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AN ABATTOIR STUDY ON THE PREVALENCE OF SOME HELMINTHS AMONG SLAUGHTERED CATTLE, SHEEP AND GOATS FROM MWANZA CITY, LAKE VICTORIA BASIN, TANZANIA.

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Abstract

The main source of animal protein for humans is livestock and their products. Parasitism is one of the main constraints limiting livestock production. A study was conducted to determine the prevalence of helminths in slaughtered cattle, sheep and goats in Mwanza City, Tanzania. The period of study was from 2008 to 2011 within which a total of 191,033 and 107,498 sheep and goats were respectively slaughtered. The prevalence rate of helminths infections in cattle was: Fasciolosis 7.92%, *oesophagostomosis* (pimply gut) 4.38%, Hydatidosis 2.15% and *Stilesia hepatica* 1.86%. In sheep and goats *oesophagostomosis* was 7.13%, *Stilesia hepatica* 4.88%, Fasciolosis 1.23% and Hydatidosis 1.05%. Other parasitic conditions recorded in sheep and goats at low prevalence are: *Cysticercus tenuicollis* and *Haemonchus contortus*. Other pathologic conditions recorded in cattle which may have a bearing on helminth infections were fatty degeneration, melanosis, hepatitis, and liver cirrhosis. Among the observed parasitic infections some were of zoonotic nature and these included hydatidosis, fasciolosis and C. tennuicollis. It is recommended that efforts should be made to control these infections as they are both of economic and public health importance.

Key words: Abattoir, helminths, parasites, zoonotic,

ETUDE SUR LA PRÉVALENCE DE CERTAINS HELMINTHES CHEZ LES BOVINS, OVINS ET CAPRINS ABATTUS DANS DES ABATTOIRS DE LA VILLE DE MWANZA SITUÉE DANS LE BASSIN DU LAC VICTORIA EN TANZANIE

Résumé

Les animaux et les produits animaux constituent la principale source de protéines animales pour les êtres humains. Cependant, le parasitisme est l'une des principales contraintes qui limitent la production animale. Une étude a été menée afin de déterminer la prévalence des helminthes chez les bovins, ovins et caprins abattus dans la ville de Mwanza en Tanzanie. Au cours de la période de l'étude (2008 à 2011), 191 033 ovins et 107 498 caprins ont été abattus. Les taux de prévalence des helminthiases chez les bovins se présentaient ainsi : fasciolose 7,92%, oesophagostomose (lésions nodulaires sur l'intestin) 4,38% ; hydatidose 2,15% et Stilesia hepatica 1,86%. Chez les ovins et les caprins, l'oesophagostomose représentait 7,13%, Stilesia hepatica 4,88%, la fasciolose 1,23% et l'hydatidose 1,05%. Les autres parasitoses, observées chez les ovins et les caprins, mais qui avaient un faible taux de prévalence sont : Cysticercus tenuicollis et Haemonchus contortus. Les autres pathologies notées chez les bovins, susceptibles d'avoir un rapport avec les helminthiases, étaient la dégénérescence graisseuse, la mélanose, l'hépatite et la cirrhose du foie. Quelques-unes des parasitoses observées avaient un caractère zoonotique, et elles comprenaient l'hydatidose, la fasciolose et C. tennuicollis. Il est donc recommandé de déployer des efforts pour contrôler ces infections car elles ont des conséquences économiques et revêtent une importance pour la santé publique.

Mots-clés : abattoir, helminthes, parasites, zoonose

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Introduction

Tanzania has a cattle population of 17.7 million of which 95% are of the indigenous Tanzanian Shorthorn Zebu (TSZ) genotype kept by the traditional sector (Ministry of Agriculture and Food Security (MAFS, 2000). Goat and sheep population is estimated at 12.5 million and 3.5 million respectively and are also kept by pastoral communities as a source of food and income generation thereby improving socio-economic status (MAFS, 2002). The contribution of livestock to the national economy and food security is 18% of total GDP. The indigenous cattle provide more than 70% of the meat as well as 67% of milk consumed in Tanzania (Mellau, *et al.,* 2010).

Livestock is the main source of proteins and other animal products for the human population. Parasitism is one of the main constrains limiting livestock production. Mortality of animals due to parasitic infections may not be alarming at times but their direct effects in terms of reduced milk, meat, hide production, infertility and losses of stamina of working animals and especially zoonotic impact on human health are considerably greater (Baker and Muller, 1988). Control of diseases communicable from animals to humans under natural conditions is an important task of a Veterinary professional. There are many important zoonotic parasitic diseases such as Fascioliasis, Hydatidosis, Trichinellosis (Swabe *et al.,* 1984). These diseases are important public health hazards, particularly in rural areas where there is a close association between humans and domestic animals. Supervision of the slaughter houses and organization of hygienic precautions are unsatisfactory in rural areas of Tanzania. Many slaughter houses and village markets have no qualified meat inspectors and have become the places where stray dogs, cats and carnivorous birds congregate.

In Mwanza city and other urban areas in Tanzania livestock brought for slaughter come from pastoral and agro-pastoral community villages where disease control regimens are limited to endemic tick borne diseases and trypanosomiasis (Swai *et al.,* 2005). Veterinary services are rarely extended to villages for many reasons including shortage of veterinary staff, poor transport infrastructure and limited diagnostic facilities and drugs (Kambarage *et al.,* 1995). Lack of veterinary services to these livestock rearing areas suggests a possible widespread occurrence of diseases in traditional cattle herds, sheep and goats. This further suggests that most cattle, sheep and goats brought for slaughter may harbour chronic or sub-clinical infections which can rarely be detected during ante-mortem examination (Kambarage *et al.,*1995).

There is a need to document problems concerning meat hygiene and possible health risks during both ante-mortem and post-mortem examinations. In this context, meat-inspection data are a potential source of information and have an important role to play in epidemiology and preventive veterinary medicine (Gracey *et al.,* 1999). Monitoring disease and other conditions at slaughter has been recognized by (Herenda and Jackel, 1994) as one way of assessing the disease status of a herd. However this source of information is not available for livestock which originate from the Lake Victoria basin areas as exemplified by Nyakato abattoir in Mwanza City, Tanzania. The purpose of the present study was to investigate the occurrence and prevalence of parasitic infections detected in food animal carcasses slaughtered at Nyakato abattoir in Mwanza, during a four year period from 2008 to 2011.

Materials and Methods

Study Area

The study was undertaken at Nyakato abattoir within urban Mwanza City in Ilemela and Nyamagana Districts (Figure 1). Mwanza is the second largest urban and peri-urban establishment in Tanzania after Dar es Salaam and is fast growing. It is located on the southern shores of Lake Victoria in north western Tanzania between latitudes 2.15° and 2.45° south of the Equator and longitudes 32.45° and 33° East of Greenwhich. It comprises of Ilemela and Nyamagana districts. It covers a total area of approximately 13337 Km^2 of which 900 Km^2 is covered by water from Lake Victoria and the remaining portion is land covering approximately 437 Km^2 (32.7%). The city covers only about 3.8% of the total area and has a

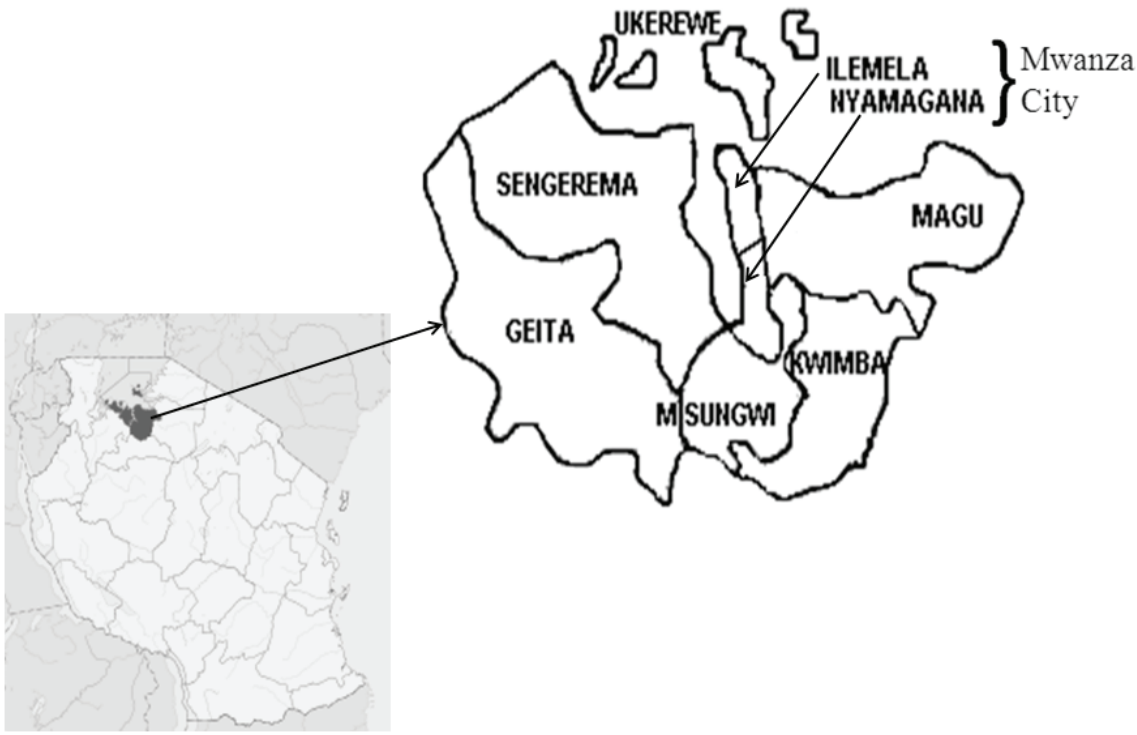


Figure 1: Map of Tanzania showing Lake Victoria and Position of Mwanza City and adjoining Districts.

population of 537547 Mwanza City Master Plan (2008/2028) the biggest port to Lake Victoria in Tanzania. Other districts involved in this study were Ukerewe, Sengerema, Geita, Misungwi, Kwimba and Magu all within the Lake Victoria Basin.

Source of Animals

With regards to the set up of livestock sector in Tanzania almost all animals sent for slaughter are adult and come from the rural traditional sector. It is clear that more than 98% of the livestock population sent for slaughter in the country are of indigenous types kept in the rural areas. These breeds are the Tanzania Short Horn Zebu (TSZ), Ankole breeds and local breeds of goats and sheep. These local indigenous breeds of livestock originated not only from Mwanza city districts, but also from other adjoining districts namely: Ukerewe, Geita, Sengerema, Misungwi, Kwimba and Magu (Figure 1).

Data Collection

Data for the years 2008 to 2011 was collected from Nyakato abattoir records and was made up of a total of 191,033 cattle and 107,498 goats and sheep slaughtered and inspected during the period under study. Veterinarians and meat inspectors conducted ante-mortem and post-mortem examinations. They routinely physically examined all live animals brought for slaughter a day before slaughter and only clinically healthy animals were allowed to be slaughtered. All animals which were found unhealthy during ante-mortem examination were isolated and kept in isolation compound for further observation. Day to day routine post-mortem meat inspection, a thorough visual examination and a systematic incision of carcasses and visceral organs was carried out according to procedures described by (Gracey *et al.,* 1999) and the Tanzania General Guidelines on Meat Inspection. Intestinal and liver lesions were diagnosed according to pathological changes of organ size, colour, consistence and presence of parasites and lesions. At the end of the day's meat inspection all condemned livers and intestines were counted and recorded.

Data analysis

Data was entered in a computer and Statistix© 2000 Analytical software was employed to carry out descriptive statistics where the prevalence rate of each parasite species was determined. A proportion test procedure was used to perform sample hypothesis tests and confidence intervals for the prevalence rates of each parasite species was computed using the normal approximation with the correction for continuity. Graphpad prism software was used in drawing graphs for prevalence rates of each parasite species.

Results

Between 2008 and 2011, a total of 4002, 42921, 46099, and 61991 cattle were slaughtered respectively (Tables 1). For the four years the total slaughter figures for cattle were 191,033. On parasitic infestations, overall, fasciolosis (7.92%), and *oesophagostomosis* (4.38) were the leading helminthes infections in cattle, followed by *hydatid cysts* (2.15%), and *Stilesia hepatica* (1.3%), (Table 1). There were other disease conditions which were recorded at low prevalence included, melanosis, hepatitis, fatty degeneration, and liver cirrhosis.

Table 1: Prevalence (%) of parasitic infections in slaughtered cattle at Nyakato abattoir in Mwanza City, Tanzania between 2008 - 2011.

Year	Number cattle slaughtered	*Fasciola spp* (Liver flukes)		*Stilesia hepatica*		*Hydatid cysts*		*Oesophagostomosis* (pimply gut)	
		Total	%	Total	%	Total	%	Total	%
2008	40,022	3,874	9.67	616	7.76	238	0.5	957	2.39
2009	42,921	4,571	10.64	705	1.64	1488	3.46	2296	5.34
2010	46,099	3,252	7.05	794	1.72	984	2.13	1270	2.75
2011	61,991	3,450	5.56	1455	2.35	1400	2.25	3856	6.22
Total	**191,033**	**15,147**	**7.92**	**3570**	**1.86**	**4110**	**2.15**	**8376**	**4.38**

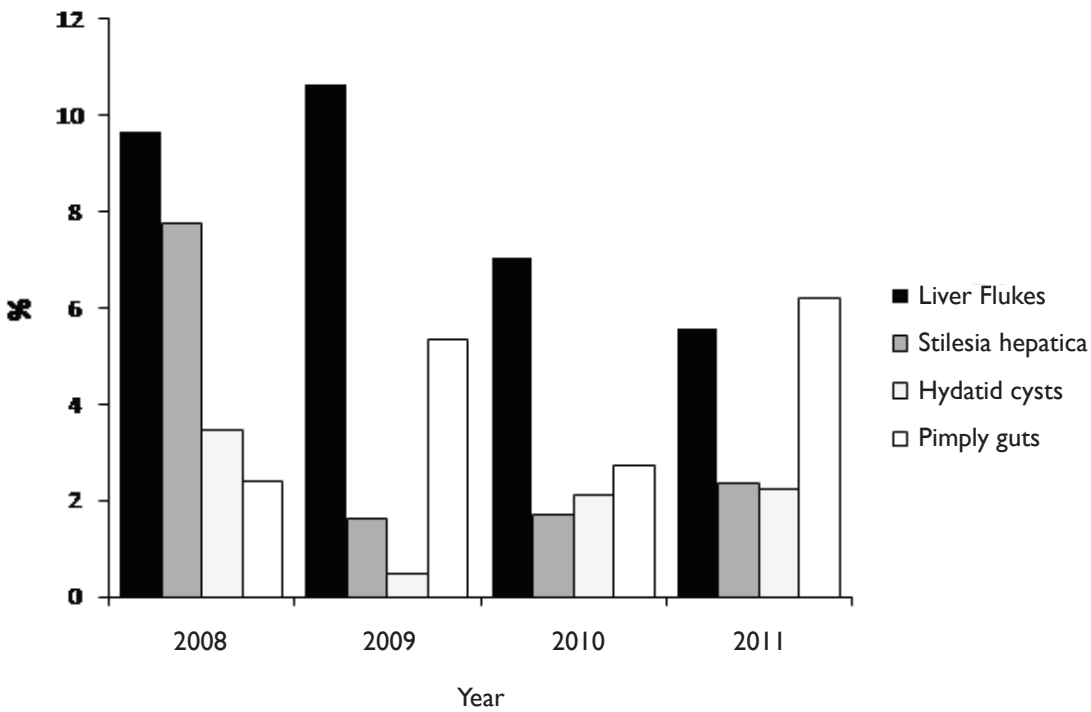


Figure 2: Prevalence (%) of parasitic infections in slaughtered bovine at Nyakato abattoir in Mwanza City, Tanzania between 2008 – 2011.

