47 $\pm$ 3,13 <sup>b</sup>	17,07 $\pm$ 2,38 <sup>a</sup>	15,08 $\pm$ 1,68 <sup>a</sup>
copépode	<i>Nauplii</i>	10,92 $\pm$ 0,42 <sup>a</sup>	9,49 $\pm$ 0,76 <sup>a</sup>	18,75 $\pm$ 1,16 <sup>b</sup>	29,08 $\pm$ 3,30 <sup>c</sup>
	<i>Cyclops sp</i>	4,007 $\pm$ 0,42 <sup>a</sup>	4,79 $\pm$ 0,24 <sup>a</sup>	5,07 $\pm$ 0,61 <sup>b</sup>	14,56 $\pm$ 0,21 <sup>c</sup>
	Total copépode	14,94 $\pm$ 0,49 <sup>a</sup>	14,29 $\pm$ 0,98 <sup>a</sup>	23,82 $\pm$ 1,67 <sup>b</sup>	43,64 $\pm$ 3,28 <sup>c</sup>
Total	zooplancton	262,11 $\pm$ 19,23 <sup>a</sup>	337,32 $\pm$ 24,52 <sup>b</sup>	572,06 $\pm$ 3,31 <sup>c</sup>	597,28 $\pm$ 4,29 <sup>c</sup>

D0; D450; D600 et D750: bacs fertilisés respectivement à 0, 450, 600 et 750g/m<sup>3</sup> de fiente de poule

<sup>a,b,c</sup> les moyennes portant des lettres identiques sur la même lignes ne sont pas significativement ( $P>0,05$ ) différents.

*Effet du niveau de fertilisation à la fiente de poules sur le taux d'accroissement de la biomasse des différents groupes zooplanctonique*

L'analyse de la variance a montré que le niveau de fiente a eu un effet significatif sur le taux d'accroissement de la biomasse de tous les groupes zooplanctonique. Ainsi, l'effet du niveau de fiente de poule sur le taux intrinsèque d'accroissement de la biomasse zooplanctonique est résumé ci-dessous (tableau 6).

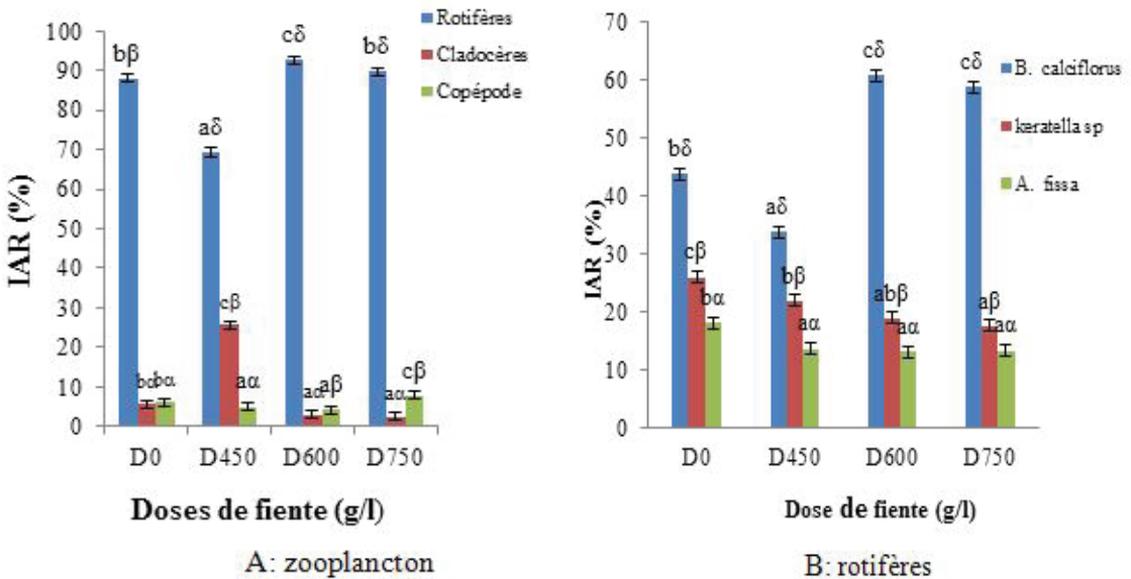
Il ressort du tableau 6 que, le taux d'accroissement de la biomasse de toutes

les espèces de rotifères et des copépodes a augmenté significativement ( $p<0,05$ ) de manière générale d'un niveau de fertilisation à l'autre. Ainsi, les plus faibles valeurs ont été obtenues avec les niveaux les plus faibles de fiente. Pour ce qui est des cladocères, le taux d'accroissement de la biomasse a été significativement plus élevé dans les bacs ayant reçus 450g/m<sup>3</sup> de fiente de poule. En ce qui concerne le total de zooplanctons, les valeurs du taux d'accroissement de la biomasse ont été significativement plus élevées dans les plus hauts niveaux de fientes.

**Tableau 6:** Taux d'accroissement de la biomasse (Kr) des différents groupes de zooplanctons en fonction des niveaux de fertilisation à la fiente de poule

Groupes zooplanctoniques	espèces	Traitements			
		D0	D450	D600	D750
Rotifères	<i>B. calciflorus</i>	1,19 ± 0,010 <sup>a</sup>	1,180 ± 0,030 <sup>a</sup>	1,370 ± 0,002 <sup>b</sup>	1,370 ± 0,001 <sup>b</sup>
	<i>keratellasp</i>	1,270 ± 0,030 <sup>a</sup>	1,280 ± 0,019 <sup>a</sup>	1,34 ± 0,004 <sup>b</sup>	1,34 ± 0,001 <sup>b</sup>
	<i>A. fissa</i>	1,170 ± 0,016 <sup>a</sup>	1,170 ± 0,016 <sup>a</sup>	1,25 ± 0,007 <sup>b</sup>	1,25 0 ± 0,001 <sup>b</sup>
	Total Rotifères	1,204 ± 0,014 <sup>a</sup>	1.204 ± 0,016 <sup>a</sup>	1,343 ± 0,001 <sup>b</sup>	1,344 ± 0,001 <sup>b</sup>
Cladocères	Daphniasp	0,63 ± 0,021 <sup>a</sup>	0,93 ± 0,01 <sup>b</sup>	0,66 ± 0,02 <sup>a</sup>	0,64 ± 0,02 <sup>a</sup>
	Nauplii	0,68 ± 0,01 <sup>a</sup>	0,67 ± 0,01 <sup>a</sup>	0,77 ± 0,01 <sup>b</sup>	0,84 ± 0,03 <sup>c</sup>
copépo	Cyclops sp	0,371 ± 0,016 <sup>a</sup>	0,398 ± 0,008 <sup>b</sup>	0,406 ± 0,018 <sup>b</sup>	0,573 ± 0,002 <sup>c</sup>
	Total copépo	0,53 ± 0,01 <sup>a</sup>	0,52 ± 0,01 <sup>a</sup>	0,60 ± 0,01 <sup>b</sup>	0,70 ± 0,01 <sup>c</sup>
Total	zooplancton	0,861 ± 0,012 <sup>a</sup>	0,903 ± 0,012 <sup>b</sup>	0,991 ± 0,001 <sup>c</sup>	0,998 ± 0,001 <sup>c</sup>

<sup>a, b, c</sup> les moyennes portant des lettres identiques sur la même ligne ne sont pas significativement ( $P > 0,05$ ) différents  
D0; D450; D600 et D750: bacs fertilisés respectivement à 0, 450, 600 et 750g/m<sup>3</sup> de fiente de poule



D0; D450; D600 et D750: bacs fertilisés respectivement à 0, 450, 600 et 750g/m<sup>3</sup> de fiente de poules

a, b, c: Les barres de même couleur affectées des mêmes lettres ne sont pas significativement ( $p < 0,05$ ) différent.

α, β, δ : Les barres affectées de la même lettre et faisant partir du même traitement ne sont pas significativement ( $p < 0,05$ ) différent.

**Figure 2:** Abondance relative (%) des groupes de zooplanctons (A) et des rotifères (B) en fonction du niveau de fertilisation

*Effet du niveau de fiente de poules sur l'abondance relative des groupes de Zooplanctons*

L'abondance relative des groupes zooplanctoniques en fonction du niveau de fertilisation est illustrée par la figure 2.

Il découle de la figure 2A que les rotifères ont été significativement ( $p < 0,05$ ) et relativement plus élevés quelque soit le niveau de fiente de poule. Ainsi, les bacs ayant reçus 600g/m<sup>3</sup> de fiente ont présentés les valeurs significativement plus élevées d'indice

d'abondance de rotifères. Cependant, chez les cladocères, les valeurs significatives les plus faibles ont été obtenues avec les plus forts niveaux. Quant à l'indice d'abondance relative des copépodes, les valeurs significatives les plus élevées ont été obtenues avec les plus hauts niveaux.

En ce qui concerne particulièrement l'indice d'abondance relative des espèces de rotifères, (figure 2B), quelle que soit le niveau de fiente de poules, l'espèce *Brachionus calyciflorus* a présenté les indices significativement plus élevées. Ainsi, les plus hauts niveaux ont présenté des valeurs d'indices d'abondance spécifique significativement plus élevées contrairement à *Keratella* sp et *A. fissa*.

#### *Corrélations entre différents groupes de zooplancton*

Les relations entre groupes zooplanctoniques sont mises en évidence par le tableau 7si dessous

Il ressort du tableau 6 de manière générale que les rotifères ont été significativement corrélés de manière positive et forte ( $P < 0,05$ ) avec les copépodes. Cependant elle a été négative et non significative ( $P < 0,05$ ) avec les cladocères (*Daphniasp*). Ainsi, on constate donc que *B. calyciflorus* a eu une corrélation positive et forte ( $P < 0,05$ ) avec *keratellasp*, *A. fissa* et les Nauplii et très forte ( $P < 0,01$ ) avec *Cyclops* sp. Quant à *keratellasp*, on note également une corrélation positive et forte ( $P < 0,05$ ) avec *A. fissa* et les Nauplii et très forte ( $P < 0,01$ ) avec *Cyclops* sp. Toutefois une corrélation négative et non significative ( $P < 0,05$ ) a été observée entre *Daphniasp*, et toutes les autres espèces.

### **Discussion**

Les résultats de cette étude ont montré que, la densité zooplanctonique a augmenté avec les niveaux croissants de fiente de poules, les meilleures valeurs ayant été obtenues en fertilisant les bassins avec les niveaux les plus élevés de fiente. Des résultats similaires ont été trouvés par Agadjihouédé et al. (2011a). Les valeurs de densité zooplanctonique croissantes

avec le niveau de fertilisation peuvent s'expliquer par les caractéristiques physico-chimique du milieu qui avaient des valeurs moyennes requises pour une bonne réponse à la fertilisation. En effet une bonne fertilisation conduit à une production massive d'algues, ce qui induit une multiplication abondante de zooplanctons, lesquels auraient trouvé dans leur milieu une abondance de nourriture (Seyer, 2002). Pour ce qui est de la biomasse, elle a augmenté avec le niveau croissant de fiente de poules, exception faite des bacs ayant reçu 450g/m<sup>3</sup> qui ont montré les valeurs les plus élevées malgré une densité de zooplanctons plus faible que les plus forts niveaux. Cela pourrait être lié à la différence de poids individuel entre les groupes zooplanctoniques (allant de 0,07µg/ind pour les rotifères, 0,08µg/ind pour les copépodes au stade nauplius, 0,47µg/ind pour les mélanges de copépodes et jusqu'à 2,7µg/ind adultes pour les cladocères, telle que rapportée par Dabbadie (1996). En effet le niveau 450g/m<sup>3</sup> a montré une plus forte densité de cladocères, lesquelles ont un plus grand poids individuel.

Les rotifères, comparativement aux copépodes et aux cladocères ont montré un développement rapide avec un pic atteint entre les jours 6 et 8 après insémination (ce qui correspond en fait à au 12ème-14ème jour après fertilisation). Cependant, les copépodes avec un développement lent n'ont atteint leur pic qu'entre le 12ème et le 14ème jour. Cela s'expliquerait par leur mode de reproduction parthénogénétique (donc un cycle de développement court) chez les rotifères et sexuée (rendant plus long le cycle de développement) chez les copépodes et les cladocères. Ces observations rejoignent celles de ACF (2010) qui rapportent qu'une semaine après la fertilisation les rotifères pullulent dans le milieu, tandis que les copépodes abondent seulement après deux semaines. La baisse rapide de la densité des rotifères quelque temps après le pic dans tous les bassins serait liée à l'augmentation de la densité des copépodes prédateurs de ces derniers. Ce résultat est comparable à ceux obtenus par Legendre et al. (1987) lors d'une étude de recolonisation des étangs de Layo par les zooplanctons ainsi

que ceux de Agadjihouédé et al. (2011a; 2011b). Le taux d'accroissement de la biomasse dans les bassins au cours de l'essai est bien plus élevé comparé à ceux rapportés par Saint-Jean et Bonou (1994) et Awaïss et Kestemont (1997) cependant il reste très proche de celui rapporté par Agadjihouédé (2011b) lors d'une production mono-spécifique de zooplanctons. Ce taux élevés'expliquerait par la richesse plus grande en azote des fientes de poules utilisées lors de cet essai. La plus forte abondance relative des rotifères quelle que soit le niveau s'expliquerait par la richesse phytoplanctonique de ces milieux. Cette assertion rejoint celle de Zébazé et al. (2004) qui stipule que la forte représentativité des rotifères dans un milieu indique biologiquement son niveau trophique, ce résultat est cependant contradictoire à celui obtenu par Agadjihouède et al. (2011a).

### Conclusion

Au terme de cette étude les conclusions majeures suivantes peuvent être dégagées: Le niveau de fertilisation à la fiente de poule a eu un effet significatif sur la production du zooplancton. Ainsi, la densité, la biomasse et l'indice d'abondance relative des différents groupes zooplanctoniques ont été affectés significativement par le niveau de fertilisation à la fiente de poules. Ces valeurs ont augmenté avec le niveau de fertilisation. La production du zooplancton quel que soit le groupe et le niveau, a été optimale entre les jours 6 et 10 après introduction du zooplancton

Dans les conditions du milieu écologique de la station IRAD de Fouban, La dose 600g/m<sup>3</sup> de fiente de poule est recommandée pour la production optimale de zooplanctons. La fertilisation aux niveaux 600, 450 et 750g/m<sup>3</sup> de fiente de poule est optimale pour la production des rotifères, cladocères et copépodes respectivement. De plus, Il conviendrait d'introduire les larves de poisson dans un milieu de production environ 6 à 8 jours après insémination du zooplancton (12-14 jours après fertilisation: date de production optimale de zooplancton).