Table 2: Prevalence (%) of parasitic infections in slaughtered caprine and ovine species at Nyakato abattoir in Mwanza City, Tanzania between 2008-2011.

Year	Number cattle slaughtered	Liver flukes		*Stilesia hepatica*		*Hydatid cysts*		*Oesophagostomosis* (pimply gut)	
		Total	%	Total	%	Total	%	Total	%
2008	11,010	192	1.7	598	5.43	6	0.05	805	7.31
2009	25,811	333	1.29	705	2.73	59	0.22	1584	6.13
2010	28,177	347	1.23	1455	5.16	376	1.33	1056	3.77
2011	42,500	454	1.06	2488	5.85	690	1.62	4223	9.93
Total	**107,498**	**1,326**	**1.23**	**5246**	**4.88**	**1131**	**1.05**	**7668**	**7.13**

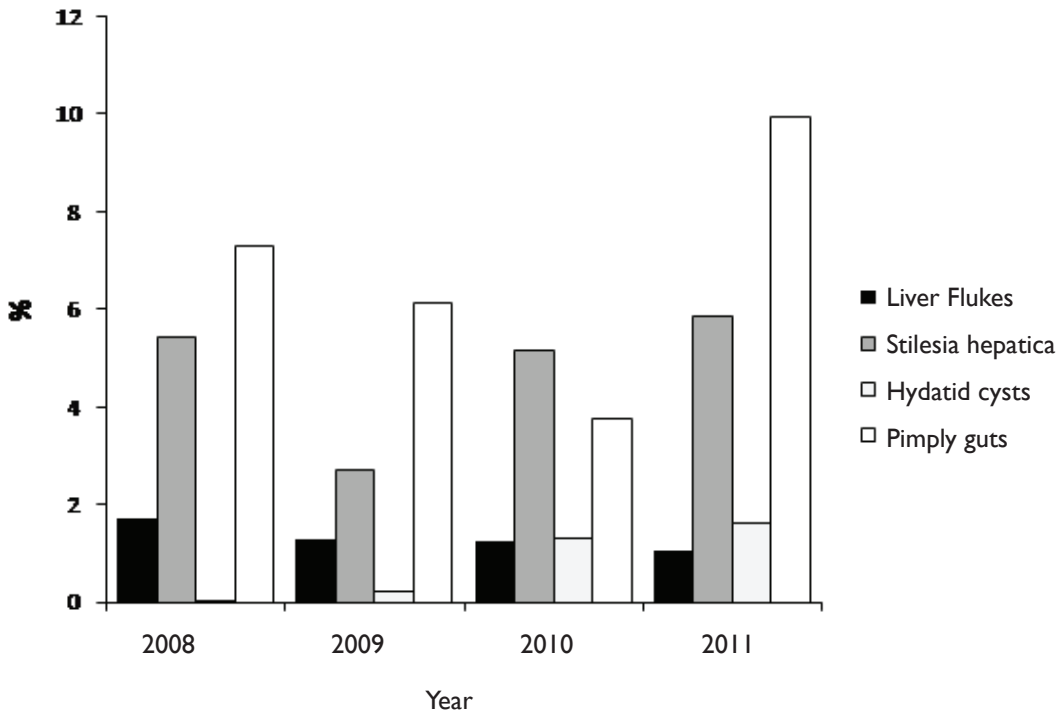


Figure 3: Prevalence (%) of parasitic infections in slaughtered caprine and ovine species at Nyakato abattoir in Mwanza City, Tanzania between 2008 – 2011.

Among sheep and goats, a total of 11010, 25811, 28177, 42500, were slaughtered in 2008, 2009, 2010, and 2011 respectively (Table 2).

The leading parasitic infestations were esophagostomosis (7.13), *Stilesia hepatica* (4.88%) followed by liver flukes (1.23%) and *hydatid cysts* (1.5%), (Table 2). Cysticercus tenuicolli, Haemochus contortus infections were also recorded in sheep and goats.

Discussion

Cattle, sheep and goats play an important role in the nutrition and income to local farmers in the rural areas in Tanzania (Mutenga *et al.,* 1986, Connor *et al.,* (1990) They provide meat, milk, skins, manure and they also serve as an investment that can easily be converted into cash when need arises (Njombe, 1993). Parasitic diseases constitute a major threat to livestock production in sub –Saharan Africa owing to the direct and indirect losses they cause (Kagira and Kanyari, 2001). The

epidemiology of helminth diseases is complex and involves a balance between the infection rate and the resistance of the host.

The results of the current study which is the first to document the prevalence of helminthes among slaughtered cattle, sheep and goats from the lake Victoria Basin areas in Tanzania, showed that cattle are the most common ruminants slaughtered at Nyakato slaughter house, numbering to 191, 033 cattle in the four years from 2008 to 2011. Sheep and goats totaled to 107,498 slaughtered in the same period. The types of parasitic infections found in this study included Fascioliasis, Pimpy guts, *Stilesia hepatica*, *hydatid cysts* and other randomly recorded infections such as fatty degeneration, melanosis, hepatitis, and liver cirrhosis. The most prevalent infections in cattle were the liver flukes, but this condition was lowest among sheep and goats. Fasciolosis as the leading liver condemnation has been previously reported by (Kambarage *et al.,* 1995) in Short Zebu cattle slaughtered in Tanzania and by (Mellau et al 2010) in a slaughterhouse survey of liver lesions in slaughtered cattle, sheep and goats at Arusha Tanzania respectively. Findings of fasciolosis of similar magnitude have been reported from Taita Taveta in Kenya by (Mungube *et al.,* 2006). However, a survey in Hai district in Tanzania of an evaluation of the economic losses resulting from condemnation of cattle livers due to fasciolosis was found to be higher than the present results. Mhoma *et al.,* 2010 carried out a survey on cattle parasitic infections in urban and peri urban areas of Mwanza city and found the prevalence of fasciolosis to be lower than the present findings this might be due to the fact that these animals used in the present study came from rural areas of different districts with different animal husbandry practices. Other surveys on the prevalence of fasciolosis conducted in Ethiopia by (Berhe *et al.,* 2009), in Kenya a survey carried out by (Kanyari *et al.,* 2010) to determine the prevalence of endoparasites in cattle within urban and peri-urban areas of Lake Victoria Basin indicated high incidences of fasciolosis. In the present study the prevalence of Fasciolosis infection of 7.98% was the leading parasitic infection in cattle compared to 1.2% among sheep and goats. This might be due to the different feeding habits of the two hosts. Goats browsing on leaves on top of trees and shrubs while cattle graze on grass which is close to the ground and hence easily encounter metacercarie encysted on wet herbage. Fasciolosis has been reported to be associated with areas having plenty of water. The snail intermediate host Lymnaea trunculatula is well adapted to aquatic life (Soulsby, 1982). In the present study it can be postulated that the occurrence of flooding in some areas in the Lake Victoria basin provides favourable habitats for the reproduction of these snails. Fasciolosis apart from being of great economic importance among ruminants through condemnations of livers at slaughter, has been shown to be a re-emerging zoonotic disease with 2.4 to 17 million people infected world wide (WHO 1995, Mas-Coma, 2005). It is obvious that the communities living in the Lake Victoria basin is in danger of this zoonotic infection.

The prevalence of pimply gut in this study was higher in sheep and goats at 7.13% compared to cattle at 4.38%. This condition is caused by Oesophagostomum columbianum (nodular worm) in sheep and goats and Oesophagostomum radiatum in cattle. These worms cause anorexia severe and persistent diarrhoea, loss of weight, anemia and finally death, (Soulsby, 1982). The results from this study agree with those of (Kusiluka *et al.,*1994) who carried out the epidemiology of gastrointestinal helminths in goats in Morogoro district in Tanzania, and those of (Ng'ang'a *et al.,* 2004) who reported similar findings in Kenya. To diagnose the lesions of pimply guts in sheep and goats requires experience; meat inspectors with limited experience may easily miss it. It is quite likely the magnitude of the disease is higher than that recorded in this study.

Stilesia hepatica was higher in sheep and goats (4.88%) than in cattle (1.3%). This infestation occurs in all ages among its hosts. It is generally non-pathogenic, but bile ducts form into sac-like dilations filled with worms. Such livers are condemned at meat inspection. A study by (Mungube *et al.,* 2006) in Kenya reported a prevalence of 28% and 22% in sheep and goats respectively. A survey carried out in Ethiopia by (Sissay *et al.,* 2008) revealed a stilesiosis prevalence of 40% and

46% in sheep and goats respectively. Similarly a slaughterhouse survey of liver lesions in cattle, sheep and goats at Arusha, Tanzania by (Mellau *et al.,* 2010), reported stilesia infection contributed significantly to sheep and goat liver condemnations. The findings from Ethiopia and Kenya are higher than the ones from the present study and this might be due to various environmental conditions such as time of investigation that is conducive to parasite reproduction and infection and the number of animals examined.

Hydatidosis infestation from this study showed 2.15% of cattle and 1.55% of sheep and goats were infected. Similar low levels of infection were reported by (Njoroge *et al.,* 2002) in Kenya, (Ansari-Lari 2005) in Iran and (Mellau *et al.,* 2010) in Tanzania. In contrast, a high prevalence of hydatidosis in cattle, sheep and goat was reported in Sudan by (Elmahdi *et al.,* 2004), in Morroco by (Azilaf and Dakkak 2006), in Ethiopia by (Kebede *et al.,* 2009) and (Md.Hazzaz Bin Kabir *et al.,* 2010) in Bangladesh. The differences in prevalence of this condition may arise due to differences in environmental conditions that are conducive to the perpetuation of the parasite, abundance of infected definitive host, livestock husbandry, stocking rate, nature of the pasture and grazing patterns of animals. Hydatidosis is an important parasitic zoonosis and the disease has been reported in almost all parts of the world during meat inspection in abattoirs (El-Badaw et al 1980, Chermette, 1983, Petkov et.al 1987, Ashraf *et al.,* 1987 and Anwar *et al.,* 1993). This zoonosis is a threat to humans not only in Tanzania but also to other countries in the Lake Victoria Basin. Due to the presence of large numbers of stray dogs and lack of proper meat inspection at the villages, there is every likelihood of the disease spreading to humans.

Other conditions rarely recorded at the abattoir included fatty degeneration, melanosis, hepatitis, and liver cirrhosis. C. tenuicolli, Haemochus contortus, infections were also recorded in sheep and goats. The rarity of these conditions suggests that they are likely to be of little concern and only appear sporadically.

Slaughter house survey is a complex way of gathering information on livestock diseases particularly subclinical conditions (Md. Hazzaz Bin Kabir *et al.,* 2010). Meat inspection practices are predominantly intended for the safety and wholesomeness of meat for human consumption. Abattoir records are also useful in the trace back, in case of finding disease during a control programme. This latter role provides records which can be used for animal disease planning and control strategies as well. For diseases which produce easily recognizable lesions at post-mortem such as fascioliosis and hydatidosis, abattoir records can indicate the prevalence of diseases as well as evaluate a disease control programme.

The present study was based entirely on information that was recorded in a slaughterhouse; the results indicate that among the helminths infections were of great public health concern and economic importance due to condemnation of organs and carcasses which are the sources of meat and animal protein. In addition, there is loss of production and performance of affected animals. Therefore efforts should be made to control the helminths infections of economic and public health importance.

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RÉSULTATS DE LA CAMPAGNE DE VACCINATION MASSIVE DES CHIENS ET AUTRES ANIMAUX DE COMPAGNIE CONTRE LA RAGE DANS LA VILLE DE NDJAMENA, 2012

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Résumé

La rage est une zoonose virale grave. Elle constitue autant de menace pour la Santé Publique que les autres infections. Elle est causée par un virus du genre *Lyssavirus* et de la famille *rhabdoviridae*. Pour l'exécution de la campagne antirabique 2012, 30 agents vaccinateurs et 3 superviseurs ont été recrutés, vaccinés contre la rage et formés sur les techniques de vaccination. Aussi, 50.000 doses du vaccin antirabique, des seringues, des carnets de vaccination et des aiguilles ainsi que des glacières (10 de 5 litres et 3 de 50 litres) ont été achetées. Les séances de vaccination des chiens et autres animaux de compagnie se sont déroulées uniquement pendant les week-ends (vendredi, samedi et dimanche) et se sont réalisées aux endroits (postes) fixes prédéterminés en commun accord avec les Délégués des quartiers. Pour mener à bien la campagne de vaccination, des séances de sensibilisation de la population de la ville de N'djamena ont été organisées. Elles consistaient en l'affichage des posters dans les lieux publics (les hôpitaux, les centres de santé, les établissements scolaires, les bureaux des arrondissements, etc.), la distribution des dépliants dans les hôpitaux et les centres de santé et l'organisation d'un atelier d'information à l'intention des Délégués de différents quartiers de Ndjamena. La campagne de vaccination antirabique édition 2012 a été lancée officiellement lors de la manifestation de la journée mondiale de lutte contre la rage le 28 Septembre à l'Ecole Nationale des Techniques d'Elevage (ENATE). La campagne de vaccination de masse a duré au total 13 semaines. Selon la méthode développée par Thomas Bayes au 18ème siècle citée par Goodman (1999), la population canine dans la ville de Ndjamena a été estimée à 24.989 têtes. Au total 17.701 chiens avec propriétaires ont été vaccinés lors de cette campagne soit une couverture vaccinale de 70,83%. Hormis les chiens, 1.484 chats et 104 singes ont aussi été vaccinés. Malgré les difficultés rencontrées durant la campagne de vaccination antirabique 2012, les résultats de la campagne antirabique de 2012 peuvent être considérés comme succès car plus de 70,% de taux de couverture vaccinale est obtenue, ce qui est légèrement au dessus du seuil préconisé par l'Organisation mondiale de la santé animale qui est de 70%. Pour la prochaine campagne, il est recommandé d'impliquer suffisamment les maires d'arrondissements afin d'amener les délégués des quartiers et les chefs des carrés à mieux sensibiliser leurs populations respectives.