Cependant il serait envisageable dans nos futurs travaux d'évaluer l'effet de la fréquence de fertilisation à la fiente de poule sur la production zooplanctonique ainsi sur la survie et la croissance des larves de carpe, puis d'évaluer la productivité en conditions monospécifiques des groupes zooplanctoniques ainsi que leurs effets sur la production des larves durant un cycle complet d'un an.

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## PRIORITISATION AND CATEGORISATION OF TRANSBOUNDARY ANIMAL DISEASES AND ZONOSSES FOR EFFECTIVE SURVEILLANCE AND CONTROL IN RWANDA

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### Abstract

Despite current efforts and political will in Rwanda, livestock sector is facing serious limiting factors to this increase among others animal feeding (quantity and quality), genetic improvement and TADs and zoonoses control and management. A ranking of animal diseases is important in order to prioritise actions to be done and to allocate available resources according to the priorities. The objectives of the prioritisation process was to: assess the real or potential impact of p15 to 17 diseases at country and regional level; to define a short list of prioritised diseases for every member state that could be part of public animal health policy; to define a short list of prioritised diseases for the EAC region that could be part of public animal health policy; to identify some strategic priorities to control the diseases, including the roles of different stakeholders; and to provide evidence for decision making on policies, strategy development and appropriation of resources. The Phylum tool was used in conducting this exercise to list and categorise priority animal diseases, including TADs and zoonoses. Fifteen animal diseases and zoonosis were assessed and ranked using the tool. These were RVF, FMD(Absent), FMD(Present), ASF, *Brucellosis*, CBPP, CCPP, ECF, HPAI, LSD, ND, PPR, Rabies, G&S Pox, *Tuberculosis* and Trypanosomiasis. The importance of all these diseases was assessed based on specific criteria including local economic impact, local public health impact, and local societal, local environmental impact, and local feasibility, local economic and local societal & environmental impact of control measures. Assessment considered both present and absent diseases. Priority was often given to diseases severely affecting the livestock industry with high morbidity ( eg ND, FMD and, ASF) with additional importance being given to those causing massive epizootics compared to more chronic diseases. This national surveillance and control strategy should be translated into concrete actions, especially at the level of livestock owners, processors, traders, veterinary services, livestock production, food safety and wildlife conservation with an appropriate action plan. Constraints to livestock and wildlife production that include diseases are interrelated and need an integrated approach combining health, genetics, feeding, husbandry practices and organisation at producers' level.

**Key words:** transboundary animal diseases, zoonoses, surveillance, control, Rwanda

## PRIORISATION ET CATÉGORISATION DES MALADIES ANIMALES TRANSFRONTALIÈRES ET DES ZONOSSES POUR UNE SURVEILLANCE ET UN CONTRÔLE EFFICACES AU RWANDA

### Résumé

En dépit des efforts actuellement déployés et de la volonté politique du Rwanda, le secteur de l'élevage est confronté à de sérieux facteurs qui entravent sa croissance, notamment l'alimentation animale (quantité et qualité), l'amélioration génétique et le contrôle et la prise en charge des maladies animales

transfrontalières et des zoonoses. La classification des maladies animales est importante car elle permet de prioriser les actions à mener et d'allouer les ressources disponibles en fonction des priorités. Les objectifs du processus de priorisation étaient les suivants : évaluer l'impact réel ou potentiel des maladies p15 à 17 aux niveaux national et régional ; définir une liste restreinte de maladies prioritaires pour chaque État membre qui pourrait faire partie de la politique de santé animale publique ; définir une liste restreinte de maladies prioritaires pour la région de l'EAC susceptible de faire partie de la politique publique de santé animale ; identifier certaines priorités stratégiques pour le contrôle des maladies, y compris les rôles des différentes parties prenantes ; et fournir des informations factuelles pour la prise de décision sur les politiques, la stratégie de développement et l'appropriation des ressources.

L'outil Phylum a été utilisé dans le cadre de cet exercice pour répertorier et classer les maladies animales prioritaires, y compris les maladies animales transfrontières et les zoonoses. Quinze maladies animales et zoonoses ont été évaluées et classées en utilisant l'outil. Il s'agit de la FVR, de la fièvre aphteuse (absente), de la fièvre aphteuse (présente), de la peste porcine africaine, de la péripneumonie contagieuse bovine, de la péripneumonie contagieuse caprine, de la FCO, de la DNC, de la MNC, de la PPR, de la rage, de la clavelée du mouton et variole caprine, de la tuberculose et de la trypanosomiase. L'importance de toutes ces maladies a été évaluée sur la base de critères spécifiques tels que l'impact économique local, l'impact local sur la santé publique et l'impact sociétal local, l'impact environnemental local, et la faisabilité des mesures locales, l'impact économique et social local et environnemental des mesures de contrôle. L'évaluation a pris en compte à la fois les maladies présentes et absentes. La priorité a été souvent donnée aux maladies affectant gravement l'industrie de l'élevage - avec une morbidité élevée (par exemple la MNC, la FA et la FAA), une importance accrue étant accordée à celles qui causent des épizooties massives par rapport aux maladies plus chroniques. Cette stratégie nationale de surveillance et de contrôle devrait se traduire par des actions concrètes, notamment au niveau des éleveurs, des transformateurs, des commerçants, des services vétérinaires, de la production animale, de la sécurité alimentaire et de la conservation de la faune sauvage avec un plan d'action approprié. Les contraintes à la production animale et faunique incluant les maladies sont interdépendantes et nécessitent une approche intégrée combinant la santé, la génétique, l'alimentation, les pratiques d'élevage et l'organisation au niveau des producteurs.

**Mots-clés :** maladies animales transfrontalières, zoonoses, surveillance, contrôle, Rwanda

## Introduction

Livestocks play a vital role in the economy of the African Countries. It provides food, income and employment. In Rwanda, the livestock sub-sector contributes to 12% of the national GDP with a growth rate estimated at 6% in 2007 for the entire agricultural sector (Indexmundi.com, 2011; MINAGRI, 2012).

For the development of livestock sector, the current national policy is aiming to the intensification of farming, is not only essential for survival of the sub-sector, but also the only likely way to ensure its participation in meeting the needs of the population in the context of food security.

Despite this efforts and political will, livestock sector is facing serious limiting factors to this increase among others animal feeding (quantity and quality), genetic improvement and TADs and zoonoses control and management. Transboundary Animal Diseases (TADs) and

zoonosis have a negative impact on livestock productions. The damage can be economic (loss of output, income and investment), and psychological such as shock and panic (FAO, 2004). The impact of animal diseases on public health and human welfare is being increasingly considered as sixty percent of emerging diseases that affect humans are zoonoses and most of them (about 75%) originate from wildlife.

Therefore, direct impact of Transboundary Animal Diseases in agriculture and public health, constitute a serious limitation to export living animals and their products, as well for international trade ( ROJAS,2009)

A ranking of animal diseases is important to prioritise actions to be done and to allocate available resources according to the priorities. The objectives of the prioritisation process is to conduct surveillance that will result in controlling and eradicating animal diseases in Rwanda, to prevent the introduction

from infected areas within the country and from infected countries and stop the spread of the disease in the country and to manage the diseases once they occur in the country.

The East Africa Community (EAC) and AU-IBAR have been working in collaboration to strengthen the control of animal diseases in the region, with efforts mainly targeted at the establishment of integrated regional (inter-country) coordination mechanisms for the prevention and control of TADs and zoonoses, strengthening of veterinary governance, capacity building in different areas of animal health service delivery, strengthening stakeholder engagement in policy processes and disease control; and resource mobilisation to support regional integration and disease control initiatives among others.

The regional disease prioritisation exercise was organized with the financial support from the EU funded Veterinary Governance Programme and used OIE PHYLUM tool for the “Listing and categorisation of priority animal diseases including those transmissible to humans”

The regional disease prioritisation exercise was intended to support the 5 member states and EAC secretariat to identify, prioritise and categorize the key transboundary and zoonoses for public policy and animal health programs. The specific objectives were:

- To assess the real or potential impact of 15 to 17 diseases at country and regional level;
- To define a short list of prioritised diseases for every member state that could be part of public animal health policy;
- To define a short list of prioritised diseases for the EAC region that could be part of public animal health policy;
- To identify some strategic priorities to control the diseases, including the roles of different stakeholders, and
- To provide evidence for decision making on policies, strategy development and appropriation of resources.

## Materials and Methods

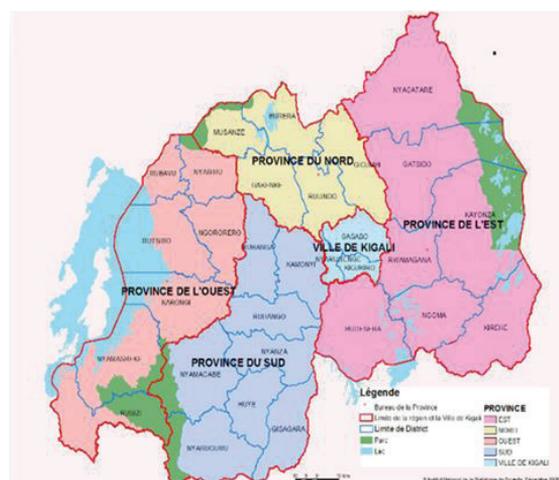
### Study Area

The study used data from Rwanda for analysis. Rwanda is a landlocked East African country in the Great Lakes Region. It borders Uganda to the North, Tanzania to the East, Burundi to the South and the Democratic Republic of Congo (DRC) to the West (Figure 1). The country has an area of 26,338 km<sup>2</sup> (MINAGRI, 2012). Its population is approximately 10.5 million people with a density of 416 inhabitants per km<sup>2</sup> (NISR, 2012). Over 90% of the population is occupied by subsistence agriculture; the urban population is only estimated at 19.1% and about 44.9% of the adult population lives below the poverty line (indexmundi.com, 2011).

Since the implementation of the decentralization policy, the country is currently divided into four provinces namely East, North, South, West and the City of Kigali. Each province is divided into districts, sectors, cells and villages. In total, Rwanda has 30 districts, 416 sectors, 2148 cells and 14 837 villages.

### The tool for data collection and data analysis

The exercise to list and categorize priority animal diseases, including TADs and zoonoses was conducted using a tool developed by PHYLUM, a consulting firm. The tool was developed based on a study co-financed by the



**Figure 1:** Administrative map of Rwanda ( Source: Google 2016)

European Commission and the World Bank, through the OIE World Animal Health and Welfare Fund. It is a tool for risk management that is based on balanced criteria among other descriptive criteria (epidemiology, control measures) and impact criteria (economy, trade and social aspects). This tool consists of an Excel files, organized in different modules. Each module corresponds to a separate spreadsheet, assessing a particular impact or type of data. The different spreadsheets in the tool are arranged in a chronological sequence.

#### *Process of prioritisation and categorisation of animal diseases*

Under the technical and financial support of AU-IBAR animal health unit and Phylum, a regional training was organised in November 2014 in Gaborone for East and South African countries including Rwanda. Four experts from the animal health, wildlife and public health were involved in the whole process of prioritisation and categorisation of TADs and zoonoses. The training was aimed to train trainers and to identify the common priority diseases at the Regional Economic Community (REC) level that could justify common diseases prevention and control programs.

After the training of trainers in November 2014, a workshop was organised in May 2015 in Kigali. A list of priority TADs and zoonosis at national level, categorisation of TADs and zoonoses disease control interventions and proposition of roles of different stakeholders in the management of TADs and zoonoses were elaborated

#### *Data analysis procedures*

The listing and categorisation of priority animal diseases method consisted of two sequential steps:

- A global characterisation of the disease, aimed at assessing inherent aspects of the disease, independently of any particular local context
- A local approach, aiming at refining the study of the disease within the specific context of the country

Before starting the analysis, prerequisites for the exercise were gathered. Initially, the appropriate weighting between the different types of indicators was determined. This weighting was based on the country's political objectives for farming sectors, especially the livestock sector and objectives for human health systems. Then after, diseases to be included in the analysis were defined by focusing on the most important diseases with the heaviest impact in country.

The pre-analysis preparation was completed by collecting all required data and characterisation of the country.

The listing and categorisation of priority animal diseases proceeded with the global characterisation of diseases. It consisted of recording data for each disease in a summary spreadsheets of: Epidemiological profile of the disease, Economic profile of the disease, Zoonotic profile and Profiling of the control measures in animals. This global characterisation considered the nature of the disease (whether or not it is a potential zoonosis) and the possibility of vector-borne transmission of the pathogen. There were addressed through several criteria, regarding epidemiology (transmissibility and persistence in animals), human health (transmissibility in humans), and possible control measures. This profiling step provided an overview of the disease and its control options.