Mots clés : Rage, vaccin, vaccination, chiens, Ndjamena, Tchad

RESULTS OF MASS VACCINATION CAMPAIGN AGAINST RABIES OF DOGS AND OTHER PETS IN 2012 IN THE CITY OF N'DJAMENA (CHAD)

Abstract

Rabies is a serious viral zoonosis. It is as much a threat to public health than other infections. It is caused by a virus of the genus *Lyssavirus* and the family *Rhabdoviridae*. For carrying out rabies vaccination campaign in 2012, 30 vaccinators and 3 supervisors were recruited, vaccinated against rabies and trained on vaccination techniques. Also, 50,000 doses of rabies vaccine, syringes, vaccination records, needles and cool- boxes (10 of 5 liters and 3 of 50 liters) were purchased. Vaccination sessions of dogs and other pets were held only during weekends (Friday, Saturday and Sunday) and are made in fixed places (positions) predetermined in agreement with the neighborhoods Deputies. To carry out the vaccination campaign,

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awareness sessions of the population of the city of N'djamena were organized. They consisted of the display of posters in public places (hospitals, health centers, schools, borough offices, etc.), the distribution of leaflets in hospitals and health centers and the organization an information workshop for Delegates from different parts of Ndjamena. The vaccination campaign against rabies in 2012 was officially launched at the event of World Day against rabies on September 28 at the National School of Breeding Techniques (ENATE). The mass vaccination campaign lasted a total of 13 weeks. According to the method developed by Thomas Bayes in the 18th century cited by Goodman (1999), the canine population in the city of N'Djamena was estimated at 24,989 heads. In total 17,701 dogs with owners were vaccinated during the campaign for a coverage of 70.83 %. Apart from dogs, 1,484 cats and 104 monkeys were also vaccinated. Despite the difficulties encountered during the vaccination campaign against rabies in 2012, the results of the anti-rabies vaccination campaign in 2012 can be considered as successful as over 70 % of vaccination coverage is obtained which is slightly above the threshold recommended by the World Organization for animal Health, which is 70%. For the next season, it is recommended to sufficiently involve the borough mayors to bring delegates and heads of districts squares awareness their respective populations

Key words: rabies, vaccine, vaccination, dogs, N'djamena, Chad.

Introduction

La rage, zoonose virale, est un grave problème et constitue autant de menace pour la Santé Publique que les autres infections. Elle est causée par un virus du genre *Lyssavirus* et de la famille *rhabdoviridae*. Chez l'homme, la rage résulte généralement de la morsure d'un animal infecté. Selon Cleaveland *et al.,* (2002), Plus de 95% de cas de rage sont dus à la morsure, au griffage et léchage sur les plaies par des chiens enragés.

Selon l'OMS (2010), la rage est la 10e cause de mortalité humaine parmi les maladies infectieuses au monde car elle tue plus de 55000 personnes par an dans le monde (Knobel *et al.,* 2005). L'Afrique et l'Asie sont les Zones les plus touchées avec environ 24.000 et 70.000 de morts par an respectivement.

Entre 2000 et 2002 d'autres études menées à N'Djamena par le LRVZ, le Swiss-TPH et le CSSI ont confirmé l'existence endémique de la rage canine dans la ville de N'Djamena (Kayali *et al.,* 2003a). Chaque année, de nombreuses victimes des morsures par des chiens sont enregistrées dans cette ville. Sur la base des résultats des études menées de 2000 à 2002 et de la campagne pilote de vaccination antirabique de masse menée par Kayali *et al.,* En 2001 (2003b), les Directeurs du LRVZ, de du Swiss- TPH et du CSSI ont signé le 28 septembre 2012 un protocole d'Accord dans lequel s'inscrit la campagne de vaccination de masse antirabique 2012-2013 à N'Djamena.

Les Objectifs de la campagne de vaccination sont de :

- Rompre le cycle de transmission du virus rabique au sein de la population canine à N'Djamena
- Vacciner au moins 70 % de la population canine de N'Djamena durant deux (2) ans consécutifs.

Matériels et Méthodes

Organisation administrative de la ville de Ndjamena

La ville de Ndjamena est composée de 10 arrondissements ayant chacun plusieurs quartiers. Les quartiers sont composés de plusieurs carrés. Les arrondissements sont gérés par les maires des communes et les quartiers par les délégués. Les carrés sont sous la tutelle des chefs des carrés. Les responsables de ces différentes entités administratives ont été impliqués largement dans l'organisation de la campagne de vaccination antirabique.

Structure organisationnelle

L'organisation de la campagne de vaccination contre la rage était réalisée par un comité de coordination constitué des représentants des trois Institutions partenaires (LRVZ, Swiss-TPH et CSSI) et des superviseurs. Parmi les compétences et responsabilités de ce comité étaient comptées la décision sur la progression spatiale de la campagne de vaccination, l'organisation de la sensibilisation, la logistique et la communication avec les autorités administratives de l'arrondissement où se déroule la campagne de vaccination. Ce

comité se réunit deux fois par semaine, le mardi et le jeudi et en cas de difficulté majeure, la rapporter au comité de suivi. Ce comité de suivi est constitué des directeurs des trois Institutions, LRVZ, Swiss-TPH et CSSI ou leurs représentants.

Recrutement et formation des agents vaccinateurs

Les agents vaccinateurs étaient en majorité des techniciens de l'Elevage. Leur niveau de formation est très varié (vétérinaires, ingénieurs de techniques d'Elevage et agents techniques d'Elevage). Ils ont été recrutés parmi les agents du Laboratoire de Recherches Vétérinaires et Zootechniques de Farcha (LRVZ), de l'Ecole Nationale de Techniques d'Elevage (ENATE), de la Délégation Régionale de l'Elevage de Ndjamena (DREN) et de la Clinique Vétérinaire Urbaine de Ndjamena (CVUN). Ils ont été au préalable vaccinés contre la rage et formés. Le programme de formation a été axé sur l'organisation des activités dans le poste de vaccination, l'accueil des propriétaires des chiens, la manipulation des chiens et du vaccin antirabique, l'enregistrement des coordonnées géographiques des propriétaires des chiens vaccinés, le remplissage des carnets de vaccination et les mesures à prendre pour éviter les morsures et les griffures par des animaux présentés aux postes de vaccination. Au total trente (30) agents vaccinateurs et trois (3) superviseurs ont été recrutés et formés pour la campagne de vaccination antirabique à Ndjamena. Pour l'exécution de la campagne antirabique 2012, 50.000 doses du vaccin antirabique, des seringues, des carnets de vaccination et aiguilles ont été achetés. Pour le transport et la conservation des vaccins, des glacières (10 de 5 litres et 3 de 50 litres) ont été achetées.

Les séances de vaccination des chiens et autres animaux de compagnie se sont déroulées uniquement pendant les week-ends (vendredi, samedi et dimanche) et se sont réalisées aux endroits (postes) fixes prédéterminés en commun accord avec les Délégués des quartiers.

Logistique

Pour l'exécution de la campagne de vaccination antirabique 2012, d'importants moyens matériels ont été mobilisés. L'enquête démographique réalisée par R. Mindekem et collaborateurs en 2001 (2005) a montré un ratio chien-homme de 1 : 20 et le nombre de chiens était à l'époque estimé à 23000. L'extrapolation de ces chiffres pour avoir une idée de la population actuelle a donné l'estimation de 45.000 têtes de chiens dans la ville de Ndjamena en 2012, alors 50 000 doses de vaccin antirabique, 50 000 seringues de deux ml, 50 000 aiguilles et 50.000 colliers pour le marquage. 10 registres ont été déployés. 10 glacières de 5 litres pour le transport et la conservation du vaccin et 3 glacières de 50 litres ont aussi été achetées. A noter que les 50 000 carnets de vaccination ont été gracieusement offerts par Merial. Les vaccins, 10 cachets avec le logo de la campagne pour authentifier les carnets de vaccination étaient achetés. Trois véhicules 4 x 4 (deux du LRVZ et un du CSSI) ont été mis à disposition pour faciliter le transport des agents vaccinateurs, le ravitaillement des équipes de vaccination en cas de rupture de vaccin ou des consommables et la distribution de la collation à midi. Chaque superviseur était doté d'un véhicule.

Sensibilisation de la population de Ndjamena (propriétaires des chiens et autres animaux de compagnie)

La campagne de sensibilisation de la population de Ndjamena sur le danger de la rage a été organisée en deux phases. La première phase a précédé le lancement officiel de la campagne. Elle a consisté en l'affichage des posters dans les lieux publics (les hôpitaux, les centres de santé, les établissements scolaires, les bureaux des arrondissements, etc.), la distribution des dépliants dans les hôpitaux et les centres de santé et l'organisation d'un atelier d'information le 25 septembre 2012 au Centre d'Etude et de Formation pour le Développement (CEFOD) à l'intention des Délégués de différents quartiers de Ndjamena. A l'issue de cette rencontre, les Délégués des quartiers en collaboration avec les chefs des carrés ont été chargés de sensibiliser à leur tour leur population. A cet effet, des posters annonçant l'arrivée des équipes de vaccination dans les quartiers leur ont été remis.

La deuxième phase de sensibilisation a été menée conjointement avec les autres activités de la campagne. Chaque mercredi, le comité d'organisation de la campagne organise une réunion de travail avec les délégués des quartiers de l'arrondissement ciblé. Ensemble, ils établissent la liste des lieux où les équipes de vaccination devraient être installées. Ensuite, par le biais des stations de radios les plus écoutées dans l'arrondissement concerné, le comité d'organisation fait passer un communiqué pour informer les propriétaires des animaux de compagnie (chiens, chats et singes) du démarrage de la campagne de vaccination antirabique dans leur zone et les inviter à sortir massivement pour faire vacciner gratuitement leurs animaux. Le communiqué radiodiffusé passe généralement le jeudi, le vendredi et le samedi deux fois par jour en Français, en Arabe et en Sara pour préparer les propriétaires des chiens à amener leurs animaux aux postes de vaccination. En plus du communiqué radiodiffusé, deux équipes dotées de mégaphones sillonnent en véhicule le jeudi soir les différents quartiers de l'arrondissement pour indiquer aux propriétaires des chiens et autres animaux de compagnie les lieux où ils peuvent trouver les équipes de vaccination. Les mêmes mégaphones sont utilisés le jour de la vaccination quand un poste de vaccination est mal fréquenté. Dans ce cas, un agent vaccinateur ou un représentant du chef de carré chemine dans la zone autour du poste pour mobiliser les riverains.

Déroulement de la campagne

La campagne de vaccination antirabique édition 2012 a été lancée officiellement avec la vaccination de quelques chiens lors de la manifestation de la journée mondiale de lutte contre la rage le 28 Septembre à l'Ecole Nationale des Techniques d'Elevage (ENATE). Les activités de terrain ont commencé le 5 Octobre et se sont terminées le 30 Décembre 2012. La campagne a duré au total 13 semaines. Les équipes de vaccination ont procédé arrondissement par arrondissement. La durée de vaccination dans les différents arrondissements était très flexible. Elle variait d'une à trois semaines en fonction de la densité de la population canine dans les arrondissements concernés. La campagne de vaccination a progressé de l'Ouest à l'Est. Les équipes de vaccination sont constituées de trois vaccinateurs chargés d'administrer le vaccin aux chiens, de marquer les chiens vaccinés avec un collier de couleur bleue, de noter les renseignements sur les chiens et leurs propriétaires dans le registre ainsi que de remplir les carnets de vaccination de chiens vaccinés. Elles ont été assistées des chefs de carré dont le rôle est d'assurer la discipline au poste de vaccination et de sensibiliser les habitants riverains. Les équipes de vaccination sont encadrées par des superviseurs. Chaque superviseur est responsable d'au moins trois postes de vaccination. Le rôle des superviseurs est de contrôler la qualité du travail effectué par les vaccinateurs, d'en rendre compte au comité d'organisation, de ravitailler les équipes en cas de rupture de vaccin ou d'autres consommables, d'installer ses postes de vaccination en collaboration avec le délégué du quartier concerné et les chefs de carrés et de distribuer la collation à ses équipes. Les superviseurs ont aussi pour mission d'assurer la gestion efficiente des vaccins et de matériels mis à la disposition des équipes. Pour cette tâche, les superviseurs ont reçu chaque jour une feuille de contrôle de poste et une feuille de contrôle de matériels pour chaque poste ou entre outre était noté le total des animaux vaccinés par espèce, le total des doses de vaccin utilisées, le nombre des flacons donnés (entamés et pleins) le matin et le nombre de flacons (vide, pleins, entamés) restant le soir.

Résultats

Sur l'ensemble des arrondissements couverts par la campagne de vaccination antirabique 2012, au total 17.701 chiens, 1.484 chats et 104 singes ayant des propriétaires ont été vaccinés. Le tableau 1 ci-dessous donne la répartition des résultats de vaccination par arrondissement couvert. Selon la méthode développée par Thomas Bayes au 18ème siècle citée par Goodman (1999), la population canine dans la ville de Ndjamena est estimée à 24.989 têtes. Au total 17.701 chiens, ont été vaccinés lors de cette campagne soit une couverture vaccinale de 70,83%. Hormis les chiens, 1.484

chats et 104 singes ont aussi été vaccinés lors de cette campagne (tab.1).

Les résultats de la campagne de vaccination des chiens sont compilés dans le tableau 2 duquel on remarque que quatre (4) Arrondissements (2 ; 4 ; 8 et 10) ont eu un taux de couverture vaccinale inferieure à 50%.