Once the first global characterisation of diseases was done, the local approach was performed. Data was filled and the absence or presence of the disease and any disease-related societal impact was assessed using the spreadsheets of the economic impact of the control measures, the local feasibility of the control measures, and the societal and environmental impact of the control measures. The results for all the diseases were compared in the table summarising the following:

- First, the results for each kind of indicator were compared to validate the local characteristics of the diseases.
- Second, a global prioritisation of diseases that are present or absent was assessed with a different weighting for each indicator

**Table 1:** Epidemiological status of priority diseases

	RVF	FMD Circulating	FMD exotic	CBPP	CCPP	PPR	S& G Pox	ASF	LSD	ND	ECF	Brucellosis	HPAI	TB	Rabies	Tryps
Epidemiological status of the disease in the country	A	P	P	A	A	P	A	A	A	P	P	P	P	A	A	P

**Table 2:** Scores of priority TADs and Zoonoses

	RVF	FMD Circulating	FMD exotic	CBPP	CCPP	PPR	S& G Pox	ASF	LSD	ND	ECF	Brucellosis	HPAI	TB	Rabies	Tryps
Local Economic Impact Criteria	1.85	3.7	2.71	1.29	0.1	0.00	0.4	0.0	3.9	2.8	1.9	2.6	1.5	3.23	1.5	2.06
Local on Human Health	1.67	1.67	0.83	1.67	0.0	1.67	5.0	0.83	4.0	1.5	2.41	2.3	0.33	0.83	0.83	3.20
Local Societal Impact	3.52	6.27	5.15	4.44	5.42	6.18	6.4	5.29	5.31	6.39	7.29	4.9	3.3	6.18	5.5	4.87
Local Environmental Impact	0.0	5.63	1.25	0.0	0.0	0.63	4.38	3.75	6.8	4.38	0.63	0.63	0.63	5.63	5.0	8.1
Local feasibility of CM	5.1	5.8	5.1	3.63	2.2	5.2	4.4	5.1	5.8	5.2	3.7	2.8	2.6	3.2	3.9	2.6
Local Economic Impact on CM	4.3	8.6	7.1	5.0	7.1	7.1	4.3	7.1	10	7.1	2.1	8.6	2.1	5.7	7.9	8.1
Local societal & Environment CM	5.0	6.5	6.0	5.5	6.0	4.5	4.5	5.5	6.5	5.5	4.5	4.5	1.5	6.5	6.5	

of impact.

- Third, a comparison was performed with the control measures.

At the end of this step, a first prioritisation list of TADs and zoonoses at national level was available.

### Results

Fifteen (15) animal diseases and zoonosis were assessed using the tool, these were Rift Valley Fever, Foot and Mouth Disease (Absent), Foot and Mouth Disease (Present),

African Swine Fever, *Brucellosis*, *Contagious Bovine Pleuro-Pneumonia* (CBPP), *Contagious Caprine Pleuro Pneumonia* (CCPP), East Coast Fever (ECF), *Highly Pathogenic Avian Influenza* (HPAI), *Lumpy Skin Disease* (LSD), *New Castle Disease* (ND), *Peste de Petit Ruminants* (PPR), *Rabies*, *Goat and Sheep Pox*, *Tuberculosis* and *Trypanosomiasis*.

The importance of all these diseases was assessed based on specific criteria including local economic impact, local public health impact, and local societal, local environmental impact, and local feasibility, local economic

**Table 3:** Ranking/scores of priority diseases

Local Economic Impact	Local impact on Human Health	Local Societal Impact	Local Environmental Impact	Local Economic Impact of control	Local Environment and societal CM	Final Disease Ranking of zoonoses
1. HP AI	1. Rabies	1. HP AI	1. HP AI	1. FMD present	1. HP AI	1. FMD
2. FMD absent	2. HP AI	2. TB	2. FMD present	2. HP AI	2. ASF	2. ASF
3. FM D Present	3. TB	3. Rabies	3. FMD absent	3. PPR	3. FMD Absent	3. HP AI
4. ND	4. <i>Brucellosis</i>	4. ND	4. ASF	4. ND	4. FMD Present	4. CBPP
5. LSD	5. RVF	5. FMD present	5. Rabies	5. LSD	5. LSD	5. PPR
6. <i>Brucellosis</i>	6. FMD present	6. FMD Absent	6. ND	6. RVF	6. CBPP	6. NCD
7. TB	7. CBPP	7. PPR	7. S&G Pox	7. S&G Pox	7. ND	7. <i>Brucellosis</i>
8. RVF	8. PPR	8. ASF	8. LSD	8. Rabies	8. CCPP	8. RVF
9. ASF	9. ND	9. CBPP	9. <i>Brucellosis</i>	9. TB	9. RVF	9. Rabies
10. ECF	10. FMD Absent	10. CCPP	10. ECF	10. CBPP	10. PPR	10. LSD
11. CBPP	11. S&G Pox	11. S&G Pox	11. PPR	11. FMD Absent	11. Rabies	
12. Rabies	12. LSD	12. LSD	12. CBPP	12. <i>Brucellosis</i>	12. TB	

and local societal & environmental impact of control measures. Assessment considered both present and absent diseases.

#### *Categorisation of priority diseases*

Table 1, shows the epidemiological status of the diseases, the absent diseases (CCPP, S&G pox, FMD exotic, HPAI, PPR, ASF) were also assessed as their risk of introduction is high as well as the present and clinically absent diseases (RVF, FMD circulating, LSD, NCD, Rabies, TB, *Brucellosis*, ECF and Trypanosomiasis).

### **Discussion**

Prior to this TADs and zoonosis prioritisation exercise, animal diseases in Rwanda had been assessed and ranked during the elaborating the national animal disease surveillance and control strategy. This was the first prioritisation with the assistance of the expert from FAO-ECTAD Nairobi, and basic prioritisation was done based on different criteria, weights and scores as parameters for effective qualification and quantification. The criteria that guide prioritisation included: public health impact, production impact, and feasibility of control, impact on trade / animals, impact on trade / animal products, public perception, social impact and the likelihood of transmission. The evaluation criteria are almost the same, but the Phylum tool is more detailed, more inclusive and designed in statistic modelling. After the TADs and zoonosis assessment in Rwanda, 10 diseases namely FMD, ASF, HPAI, CBPP, PPR, NCD, *Brucellosis*, RVF, Rabies and LSD were identified as priority for national surveillance and control programmes. This was mainly due to their probable impact in case of their occurrence in Rwanda.

Priority was often given to diseases severely affecting the livestock industry with high morbidity (FMD, ASF) with additional importance being given to those causing massive epizootics compared to more chronic diseases. Even in the case where an epizootic disease and a chronic disease have equivalent consequences, the epizootic disease will always be more “obvious”, and seem more problematic

than a relatively “cryptic” disease.

In recent years, Rwanda has experienced several outbreak of TADs especially FMD, RVF CBPP, LSD and others with severe public health concerns. Control measures were put in place thus FMD, PPR, ASF and CBPP are clinically absent in Rwanda, but still the risk of introduction is high since neighbouring countries.

NCD is one of the main constraints to increasing small-scale poultry which is growing fast and making a substantial contribution to household food security and income.

### **Conclusion and Recommendations**

Based on the results of this disease prioritisation exercise, current the national surveillance and control strategy should now be translated into concrete actions, especially at the level of livestock owners, processors, traders, veterinary services, livestock production, food safety and wildlife conservation with an appropriate action plan. Such action plan should not only articulate short, medium and long term possible streams of actions, but also to identify the key stakeholders that ought to be engaged in the prevention and control of livestock and wildlife diseases in Rwanda. Although the formulation of a national strategy is an obligatory and mandatory requirement, it needs to be accompanied by a control programmes/projects to achieve the expected outputs to attain the final outcomes. Constraints to livestock and wildlife production that includes diseases are interrelated and need an integrated approach combining health, genetics, feeding, husbandry practices and organisation at producers’ level. Different other emerging and re-emerging diseases are a serious concern, especially as reported in neighboring countries of Rwanda; Ebola, Zika, Marburg, Yellow fever. as certain pathogenic microorganisms can undergo rapid genetic changes, leading to new phenotypic properties that take advantage of changing host and environmental opportunity. Another important issues is the control of antimicrobial resistance, drugs have been used so widely and

for so long that the infectious organisms the antibiotics are designed to kill have adapted to them, making the drugs less effective. The livestock and wildlife sector development depends on the ability of stakeholders to participate in solutions addressing the major constraints facing animal health, production, processing and marketing..

### **Conflict of interest**

There is no conflict of interest identified.

### **Ethical standards**

The study involved review of secondary data and reports generated during routine animal and human disease surveillance. There was no direct contact with human and animal subjects. Because this was part of the routine surveillance activities which are exempted from Institution Review Boards (IRBs) and National Council of Science and Technology (NCST) permission to conduct the study was not sought. The study has many benefits to the human population and to the animal by guiding policy and resource allocation for prevention and control of the diseases.

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## PRIORITISATION OF TRANSBOUNDARY ANIMAL DISEASES (TADS) AND ZONOSSES FOR EFFECTIVE CONTROL IN SUDAN

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### Abstract

Prioritisation of TADs and zoonoses in the country was conducted using the OIE PHYLUM tool. Livestock diseases were prioritised based on economic, human health, social and environmental impacts and the feasibility, economic, social and environmental impacts of the control measures. Based on all criteria mentioned a final proposed list of prioritised diseases was obtained including present list of diseases in the country namely: FMD, PPR, CBPP, Shoaat pox, *Brucellosis* and Rabies and absent list of diseases namely: introduction of new FMD strains in the country, RVF, Ebola, BT, TB and HPAI.

Diseases like FMD, PPR, CBPP, CCPP and NCD which scored moderately high in their impact of the control measures, still their feasibility to be controlled is high, due to their effects on local and international trade, food security issues and the livelihoods of small livestock producers.

The prioritisation constitutes baseline data and provides a good opportunity for further assessment and reviewing of diseases list to better understand the diseases map in the country, their epidemiology and their economic implications on livestock, livelihood and the national economy.

**Keywords:** Disease prioritisation, Effective Control, Sudan

## PRIORISATION DES MALADIES ANIMALES TRANSFRONTIÈRES (MAT) ET DES ZONOSES POUR UN CONTRÔLE EFFICACE AU SOUDAN

### Résumé

La priorisation des maladies animales transfrontières et des zoonoses dans le pays a été réalisée à l'aide de l'outil PHYLUM de l'OIE. Les maladies animales ont été classées par ordre de priorité en fonction des impacts économiques, sanitaires, sociaux et environnementaux, ainsi que de la faisabilité, des impacts économiques, sociaux et environnementaux des mesures de contrôle. Sur la base de tous les critères mentionnés, une liste définitive proposée de maladies prioritaires a été obtenue, incluant la liste actuelle des maladies dans le pays, laquelle comporte la fièvre aphteuse, la PPR, la PPCB, la variole, la brucellose et la rage, et la liste des maladies absentes, à savoir l'introduction de nouvelles souches de fièvre aphteuse dans le pays, la FVR, l'Ebola, la trypanosomose bovine, la TB et l'IAHP.

Les maladies comme la fièvre aphteuse, la PPR, la péripneumonie contagieuse bovine, la péripneumonie contagieuse caprine et la MNC ont été classées modérément importantes pour les mesures de contrôle, cependant, la faisabilité des mesures pour leur contrôle est élevée, en raison de leurs effets sur le commerce local et international, la sécurité alimentaire et les moyens de subsistance des éleveurs.

La priorisation offre des données de base et une bonne d'évaluer et de réviser davantage la liste des maladies afin de mieux comprendre la carte des maladies dans le pays, leur épidémiologie et leurs implications économiques sur l'élevage, les moyens de subsistance et l'économie nationale.

**Mots-clés :** priorisation des maladies, contrôle efficace, Soudan

## Introduction

The Republic of the Sudan has a total area of 1,861,484 sq km and is shared the political boundaries with the Central African Republic, Chad, Egypt, Eritrea, Ethiopia, Libya and South Sudan. The country is divided into 18th states (administrative boundaries). Although Sudan lies within the tropics, the climate ranges from arid in the north to tropical wet-and-dry in the far southwest. Temperatures do not vary greatly with the season at any location; the most significant climatic variables are rainfall and the length of the dry season. Variations in the length of the dry season depend on which of two air flows predominates, dry northeasterly winds from the Arabian Peninsula or moist southwesterly winds from the Congo River basin, (globalsecurity.org, 2016).

The production systems for Sudan consists of nomadic and transhumance cattle and small ruminant that are raised extensively on natural rangelands and comprise 80% of the national herd; agro-pastoralism (cattle and small ruminants) that is practiced around agricultural schemes and traditional crop plantations; and dairy and trade beef cattle that are intensively raised on cereals, wheat bran, green fodder, oil seed cakes and molasses. Camels are kept in desert and semi-desert areas of the country. This climatic diversity governs the livestock distribution throughout the country.

Transboundary animal diseases pose a serious risk to the world animal agriculture and food security and jeopardise international trade. The world has been facing devastating economic losses from major outbreaks of transboundary animal diseases (TADs) such as foot-and mouth disease, classical swine fever, rinderpest, *Peste des Petits Ruminants* (PPR), and Rift Valley Fever (RVF). Lately the highly pathogenic *Avian Influenza* (HPAI) due to H5N1 virus has become an international crisis as all regions around the world can be considered at risk, Domenech et al (2010).

The livestock population figures in the country are estimated to be 105,585,000 Million heads. Sheep and goats account for 37.64% and 29.31%, respectively, of the total

livestock population and contribute significantly to the national economy. The agriculture sector presently contributes approximately 35 to 40 percent of gross domestic product (GDP). Live export earnings for the years 2009 and 2010 for sheep were US\$ 181,314,000 and US\$ 164,072,000 and US\$ 4,445,000 and US\$ 5,089,000 for goats, respectively (Customs Department, 2012). For the same period, sheep meat exports brought in US\$ 9,191,000 and US\$ 38,712,000 respectively.

Other than their contribution to the national economy, sheep and goats in themselves are a coping mechanism that reduces the vulnerability of livestock keepers due to their high fertility rate, ability to reproduce and produce in very harsh and dry environments and recover faster than other species from natural disasters (droughts, epidemics).

However, the sector is seriously constrained by the continued presence of trans-boundary animal diseases (TADs) such as *Peste des Petites Ruminants* (PPR) and sheep pox and goat pox (SGP), FMD, CBPP and *Brucellosis* that limit productivity and access to domestic and export markets and are therefore key obstacles to improving food security and the national economy (MLFR, 2012). Tamador E. Angara (2004) reported the annual total cost of control of *Brucellosis* in both dairy and health sectors to be 65,833,570 SDG, out of which 65,617,120 SD was the cost of the dairy sector and 216,450 SDG was the cost of health sector. The most recent evidence for the RVF virus in Sudan was in the region was in 2007/2008 in which Kenya, Tanzania Somalia, Sudan and later Madagascar and South Africa were affected. Since then there is no report of the disease in the country.