Discussion

De manière générale, les résultats de la campagne de vaccination antirabique 2012 restent en deçà de l'objectif initial qui est de vacciner 45.000 chiens en 2012 car les résultats du calcul de la population canine pendant

Tableau 1: Nombre de chiens, chats et singes vaccinés par arrondissement de Ndjamena

Arrondissement de Ndjamena	Nombre de chiens vaccinés	Nombre de chats vaccinés	Nombre de singes vaccinés
1 er	951	135	5
2 eme	64	49	3
3eme	376	79	12
4eme	24	108	2
5eme	311	87	1
6eme	919	80	12
7eme	10 628	310	41
8eme	413	107	3
9eme	3858	372	24
10eme	157	157	1
TOTAL	**17701**	**1 484**	**104**

Tableau 2: Résultats de la vaccination par arrondissement

Arrondissement	Population canine estimée	Taux de chiens errants	Nombre de chiens vaccinés	Couverture vaccinale
1	1290	15%	951	73,72%
2	185	1%	64	34,59%
3	511	21%	376	73,58%
4	55	46%	24	43,63%
5	510	10%	311	60,98%
6	1479	8%	919	52,26%
7	1745	12%	10628	73,03%
8	1032	30%	413	40,01%
9	4713	12%	3858	81,85%
10	395	28%	157	39,74%
Total Ndjamena	**24989**	**13%**	**17701**	**70,83%**

l'analyse de couverture montrent que la proportion chien-homme adoptée au préalable pour avoir une idée du nombre de doses à procurer de 1 : 20 était une surestimation. Le nombre total estimé des chiens pour la ville de N'djamena par les analyses menées pendant la campagne est de 24 989 têtes (tabl.2). De ce nombre, 13.% chiens sont sans propriétaires et ne sont donc pas accessibles à la vaccination parentérale. Ces résultats corroborent ceux obtenus par Kayali *et al.,* (2003b) qui ont trouvé que le taux des chiens sans propriétaires varie entre 10 et 15%.

Les résultats des travaux scientifiques effectués par le LRVZ, le Swiss-TPH et le CSSI ont montré qu'en début de l'année 2012, Quatre (4) nouveaux cas de rage canine par mois étaient enregistrés dans la ville de N'Djamena.

Grâce à la campagne de vaccination massive des chiens et autres animaux de compagnie menée d'octobre à décembre 2012, seulement un cas par mois est registré, ceci démontre l'effet positif de la vaccination massive et montre qu'on peut éliminer la rage dans un pays ou sur un territoire donné par la vaccine massive des chiens.

Bien que le taux de couverture vaccinale globale soit environ de 71%, l'analyse des résultats par arrondissement fait ressortir une hétérogénéité de la densité des chiens vaccinés. Les résultats obtenus au Tchad sont comparables à ceux obtenus par Townsend *et al.,* (2013) qui écrivent que Pendant une pareille campagne de vaccination de masse des chiens à Bali (Indonésie), une modélisation a montré que des zones avec faible couverture vaccinale peuvent être à l'origine des foyers ré-émergents de la maladie même si la population canine de ces zones constitue seulement un petit pourcentage du total de la population des chiens. Pour détecter des cas ré-émergents ou d' éventuelles réintroductions du virus, il faut que la surveillance intensive puisse être assurée dans les années à venir.

Nos résultats relatifs au taux de couverture vaccinale corroborent ceux obtenus par De Balogh *et al.,* (1993), Durr S. *et al.,* (2009), Estrada R. *et al.,* (2001) et Kayali *et al.,* (2003b) qui trouvé un taux de couverture vaccinale égale ou supérieure à 70%. Par ailleurs, il a été démontré que même si le taux de couverture vaccinale n'atteint pas les 70%, mais compris entre 60 et 70%, cela induit une réduction substantielle de l'incidence de la rage humaine (Cleaveland *et al.,* 2003).

Contraintes

Quelques contraintes rencontrées ça et là lors du déroulement de la campagne sont ci-après énumérées:

- Indifférence de certains propriétaires des chiens ;
- insuffisance de sensibilisation de la population sur le bienfait de la vaccination antirabique ;
- faible participation de quelques chefs des carrés;
- faible participation des propriétaires des chiens dans la campagne dans quelques arrondissements;
- accès difficile dans certains quartiers de N'Djamena;
- manque de motivation des vaccinateurs (faible montant de per diem);
- retards dans l'installation des postes de vaccination dus à la difficulté d'identification des carrés.

Conclusion

Malgré les difficultés rencontrées durant la campagne de vaccination antirabique 2012, les résultats de la campagne antirabique de 2012 peuvent être considérés comme succès car plus de 70,% de taux de couverture vaccinale est obtenue, ce qui est légèrement au dessus du seuil préconisé par l'Organisation mondiale de la santé animale (OIE) qui est de 70%. Toutefois, il va falloir donc redoubler d'efforts pour accroître ce taux.

Pour la prochaine campagne, il est recommandé d'impliquer suffisamment les maires d'arrondissements afin d'amener les délégués des quartiers et les chefs des carrés à mieux sensibiliser leurs populations respectives.

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DIGESTIBILITÉ IN VIVO DES CHAUMES DE MAÏS TRAITÉS À L'URÉE ASSOCIÉS À DIFFÉRENTS NIVEAUX DE MÉLASSE CHEZ LA CHÈVRE NAINE DE GUINÉE (CAPRA HIRCUS)

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Résumé

Une étude portant sur l'ingestion et la digestibilité in vivo des chaumes de maïs traités à 5% d'urée associés à 0 ; 5 et 10% de mélasse chez la chèvre naine de guinée a été menée entre Septembre 2012 et Janvier 2013. Neuf chèvres ont été réparties en trois lots de trois animaux chacun et logées dans des cages métaboliques individuelles. Les périodes d'adaptation et de collecte de données (urines et fèces) étaient respectivement de 8 et 6 jours. Chaque chèvre recevait par jour, une ration de 700 g de chaumes de maïs traités à 5% d'urée avec inclusion de 0% de mélasse (CM5+M0) pour le lot 1 ; 5% de mélasse (CM5+M5) pour le lot 2 et 10% de mélasse (CM5+M10) pour le lot 3. Chaque matin, les refus de chaque ration étaient pesés. De même, la quantité d'urine et de fèces produit par chaque animal était évaluée. Les échantillons de 100g de chaque ration, de fèces et 100 ml d'urine ont été collectés (chaque matin pendant la période de collecte des données) pour les analyses de laboratoire. Les résultats de cette étude ont montré que le traitement à la mélasse a amélioré la dMO des chaumes de maïs traités à 5% d'urée. L'ingestion de la MS et de la MO des rations CM5+M0 et CM5+M5 étaient comparables et significativement ($p<0,05$) plus élevées que celle de la ration CM5+M10. Les digestibilités apparentes de la MS et de la MO des rations CM5+M0 et CM5+M10 ont été comparables et significativement ($P<0,05$) plus faibles que celles de la ration CM5+M5. La digestibilité des parois cellulaires (NDF) de la ration CM5+M5 a été significativement ($p<0,05$) plus élevée que celle obtenue avec la ration CM5+M10. L'ajout de la mélasse a significativement ($P<0,05$) amélioré la digestibilité de l'azote des rations CM5+M5. Ces résultats nous montrent que l'ajout de 5% mélasse aux chaumes de maïs traités à 5% d'urée améliore leur ingestion et leur digestibilité.

Mots clés: chaumes de maïs, digestibilité, mélasse, petits ruminants.

IN VIVO DIGESTIBILITY OF MAIZE STOVER TREATED WITH UREA ASSOCIATED WITH DIFFERENT LEVELS OF MOLASSES IN WEST AFRICAN DWARF GOAT (CAPRA HIRCUS)

Abstract

A study on intake and in vivo digestibility of maize stover treated at 5% urea associated with 0, 5 and 10 % molasses in West African dwarf goats was conducted between September 2012 and January 2013. Nine West African dwarf goats were divided into three groups with three animals per group and housed in individual metabolic cages. The adaptation and data collection (urine and feces) periods were 8 and 6 days respectively. Each goat received a daily ration of 700 g chopped maize stover treated at 5% urea associated with 0% molasse (CM5+M0) for group 1; 5% molasse (CM5+M5) for group 2 and 10% molasse (CM5+M10) for group 3. Every morning, the refusals (leftovers) of each ration were weighed. Similarly, the amounts of feces and urine from each goat were assessed, collected and weighed. Samples of each treatment, urine and

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feces were collected and taken to the laboratory for chemical composition analysis. Results of this study show that the addition of molasses has increased the level of organic matter digestibility of maize stover treated at 5% urea. The dry matter and organic matter intake of the diet CM5+M0 and CM5+M5 were comparable and significantly (P<0.05) higher than that of the diet CM5+M10. The apparent digestibilities of the dry matter and organic matter of the diets CM5+M0 and CM5+M10 were comparable, and significantly (P<0.05) lower than that of the diet CM5+M5. The digestibility of cells wall (NDF) of the diet CM5+M5 were significantly (P<0.05) higher than that of the diet CM5+M10. The addition of molasses has significantly (P<0.05) increased the digestibility of nitrogen of the diet CM5+M5. These results show that the addition of 5% molasses in maize stover treated at 5% urea increases its intake and digestibility.

Key words: digestibility, maize stover, molasses, small ruminants

Introduction

Au Cameroun, les petits ruminants jouent un rôle socio-économique considérable dans la vie des populations. Ils interviennent dans les rites culturels, traditionnels et religieux des différents peuples (Tendonkeng *et al.,* 2013), constituent une forme d'épargne et sont une source de fertilisant organique pour de nombreux agro-éleveurs (Tendonkeng *et al.,* 2013). Malgré cette importance, la productivité de ces animaux reste médiocre à cause de contraintes principalement sanitaires et alimentaires (Pamo *et al.,* 2004). Dans la région Ouest du Cameroun, le déficit alimentaire pour les petits ruminants est surtout marqué en saison sèche (Pamo *et al.,* 2007). Au cours de cette période, les pâturages naturels qui constituent la base de l'alimentation de ces animaux sont essentiellement constitués de graminées dont la valeur nutritionnelle se détériore rapidement avec l'âge et l'avancée de la saison sèche (Pamo *et al.,* 2007). Cette faible valeur nutritive des fourrages réduit considérablement leur digestibilité et ne permet pas aux animaux d'extérioriser leurs potentialités (Chesworth 1996 ; Pamo *et al.,* 2006 ; Tendonkeng *et al.,* 2011). La productivité demeure par conséquent toujours très faible tant en viande qu'en lait (Tendonkeng *et al.,* 2014).

En outre, l'agriculture pratiquée presque partout dans cette zone produit chaque année des quantités énormes de résidus agricoles comme les chaumes de maïs, les fanes d'arachide et de haricot. Ces résidus de récolte parmi lesquels les chaumes de maïs, qui représentent la majeure partie de l'ensemble de la biomasse végétale produite, peuvent être valorisées dans l'alimentation des ruminants (Aregheore, 1994 ; Boukila *et al.,* 2005 ; Pamo *et al.,* 2007). Malheureusement, les chaumes de maïs sont traitées comme les nuisances environnementales et de ce fait sont brulées ou enfouies dans le sol lors du labour (Aregheore, 1994) et très peu utilisées dans l'alimentation des ruminants. Pourtant, bien traitées ou bien complémentées, ces chaumes de maïs constituent une bonne ressource alimentaire pour les ruminants, particulièrement pendant la saison sèche. Les études antérieures ont montrées que le traitement des fourrages pauvres (chaumes de maïs) à l'urée à un taux de 5% améliore la teneur en azote du fourrage, son ingestion et sa digestibilité (Chenost et Kayouli, 1997 ; Zhang et Yan, 2002; Tesfaye *et al.,* 2006). D'autres études montrent que l'addition de la mélasse aux rations améliore l'ingestion et la digestibilité apparente des principes nutritifs chez les chèvres, augmente la production laitière chez les vaches et assure le maintien d'un bon état corporelle chez des génisses nourries à base de chaume de maïs pendant la saison sèche (Aregheore et Perera, 2004 ; Broderick et Radloff, 2004 ; Dawit *et al.,* 2013). Cependant, bien que de nombreuses études aient montrées que le traitement des chaumes de maïs à 5% d'urée modifierait positivement la valeur nutritive, l'ingestion et la digestibilité de ce fourrage particulièrement pendant les périodes de soudure, aucune étude n'a été menée sur l'effet de l'inclusion de différents niveaux de mélasse sur l'ingestion et l'utilisation digestive de ces chaumes traitées à 5% d'urée. C'est donc pour pallier cette lacune que le présent travail a été initié avec pour objectif d'évaluer l'ingestion et la digestibilité in vivo des chaumes de maïs traitées à 5% d'urée associées

à différents niveaux de mélasse chez la chèvre naine de Guinée.

Matériel et méthodes

Zone d'étude

La présente étude a été réalisée à la Ferme d'Application et de Recherche (FAR) de l'Université de Dschang. La FAR est située à 05°26' latitude Nord et 10°03' longitude Est et à une altitude moyenne de 1410 m. Le climat de la région est équatorial de type camerounien, modifié par l'altitude. Les précipitations varient entre 1500 et 2000 mm par an, et les températures oscillent entre 10°C (juillet- août) et 25°C (février). La saison sèche s'étend de mi-novembre à mi-mars et la saison des pluies de mi-mars à mi-novembre et correspond à la principale période des cultures.

Matériel végétal

Récolte des chaumes de maïs

Les chaumes de maïs ont été récoltés en septembre 2012 dans les parcelles de production de la FAR de l'Université de Dschang, puis hachés à une dimension de 2-3 cm et stockés dans un magasin, avant d'être traités à l'urée.

Traitement des chaumes de maïs à l'urée

A partir du poids des chaumes de maïs à traiter (32 kg), de la matière sèche (MSp = 94,3%), de la quantité d'urée à apporter (N = 1,6 kg) et de la matière sèche finale souhaitée sur le fourrage (MSf = 62%), nous avons déterminé la quantité d'eau (E) nécessaire pour le traitement à l'aide de la formule ci-dessous proposée par Chenost et Kayouli (1997).

$$E = \frac{(100(MSp+N)-MSf(P+N))}{MSf}$$

Où : E = quantité d'eau (en litre) pour dissoudre l'urée nécessaire pour 100 kg de fourrage ;
MSp = matière sèche (%) des chaumes à traiter déterminée après séchage ;
N = quantité d'urée (%) à inclure ;
MSf = matière sèche finale (%) désirée dans les chaumes ;
P = poids de la paille à traiter.

La solution obtenue à partir de l'urée et de l'eau a été mélangée aux chaumes de maïs et emballé à l'aide du papier en polyéthylène puis, incubé pendant trois semaines.

La mélasse liquide était diluée dans une quantité d'eau en fonction de la quantité de chaumes à traiter, à savoir 250 ml d'eau pour 600 g de chaumes (Chenost et Kayouli 1997). Soit 292 ml d'eau pour la dilution de la mélasse à ajouter à la ration quotidienne (700 g de chaumes).

Mesures biologiques

Animaux

Neuf chèvres naines de Guinée adultes vides (17,53 ± 2,25 kg) âgées d'environs deux ans ont été utilisées. Un mois avant le début des essais, tous les animaux ont été déparasités à l'Ivermectine® 1%.

Digestibilité in vivo

Les neuf chèvres étaient réparties en trois lots de trois animaux équilibrés sur le poids. Elles ont été placées dans des cages de digestibilité individuelle munie d'un dispositif permettant de collecter séparément les urines et les fèces. Les urines étaient collectées dans des flacons en verre de 500 ml dans lequel on avait au préalable introduit de l'acide sulfurique (10 %) pour stabiliser l'azote. Chaque lot recevait une des rations suivantes :

CM5+M0 : chaumes de maïs traités à 5% d'urée + 0% de mélasse (Lot 1);

CM5+M5 : chaumes de maïs traités à 5% d'urée + 5% de mélasse (Lot 2) ;

CM5 +M10: chaumes de maïs traités à 5% d'urée + 10% de mélasse (Lot 3).

L'essai était précédé par une période d'adaptation de 8 jours au cours de laquelle chaque chèvre a reçu le premier jour 350 g de ration en fonction du lot. Cette quantité a progressivement augmentée jusqu'à atteindre la totalité de leur ration (700 g) le dernier jour d'adaptation. Pendant la phase de mesure (6 jours), chaque animal recevait 700 g de chaumes de maïs traitées à 5% d'urée et associée à 0, 5 ou

10% de mélasse, correspondant respectivement au lot 1, 2 et 3 et de l'eau à volonté.

Les ingestions des différentes rations ont été calculées par différence entre la quantité offerte et la quantité refusée. Les refus de ont été pesés et retirés chaque matin avant la distribution de la nouvelle ration. Un échantillon de chaque de refus a été prélevé pour déterminer la teneur en MS.

Tous les matins, les fèces produites par chaque animal étaient pesées et les urines mesurées à l'aide d'une éprouvette en verre graduée de 500 ml. Un échantillon de 100 g de fèces était ensuite prélevé et séché à 60°C jusqu'à poids constant dans une étuve ventilée, puis broyé à l'aide d'un broyeur à marteaux munit d'une grille de mailles de 1 mm et conservé pour analyses. Un échantillon de 10 ml d'urine était par la suite collecté et introduit dans des flacons puis conservé à 4°C dans un réfrigérateur en vue de l'analyse de l'azote. Par ailleurs, chaque matin, durant la période d'essai, des échantillons de chaque ration (100g) étaient collectés et séchés à 60°C jusqu'à poids constant dans une étuve ventilée, broyés et conservés pour des analyses chimiques. Les échantillons de chaque ration ont été analysés pour déterminer les teneurs en matière sèche, matière minérale totale, matière organique et matières azotées totales (MAT) par les méthodes AOAC (2000). Les teneurs en NDF et ADF ont été déterminés par la méthode de Van Soest *et al.,* (1991).

Le coefficient d'utilisation digestive (CUDa) de la matière sèche (MS), de la matière organique et des parois cellulaires (NDF) a été calculé avec la formule suivante (Roberge et Toutain (1999) :

$$\text{CUDa MS (\%)} = \frac{(\text{MS ingérée-MS excrétée})}{(\text{MS ingérée})} \times 100$$

$$\text{CUDa MO (\%)} = \frac{(\text{MO ingérée-MO excrétée})}{(\text{MO ingérée}) \times 100} \times 100$$

$$\text{CUDa NDF (\%)} = \frac{(\text{NDF ingéré-NDF excrété})}{(\text{NDF ingéré}) \times 100} \times 100$$

La digestibilité de l'azote a été calculée avec la formule :

$$\text{Digestibilité de l'azote (\%)} = \frac{(\text{Azote ingéré-Azote(fecal+urinaire)excrété})}{(\text{Azote ingéré})} \times 100$$

Les analyses ont également permis de calculer :
dMO (% MS) = - 2,10CB (%MS) + 96,8 (Jarrige, 1980) ;

MAD (g/kgMO) = 0,917MAT (g/kgMO) – 0,0055CB (g/kgMO) – 17,6 (Jarrige, 1980) ;

UFL = 121,80 + 0,11MAT – 1,81CB + 1,26MG (Sauvant, 1981) ;

UFV = 124,15 + 0,06MAT – 2,20CB + 1,22MG (Sauvant, 1981).

Analyses statistiques

Les données d'ingestion et la digestibilité des rations ont été soumises à une analyse de la variance à un facteur (traitement à la mélasse). Lorsque les différences entre les traitements étaient significatives, leurs moyennes étaient séparées par le test de Waller Duncan au seuil de 5%.

Résultats

Composition chimique des différentes rations

L'analyse de la composition chimique des différentes rations montre que les teneurs en parois cellulaires (NDF), lignocellulose (ADF) et matière azotée totale (MAT) ont peu varié avec l'augmentation du niveau de la mélasse dans les rations (Tableau 1). Les teneurs en matière minérale totale et en matière organique (MO) les plus élevées ont été obtenues respectivement avec les rations CM5+M10 et CM5+M5.

Teneur en nutriments des différentes rations

La dMO des chaumes de maïs traités à 5% d'urée a augmenté avec le niveau croissant de traitement à la mélasse (Tableau 2). Par contre, la matière azotée digestible (MAD), les teneurs en UFL et en UFV des chaumes de maïs traités à 5% d'urée a peu varié avec le niveau de traitement à la mélasse.

Ingestion de la MS et de la MO des différentes rations

L'ingestion de la MS et de la MO de la ration CM5+M5 et CM5+M0 ont été comparables (P>0,05) et significativement (P<0,05) plus élevée que celle de la ration CM5+M10 (Figures 1 et 2).

Digestibilité de la MS, de la MO et des parois cellulaires (NDF) des différentes rations

Les digestibilités apparentes (CUDa) de la MS et de la MO des rations CM5+M0 et CM5+M10 étaient comparables (P>0,05) et significativement (P<0,05) plus élevés que celles de la ration CM5+M5 (Tableau 3). La digestibilité des parois cellulaires de la rations CM5+M0 (59,36%) était comparable (P>0,05) à celle de la ration CM5+M5. Par contre, les digestibilités des parois cellulaires des rations CM5+M5 et CM5+M0 ont été significativement (P<0,05) plus élevées que celle de la ration CM5+M10.

Digestibilité de l'azote des différentes rations

La quantité d'azote ingéré et retenu de la ration CM5+M5 a été plus élevée que celle obtenue avec les rations CM5+M0 et CM5+M10 (Tableau 4). Les quantités d'azote ingéré et fécal des rations CM5+M0 (2,44 et 1,46 g/j) et CM5+M5 (2,35 et 1,11 g/j) ont été comparables (P > 0,05) et significativement plus élevées (P < 0,05) que celle de la ration CM5+M10. L'ajout de la mélasse n'a pas influencé de manière significative (P>0,05) les quantités d'azote urinaire excrétés. La quantité d'azote retenu et la digestibilité de l'azote de la ration CM5+M5 (0,87 g/j et 69,87% respectivement) ont été significativement plus élevées (P < 0,05) que celle des rations CM5+M0 (0,31 g/j et 40,60% respectivement) et CM5+M10 (0,36 g/j et 46,31% respectivement) qui étaient par ailleurs comparables (P > 0,05).

Tableau 1: Composition chimique des différentes rations

	Rations		
	CM5+M0	**CM5+M5**	**CM5+M10**
MS (%)	94,9	94,8	95,4
% MS			
Matière minérale totale	11,4	10,5	12,7
Matière organique (MO)	83,5	84,3	82,8
Matière azotée totale (MAT)	9,6	9,2	9,1
Parois cellulaires (NDF)	77,3	70,9	68,2
Lignocellulose (ADF)	54,6	51,9	47,8

Tableau 2: Teneurs en nutriments des différentes rations

	Rations		
	CM5+M0	**CM5+M5**	**CM5+M10**
dMO (%MS)	23,9	25,3	38,4
MAD (g/100gMOD)	8,6	8,0	8,2
UFL/kgMS	0,6	0,6	0,7
UFV/kgMS	0,5	0,5	0,6

Tableau 3. Digestibilité de la MS, de la MO et des NDF des différentes rations.

Digestibilité apparente (%)	Rations				
	CM5+M0	**CM5+M5**	**CM5+M10**	**SEM**	**P**
Matière sèche (MS)	40,1^{b}	56,3^{a}	28,2^{b}	4,66	0,014
Matière organique (MO)	42,5^{b}	59,8^{a}	31,9^{b}	4,67	0,014
Paroi cellulaire (NDF)	59,4^{a}	67,9^{a}	44,9^{b}	3,73	0,006

^{a,b,c} les moyennes portant la même lettre sur la même ligne sont statistiquement comparables au seuil de 5%. SEM = standard error of means; P = probabilité.

Tableau 4: Digestibilité de l'azote des différentes rations chez la chèvre naine de Guinée

Bilan azoté (g/j)	**Rations**				
	CM5+M0	**CM5+M5**	**CM5+M10**	**SEM**	**P**
Azote ingéré	2,44[a]	2,35[a]	2,07[b]	0,06	0,003
Azote fécal	1,46[a]	1,11[a]	0,71[b]	0,12	0,009
Azote urinaire	0,67[a]	0,78[a]	0,59[a]	0,05	0,371
Azote retenu	0,31[b]	0,87[a]	0,36[b]	0,10	0,017
Digestibilité de l'azote	40,60[b]	69,87[a]	46,31[b]	4,99	0,008

[a,b]: les moyennes portant la même lettre sur la même ligne sont statistiquement comparables au seuil de 5%. SEM = standard error of means; P = probabilité.

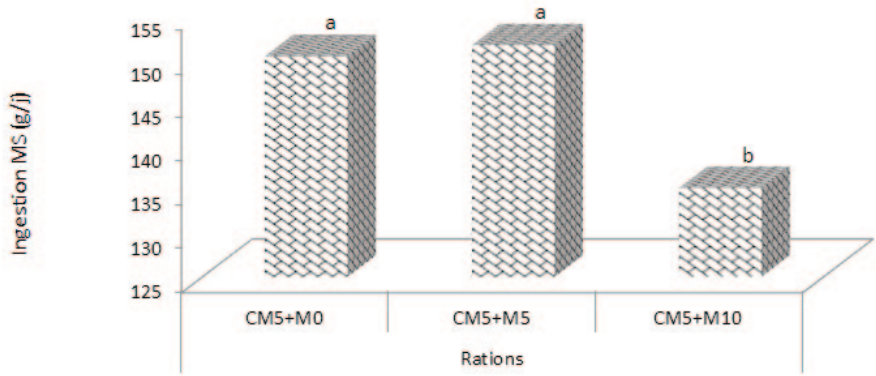


Figure 1: Ingestion de MS des différentes rations.

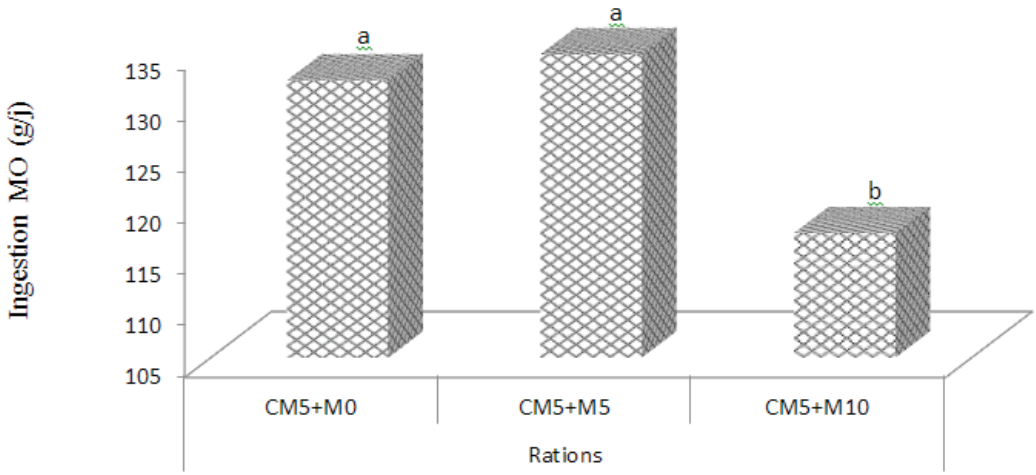


Figure 2: Ingestion de la MO des différentes rations.

Discussion

Les teneurs en MO et MAT ont peu varié avec l'ajout de la mélasse aux chaumes de maïs traitées à 5% d'urée. Ces résultats sont semblables à ceux obtenus par Matumuini *et al.,* (2013) et Lemoufouet *et al.,* (2014). L'ajout de la mélasse a légèrement amélioré les teneurs en UFL et UFV des chaumes de maïs traitées à 5% d'urée. Ces résultats corroborent l'assertion selon laquelle, la mélasse améliore la valeur énergétique des rations (Devun *et al.,* 2011, Dawit *et al.,* 2013 ; Matumuini *et al.,* 2013).

La quantité de MS ingérée de la ration CM5+M5 a été supérieure à celle obtenue par Woyengo *et al.,* (2004) chez le mouton et Aregheore et Perera (2004) et chez la chèvre, lorsque ces auteurs traitaient les chaumes de maïs respectivement à 4 et 3,6% d'urée. Elle est par contre inférieure à celle obtenue Lemoufouet *et al.,* (2014) avec les chaumes de maïs traités à 5% d'urée additionnées à 5% de mélasse chez les moutons. Ce taux d'inclusion de mélasse qui donne la meilleure ingestion (5%) se trouve dans la marge recommandée (4 - 6%) par Broderick et Radloff (2004). En effet, la mélasse est un concentré d'extrait de canne à sucre riches en minéraux (Ca, P, K…), vitamines et sucres (saccharose, glucose et fructose); ces nutriments auraient contribués à améliorer l'appétence de la ration (Aregheore et Perera, 2004 ; Matumuini *et al.,* 2013). Il semble en effet que les sucres apportés par la mélasse et l'azote fournit par les chaumes de maïs ont fermenté rapidement dans le rumen, fournissant ainsi une plus grande quantité d'énergie et d'azote aux micro-organismes, ce qui aurait accéléré la dégradation des chaumes et facilité le passage des digesta du rumen-réseau vers le feuillet, augmentant ainsi l'ingestion (Azizi-Shotorkhoft *et al.,* 2013 ; Lemoufouet *et al.,* 2014 ; Tendonkeng *et al.,* 2014). Par contre, la faible ingestion de la ration CM5+M10 pourrait s'expliquer par le séjour prolongé de ces dernières rations dans le rumen, à cause d'un apport excessif d'énergie. En effet de nombreux auteurs (Morales *et al.,* 1989 ; Broderick et Radloff, 2004) rapportent qu'un apport de mélasse au-delà de 6 – 8% de la ration contribue à réduire l'ingestion de cette dernière.