A study on the assessment of the relative importance of priority livestock diseases (Baumann 2009) has prioritized FMD, CBPP, *Brucellosis*, RVF, Tick-borne diseases in addition to SGP and PPR as the top rank of small ruminant diseases in the country. The development of economically and technically sound plans for the control of priority livestock diseases is a major activity of the Livestock Epidemio-Surveillance Programme (LESP) in

Sudan with the overall objective to contribute to a significant long-term reduction in the level of poverty and food insecurity of those involved in livestock farming by improving animal health, productivity, trade and food security. On this basis, the ministry through AH&EDC General Directorate has prepared national control strategies for six of those TADs as integral components of the Sudan National Veterinary Emergency Plan (SUDNAVETPLAN). Later on harmonised Action Plans, including the cost estimate to implement the control strategies for those diseases was put in place namely: FMD, PPR and sheep & goat pox, CBPP, RVF and *Brucellosis* (Dickens Chibau, 2012).

All of Sudan's neighbors to the west, south and east are infected with TADs.

## Material and Methods

### Description of the Study Area

The study area comprises the 18th states of the whole country, which bordered by Egypt in the far North, Libya in the North West part, Chad and Central African Republic in the West, Eritrea and Ethiopia in the East, and Republic of South Sudan in the long South border. Livestock estimated to be for 2014, 105 M head approximately including: cattle, shoats and camels, Table (I) shows the total livestock population per state.



Figure 1: Study Area ( Source: Google, 2016)

**Table 1:** Estimates of livestock population 2014:

State	Cattle	Sheep	Goats	Camels	Total
North Kordofan	754775	4104138	2637465	920064	8416442
South Kordofan	4416943	2151684	2109972	247267	8925867
West Kordofan	3381392	4243599	2358204	627752	10610947
North Darfour	703450	3825640	2943550	599479	8072119
South Darfour	2374522	2151684	1679289	88820	6294315
East Darfour	1942791	1757209	1373964	72671	5146634
Center Darfour	1865804	1793070	2010679	195034	5864587
West Darfour	2282440	2183561	2460600	238162	7164763
Elgedarif	1062723	2172455	1075613	346941	4657732
Kassala	863463	2055884	1699734	699153	5318234
Red sea	138879	423894	730354	290395	1583522
Blue Nile	2062045	3974003	460345	14376	6510769
Sennar	1618238	1398849	1664323	118362	4799772
Elgezira	2539063	2516868	2177784	125071	7358787
White Nile	3577634	2596348	2598291	35940	8808213
Northern	256624	996150	1168567	49837	2471177
River Nile	102649	1049137	1226110	115966	2493863
Khartoum	247566	450387	655106	6709	1359768
Total	30191000	39846000	31029000	4792000	105858000

Source: MLFR 2014

**Prioritisation and Ranking Tool:**

A comparative table (spreadsheet) been designed in Microsoft excel program is used, which assigned to PHYLUM in collaboration with OIE and World Bank and European Union. The method aim to define the main characteristics of the disease, enabling its profile to be described in terms of three aspects and their impact on the control measures: (PHYLUM, 2015).

The general sequence of the method consists of two sequential steps in the analysis of a disease:

- i. A global characterisation of the disease, aimed at assessing inherent aspects of the disease, Independently of any particular local context.
- ii. A local approach, aiming at refining the study of the disease within the specific context of the country or region in question.

The objective of *global characterisation* of the disease is to find out the characteristics of a disease that will enable a description to be made of its profile and potential nuisance in terms of three main aspects:

- Epidemiology;
- Economic consequences;
- Human health issues.

The objective of *local approach* is to refine the previous analysis by considering the disease in the particular context of a given country or region. The impact of a disease is highly dependent on local geography, production and trade systems, socio-cultural background, etc. On this basis, it will be possible to prioritise the different diseases with regard to their respective level of concern at the local level. Local analysis sequence depending on:

- Presence of the disease
- Absence of the disease
- Unknown situation of the disease

## Results:

The results of prioritisation obtained based on: Economic, Human health, societal, Environmental impacts, Feasibility of Control Measures, Economic impact of Control Measures, and the Societal and Environmental Impact of control measures. The following findings demonstrate the results obtained:

### *Disease prioritisation based on Economic impact*

Based on the data fed into the comparative table and given the coefficient one in the economic impact criteria of the disease, the following diseases were selected and scored according to data processed by the tool: FMD 6.1, ND 5.9, PPR 5.1, *Brucellosis* 5.1, LSD 4.6, which then ranked according to their economic impacts into 1, 2, 3, 4, 5 and 6 respectively, Table 2.

### *Disease prioritisation based on Human Health impact*

Rabies the only disease been selected in which has human health impact and had scored of 5.8 (Table 3)

### *Disease prioritisation based on societal impact*

When the coefficient one been moved the social impact criteria the 10 diseases were prioritized and given the following scores and ranked accordingly: PPR 6.3, Rabies 5.5, CCPP 5.2, AHS 4.9, *Brucellosis* 4.8, ND 3.7, CBPP 3.6, LSD 2.4, FMD 1, Camel Pox 0.4, Table 4.

### *Disease prioritisation based on Environmental impact*

In the environmental impact criteria the following diseases were scored and ranked accordingly FMD 10, PPR 3.8, CBPP 3.8, CCPP 3.8, *Brucellosis* 2.5, LSD 2.5, Shoat pox 2.5, AHS 2.5, Camel Pox 2.5, ND 2.5, Rabies 1.3, Table 5.

### *Disease prioritisation based on Feasibility of Control Measures*

Nine disease were listed based on their feasibility of control measures and ranked accordingly CCPP 6.2, FMD 4, AHS 3.6, LSD 2.9, Camel Pox 2.9, ND 2.9, Shoat Pox 1.9,

*Brucellosis* 1.8, Table 6 demonstrate the results obtained.

### *Disease prioritisation based on Economic impact of Control Measures:*

FMD 7, CBPP 3, CCPP 3, LSD 3, PPR 2, Camel Pox 1, AHS 1, ND 1, *Brucellosis* 1, Rabies 0, Shoat Pox 0, refer to Table 7.

### *Disease prioritisation based on Societal and Environmental Impact of Control Measures:*

The list of disease prioritised based on their social and environmental impacts of the control measures were given the following scores and ranked accordingly: Rabies 7.14, AHS 7.14, *Brucellosis* 5.7, ND 5.7, FMD 2.9, CBPP 2.9, CCPP 2.9, LSD 2.9, Shoat Pox 1.4, PPR 0, Camel Pox 0, Table 8.

### *Final proposed prioritisation:*

When the disease prioritisation criteria combined with the control measures criteria giving them the same coefficient (1) the results obtained were two categories of disease: Absent disease (risk of introduction to the country) and present diseases these illustrated in Table (9) and Table (10):

### *Present diseases:*

Six diseases were prioritised as present in the country when diseases criteria combined with the control measures criteria giving all criteria coefficient one in the comparative table, the following diseases were scored and ranked according to the score obtained: FMD 4.4, PPR 2.5, Shoat pox 1.2, CBPP 2.5, *Brucellosis* 3.0 and Rabies 2.04, refer to Table 9. The obtained list of selected diseases is relevant to the current national prioritization list of diseases with the exception of Rabies. The justification for the scores ranking is for their moderately high feasibility, economic, social and environmental impact of control measures, the existing national control plans, and high demands of exports. FMD is always on top of the list of most of the criteria used.

**Table 2:** Disease prioritisation based on Economic impact

Rank	Score	Diseases	Justification
1	6.1	FMD	TADs are potential risks to livestock, being OIE Listed diseases which prevent livestock exports, make them very important to be controlled specially in Sudan, they have great impacts on food security, nutrition, and health besides the financial costs of control.
2	5.9	ND	
3	5.1	PPR	
4	5.1	<i>Brucellosis</i>	
5	4.6	LSD	

**Table 3:** Disease prioritisation based on Human Health impact

Rank	Score	Diseases	Justification
1	5.8	Rabies	Fatal, bite wounds can cause serious injury. Cost of control

**Table 4:** Disease prioritisation based on societal impact

Rank	Score	Diseases	Justification
1	6.3	PPR	Majority of SR owned by small producers who depend on them solely on their social life.
2	5.5	Rabies	direct medical costs fear of being bitten and infected again has left the bitten to be unable to work, causing prolonged income loss
3	5.2	CCPP	
4	4.9	AHS	Race horses and horse and donkey-carts
5	4.8	<i>Brucellosis</i>	Food-borne
6	3.7	ND	The majority of the people in the country depend on poultry meat and eggs
7	3.6	CBPP	Majority of livestock owned by pastoralists which use livestock as social welfare.
8	2.4	LSD	
9	1	FMD	
10	0.4	Camel pox	Social prestige and international trade

**Table 4:** Disease prioritisation based on societal impact

Rank	Score	Diseases	Justification
1	10	FMD	<ul style="list-style-type: none"> <li>Animal waste is a leading factor in the pollution of land and water resources.</li> <li>Negative impact of use of chemical and pesticides on untargeted organisms hence disrupting functioning of ecosystems</li> </ul>
2	3.8	PPR	
3	3.8	CBPP	
4	3.8	CCPP	
5	2.5	BRU	
6	2.5	LSD	
7	2.5	Shoat pox	
8	2.5	AHS	
9	2.5	Camel Pox	
10	2.5	ND	
11	1.3	Rabies	

**Table 6:** Disease prioritisation based on Feasibility of Control Measures

Rank	Score	Diseases	Justification
1	6.2	CCPP	<ul style="list-style-type: none"> <li>• Small Ruminants are key livelihood assets of small livestock producer;</li> <li>• Local and international trade</li> <li>• Food security issues (poultry meat and egg, beef, milk)</li> </ul>
2	4	FMD	
3	3.6	AHS	
4	3	LSD	
5	2.9	C. pox	
6	2.9	ND	
7	1.9	Shoat pox	
8	1.8	<i>Brucellosis</i>	

**Table 7:** Disease prioritisation based on Economic impact of Control Measures

Rank	Score	Diseases	Justification
1	7	FMD	<ul style="list-style-type: none"> <li>• High cost of control of FMD beyond the N.Vet. Authority call for regional and international initiatives,</li> <li>• High mutant nature of the virus/ vaccine matching</li> <li>• Local and international trade disruption (livestock markets)</li> <li>• Sheep and goats account for 37.64% and 29.31% respectively of the total livestock population and contribute significantly to the national economy</li> </ul>
2	3	CBPP	
3	3	CCPP	
4	3	LSD	
5	2	PPR	
6	1	Camel pox	
7	1	AHS	
8	1	ND	
9	1	<i>Brucellosis</i>	
10	0	Rabies	
11	0	Shoat pox	

**Table 8:** Disease prioritisation based on Societal and Environmental Impact of Control Measures:

Rank	Score	Diseases	Justification
1	7.14	Rabies	<ul style="list-style-type: none"> <li>• Shooting stray dog instantly in some areas may create security problem</li> <li>• Testing and slaughter without policy of compensation may not be accepted socially,</li> </ul>
2	7.14	AHS	
3	5.7	<i>Brucellosis</i>	
4	5.7	ND	
5	2.9	FMD	
6	2.9	CBPP	
7	2.9	CCPP	
8	2.9	LSD	
9	1.4	Shoat pox	
10	0	PPR	
11	0	Camel pox	

**Table 9:** Final proposed prioritisation

**9.1. Present diseases**

Disease	Rank	Justifications	Proposed Control strategy	Current Country control strategy
FMD	1	<ul style="list-style-type: none"> <li>The moderately high feasibility, The moderately high economic impact and social and environmental impact of control measures.</li> </ul>	Review the zoning strategy	Zoning, risk-based control
PPR	2		Review the zoning strategy to be based on epidemiological status of the disease and to align the strategy with the global one	Zoning based on production system, risk-based control
Shoat pox	3		Revise existing strategy	risk-based control based on production systems
CBPP	4	<ul style="list-style-type: none"> <li>Existing NCS aligned with OIE, PVS</li> </ul>	Review existing strategy based on the achievement progress	Zoning, risk-based control
<i>Brucellosis</i>	5	<ul style="list-style-type: none"> <li>High demands of SR exports</li> <li>FMD selection based on it is high impact on livestock exports</li> </ul>	Testing and slaughter (in the zoning of FMD) based on the PVS gab analysis	<ul style="list-style-type: none"> <li>risk-based control</li> </ul>
Rabies	6		<ul style="list-style-type: none"> <li>risk-based control</li> <li>euthanizing stray dogs</li> </ul>	<ul style="list-style-type: none"> <li>test and slaughtering</li> </ul>

**9.2. Exotic diseases**

Given the low scores of control measures and the economic, social, and environmental impact of the diseases the following exotic disease were selected and ranked based on the scores obtained:

Disease	Rank	Justifications	Proposed Control strategy	Current Country control strategy
FMD	1	High spread among naïve population	Risk-based control along the border with neighboring countries	<ul style="list-style-type: none"> <li>Zoning</li> <li>Risk-based</li> </ul>
RVF	2	Limited geographical areas	Strengthen the use of: <ul style="list-style-type: none"> <li>Sentinel herd</li> <li>Using the RVF-RBDF</li> </ul>	<ul style="list-style-type: none"> <li>Zoning</li> <li>Risk-based</li> <li>Sentinel herd</li> <li>Using the RVF-RBDF</li> </ul>
BT	3	Been vector-borne and un-availability of vaccine	Active surveillance Culling of infected animals Risk-based control	General control policy of livestock diseases
Ebola	4	Public health hazard	Risk-based surveillance jointly with Ministry of Health	Working in close collaboration with Ministry of Health
TB	5	Public health hazard		
HPAI	6	Public health hazard	Active surveillance, PDS	Active surveillance, PDS (migrating birds historical water sources/routes)

### Exotic diseases:

Six diseases were selected when combining all criteria, scored and ranked according to their risk of introduction into the country as exotic. Given their nature of high spreading and public health concern, the high scores of control measures and the economic, social, and environmental impacts ensure the importance of control measures should be implemented in time, they are namely: FMD 4.6, RVF 5.04, BT 4.67, Ebola 4.19, TB 5.7, & HPAI 7.0, refer to Table 10. The justification of their ranking is mainly due to their effects on local and international trade, public health concerns and presence of preparedness plan for HPAI.