Les digestibilités de la MS et de la MO de la ration CM5+M5 ont été similaires à ceux obtenus par Aregheore et Perera (2004) avec les chaumes de maïs traités à 3,6% d'urée avec addition de 4% de mélasse chez les chèvres. La digestibilité des parois cellulaires de la ration CM5+M5 a été supérieure à celle obtenue par Aregheore et Perera (2004) chez les chèvres et Lemoufouet *et al.,* (2014) chez les moutons. La digestibilité de l'azote de la ration CM5+M5 a été supérieure à celle observé par Aregheore et Perera (2004) avec les chaumes de maïs traités à 3,6% d'urée avec l'addition de 4% de mélasse chez les chèvres. Cette différence s'expliquerait par l'inclusion des taux différents d'urée et de mélasse (5 et 4% d'urée et, 4 et 5% de mélasse respectivement). En effet, que l'ajout de mélasse (5%) induit une meilleure activité cellulolytique de la microflore du rumen, ce qui améliore la dégradation des chaumes de maïs et le passage des digesta du rumen-réseau vers le feuillet (Chenost et Kayouli, 1997 ; Matumuini *et al.,* 2013, Lemoufouet *et al.,* 2014). En outre, l'inclusion de la mélasse fournit l'énergie et le squelette carboné nécessaire aux micro-organismes du complexe rumen-réseau pour assurer la dégradation des fourrages (Chestworth, 1996), avec pour conséquence, une amélioration de la digestibilité des nutriments. La digestibilité de la MS, MO et des parois cellulaires des chaumes de maïs traitées à 5% d'urée à baissée avec l'ajout de 10% de mélasse. La faible digestibilité cette ration CM5+M10 est probablement due à l'excès de sucre apporté par la ration qui limiterait l'utilisation optimal de l'azote. En effet, ce taux élevé de mélasse aurait probablement neutralisé l'ammoniac issu de la transformation de l'urée (uréolyse) et par conséquent, aurait contribué à réduire le degré de rupture de liaisons lignine-hémicellulose-cellulose de cette ration. La même observation a été faite par Lapierre et Bernier (1996), Broderick et Radloff (2004), Dawit *et al.,* (2013) et Lemoufouet *et al.,* (2014).

Conclusion

Au terme de cette étude, il apparait que l'ajout de la mélasse aux chaumes de maïs traités à 5% d'urée a amélioré la dMO. L'ingestion des chaumes de maïs traitées à 5% d'urée a été meilleure avec l'ajout de 5% de mélasse. La digestibilité de la MS, de la MO et des parois cellulaires des chaumes de maïs traitées à 5% d'urée a été significativement ($P<0,05$) améliorée lorsque la mélasse a été ajoutée à 5%. L'ajout de 5% de mélasse aux chaumes de maïs traitées à 5% d'urée a significativement ($P<0,05$) améliorée la rétention d'azote et sa digestibilité chez la chèvre naine de Guinée.

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PRESUMPTIVE DIAGNOSIS OF *AVIAN ENCEPHALOMYELITIS* IN JAPANESE QUAIL IN IBADAN, NIGERIA

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Abstract

A report of *Avian encephalomyelitis* outbreak in two flocks of adult Japanese quail is presented. High mortalities, tremor, ataxia and lateral recumbency were the prominent clinical signs observed. Absence of gross pathology and microscopic lesions of gliosis, neuronal degeneration, meningitis, congested blood vessel with perivascular cuffing, suggestive of acute to subacute encephalitis gave a presumptive diagnosis of Avian encephalomyelitis. However, this outbreak in adult birds, being the first in Japanese quail in Nigeria, is at variance with previous reports on the disease occurring in young flocks. It is believed that the relative small body size of the Japanese quail is a contributory factor to their susceptibility even as adult birds. Routine vaccination is therefore recommended.

Keywords: Avian Encephalomyelitis, Japanese quail, Clinical signs, Pathology, Nigeria.

DIAGNOSTIC PRÉSOMPTIF DE LA GRIPPE ENCÉPHALOMYÉLITE CHEZ DES CAILLES JAPONAISES À IBADAN (NIGERIA)

Résumé

Le présent rapport fait état d'un foyer d'encéphalomyélite aviaire chez deux troupeaux de cailles japonaises adultes. Les principaux signes cliniques observés chez ces troupeaux étaient les suivants : de fortes mortalités, des tremblements, une ataxie et un décubitus latéral. L'absence d'observations pathologiques macroscopiques et de lésions microscopiques de gliose, la dégénérescence neuronale, la méningite, la congestion des vaisseaux sanguins avec manchon périvasculaire, évocatrices d'encéphalite aiguë et subaiguë, ont conduit à un diagnostic présomptif d'encéphalomyélite aviaire. Cependant, cette épizootie des oiseaux adultes, apparue pour la première fois chez des cailles japonaises au Nigeria, est en contradiction avec les rapports antérieurs selon lesquels la maladie affecte normalement de jeunes troupeaux. L'on pense que la taille relativement petite du corps de la caille japonaise est un facteur qui contribue à sa sensibilité même chez l'oiseau adulte. La vaccination systématique est donc recommandée.

Mots-clés : encéphalomyélite aviaire, caille japonaise, signes cliniques, pathologie, Nigeria.

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Introduction

Japanese quials (Coturnix japonica) are hardy small birds that are reared in cages and are susceptible to common diseases of poultry but are believed to be fairly disease resistant (Randall and Bolla, 2008). They mature in about six weeks, an age when other poultry species such as chickens and turkeys and ducks are considered as young. The Coturnix is highly prolific, it is able to produce three to four generations per year, making it an interesting breeding bird as is currently observed in Nigeria.

Avian encephalomyelitis (AE) is an infectious viral disease of young chickens, pheasants, pigeons, Japanese quail, and turkeys, ducklings, patridges (Bodin *et al.,* 1981; Toplu and Alcigir, 2004; Welchman *et al.,* 2009). It is a disease of the central nervous system exhibiting clinical signs of ataxia and tremors of the head, neck and limbs, thus the name, epidemic tremor. Clinical signs are usually accompanied by high morbidity and variable mortality. Drop in egg production is usually the only clinical sign observed in layers (Calnek, 2008). *Avian encephalomyelitis* virus is a member of the Picornaviridae family and was temporarily classified as an Hepatovirus due to its relatedness to hepatitis A virus (Marvil *et al.,* 1999; Calnek, 2008). However, the virus has recently been re-classified as a Tremovirus and only one serotype exists i.e. AEV-1 (Calnek, 2008).

AE occurs worldwide including Africa (Adene *et al.,* 1976; Cadman *et al.,* 1994; Abdellah *et al.,* 2007). In Nigeria, Adene *et al.,* (1976) isolated the virus while Oladele and Onwuka (2013) reported antibody detection in chicken breeder flocks post-vaccination. Natural infections has been reported in turkey flocks (Dovadola *et al.,* 1973) and in quail chicks that were hatched from eggs laid during an outbreak (Hill and Raymond, 1962).

Case Report

History

A report of high mortalities was made at the Avian Clinic of the Veterinary Teaching Hospital, University of Ibadan, Ibadan, Nigeria by the management of a Japanese quail (Couturnix japonica) farm located also at Ibadan in November, 2013. The farm had two flocks of quail aged 12 and 78 weeks with flock sizes of 760 and 525 respectively. Farmer reported persistent mortalities of between 15 and 20 daily from both flocks for up to one week.

Clinical findings

On visiting the farm, morbidity of about 30% on the average was observed in both flocks with anorexia. Ataxia, prostration and fine tremors of head and limbs of quails were observed in about 20% the birds. Test for landing reflex on the recumbent birds revealed leg paralysis and there was about 22% reduction in egg production in both flocks (Plate 1).

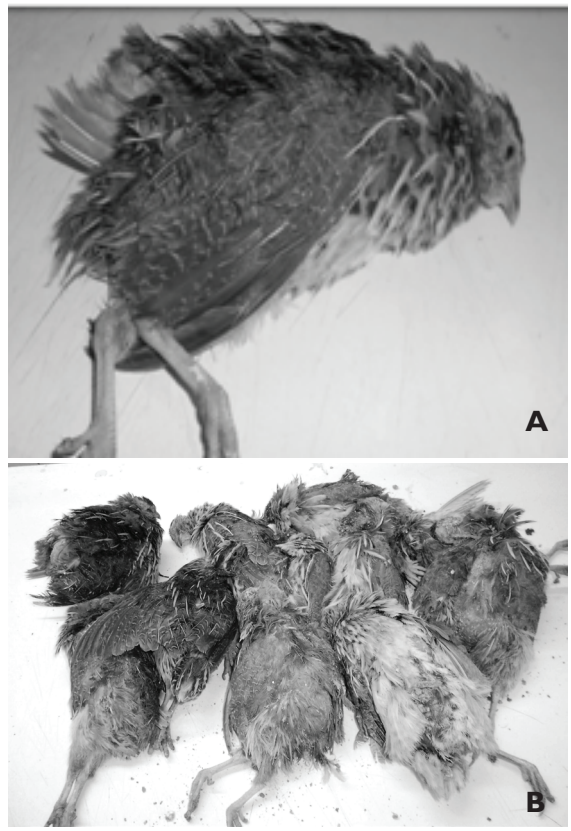


Plate 1: A, moribund Japanese quail presented by farmer showing lateral recumbency and paralysis; B, some mortalities experienced on farm.

Pathology

Post mortem examination of the carcasses revealed no visible lesion. A tentative diagnosis of *Avian encephalomyelitis* was made with a differential diagnosis of Vitamin E deficiency. Tissue samples were harvested from the brain,

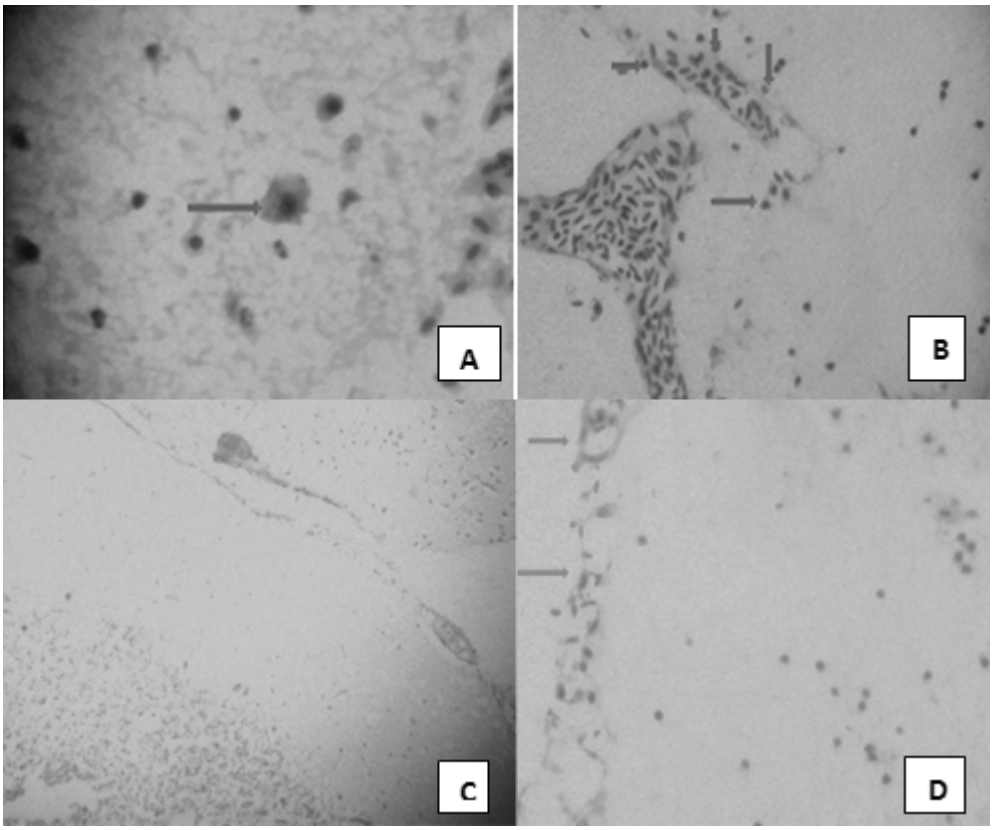


Plate 2: Photomicrographs of cerebrum and cerebellum. H&E stain.
A, neuronal chromatolysis (arrow) with diffuse gliosis in cerebrum (x400); B, congested blood vessels(arrows) in the meninges of cerebellum(x100); C, congested meninges and diffuse gliosis in cerebellum (x100); D, lymphocytic infiltrations into cerebellar meninges (arrows) and aggregates of lymphocytes in molecular layer - (x100).

fixed in 10% neutral buffered formalin and routinely processed for paraffin embedding. Sections of 4µm thickness were stained with hematoxylin and eosin for microscopic examination.

Microscopically, there was evidence of acute to subacute encephalitis, foci of loose gliosis, moderately congested vessels with lymphocytic perivascular cuffing and central chromatolysis of neuronal cell bodies in the cerebrum and cerebellum. There was also focal necrosis of the purkinje layer of the cerebellum involving purkinje cells as well as infiltration of lymphocytes into the meninges. (Plate 2).

Discussion

Avian encephalomyelitis (AE) is known to be a disease of young chickens, pheasants, pigeons, Japanese quail, and turkeys, ducklings, patridges (Bodin *et al.,* 1981; Toplu and Alcigir, 2004; Welchman *et al.,* 2009). Clinical disease has not been reported in adult birds but they could be infected showing reduction in egg production. The history, clinical findings, absence of gross pathology and histopathological lesions observed in this outbreak are characteristic of horizontally transmitted *Avian encephalomyelitis* (Calnek, 2008) except for the age of outbeak. Findings from this outbreak are similar to those reported by Hill and Raymond (1962) in quail chicks. It is worthy of note that this is the first report of the disease in Japanese quail in Nigeria.

Recently, there has been an upsurge in the domestication of Japanese quail in Nigeria after the initial introduction by the Nigeria Veterinary Research Institute, Vom, in 1992. Intensification in rearing has been accompanied by health challenges with little or no disease

control measures instituted by either Veterinary authorities or individual farmers in the country. However, the age of the flocks at the time of this outbreak is worthy of consideration. It is believed that the dose of infection relative to the size of the birds played a major role in this outbreak. A recent study in Southwest Nigeria by Oladele and Onwuka (2013) revealed inefficiency of AE virus maternal antibody transfer to progeny among breeder flocks which could be responsible for the apparent poor control of *Avian encephalomyelitis* in chicken flocks in this region, evident by incessant outbreaks. The Japanese quail is a small-sized bird even as adults. The adult male weighs between 100 and 140g while the female weighs between 120 and 160g (Randall and Bolla, 2008). This genetic trait coupled with apparent endemicity of the disease in southwest Nigeria is believed to be responsible for this outbreak.