## Discussion

TADs are potential risks to livestock, being OIE Listed diseases which prevent livestock exports, make them very important to be controlled specially in Sudan, exert broader impacts on food security, nutrition, and health besides the financial costs of control. Prioritisation exercise using the PHYLUM tool indicates that, Foot and Mouth Disease, *Brucellosis* and Pest des petites Ruminants are of economic importance in the country which coincided with the study results on the assessment of the relative importance of priority livestock diseases (Baumann, 2009), and (Tamador, 2004). The three diseases have control strategies and Action plans in place which aligned with OIE standards (2011) and PVS (2013) and the PCP-FMD roadmap and the global control strategies for PPR and FMD. The challenges are the zonal vaccination for FMD in line with the Action plan for the control of the disease (vaccine imported and operated on cost recovery bases), for *Brucellosis* control the non existence of a national compensation policy in the ministry to compensate the livestock owners when applying the testing and slaughter policy.

Rabies has been the only disease prioritised in the human health impact due to it is fatal nature and there is no routine vaccination against the disease. Instantly a risk-based control measure been applied in hot

spots concerning vaccination of at risk animals and shooting of stray dogs, with the exception of the areas where insecurity is present.

In prioritisation of the diseases based on their social impact, the small ruminants' diseases come on top of the priority diseases list specially: PPR and CCPP. The majority of small ruminants in the country owned by small farmers whose livelihoods depend solely on the incomes derived from the sale of live animals and their products to meet their daily expenses, in addition to the significant contribution in the exports earnings (National economy) from live animal. This necessitate the need for implementing strict control measures which clearly demonstrated on the list of the prioritisation of diseases based on economic impact of control measures. This result is coincided with the livestock earnings for 2009, 2010 (Customs Department, 2012).

A long list ( $n = 11$ ) had been established when diseases prioritised based on environmental impact having FMD, PPR, CBPP, *Brucellosis* been on top of the list, this mainly due to poor implementation of bio-security measured applied.

When the diseases prioritised based on feasibility and economic impact of control measures. Most of the diseases been found in the list namely: FMD, PPR, CBPP, *Brucellosis*, CCPP and NCD, which all impacted the control measures in a way or another due to high cost of FMD vaccine (imported) regular surveys for circulating new strains for vaccine matching, and their effects on the Local and international trade (small ruminants diseases mainly) and Food security issues (NCD) these results are relevant to the study on the assessment of the feasibility and economic impact on control measures (LESP, 2012) .

The final prioritisation of the present and absent diseases comes with 6 major diseases namely: the proposed list of the present diseases in the country FMD, PPR, CBPP, shoaft pox, *Brucellosis* and Rabies which have currently control strategies and Action plan for their control in place and aligned with OIE standards, PVS, 2013 and PCP-FMD, regional and global strategy of PPR and global strategy of FMD.

These results of prioritisation are very relevant to the current national priority list of diseases and in partial coincided with the current STSD project prioritisation, 2014 (FMD, CBPP, PPR, RVF). Concerning the exotic list of diseases, the HPAI has a contingency plan in place which needs to be updated; a plan for introduction of FMD new strains and Ebola should be formulated.

This prioritisation exercise is not far from the country prioritisation list of diseases concerning present and exotic list of diseases and will be useful baseline review for Sudan existing list of priority to be in line with the region.

### **Conclusion:**

The OIE PHYLUM tool is appreciated and should be adopted as a routine exercise in assessing some other diseases with national importance, and in updating diseases prioritisation list at national level.

Sudan is a large country with different geo-climatic zones and a large number of animals. A large part of the livestock is raised through nomadic systems with high number of movements. Has an open border with 7 countries in the region. A large part of the production is dedicated to the Middle East market through the export of live animals this make it very sensitive to the risk of ban in case of outbreaks. This necessitated the need to strengthen the bilateral agreements with neighboring counties to harmonise TADs control activities at borders. A need for call to a joint livestock epidemio-surveillance projects at regional and international levels will add value to the reduction of disease incidence and outbreak in the region.

The findings from the study/ prioritisation constitute baseline data and provide a good opportunity for further assessment and reviewing of diseases list to better understand the epidemiology of the diseases in the country and it is economic implications on livestock, livelihood and the national economy.

There was significant difference found

between geographical areas/ countries which may provide different interests for effective disease control, this need to strengthens the coordination and harmonise control activities among countries in the region.

### **Conflict of interest**

There is no conflict of interest identified.

### **Ethical standards**

The study involved review of secondary data and reports generated during routine animal and human disease surveillance. There was no direct contact with human and animal subjects. Because this was part of the routine surveillance activities which are exempted from Institution Review Boards (IRBs) and National Council of Science and Technology (NCST) permission to conduct the study was not sought. The study has many benefits to the human population and to the animal by guiding policy and resource allocation for prevention and control of the diseases.

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## PREVALENCE OF BOVINE TRYPANOSOMOSIS IN AND AROUND BAHIR DAR, ETHIOPIA

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### Abstract

*Trypanosomosis* is a parasitic disease that causes serious economic losses in livestock, in sub-Saharan African countries. Cross sectional study was conducted from January 2012 to September 2012 on a total of 384 cattle to determine the prevalence of bovine *trypanosomosis* and to assess the risk factors associated with the disease. Blood samples were collected from cattle's of both sexes in different age group and body condition from their ear vein using simple random sampling techniques and thin smear was used as a diagnostic technique. An overall prevalence of *trypanosomosis* infection in the study area was 5.73% and the most frequently identified *Trypanosoma* species were *T.congolense* (4.4%) followed by *T.vivax* (1.3%). This study revealed that there was no statically significant difference between male (5.41%) and female (6.03%) and statistically significance ( $\chi^2=11.43$ ,  $p=0.002$ ) difference was recorded among age groups. The prevalence of *trypanosomosis* infection among the three body condition category (poor, medium, good) also statically significant ( $\chi^2=89.34$ ,  $P=0.04$ ). Statistically significant difference ( $\chi^2=36.23$ ,  $P=0.001$ ) was observed when overall prevalence of trypanosome infection was compared with different altitude. A mean PCV of 20.16% and 26.04 % was found for infected animals and non-infected animals respectively. The difference was statistically significant ( $P = 0.000$ ). *Trypanosoma congolense* caused statistically significant reduction in PCV ( $P = 0.0000$ ) as compared to *Trypanosoma vivax* infection. In conclusion, *trypanosomosis* was found to be important disease in the study area. Therefore, proper intervention strategies should be put in place and implemented to minimize the burden of the disease.

**Key words:** Bahir Dar, Bovine, PCV, Prevalence, *Trypanosomosis*, Thin smear,

## PRÉVALENCE DE LA TRYPANOSOMOSE BOVINE DANS ET AUTOUR DE BAHIR DAR EN ÉTHIOPIE

### Resume

La trypanosomose est une maladie parasitaire qui est à l'origine de graves pertes économiques dans le secteur élevage, dans les pays d'Afrique subsaharienne. Une étude transversale a été menée de janvier 2012 à septembre 2012 sur un total de 384 bovins dans le but de déterminer la prévalence de la trypanosomose bovine et d'évaluer les facteurs de risque associés à la maladie. Des échantillons de sang ont été prélevés sur des bovins des deux sexes de différents groupes d'âge et différentes conditions corporelles à partir de leur veine auriculaire en utilisant des techniques d'échantillonnage aléatoire simple, et un frottis mince a été utilisé comme technique diagnostique. La prévalence globale de l'infection trypanosomienne était de 5,73%. Les espèces de *Trypanosoma* les plus fréquemment identifiées étaient *T.congolense* (4,4%) suivie de *T.vivax* (1,3%). Cette étude a révélé qu'il n'y avait pas de différence statistiquement significative entre mâles (5,41%) et femelles (6,03%) ni de différence statistiquement significative ( $\chi^2 = 11,43$ ,  $p = 0,002$ ) entre les groupes d'âge. La prévalence de l'infection trypanosomienne parmi les trois catégories d'affections (pauvres, moyennes, bonnes) est également statistiquement significative ( $\chi^2 = 89,34$ ,  $p = 0,04$ ). Une différence statistiquement significative ( $\chi^2 = 36,23$ ,  $P = 0,001$ ) a été observée lorsque la prévalence globale de l'infection trypanosomienne a été comparée à une altitude différente. Un taux d'hématocrite moyen de 20,16% et de 26,04 % a été trouvé respectivement pour les animaux infectés et non infectés. La différence était statistiquement significative ( $P = 0,000$ ). *Trypanosoma congolense* a provoqué une réduction statistiquement significative de l'hématocrite ( $P = 0,0000$ ) par rapport à l'infection à *Trypanosoma vivax*. En conclusion, la trypanosomose s'est révélé une maladie importante dans la zone d'étude. Par conséquent,

des stratégies d'intervention appropriées doivent être mises en place et exécutées pour minimiser la charge de la maladie.

**Mots-clés :** Bahir Dar, bovin, hématocrite, prévalence, trypanosomose, frottis mince

## Introduction

*Trypanosomosis* is serious disease in domestic livestock that causes significant negative impact in food production, economic growth in sub Saharan Africa and has profound effect on rural development over vast areas. Ethiopia is one of the counties with the large number of livestock population in Africa which is estimated to be 35.5million (FAO, 2003). In Ethiopia this sector contributes 12% of total GDP and over 30% of agricultural GDP (MOA, 1998). However the animal product is due to a number of factors such as poor genetic potential, high disease prevalence, malnutrition and poor management (Radostitis, 2000). Tsetse transmitted trypanosomes affect 37% of sub Saharan countries out of the estimated 160 million head of cattle and 260 million of sheep & goat are at risk of *trypanosomosis* over 10 million km<sup>2</sup> of land.

In Africa the most important species of *trypanosomosis* in terms of economic loss in domestic livestock includes *T.congolense*, *T. vivax*, *T.brucie* and closely related species of *T.rhodensie* and *T.gambiense* which cause human sleeping sickness. The disease also found outside the tsetse belt area transmitted mechanically by flies of genus *tabana*, stomoxy another biting flies which cause the spread of *T.evansi* and *T.vivax* outside tsetse infested area (Abebe and Jobre, 1996). In Ethiopia it has been estimated that 120000km<sup>2</sup>of land infested by tsetse and associated disease *trypanosomosis* and in Ethiopia 14.8 million cattle, 6122 million sheep and goat, 1 million of camels are at risk of contracting *trypanosomosis* (MOA, 1995). In Ethiopia *trypanosomosis* is widely spread in domestic livestock southwest and northwest low land. The risk of *trypanosomosis* in southwest and northwest region of Ethiopia in an indication of sever problem of drug resistance. Economic importance *trypanosomosis* and existing drug resistance problem currently

affecting the livestock, productivity as a result of mortality, morbidity and infertility, when the impact on crop productivity due to reduced animal draught power and manure is considered the true economic loses could be much higher (Budd, 1999). The prevalence of bovine *trypanosomosis* in different study area of Ethiopia shows different result in Quara 6.5% by (Tenagne, 2006), 11.7% Jabitehena by (Molalegn *et al.*, 2010), 23.7% Dermallo by (Kalkidan 2009), 21% Arbaminch by (kenenet 2003) 19.05% Gore by (Abay, 2002), 32% Gamogofa by (Takele *et al.*, 2006), and 35.5 North Omo by (Haile, 1996). However there was no quantified and documented research output in the study area as a result it is important to quantify the extent of the problem for the implementation of appropriate intervention measures. Therefore, the objectives of this study were:

- To determine the prevalence of bovine *trypanosomosis* and associated risk factors
- To identify *Trypanosomoses* species involved in the area

## Materials and Methods

### Study area

The study was conducted in and around Bahir Dar from January 2012 to September 2012. Bahir Dar is located 578 k.m north west of the capital Addis Ababa having an elevation of 1840 m.a.s.l , mean annual rain fall of 1170-1600 mm and mean annual average maximum and minimum temperature is 29.36oc and 9.24oc) respectively, most area is part of mid low land, however along the Blue Nile river valleys lies high land. The live stock population in and around Bahir Dar was 7839 cattle, 2152 equine, 23463 sheep and goat and 62012 poultry which are owned by the farmers and lesser extent by the small holders. The major management system is extensive type in farmers and semi intensive in small holders (CSA, 2007).

### Study animals

This study was carried out on a total of 384 heads of naturally grazing local cattle in four kebeles. The number of animals sampled from each kebele was purposively based on the animal population of the study area and information obtained from both sex, animals comes both from lowlands and midlands, in age groups of <1 year, 1-3 year and > 3 year as described by (Kalkidan, 2009), body condition of poor, medium & good were included in this study (Nicholson and Butterworth, 1986) and PCV 24%-42% as normal from selected animals was recorded (Coles, 1986).

### Study design and Sampling

Cross sectional study was conducted from January 2012 to September 2012 on a total of 384 bovines to study the prevalence of bovine *trypanosomosis*. Sampling method employed was simple random sampling techniques. The desired sample for estimation of the disease occurrence was calculated and decided in the study area 50% expected prevalence was considered in the sample size determination. And also the other determinants considered in the sample size determination are 95% confidence interval and 5% desired absolute precision (Thrusfield, 2005). Therefore

$$N = \frac{(Zx)^2 Pexp (1-Pexp)}{D^2}$$

Where N=required sample size

Zx<sup>2</sup> =the required Value of CI (1.96)

Pexp =the expected prevalence rate (50%)

D<sup>2</sup>= Desired absolute level of precision (5%)

Hence the sample size required as per the above formula was 384 heads of cattle were sampled.

### Sample Collection, Packed Cell Volume Determination and Parasite Detection

Blood samples were collected by puncturing of the marginal ear vein of each animal with a lancet and drawn directly in to heparinized capillary tube. The packed cell volume (PCV) was measured after the

heparinized capillary tubes containing blood were centrifuged for 5 min at 12,000 rpm in microhematocrit centrifuge. The capillary tube was then cut, the buffy coat and upper most layers of the red blood cells discharged onto a microscopic slide, covered with cover slip, and examined under microscope for the presence of motile Trypanosomes (Paris *et al.*, 1982; MORAD, 2005). Thin smear were made and stained with Giemsa staining technique from buffy coat positive samples for identification of Trypanosoma species.

### Data Management and Analysis

Data on individual animals and parasitological examination result was entered into MS-Excel spread sheets program. The prevalence of *trypanosomosis* and PCV, with corresponding 95% confidence intervals, was determined for different categories of study animals. The trypanosome infection rates with different variables like altitude, age, sex and body condition score were compared by chi-square test. The mean PCV of infected and non-infected animals were compared using student t-test. SPSS version 17 software was used to conduct the statistical analyses

## Results

### Overall Prevalence of Trypanosomosis

From the total of 384 cattle examined with a Buffy coat technique, 22 were positive for trypanosomes giving an overall prevalence of 5.73%. *T.conglonse* and *T.vivax* was the only trypanosome species identified by Giemsa stained thin blood smear examination in the study area with a prevalence rate of 4.4% and 1.3%, respectively.

### Prevalence of Trypanosomosis in Sex and Age Category

In the present study the effect of sex on the occurrence of *trypanosomosis* was analyzed. Accordingly the rate was found to be 5.41% in male and 6.03% in female. There was no statistically significance difference detected in male and female for the overall occurrence of *trypanosomosis* in the study area ( $\chi^2=0.618$ ,

$P > 0.05$ ). Two species of Trypanosomoses were identified; the prevalence of *T. congolense* in male was 3.8% and in female 5.0% and *T. vivax* in male 1.6% and in female 1.0%. There was no statistically significant difference between male and female for the two identified species of *Trypanosoma* (Table 1).

Attempts were also made to assess the effect of age category on the occurrence of *trypanosomosis* infection. Consequently, based on age group the highest prevalence of *trypanosomosis* was observed in adult (9.86%) followed by young (3.51%) and calf (1.031%). The statistical analysis result showed that there was a statistically significant difference in trypanosome infection rate among the three age categories ( $\chi^2 = 11.430$ ,  $p < 0.05$ ) as indicated in (Table 1).

#### Prevalence of Bovine Trypanosomosis among Body Condition Score

In the present study statistically significant association ( $P = 0.004$ ) was observed between trypanosome infection and body condition score. The highest prevalence of *trypanosomosis* was recorded in poor body condition animals and followed by medium. (Table 2).

Statistically significant difference ( $P = 0.001$ ) was observed when overall prevalence of trypanosome infection was compared with different altitude (Table 3).

#### Hematological Findings

The PCV of individual animals was measured for the assessment of degree of anemia. A mean PCV of 20.16% and 26.04% was found for infected animals and non-infected animals respectively. The difference was statistically significant ( $P = 0.000$ ) (Table 4).

**Table 1:** Prevalence of bovine *trypanosomosis* sex and age categories.

Risk factors	Total No of examined animals	No (%) of positive for Total			$\chi^2$ (P- value)
		<i>T. congolense</i>	<i>T. vivax</i>	Total	
<b>Sex category</b>					
Male	185	7(3.8%)	3(1.6%)	5.41%	$\chi^2 = 0.618, P = 0.15$
Female	199	10(5.0%)	2(1.0%)	6.03%	
Total	384	17(4.4%)	5(1.3%)	5.73%	
<b>Age category</b>					
Calf	97	0(0%)	1(1.0%)	1(1.031%)	$\chi^2 = 11.43, p = 0.002$
Young	114	3(2.6%)	1(0.9%)	4(3.51%)	
Adult	173	14(8.1%)	3(1.7%)	17(9.86%)	
Total	384	17(4.4%)	5(1.3%)	22(5.73%)	

**Table 2:** Prevalence of bovine *trypanosomosis* among body condition

Total No of examined animals		No (%) of positive			$\chi^2$ (P- value)
		<i>T. congolense</i>	<i>T. vivax</i>	Total	
Poor	176	13(7.4%)	4(2.3%)	17(9.66%)	$\chi^2 = 89.34, P = 0.04$
Medium	137	3(2.2%)	1(0.7%)	4(2.92%)	
Good	71	1(1.4%)	0(0%)	1(1.41%)	
Total	384	17(4.4%)	5(1.3%)	22(5.73%)	

**Table 3:** The prevalence of *Trypanosoma* species at different altitudes in the study area

Altitude	No of animals examined	Species of <i>Trypanosoma</i>		Total prevalence	P-Value
		<i>T. vivax</i>	<i>T. congolense</i>		
Midland	113	2(1.8%)	6(5.3%)	8(7.07%)	$\chi^2=36.23, P=0.001$
Lowland	271	3(1.1%)	11(4.06%)	14(5.17%)	
Total	384	5(1.3%)	17(4.43%)	22(5.73%)	

**Table 4:** Mean PCV of infected and non – infected animals and Comparison of mean PCV of infected cattle within different species of *Trypanosoma*.

Status of infection	Number of animals	Mean PCV (%)	95% CI (%)	P-value
Infected	22	20.16	21.00-22.23	0.000
Non- infected	362	26.04	26.68-27.65	
<b>Species of <i>Trypanosoma</i></b>				
<i>T. vivax</i>	5	21.25	21.23-23.26	0.000
<i>T. congolense</i>	17	16.86	15.60-18.06	

Both *Trypanosoma vivax* and *Trypanosoma congolense* infections caused reduction of PCV in infected animals. *Trypanosoma congolense* caused statistically significant reduction in PCV ( $P = 0.0000$ ) as compared to *Trypanosoma vivax* infection (Table 4).

### Discussion

The result of the study which was conducted from January 2012 to September 2012 in and around Bahir- Dar, North west of Ethiopia indicate that *trypanosomosis* is one of the major disease of cattle. Of all examined cattle a total of 22 were found positive for *T.congolense* and *T.vivax* species of *trypanosomosis* showing an overall prevalence of 5.73% almost similar to earlier reports 5.66 % in Dembiya by Nigusie, (2011), 6.5 in Quara by Tenagne, (2006). However, high prevalence were reported as compared to studies conducted in other parts of the country 11.7% from west Gojjam in Jabitehinan by Molalegn *et al.*, (2010) 23.7% from Dermallo by Kalkidan, (2009 ) 21% from Arbaminch by Kenenet, (2003) and 35.5% from North Omo by Hile, (1996). The low prevalence of the present work as compared to the other may be due to frequent usage of trypanocidal drugs by the farmer to treat sick animals in the area and it may be due to the

agro ecology zone of the area.

The present study showed that *T.congolense* and *T.vivax* were the predominant species of *trypanosomosis* in the study area. The result showed that from the total trypanosome positive animals (4.43%) was found to be infected with *T.congolense* and (1.3%) is due to *T.vivax*. The result agrees with Nigusie, (2011). This could be the fact that *T.congolense* is the only found and multiply in the blood vessels of the infected animals. This increase the chance of *T. Congolese* to be highly transmissible and to be found in the blood sample (Husameldin, 2001). The other fact may due to drug resistance problems in case of *T.congolense* is higher than the rest of trypanosome species due to high number of variable glycoprotein found in *T.congolense* Kalkidan, (2009). It can be also that animals were travel to tsetse challenge area for grazing and return from that area for the recent time. The prevalence is highest in the lowland as compared to the midland.

This work revealed that distinct difference in the prevalence of bovine *trypanosomosis* between sex, age and body condition. The prevalence of *trypanosomosis* infection of 5.41% in male and 6.03% in female showed that insignificant ( $p>0.05$ ) variation that sex did not justify a relationship with occurrence of infection. This is due to the fact

that cattle are driven to pasture and watering regardless of sex and allowed in the same ecology having comparable degree to acquire infection, similarly with Dagnachew *et al.*, (2005) who demonstrated about 18.45% for male and 13.87% for female sexes without significant variation. Among the age groups categorized the highest prevalence was recorded in adult cattle 9.86% followed by young 3.51% and calf with insignificant degree of variation. There were previous reports showing higher prevalence in adult animals as compared to young animals which is believed to be due to high preference of tsetse for adult animals and less exposure of young animals to tsetse challenge as they are usually kept at homestead (Torr *et al.*, 2001; Cherenet *et al.*, 2006)

Of the 384 animals' sampled 9.66% and 2.92% prevalence of bovine *trypanosomosis* was recorded in poor and medium age groups of animals, respectively but there was low positive (1.41%) sample in those animals having good body condition. Therefore, poor animals were more infected because they have poor body condition, unable to protect tsetse fly perfectly and they have weak body resistance, anemia. And also medium body condition animals were less infected as compared to poor body condition of animals because medium body condition animals are resistant than poor body condition animals. Good body condition animals also infected slightly similar to medium body condition animals because good body condition animals were travel long distance and the place were low land area tsetse fly was more infested in this area. specially *T.congolense* is multiplied in blood vessels. and *T.vivax* was transmitted with mechanically the farmer were reared as pastoralist system

A significant reduction in PCV was observed in the trypanosome infected animals signifying anemia to be one of the important consequence of infection. This seems an established fact that it was similarly reported by many studies both at individual animal and herd level (Van den Bossche and Rowlands, 2001; Dagnachew *et al.*, 2005; Cherenet *et al.*, 2006, Alekaw Sinshaw *et al.*, 2006; Solomon Mekuria and Fitta Gadissa, 2011; Mollalign Bitew *et al.*,

2011 ). In the present study it was also noted animals which were infected with *T. congolense* have much lower PCV values than *T. vivax* suggesting the higher impact of *T. congolense* in terms of blood pathology as compared to *T.vivax*.

In conclusion bovine *trypanosomosis* caused by *T. congolense* and *T. vivax* with more prevalence of *T. congolense* was found to be an important disease of cattle in the study area. The sole presence of *T. congolense* in the lowland area indicated the importance of tsetse transmitted trypanosome in the study area. The observed association between reduction in PCV and body condition with infection showed the impact of the disease on productivity of infected animals.

## Conculsion and Recommendations

The result obtained from this study shows that *trypanosomosis* is important disease that brings about great economic losses to livestock. Totally the disease affects each house hold and the area of socioeconomic importance of disease appears a single most important constraint to improve productivity in the area which is commonly known as free of tsetse infection .but its altitude may allow for the presence of tsetse fly especially around Blue Nile river valley. The prevalence of *trypanosomosis* in infected male animal was less than female animal. In general female animal was slightly higher than in male animal infected and also in age calf was less infected and young animal was slightly less than infected than adult animal and also in body condition poor body condition of animal was highly infected and medium body condition was less infected and also good body condition was infected slightly. The major species of *trypanosomosis* in the study area were by *T.vivax* and *T.congolense* which is highly infected than *T.vivax* because of *trypanosomosis* caused by *T.congolense* to be transmitted mechanically by biting flies. Biting flies is best suited to the climatic conditions prevalent during heavy rainy season. This consequently leads to a high density of biting flies after the end of the rainy season.

Based on the above conclusion the following recommendations are for warded:-

- Modern veterinary service and resource should be supplied for the areas where animals are migrate to get free grazing pasture
- Appropriate chemotherapy should be done to reduce the prevalence of the disease in the study area
- The farmer in the study area should be made aware how to control the vector, to avoid contact with flies (vectors).
- Detail research should be conducted covering wide area to study the prevalence of *trypanosomosis* and the seasonal pattern of disease.

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## TRANSBOUNDARY ANIMAL DISEASES AND ZOOSES: PRIORITIZATION AND INTERVENTIONS IN TANZANIA

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### Abstract

The purpose of this study was to categorise and prioritise livestock diseases in Tanzania so as to enable developing action oriented strategic plans for prevention and control interventions in a more economic, effective and efficient use of limited resources available. This study adopted a Phylum disease prioritisation tool developed by OIE. A structured analytical tool was administered to a list of 15 identified diseases based on epidemiological reports found in the Director of Veterinary Services office in the country. The later was followed by putting scores as provided in the tool and the result of which was the prioritised list of diseases which were ranked accordingly in terms of their economic, public health, and control impacts. The results of this study indicate that seven out of the 15 diseases identified are of economic and public health importance and these includes in order of priority Rift Valley Fever; Foot and Mouth Disease (circulating serotypes); Contagious Bovine Pleuropneumonia, Newcastle Disease; *Pest des Petit Ruminants*, Highly Pathogenic Avian Influenza (Absent) and Foot and Mouth Disease exotic (serotypes). The Tanzanian Government needs to make use of prioritised list of TADs and zoonoses by paying attention to financial resource allocation and providing political support for the implementation of the disease prevention and control plans.

Since all the ranked diseases have huge economic and public health impacts and therefore, their prevention and or containment calls for cordial cooperation of various actors including Eastern Africa region member states, international organisations (OIE, FAO, WHO, AU-IBAR) for harmonised TADs and zoonoses control strategies.

**Key words:** Tanzania, Transboundary animal diseases, zoonoses Prioritization, Interventions

## MALADIES ANIMALES TRANSFRONTALIÈRES ET ZOOSES : PRIORISATION ET INTERVENTIONS EN TANZANIE

### Résumé

Le but de cette étude était de procéder à la catégorisation et à la priorisation des maladies animales en Tanzanie afin d'élaborer des plans stratégiques orientés vers l'action pour des interventions de prévention et de contrôle, et utiliser de manière plus économique, efficace et efficiente les maigres ressources disponibles. Cette étude a adopté l'outil Phylum de priorisation des maladies développé par l'OIE. Un outil analytique structuré a été administré à une liste de 15 maladies identifiées sur la base de rapports épidémiologiques trouvés dans le bureau du Directeur des Services vétérinaires du pays. Cet outil a été suivi par l'attribution de scores tels que fournis dans l'outil et dont le résultat était la liste des maladies classées par ordre de priorité en fonction de leurs impacts sur l'économie, la santé publique et le contrôle. Les résultats de cette étude indiquent que sept des 15 maladies identifiées sont d'importance économique et publique et elles comprennent, par ordre de priorité, la fièvre de la vallée du Rift, la fièvre aphteuse (sérotypes circulants), la pleuropneumonie contagieuse bovine, la maladie de Newcastle, la peste des petits ruminants, l'influenza aviaire hautement pathogène (IAHP), la fièvre aphteuse (sérotypes exotiques). Le

gouvernement tanzanien doit utiliser la liste prioritaire des maladies animales transfrontalières et des zoonoses en accordant une attention particulière à l'allocation des ressources financières et en apportant un soutien politique à la mise en œuvre de plans de prévention et de contrôle des maladies. Puisque toutes les maladies classées ont d'énormes impacts sur l'économie et la santé publique, leur prévention et / ou leur confinement exigent une coopération cordiale entre les différents acteurs, notamment les États membres de l'Afrique de l'Est, les organisations internationales (OIE, FAO, OMS, UA-BIRA) pour une harmonisation des stratégies de contrôle des MAT et des zoonoses.