This report is made in order to stimulate institution of health care measures for Japanese quails by veterinary authorities in tropical environment particularly in the face of intensification of rearing.

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COMPARATIVE STUDIES ON THE HAEMATOLOGICAL AND SERUM BIOCHEMICAL CHANGES IN SINGLE AND MIXED *TRYPANOSOMA BRUCEI* AND *TRYPANOSOMA CONGOLENSE* INFECTIONS IN RABBITS.

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Abstract

The haematological and serum biochemical changes were studied in rabbits infected with *Trypanosoma brucei*, *Trypanosoma congolense* and mixed infection. Twenty male chinchilla x New Zealand white cross bred rabbits were divided into 4 groups (A – D) of 5 rabbits each with group A infected with *T brucei*, group B; infected with *T congolense*, group C infected with mixed *T brucei* and T Congolese and D served as the uninfected control. The infection was well established by day 4 post-infection (PI). The infections led to decrease in packed cell volume, haemoglobin concentration and erythrocyte count and increased total leucocyte count. The *T congolense* infected group maintained higher PCV and Hb values than the *T brucei* only and mixed infected groups. The total leucocyte count increased significantly ($p<0.05$) following infection and on day 21 PI, the *T brucei* infected group was significantly ($p<0.05$) higher than other groups. However, the increase in leucocyte reversed from day 28 PI. There was decrease in mean creatinine levels with the *T brucei* infected group being significantly ($p<0.05$) lower than the control on days 14, 21 and 28 PI but did not differ significantly with mixed and *T congolense* only groups. The *T brucei* and *T congolense* infected groups had increased in mean urea values following infection and were significantly ($p<0.05$) higher than mixed and control groups on days 21 and 28 PI. The mean AST and ALT values increased in all infected groups following infection and on day 35 PI, the infected groups were significantly ($p<0.05$) higher than the control. The results showed variations in pathogenesis of trypanosome species in rabbits.

Key words: rabbit, trypanosomes, pathogenesis, haematology, serum biochemical,

ETUDES COMPARATIVES DES MODIFICATIONS HÉMATOLOGIQUES ET BIOCHIMIQUES SÉRIQUES DANS LES INFECTIONS SIMPLES ET MIXTES À TRYPANOSOMA BRUCEI BRUCEI ET TRYPANOSOMA CONGOLENSE CHEZ LES LAPINS

Résumé

Les modifications hématologiques et biochimiques sériques ont fait l'objet d'une étude chez des lapins infectés respectivement avec Trypanosoma brucei, Trypanosoma congolense, et les deux organismes. Vingt lapins mâles croisés chinchilla x blancs de Nouvelle-Zélande ont été répartis en 4 groupes (A - D) de 5 lapins chacun : le groupe A a été infecté avec T brucei, le groupe B avec T congolense, le groupe C avec les deux organismes T brucei et T congolense à la fois, le groupe D servant de témoin non infecté. Au 4ème jour post-infection (PI), l'infection était bien établie. Les infections ont engendré une diminution des taux d'hématocrite (PCV), du taux d'hémoglobine (Hb) et de la numération érythrocytaire, et une augmentation du nombre total de leucocytes. Le groupe infecté avec T congolense a maintenu des taux de PCV et de Hb plus élevés que ceux des groupes infectés uniquement avec T brucei et les deux pathogènes associés. La numération leucocytaire totale a augmenté de manière significative ($p <0,05$) après l'infection et au jour 21 PI ; celle du groupe infecté par T brucei était beaucoup ($p <0,05$) plus élevée que celles des autres groupes. Cependant, l'augmentation des leucocytes s'est inversée à partir du jour 28 PI. On a noté une diminution des taux moyens de créatinine, le groupe infecté avec T brucei ayant un taux significativement ($p <0,05$) plus faible que le groupe témoin aux jours 14, 21 et 28 PI mais sans différence significative avec les groupes soumis à l'infection mixte et à T congolense seulement. Chez les groupes infectés avec T brucei et T congolense,

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on a noté une augmentation des valeurs moyennes élevées de l'urée après l'infection, lesquelles étaient significativement (p <0,05) plus élevées que celles du groupe soumis à l'infection mixte et du groupe témoin aux jours 21 et 28 PI. Les taux moyens des enzymes hépatiques AST et ALAT ont augmenté chez tous les groupes infectés après l'infection et au jour 35 PI, les groupes infectés avaient des taux significativement (p <0,05) plus élevés que le groupe témoin. Les résultats ont montré des variations dans la pathogenèse des espèces de trypanosomes chez les lapins.

Mots-clés : lapin, trypanosomes, pathogénèse, hématologie, sérum, biochimique

Introduction

The human population growth in developed countries is stabilizing while that of developing countries like Nigeria is rapidly increasing; this leads to low animal protein intake in lesser-developed countries than in developed countries (Mailafia *et al.,* 2010). Thus, the search for alternative sources of protein to meet up with the population challenge is imperative. Economic indices indicate that as human population continues to increase, agricultural outputs also need to be increased to meet the protein needs of the growing population rather than food importation into such countries (Allen, 1993). In Nigeria, consumption of animal protein remains low at about 6.0-8.4 g/head/day which is far below the 13.5g per day prescribed by the WHO (Egbunike, 1997).

In order to maximize food production and meet protein requirements in Nigeria, viable options need to be explored and evaluated (Owen *et al.,* 2008). Among such alternatives is the use of microlivestock species that are yet to play a major role in animal production within these countries. Micro-livestock such as the rabbit, guinea pig, grass-cutter, giant rat, iguana and pigeons have been suggested (Vietmeyer, 1984) as a rapid means of obtaining animal proteins.

Rabbits are adjudged to be a convenient source of palatable and nutritious meat, high in protein, and contain low fat and cholesterol. A doe can produce more than 15 times her own weight in offspring in a year. Also, rabbits (Oryctolagus cuniculus) are fast-growing micro-livestock, and as such, appears to be the most sustainable means of producing high quality animal protein for the increasing populations of the Less Developed Countries like Nigeria. These attributes could be possible because rabbit has immense potentials and good attributes which include small-bodied size, high growth rate, high efficiency in converting forage to meat, short gestation period, and high prolificacy, relatively low cost of production they adapt over a wide range of ecological environments with high nutritional quality of meat, easily digestible, with low fat, sodium, and cholesterol levels. It also has a high protein level of about 20.8% and its consumption is bereft of cultural and religious biases (Biobaku and Oguntona, 1997, Omole *et al.,* 2005).

However, rabbit farming, especially in the tropic, is faced with myriad of problems, which has resulted in a gross shortage of meat to meet up the population challenge in our country (Nworgu, 2007). This includes climate, reproduction and diseases. African animal trypanosomosis remains a very important disease of domestic livestock in sub Saharan Africa, it affects the health and productivity of livestock (Mattioli *et al.,* 2004). The disease is most important in cattle but causes serious economic losses in other micro-livestock and macro-livestock. OIE (2005) reports that trypanosomes are able to infect a wide variety of domestic animals and about 30 species in the wild. Reports havee experimentally shown that trypanosomes can infect rabbits (Takeet and Fagbemi, 2009; Umar, 2010), with increasing commercial rabbit production; rabbit trypanosomosis will be one of the major disease problems that will hinder production.

This study was, therefore, to assess the effect of trypanosome infection as a single or mixed infection in rabbits.

Materials and Methods

Experimental animals: Twenty male chinchilla x New Zealand white cross bred rabbits aged 6-8 months and weighing between 1.5 and 2kg were used for the study. They were housed in a standard rabbit house that precluded access by flies and other haematophagous insects in the

Department of Veterinary Medicine Laboratory Animal House, University of Nigeria, Nsukka. The animals were dewormed with Albendazole (Tyll Pharm .Int .Ltd, Nigeria) at 25mg per kg body weight orally. Oxytetracycline long acting (Tetroxyl®) was administered at 22mg/kg body weight while sulphaquinoxalin was administered orally at 15mg/kg body weight for seven days. They were screened for haemoparasites within the period of acclimatization which lasted for four weeks using wet smear and buffy coat techniques (Murray *et al.,* 1977). They were fed ad libitum with grower mash and water. The study was in compliance with the ethical procedure of the Animal Use and Care Committee, Faculty of Veterinary Medicine, University of Nigeria, Nsukka which corresponds with NIH guidelines (NIH, 1996).

Experimental Design: The rabbits were assigned into four groups (A - D) of five rabbits each. They were housed separately and treated as follows; group A infected with T b. brucei; group B; with *T congolense*; group C with mixed *T brucei* and *T Congolense* and group D served as the uninfected control. The *Trypanosoma b.* brucei and *T congolense* used were obtained from the Nigeria Institute for *Trypanosomiasis* Research (NITR), Vom, Plateau State Nigeria. They were originally isolated from cattle and were maintained in liquid nitrogen. At the end of the experiment, the animals were treated with 7 mg/kg body weight Dimivet® (DD), a diminazine diaceturate, manufactured by SKM Pharmaceutical PVT ltd., Bangalore-560001, India.

Infection of experimental animals: Rabbits in groups A and B were each infected through intra-peritoneal route with 2.00 X 106 trypanosomes of *Trypanosoma b.* brucei and *T congolense* stock respectively. Rabbits in group C were each infected with 1.00 X106 *Trypanosoma b.* brucei and 1.00 X 106 *Trypanosoma congolense* mixed together as mixed infection, also through intra-peritoneal route.

Collection of blood samples: Blood was collected from each of the rabbits in each group by venipuncture of the ear vein at day 0 and subsequently at 7 days interval till the end of the experiment. About 0.3 ml of the collected blood was put in an EDTA bottle for haematology, while the remainder was allowed to clot or centrifuged at 3000 rpm to separate the serum for biochemical test.

Heaematological studies: The parked cell volume (PCV) was determined by haematocrit centrifugation technique (Jain, 1986). Haemoglobin concentration was measured spectrophotometrically by the cyanomethaemoglobin method (Jain, 1986) using SP6-500UV spectrophotometer (PYE UNICAM, England). The RBC and total WBC count were determined using the improved Haemocytometer.

Biochemical studies: The concentration of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the plasma samples were determined spectrophotometrically using the method of Reitman *et al.,* (1957). The level of alkaline phosphotase (ALP) in the plasma was determined as described by Omotainse *et al.,* (1994) .The total plasma protein was estimated using the biuret method as described by (Reinhold, 1953).

Statistical Analysis

Table 1: Weekly mean PCV (%) of rabbits infected with either T. brucei brucei or T. congolense or T. brucei brucei and T. congolense.

Groups / Days	A *T. brucei*	B *Mixed Infection*	C *T congolense*	D *Uninfected cotrol*
0	36.5 ± 0.96	37.3 ± 1.12	36.8 ± 0.35	35.8 ± 1.01
7	35.5 ± 1.50	36.0 ± 1.97	36.4 ± 1.09	35.7 ± 1.06
14	33.0 ± 1.52[a]	33.5 ± 1.07[a]	34.8 ± 0.73[a]	36.2 ± 1.09[b]
21	32.6 ±2.47	33.0 ± 2.56	34.9 ± 1.29	35.8 ± 1.48
28	29.2 ±1.14[a]	30.2 ± 2.34[a]	32.5 ± 1.66[ab]	34.9 ±2.54[b]
35	24.6 ± 2.13[a]	26.2 ±2.01[a]	29.6 ± 1.46[a]	35.0 ±1.79[b]

[a,b,c] Different superscripts in a row indicates significant difference between the means at the level of probability: $p < 0.05$; PS means post supplementation.

Table II: Weekly mean haemoglobin concentration () rabbits infected with either *T. brucei brucei* or *T. congolense* or *T. brucei brucei* and *T. congolense*.

Days \ Groups	A	B	C	D
	T. brucei	***Mixed Infection***	***T congolense***	***Uninfected cotrol***
0	10.23 ± 0.56	10.85 ± 0.71	11.63 ± 1.4	10.93 ± 0.59
7	9.55 ± 1.50	8.98 ± 0.48	9.55 ± 0.61	11.85 ± 1.28
14	7.13 ± 0.45[a]	7.73 ± 0.67[a]	9.28 ± 1.16[a]	10.85 ± 0.74[b]
21	7.43 ± 1.06[a]	8.25 ± 1.11[a]	9.33 ± 1.39[a]	10.70 ± 0.71[b]
28	8.01 ± 0.98[a]	8.21 ± 1.20[a]	8.79 ± 2.16[a]	11.04 ± 1.02[b]
35	7.12 ± 1.22[a]	7.62 ± 0.98[a]	7.04 ± 0.92a	11.24 ± 1.04[b]

[a,b,c] Different superscripts in a row indicates significant difference between the means at the level of probability: $p < 0.05$; PS means post supplementation.

Table III: Weekly mean leucocyte count (x 106) rabbits infected with either *T. brucei brucei* or *T. congolense* or *T. brucei brucei* and *T. congolense*.

Days \ Groups	A	B	C	D
	T. brucei	***Mixed Infection***	***T congolense***	***Uninfected cotrol***
0	9.93 ± 0.17	9.65 ±0.29	10.70 ±0.67	8.88 ± 1.83
7	12.19 ± 0.13[a]	11.43 ± 0.81[a]	11.62 ± 0.56[a]	9.68 ± 0.92[b]
14	12.64 ± 1.02[a]	13.22 ± 1.12[a]	12.89 ±1.28[a]	9.24 ± 1.04[b]
21	11.08 ± 1.26	11.42 ± 1.54	10.98 ± 1.25	8.98 ± 1.42
28	10.56 ± 1.98	9.96 ± 1.44	10.02 ± 1.23	9.06 ± 1.44
35	7.98 ± 1.02[a]	6.65 ± 1.42[a]	7.46 ± 1.04[a]	9.84 ± 0.96[b]

[a,b,c] Different superscripts in a row indicates significant difference between the means at the level of probability: $p < 0.05$; PS means post supplementation.

Means of all the parameters were compared by One-way analysis of variance (ANOVA) and variant means were separated by Duncan's multiple range test. Probability values less than or equal to 0.05 ($p \leq 0.05$) were considered significant

Results

The infection of rabbits with *Trypanosoma b.* brucei and *Trypanosoma congolense* as single and mixed infections was well established by day 5 post infection (PI) with the mean pre-patent period of 4.42 ± 0.18, 5.04 ± 0.24 and 4.86 ± 0.30 days for groups A, B and C respectively.. Following infection, the packed cell volume (PCV), haemogloin (hb) and erythrocyte counts decreased gradually. On day 14 and 35 PI, the PCV values (Table 1) of the infected groups were significantly ($p<0.05$) lower than the uninfected control. The *T congolense* infected group maintained relatively higher PCV values than the T b. brucei only and mixed infected groups. The Hb values (Table II) of the infected groups decreased following infection with T b. brucei only and mixed infection groups being significantly ($p>0.05$) lower than the uninfected control on days 14, 21, 28 and 35 PI respectively. The total erythrocyte count followed the same pattern with PCV and haemoglobin.