**Mots-clés :** Tanzanie, maladies animales transfrontalières, zoonoses, priorisation, interventions

## Introduction

Tanzania is a member state to East African Community with 94 million hectares (ha) of land resource out of which, 24 million ha are used for grazing of different domesticated ruminants.

The large livestock resource base with estimated livestock population of about 25 million cattle, 16.7 million goat, 8 million sheep, 2.4 million pigs, 36 million indigenous chickens, 23 million improved chickens and about 90% of the total population is indigenous flocks (Livestock Sample Survey Census, 2012) all contributes to individual and National economy.

Livestock production is one of the major agricultural activities in the country that is contributing towards achieving development goals of the National Growth and Reduction of Poverty (NSGRP). The livestock sector provides livelihood support to a total of 1,745,776 (37%) households out of 4,901,837 agricultural households (Livestock Sample Survey Census, 2012) and is one of the major economic activities in rural areas.

The livestock industry contribution to the Agricultural Gross Domestic Product is about 13%, and contributes 3.8% of the National Gross Domestic Product (NGDP). The existing livestock potential would have contributed substantially to the NGDP if some of the constraints would have been addressed. But the low contribution is attributed to low livestock growth rates, inadequate water and pasture availability, conflicts between crop producers and livestock keepers, high mortality rates due to diseases, low reproductive rates and poor quality of the final products from the industry.

Among these bottlenecks, the

livestock diseases both trans-boundary and zoonoses appear to be the most striking challenge towards development of the sector (Tanzania Livestock Modernization Initiative, 2015). The trans-boundary diseases greatly affect negatively the livestock industry both in terms of production performance and sector growth. On the other hand, zoonotic diseases are affecting both livestock and humans such that these if neglected will cause serious health, societal, trade and economical negative impacts.

The country is faced by a challenge of having little resources allocation to control these diseases accompanied with lack of strategic approach such that there has been an haphazard disease fighting without proper prioritized approach. Lack of well streamlined diseases control protocol has resulted into diseases recurrence with associated low production and productivity.

In order to guide future investment in disease control in the country, it's necessary to clearly identify and categorize priority diseases and develop specific strategies and policies which will enable to mobilise sufficient resources including political will for the disease control and progressive eradication. By doing so, the little resources available will be directed to major issues of the livestock sector such as prevention and control of trans-boundary animal diseases. This is in line with the National Livestock policy (2006).

Therefore the aim of this study was to prioritize diseases present in the country and those which can be introduced from other countries using Phylum tool.

## Material and Methods

### Location

The study area for the disease prioritisation was the whole country i.e. Tanzania Mainland.(Fig.1)



**Figure 1:** Map of Tanzania Mainland (source, Google 2016)

### Data collection

The disease status in the country was obtained by collecting data (secondary data) from the Ministry of Agriculture, Livestock and Fisheries –Department of Veterinary Services, Ministry of Health and Social welfare –Department of Public Health, Tanzania Meteorological Authority, Tanzania Bureau of Statics and National Institute of Medical Research.

### Methodology

Several organisations have proposed prioritization methods that consider different categories of criteria. Previous studies focused on a specific aspect of infectious diseases, such as the multi criteria analysis designed by Mourits *et al.*, (2010) to support discussions on control measures. Conversely, the method developed by the French Agency for Food, Environmental and Occupational Health and Safety included major aspects of a disease ANSES, (2010) and also considered two rankings, one for animal health and second for human health. Other tools which were also developed by Krause *et al.*, (2008) which applied a Delphi method for collecting opinions, on zoonotic and other common diseases while Rist (2014) developed One Health Zoonotic Disease Prioritisation tool which basically operate with different criteria on animal diseases. Carib

Vet Animal Health Networking by EPI Group (2011) developed another tool in Caribbean countries with the aim of assessing the relative importance of given diseases in a territory based on their score.

Tanzania adopted a Phylum disease prioritisation tool developed by OIE. This tool enabled the country team to identify and make a list of disease priorities as well as defining priority actions at different levels based on the disease impact criteria on its epidemiology, local economic impact, human health impact, societal impact, environmental impact, economic, societal and environmental impact of control measures, risk of the disease to be introduced in the country, characteristics of the country, local economic impact, local and international trade impacts.

This tool was effectively used throughout by first listing all diseases existing in the country, those which have possible predilections to occur in the country according to the climatic changes, human and livestock movement and associated animal products. For each disease there were considerations on its intrinsic parameters that included consideration of affected species, epidemiology in humans, persistence and its transmission.

The numerical scores as given in the tool for each criterion and later the total scores for each parameter were used to determine which disease ranked higher and which one ranked lower in that order hence getting a list of disease prioritisation for the country for the purpose of identifying the higher ranking diseases for effective, economic and efficient use of little resources available in collaboration with other Eastern African region.

### Data Analysis

Following the prioritisation exercise, the findings were analysed using the scores for each disease under each criterion as explained above. The numerical scores were assigned to each criterion depending on the realistic impact for each criterion in question. The results obtained after data analysis and stakeholders consultation are summarised in Tables 1, 2 and 3.





**Table 3:** Ranking of Prioritised diseases of Tanzania according to scoring criteria and strategy for prevention and control.

<b>Disease</b>	<b>Position</b>	<b>Reason</b>	<b>Strategy for the control</b>
RVF	1	<ul style="list-style-type: none"> <li>• has high effect on livestock production;</li> <li>• hinders live animals and their products for both local &amp; international trade;</li> <li>• being a zoonotic disease it has a bearing on the cost of controlling;</li> <li>• it has negative spillover effects on other sectors such as tourism and trade; and</li> <li>• the public shy from meat consumption and change eating behavior.</li> </ul>	<ul style="list-style-type: none"> <li>• Government to collaborate with livestock farmers and other development partners to provide and improve the vaccine supply that will facilitate intensified vaccination regime in order to restrict the spread of the disease.</li> <li>• To conduct active surveillance of the disease in animals and associated vector to those areas likely to have an outbreak before the occurrence of the disease.</li> <li>• Institute active communication with meteorological department in order to have early prediction and warning.</li> <li>• Continue to be vigilant with enforcement of the existing legislation</li> <li>• Comply with OIE sanitary prophylaxis measures such as control of animal movements (extension of disease), controls at slaughterhouses (exposure to disease) and draining of stagnant water to eliminate or reduce vectors.</li> </ul>
FMD (circulating)	2	<ul style="list-style-type: none"> <li>• hinders live animals and their products for both local &amp; international trade;</li> <li>• the vaccine is too expensive with limited accessibility and duration of immunity is short therefore imposing difficulties in carrying out mass vaccination campaign;</li> <li>• existence of the four serotypes in different parts of the country does not facilitate effective &amp; reliable vaccination campaign; and</li> <li>• control methods for the disease is difficult because of the mode of livestock production systems existing in the country.</li> </ul>	<ul style="list-style-type: none"> <li>• intensify epidemiological surveillance system in order to map the pattern of the disease; and</li> <li>• continue using strategic vaccination of animals in frequently affected areas in order to protect clean herds.</li> </ul>

<b>Disease</b>	<b>Position</b>	<b>Reason</b>	<b>Strategy for the control</b>
CBPP	3	<ul style="list-style-type: none"> <li>• has high effect on livestock production;</li> <li>• hinders live animals and their products for both local &amp; international trade; and</li> <li>• difficulties in controlling the disease attributed by inadequate supply of vaccine and movement control of the livestock.</li> </ul>	<ul style="list-style-type: none"> <li>• comply with OIE sanitary prophylaxis measures such as control of animal movements (extension of disease), controls at slaughter houses (using movement permits and animal identification);</li> <li>• engage more stakeholders in order to improve the vaccine supply and vaccination coverage that will meet the recommended standards;</li> <li>• implementation of contingency plan for the disease in order to reduce spread of the disease.</li> </ul>
ND	4	<ul style="list-style-type: none"> <li>• chicken production accounts for a large proportion of people living in rural areas and therefore has significant role to food security and household income;</li> <li>• has serious effects on commercial farms in terms of control measures (total culling, disinfection &amp; bio security);</li> <li>• in the event of occurrence it has high mortality; and</li> <li>• causative agents in free range system have capacity of persisting in the environment for prolonged period assisting in becoming source of new infection.</li> </ul>	<ul style="list-style-type: none"> <li>• take systematic and regular vaccination regime in both commercial and local chickens setting because both live and attenuated vaccines can markedly reduce the losses in poultry flocks since all types of vaccine are incapable of preventing virus circulation.</li> <li>• government to work in collaboration with commercial farmers to facilitate vaccination of local chickens around the farms and hatchery in order to create buffer for the disease; and</li> <li>• strengthen enforcement of the existing laws particularly movement controls, destruction of all infected and exposed birds and thorough cleaning and disinfection of the premises.</li> </ul>
PPR	5	<ul style="list-style-type: none"> <li>• has high effects on small ruminants production;</li> <li>• hinders live animals and their products for both local &amp; international trade;</li> <li>• production of small ruminants accounts for a large proportion of people living in rural areas and therefore has significant role to food security and household income;</li> </ul>	<ul style="list-style-type: none"> <li>• government to closely collaborate with other stakeholders including farmers and private sectors to facilitate improvement on vaccine supply;</li> <li>• undertake strategic mass vaccination campaign in affected areas in order to restrict spread of the disease in clean herds;</li> </ul>

Disease	Position	Reason	Strategy for the control
HPAI (Absent)	6	<ul style="list-style-type: none"> <li>• limited accessibility to vaccine which affects vaccination coverage; and</li> <li>• It is a new disease in the country and has geographical foci.</li> <li>• has high effect on production;</li> <li>• hinders live animals and their products for both local &amp; international trade;</li> <li>• being a zoonotic disease it has a bearing on the cost of controlling;</li> <li>• it has spillover effects on other sectors such as tourism;</li> <li>• it has an effect on control strategy because it has a stumping out procedure.</li> </ul>	<ul style="list-style-type: none"> <li>• intensify epidemiological surveillance system in order to map the pattern of the disease and take appropriate measure accordingly; and</li> <li>• strengthen disease advocacy in livestock community.</li> <li>• strengthening of surveillance and laboratory capacity;</li> <li>• strengthen capacity of epidemiological investigation and control management;</li> <li>• increase institutional capacity to control the disease;</li> <li>• build capacity for human influenza case management;</li> <li>• enhance research and development regarding to the disease;</li> <li>• improve public awareness and education;</li> <li>• continued control of imported chick to be brought in the country;</li> <li>• prepare and capacitate a National Preparedness Plan for HPAI</li> </ul>
FMD <sub>e</sub> (Absent)	7	<ul style="list-style-type: none"> <li>• hinders live animals and their products for both local &amp; international trade;</li> <li>• the vaccine is too expensive with limited accessibility and duration of immunity is short therefore imposing difficulties in carrying out mass vaccination campaign;</li> <li>• Control methods for the disease is difficult because of the mode of livestock production systems existing in the country.</li> </ul>	<ul style="list-style-type: none"> <li>• intensify epidemiological surveillance system in order to map the pattern of the disease;</li> <li>• institute a comprehensive control strategic plan especially at border posts in order to prevent introduction of new strain (exotic);</li> <li>• strengthen disease reporting system from the grass root to national level &amp; prompt response mechanism;</li> <li>• Put in place contingency plan for FMD (exotic serotypes);</li> <li>• to ensure availability of a vaccine with the new serotype ready at hand in case of presence of the disease; and</li> <li>• prepare and capacitate a National Preparedness Plan for exotic FMD.</li> </ul>

On the other hand, diseases of public health importance such as TB, *Brucellosis*, HPAI and Rabies scored higher in local human health impacts (between 3.6 – 4.7) indicating their zoonotic nature.

With regards to societal animal product intake and environmental contamination, diseases such as HPAI, RVF, ASF and ND scored higher due to the fact that once they occur in the community they influence the societal eating behavior and have a big impact on the environment.

Generally, the total scoring indicated in the table shows that those diseases ranking higher reflect scoring in the individual factors and therefore they have remarkable impact on economic, environment and public impacts.

### Discussion and conclusion

The principal objective of the prioritisation of diseases is to provide evidence to guide animal health policy issues such that the limited resources available for their prevention and control are targeted appropriately at those of most importance in the context of government and regional policies.

Prioritisation of transboundary animal diseases and zoonoses has acquired major interest within the past few years, especially from a prevention and control point of view and in the sector of public health. Our study considered important transboundary animal diseases as reported by Directorate of Veterinary Services, but also zoonotic diseases as reported the Ministry of Health and Social Welfare (department of public health). The prioritisation method used was developed by Phylum (OIE) as a tool for putting priority list of TADs and zoonotic diseases.

Disease prioritisation was based on given set of criteria, namely: local human health impact, control feasibility measures, risk of the disease to be introduced in the country, local economic impact, local and international trade impact and local societal impacts. The latter enabled us to put in place appropriate strategies and action plan for prevention and control measures of the diseases of economic

importance.

According to the results given by the Phylum prioritisation tool the ranking in descending order was as follows: Rift Valley Fever (RVF), *Brucellosis*, *Tuberculosis*, Highly Pathogenic Avian Influenza (HPAI), Rabies, FMD (circulating serotypes), FMD (exotic serotypes), Contagious Bovine Pleuropneumonia (CBPP) Newcastle Disease (ND) and *Peste des Petits Ruminants* (PPR). Prioritisation after discussions with other national stakeholders based on nature of the disease and associated control measures influenced the ranking as follows: RVF, FMD circulating, CBPP, ND, PPR, HPAI and FMD (exotic serotypes) in that order of importance.

Rift Valley Fever ranked higher than other diseases in terms of its economic and societal impacts as it has serious negative impacts on livestock production, trade (local and international) as supported by the last outbreak in 2006/2007 that covered wider areas of the country involving both human and domestic ruminants, resulting into the country to spend about US\$3.84 million to bring the disease under control. (Sendato *et al.*, 2007)

Collaboration between livestock farmers and other development partners is needed a well coordinated, facilitated and effective surveillance, effective vaccination regime and restricted movement of animals to avoid spread of the disease to spread to other Partner States of East African Community. It is therefore recommended that active communication with meteorological departments is inevitable for early prediction of the rainfall pattern and using this for early warning so that preparedness is undertaken to contain the disease in case of outbreak in the region.

Foot and Mouth Disease circulating is endemic in Tanzania since 1927 and ranked second. The disease affects cattle, pigs, small ruminants and wild ruminants causing substantial economic losses in terms of lowered production and trade. There are four serotypes of the virus in the country (SAT 1, SAT 2, O and A). This negates effective & reliable vaccination efforts (Sallu *et al.*, 2010). It is recommended that the country intensifies epidemiological

surveillance and employ strategic vaccination in frequently affected areas to protect clean herds.

In terms of both economic and disease control measures, CBPP ranked third in priority list as the disease is associated with direct losses due to animal mortality and its expensive control measures. (Msami *et al.*, 2001; Kusiluka and Sudi 2003).

Control of the disease has been attempted through restricting animal movements and undertaking a rollback mass vaccination campaign. However, these efforts have been unable to produce a sustainable disease control results due to limited financial resources which often leads to low vaccination coverage. For effective disease control compliance to OIE sanitary prophylaxis measures is inevitable. It is recommended that the developed national CBPP contingency plan be operational.

Newcastle disease is ranked fourth position in our ranking due to its economic impact as it is a major constraint to both rural and urban chicken settings causing over 90% chicken mortality rates. (Buza. and Mwamhehe., 1989). The disease has a seasonal occurrence usually between July – November annually (Yongolo, 1996). This occurrence pattern is an opportunity for developing effective prevention and control strategy of the disease. (Salum *et al.* 1999).

*Peste des Petits Ruminants* ranked fifth due to its significant economic, food security and livelihood impacts. The last outbreak (2008) caused a potential income loss to livestock keepers and a cost the country more than 200 billion TZS to control (Mtui, 2013). However, the country has now a National PPR progressive control and eradication strategy which has to be operationalised. The previously reported PPR outbreaks in neighboring Kenya, calls for the institution of an East African regional coordinated surveillance and control measures to address these challenges, and hence put in place effective and sustainable PPR control mechanisms.

A Highly Pathogenic Avian Influenza ranked sixth as it affects both domesticated

and wild birds. Although HPAI has yet to be reported in the country, still it needs serious consideration due to its economic, societal impacts and its zoonotic nature. Tanzania, due to its geographical position harbours three major flyways used by migratory birds from Europe and Asia to the South and these predisposes the country to a high risk of the disease. This requires the country to be in an alert phase at all times with strategic surveillance and prevention as stipulated in the National Avian and Pandemic Influenza Emergency Preparedness and Response Plan.

Exotic FMD ranked seventh despite being absent in the country. Since these serotypes have been reported in the Asian continent and there is a constant movement of people from the affected countries to Tanzania, this puts the country at risk of virus introduction. It is therefore recommended that zoosanitary inspection, law enforcement and state of alert be capacitated at all times in collaboration with neighbouring countries taking into consideration the very extensive porous borders.

In concluding, there is therefore a need for the Tanzanian Government to make use of prioritised list of TADs and zoonoses by paying attention in terms of financial resource allocation and providing political support for the implementation of disease prevention and control plans.

Since all the ranked diseases have huge economic and public health implications and therefore, their prevention and or containment calls for cordial cooperation of various actors including Eastern Africa region member states, international organizations (OIE, FAO, WHO, AU-IBAR) for harmonised TADs and zoonoses control strategies.

### **Conflict of interest**

There is no conflict of interest identified.

### **Ethical standards**

The study involved review of secondary

data and reports generated during routine animal and human disease surveillance. There was no direct contact with human and animal subjects. Because this was part of the routine surveillance activities which are exempted from Institution Review Boards (IRBs) and National Council of Science and Technology (NCST) permission to conduct the study was not sought. The study has many benefits to the human population and to the animal by guiding policy and resource allocation for prevention and control of the diseases.

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# AFRICAN UNION - INTERAFRICAN BUREAU FOR ANIMAL RESOURCES (AU-IBAR)

Bulletin of Animal Health and Production in Africa  
Guide for Preparation of Papers  
Notes to Authors

The Editor in Chief  
June 2018

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The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Interafrican Bureau for Animal Resources (AU-IBAR) is a scientific journal which publishes articles on research relevant to animal health and production including wildlife and fisheries contributing to the human wellbeing, food security, poverty alleviation and sustainable development in Africa. The bulletin disseminates technical recommendations on animal health and production to stakeholders, including policy makers, researchers and scientists in member states. The Bulletin is the African voice on animal resources issues specific to Africa.

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- Conclusions are presented in an appropriate fashion and are supported by the data. The results must be interpreted appropriately, such that all conclusions are justified. However, authors may discuss possible explanations for their results as long as these are clearly identified as speculations or hypotheses, rather than as firm conclusions. Inappropriate interpretation of results is a justifiable reason for rejection.
- The research meets all applicable standards for the ethics of experimentation and research integrity. Research to be published must have been conducted to the highest ethical standards. A brief description of the most common of these is described in our Editorial and Publishing Policies.
- Because the guidelines are updated as appropriate, authors should check them again before they submit their articles. Manuscripts submitted for publication will be considered for acceptance on the understanding that they present original work which has not been published or submitted for publication elsewhere and that they are subject to peer review.

## Types of contribution

Full papers providing accounts of original work: Research containing significant new findings. The material presented should be original and not have been published elsewhere, except in a preliminary form. Papers will be reviewed by three referees familiar with the subject matter of the paper.

*Short Communications:* are intended to provide quick publication of highly relevant and interesting information. Manuscripts will be peer reviewed by two reviewers and the Editor.

*Review Articles:* should cover subjects falling within the scope of the bulletin, which are of active current interest. Papers need not contain original work or ideas. They will be reviewed for completeness, accuracy, style and suitability of content by referees familiar with the subject and the Editor-in-Chief.

*Editorial:* articles are short articles describing news about the bulletin or the opinion of the editor-in-chief, the publisher or a guest editor of a thematic series.

**Letters to the Editor:** the bulletin welcomes letters to the editor. The purpose of Letters to the Editor is to provide a forum for positive and constructive views on articles and matters published in the bulletin. Letters to the Editor must not exceed 300 words. Letters to the editors include technical reports from countries or projects.

**Key notes and special calls:** The editor will, from time to time, invite selected key figures in the field of animal health and production for key notes on specific topics. Book Reviews: are accepted and should provide an overview of the work's contents and a critique of the work's value. Book reviews should be limited to 1000 words.

**Conference Proceedings:** Special Issues of the bulletin may be dedicated to publication of proceedings of key meetings/conferences

Obituary articles to honor prominent African scientists that have made significant contribution to animal resources research and development

**News and announcements:** BAHPA is pleased to publish information on animal health and production activities/meetings. Please send the following information to the Editor: Date of the event, title, organization offering the event, location and contact information.

### Submission Guidelines

Full papers of original research

All manuscripts submitted to BAHPA should include the following features:

1. On cover page of the manuscript, the following should be clearly written/inserted: the corresponding author, name of the institution, title of the manuscript, names of the authors, the addresses of the authors and the e-mail address of the corresponding author. The corresponding author should ensure that all the other authors consent to their names being included. The consent should be sent directly by co-authors to the editor via email.
2. Each original article should be divided into Abstract and Keywords, Introduction, Materials and Methods, Results, Discussion, conclusion, Acknowledgments and References. A textbox containing a public brief on the study for the benefit of policy makers should also be provided. This textbox will not be included in the published article but will be compiled and published in a separate edition at the end of the year.
3. Title, which should be concise, preferably not more than 15 words long, followed by the author(s) name(s) and institution(s) to which work should be attributed and address for correspondence, if different.
4. The Abstract should not be longer than 300 words giving a synopsis of the work and should contain the objectives, briefs description of materials and methods, highlights of significant results, conclusions and recommendations. Up to six keywords should be provided..
5. The Introduction should contain the problem statement, the hypothesis and the objective of the work and cite recent important work undertaken by others.
6. Materials and Methods should describe materials, methods, apparatus, experimental procedure and statistical methods (experimental design, data collection and data analysis) in sufficient detail to allow other authors to reproduce the results. This part may have subheadings. The experimental methods and treatments applied shall conform to the most recent guidelines on the animal's treatment and care. For manuscripts that report complex statistics, the Editor recommends statistical consultation (or at least expertise); a biostatistician may review such manuscripts during the review process. Cite only textbooks and published article references to support your choices of tests. Indicate any statistics software used.
7. Results should be presented clearly and concisely, in a non-

repetitive way. Subheadings may be accepted.

8. Discussion of significance should be focused on in the interpretation of results. Subheadings are not accepted in this section.
9. Acknowledgements. Where necessary acknowledgements of grants and technical assistance should be included under this heading. Please also include any potential conflict of interests if appropriate. Suppliers of materials should be named and their location (town, state/county, country) included.
10. State the conclusions, and any implications that may be drawn from the study.

**Short Communications:** Manuscripts should contain original data and be limited to 1500 words. The number of tables and figures are limited to two. A limited number of references should be included. Headings are not allowed in short communications.

### Sequence of Preparation

1. The data files must be PC/Windows-compatible. The text should be prepared using standard software (Microsoft Word) format; do not use automated or manual hyphenation. Please do not include footnotes.
2. Use Times New Roman 12 point font for all text except for tables and figures where Times New Roman 10 font should be used.
3. Use 1 inch margins on top, bottom, left and right margins,
4. Every line on the text should be numbered.
5. Use double line spacing for body of text. For Abstract, Figures, Tables and References use single line spacing.
6. Place page numbers in the lower right hand corner of your manuscript.
7. Run "the spell check" and "grammar check" on the entire file before submission using either the UK English or French standard.
8. Avoid using abbreviations for the names of concepts. Use ordinary words for variable names – not code names or other abbreviations. Use the same name for a variable throughout your text, tables, figures and appendices. Names of organizations and research instruments may be abbreviated, but give the full name (with abbreviation in brackets) the first time you mention one of these.
9. References should take the following form: In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's name should be mentioned, followed by 'et al.'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works. Examples: Abayomi (2000), Agindotan *et al.*, (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1995, 1993), (Kumasi *et al.*, 2001)

### The use of reference managing software is encouraged

The authors should be cited in a chronological order by year and then by a or b; in the reference list they should be listed alphabetically.

Please ensure that references in the text exactly match those in the manuscript's reference list. Check each reference in the text to see that you have the complete citation in the reference section of the paper in the desired style. In the references section, references are listed in alphabetical order.

### Examples of References

- **Journal Articles:** Ouyang D, Bartholic J, Selegean J, 2005. Assessing sediment loading from agricultural croplands in the Great Lakes basin. *Journal of American Science*, 1(2): 14-21.
- **Books:** Durbin R, Eddy SR, Krogh A, Mitchison G, 1999. *Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids*. London, Cambridge University Press.

- *Chapter in a Book*: Leach J, 1993. Impacts of the Zebra Mussel (*Dreissena polymorpha*) on water quality and fish spawning reefs of Western Lake Erie. In *Zebra Mussels: Biology, Impacts and Control*, Eds., Nalepa T, Schloesser D, Ann Arbor, MI: Lewis Publishers, pp: 381-397.
- *Reports*: Makarewicz JC, Lewis T, Bertram P, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. US EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.
- *Conference Proceedings*: Stock A, 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.
- *Thesis*: Strunk JL, 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, Unpublished PhD thesis, Michigan State University, East Lansing, MI.
- *Web links*: Cerón-Muñoz M F, Tonhati H, Costa C N, Rojas-Sarmiento D and Solarte Portilla C 2004 Variance heterogeneity for milk yield in Brazilian and Colombian Holstein herds. Livestock Research for Rural Development. Volume 16, Article #20 Visited June 1, 2005, from <http://www.lrrd.org/lrrd16/4/cero16020.htm>

### Illustrations

Please send the figures as separate files and do not import them into the text file. Put all tables, figures, diagrams and artwork on separate pages. Each figure, table, and bibliographic entry must have a reference in the text. References to tables and figures in the text should be by number and not to "table below" or "figure below". The Editor will place them in the appropriate place in the text of article during the final edit. Tables and figures should be numbered consecutively. Please submit the data for figures in black and white.

### Abbreviations, Symbols and Nomenclature

All specifications must be stated according to the S.I. system. Concentrations of chemical solutions are to be given in mol/l. All other concentrations should be given in % (volume or weight). Any abbreviations of chemical, biological, medical or other terms should only be employed when it is certain that they are internationally known. The full name must be stated in brackets when the abbreviation is first used. Names of micro-organisms and zoological names should be italicized in the manuscript.

### Ethical guidelines

BAHPA adheres to the below ethical guidelines for publication and research. Experimentation will only be published if such research has been conducted in full accordance with ethical principles. Manuscripts containing experimentations must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

1. When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort.
2. All studies using animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

### Revising your article

When you submit a revised version of your article in response to the referees' comments, you must accompany it with a detailed list of the changes made (ignoring typographical errors, but mentioning additional paragraphs, changes to figures, etc) suitable for transmission to the referee. Where changes have been made in response to the referees' remarks it is important to mention this and indicate where they can be found. You may also wish to send in a second copy of your article with the changes marked or underlined.

You should go through the referees' comments and for each comment mention whether you followed their suggestion or whether you disagree and wish to respond to the comment. If a referee has misunderstood a point, it is not necessarily their fault and may have been caused by ambiguity or lack of clarity in your article which needs to be corrected. Some authors copy out each of the referees' comments in turn and include their response immediately after. In other cases responses can be made referring back to the reports. Finally, please make sure that you send your revised article to us and not simply the original version again. This is a common mistake, especially when authors send in their work electronically. Electronic revised articles should contain all text and graphics files needed to generate the revised version, and not just those files that have changed.

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