The total leucocyte count increased significantly ($p<0.05$) following infection (Table III). From day 7 PI the infected groups maintained significantly higher leucocyte count when compared with the uninfected groups. However, on day 35 PI, the leucocyte count declined significantly, with the leucocyte count of the infected groups being lower than their pre-infection values. Also, the infected groups were significantly ($p<0.05$) lower than uninfected groups on day 35 PI.

The infection of rabbits with T b. brucei, *T*

congolense and mixed T b. brucei and *T congolense* did not lead to significant ($p > 0.05$) variation in mean creatinine levels when compared with the control (Table IV). The infection led to increase in mean urea values (Table V). The T b. brucei and *T congolense* infected groups had increased in mean urea values following infection. The two groups were significantly ($p<0.05$) higher than mixed and control groups on days 21 and 28 PI. The mixed infection maintained mean urea values comparable to the control through the experimental period.

The mean AST and ALT values increased in all infected groups following infection. This was, however not significant. However, by day 35 PI, AST values of the infected groups were higher than the control. Also, the mean AST of mixed infection was significantly lower than

Table IV: Weekly mean serum creatine (mg/dl) of rabbits infected with either *T. brucei brucei* or *T. congolense* or *T. brucei brucei* and *T. congolense*.

Groups / Days	A	B	C	D
	T. brucei	***Mixed Infection***	***T congolense***	***Uninfected cotrol***
0	0.88 ± 0.08	0.78 ± 0.05	0.86 ± 0.05	0.78 ± 0.08
7	0.71 ± 0.04	0.79 ± 0.06	0.81 ± 0.06	0.72 ± 0.02
14	0.75 ± 0.06	0.67 ± 0.10	0.76 ± 0.04	0.79 ± 0.03
21	0.95 ± 0.02	0.77± 0.16	0.80 ± 0.07	0.78 ± 0.06
28	0.74 ± 0.04	0.75 ± 0.09	0.70 ± 0.07	0.78 ± 0.04
35	0.68 ± 0.08	0.72 ± 0.04	0.78 ± 0.06	0.79 ± 0.07

[a,b,c] Different superscripts in a row indicates significant difference between the means at the level of probability: $p < 0.05$; PS means post supplementation.

Table V: Weekly mean serum urea (mg/dl) of rabbits infected with either *T. brucei brucei* or *T. congolense* or *T. brucei brucei* and *T. congolense*.

Groups / Days	A	B	C	D
	T. brucei	***Mixed Infection***	***T congolense***	***Uninfected cotrol***
0	20.00 ± 1.83	19.50 ± 2.50	20.05 ± 2.34	19.25 ± 1.18
7	22.25 ± 2.66	20.50 ± 171	21.75 ± 1.93	19.25 ± 0.48
14	22.75 ± 2.06	19.75 ± 1.99	21.75 ± 2.01	18.25 ± 1.38
21	23.25 ± 0.84[a]	20.00 ± 2.16[b]	22.25 ± 1.25[a]	20.75 ± 1.21[b]
28	24.04 ± 1.25[a]	19.50 ± 1.66[b]	23.50 ± 1.71[a]	21.00 ± 2.38[a]
35	20.00 ± 1.83	21.50 ± 2.50	19.75 ± 2.29	18.00 ± 0.82

[a,b,c] Different superscripts in a row indicates significant difference between the means at the level of probability: $p < 0.05$; PS means post supplementation.

Table VI: Weekly mean serum Aspartate Aminotransferase (IU/l) of rabbits infected with either *T. brucei brucei* or *T. congolense* or *T. brucei brucei* and *T. congolense*.

Groups / Days	A	B	C	D
	T. brucei	***Mixed Infection***	***T congolense***	***Uninfected cotrol***
0	46.28 ± 3.65	45.75 ± 2.44	47.65 ± 3.14	45.25 ± 5.85
7	44. 25 ± 2.25	47.02 ± 1.29	45.38 ± 3.18	44.35 ± 1.91
14	49.28 ± 2.44	48.14 ± 2.45	49.08 ± 2.22[a]	44.02 ±4.28[a]
21	49.25 ± 3.48	45.50 ± 2.78	48.00 ± 1.22	43.50 ± 3.25
28	50.23 ± 2.25	44.02 ± 2.70	48.64 ±2.45	47.50 ± 4.41
35	48.85 ±2.72	44.00 ± 3.34	47.40 ± 2.25	46.09 ± 3.51

[a,b,c] Different superscripts in a row indicates significant difference between the means at the level of probability: $p < 0.05$; PS means post supplementation.

Table VII: Weekly mean serum Alanine aminotransferase (IU/l) of rabbits infected with either *T. brucei brucei* or *T. congolense* or *T. brucei brucei* and *T. congolense*.

Groups / Days	A	B	C	D
	T. brucei	*Mixed Infection*	*T congolense*	*Uninfected cotrol*
0	13.50 ± 3.41	12.26 ± 3.35	12.50 ±1.95	11.50 ±2.00
7	12.15 ± 2.02	13.25 ± 2.16	14.35 ± 3.22	12.19 ± 2.32
14	13.60 ± 2.41	12.25 ± 2.26	14.23 ±2.52	12.00 ± 1.45
21	13.21 ± 1.25	12.10 ± 3.22	13.65 ± 1.45	12.85 ± 2.35
28	15.50 ± 2.20[a]	11.25 ± 1.12[b]	16.00 ±2.15[a]	11.25 ± 2.34[b]
35	13.86 ± 3.35	14.24 ± 2.23	14.02 ± 2.11	13.25 ± 2.30

[a,b,c] Different superscripts in a row indicates significant difference between the means at the level of probability: $p < 0.05$; PS means post supplementation.

Table VIII: Weekly mean serum protien (g/dl) of rabbits infected with either *T. brucei brucei* or *T. congolense* or *T. brucei brucei* and *T. congolense*.

Groups / Days	A	B	C	D
	T. brucei	*Mixed Infection*	*T congolense*	*Uninfected cotrol*
0	6.83 ± 1.55	5.98 ± 1.95	6.63 ± 1.39	6.03 ± 1.56
7	6.58 ± 1.32	5.30 ± 1.77	6.56 ±1.35	7.48 ± 1.72
14	6.68 ± 1.86	5.86 ± 1.50	6.45 ± 1.37	6.70 ± 1.38
21	6.48 ±	6.84 ±	5.98 ±	6.24 ±
28	5.89 ±	5.98 ±	5.64 ±	5.78 ±
35	7.00 ±	6.99 ±	7.02 ±	6.04 ±

[a,b,c] Different superscripts in a row indicates significant difference between the means at the level of probability: $p < 0.05$; PS means post supplementation.

Table IX: Weekly mean serum albumin (g/dl) of rabbits infected with either *T. brucei brucei* or *T. congolense* or *T. brucei brucei* and *T. congolense*.

Groups / Days	A	B	C	D
	T. brucei	*Mixed Infection*	*T congolense*	*Uninfected cotrol*
0	3.16 ± 0.51	3.22 ± 0.42	4.58 ± 0.25	3.53 ± 0.68
7	2.75 ± 0.22	2.55 ± 0.35	2.13 ± 0.25	2.10 ± 0.65
14	2.00 ± 0.26	1.93 ± 0.60	2.07 ± 0.35	2.77 ± 0.23
21	2.40 ± 0.15	2.08 ± 0.64	2.34 ± 0.40	2.78 ± 0.15
28	2.80 ± 0.25	2.58 ± 0.03	3.13 ± 0.58	2.53 ± 0.92
35	2.58 ± 0.24	2.23 ± 0.45	2.56 ± 0.15	2.18 ± 0.40

[a,b,c] Different superscripts in a row indicates significant difference between the means at the level of probability: $p < 0.05$; PS means post supplementation.

other infected groups on days 14, 21 and 28 PI. The mean ALT value of mixed infection was significantly lower than other infected groups on day 28 PI.

The infection led to a slight decrease in serum protein and albumin. By day 35 PI the protein levels of the infected groups were higher than the pre-infection values but did not differ significantly with the control. The albumin value of the *T congolense* infected group was significantly higher than other infected groups on days 14, 21 and 28 PI

Discussion

From the study the infection of rabbits with the trypanosome species; T.congolensis, T b. brucei and the mixed infection were well

established by day 4 post-infection. This is in line with reports by Jodi *et al.,* (2011) in T b. brucei infected rabbits and Da Silva *et al.,* (2011) in T evansi infected rabbit. However, Oyenusi *et al.,* (2010) observed 1st parasitaemia on day 5 post infection. In their report, Takeet and Fagbiemi (2009) detected parasitaemia on 7th day post infection in *T congolense* infected rabbits. The isolates alone or mixed infection showed marked susceptibility in rabbits as indicated by the animals showing parasitaemia by day 4 post infection. The infection with T b. brucei and *T congolense*, as mixed infection in an animal have been shown to lead establishment of both species in the host (Ezeokonkwo *et al.,* 2010).

All the infected groups of rabbit exhibited severe haematologic changes characterized by significant decrease in the packed cell count (PCV), haemoglobin (Hb) concentration and erythrocyte count. This is similar to the report of Abenga et al (2005) who reported changes in only T .congolrense and T b. brucei mixed infection in rats and Ezeokonkwo *et al.,* (2010) in dogs. Anemia indicated by a significant drop in PCV was noted more in the T b. brucei and mixed infection than the *T congolense*. This differs from the observations of Ikejiani (1946), Whitelaw *et al.,* (1980) and Abenga *et al.,* (2005), where the drop in PCV was consistent for all the groups. Anemia remains the most consistent feature of trypanposomosis caused by T vivax, *T congolense*s and T b. brucei (Anosa, 1983). The aetiology of anemia is complex but the most important factor is said to be haemolysis based on a reduction in red cell mass and life span and also on the occurrence of erythrophagocytosis, hemosierosis and sometimes hyper bilirubinemia (Anosa, 1983).

Leucocytosis was consistent for the infected groups, a finding that contradicts observations by Saror, (1975) who reported leucopenia. The leucocytosis in this study could be as a result of increased production leucocytes in response to the infection. The ability of mixed and *T brucei* infected groups to increase leucocyte count significantly could be an indication that it elicits better immune response. Lymphocytes are important in forming barriers against local disease conditions and may be involved in antibody formation (Frandson, 1981). The leucopenia that followed on day 35 PI indicates the waning immune response or decreased stimulation of the bone marrow during trypanosomosis. Leucopenia have been reported by Anosa *et al.,* (1997) and Ezeokonkwo *et al.,* (2010).

In this study, there was increase in urea levels in the *T brucei* and *T congolense*s group when compared with mixed infection. This is similar to elevated urea levels in natural T. brucei infected pigs (Anene *et al.,* 2011) and experimental canine trypanosomosis (Ezeokonkwo *et al.,* 2010). They observed increase in urea levels with *T congolense*s infection in rabbits. This is also consistent with results from monkeys infected with *T rhodeseinse* (Sadun *et al.,* 1973) and human infected with Trypanosoma gambiense (Awoboe, 2006). Urea is a product cleared from the body through the kidneys as such measurement during disease is good indicators of renal function (Ramakrishnan *et al.,* 1995). The causes of elevated urea levels induce kidney disease such as glomerulonephritis an excessive protein catabolism and febrile conditions. Fever and glomerulonephritis are common features of trypanosomosis and presumably act together to elevate the urea values.

The infection of rabbits with *T brucei*, *T congolense* and mixed *T brucei* and *T congolense* did not lead to significant increase in mean creatinine levels when compared with the control. Previous researchers have reported significant increase in creatinine in trypanosome infections (Abenga and Anosa, 2005, Ezeokonkwo *et al.,* 2012). The high level of creatinine in the infected groups might be due to severe muscle wasting that occurred in the course of the infection or to renal failure preventing the kidneys to excrete by-products (Mbaya *et al.,* 2008).

The non significant increase in the mean AST and ALT levels for all the infected groups following infection contradicts the observations of Orhue and Nwanze (2004) and Ezeokonkwo *et al.,* (2012), who reported a marked elevated serum levels of AST and ALT in *T brucei* infected rabbits. The finding in this experiment can be due to the resistant of the host or the pathogenicity of the isolates. The ability of the mixed infection group to have lower AST and ALT levels than other infected groups could be attributed to the fact that mixed infection caused less liver

and muscular damage. ALT and AST levels are usually elevated when damage is done to tissues cells, especially heart and liver and also in some muscle diseases. Since changes in biochemical constituents are important indicators of the physiological and pathological state of animals (Ezeokonkwo *et al.,* 2012).

The slight decrease in serum protein and albumin levels of infected groups did not differ significantly ($p>0.05$) from other groups in this study which agrees with previous finings of Kunguka and Rwakishaya, (1996), Biryomummaisho *et al.,* (2003) and Eze *et al.,* (2013), but contradict with observations made in rabbits infected with *T congolense*s by Orhue *et al.,* (2005) who reported increase in the serum total protein. According to Anosa, (1988), the total protein is normal, increased or decreased in African trypanosomosis. In this study, it was noted that by day 35 PI the protein levels of the infected groups were higher than the pre-infection values but did not differ significantly with the control. This agrees with observations made in sheep infected with *T brucei* by Taiwo *et al.,* 2003 who observe increase levels of total protein above pre-infection levels at a later stage in the infection and no change in level from pre infection values at the initial stage. Proteins usually drop in trypanosome infections as a result of excess albumin levels. The increase in protein levels during the chronic phase of the infection is usually due to increase in globulin levels this is as a result of immune response by the animals to the infection (Anosa and Isoun, 1976, Singh and Gaur, 1983, Rajorar *et al.,* 1986)

The decrease in the albumin value of the *T congolense* infected group than other groups on days 14, 21 and 28 PI may be due to the fact that uptake of albumin-bound fatty acids and lipoproteins (Vickerman and Tetley, 1979) may lead to decrease in plasma albumin concentrations in trypanosome infected animals. The decline in albumin could also be due to initiation of the immune response and synthesis of immunoglobulins (Katunguka-Rwakishaya *et al.,* 1999).

It could be concluded that Trypanosome infection in rabbit as single or mixed infection are pathogenic in rabbits and significantly alter both the haematological and biochemical levels of their host.

Conflicts of interests

There are no conflicts of interests related to this work

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INCIDENCE AND RISK FACTORS OF MILK FEVER AMONG CROSS-BRED DAIRY COWS IN KHARTOUM STATE, SUDAN

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Abstract:

The study was conducted on 206 cross-bred dairy cows in different dairy herds in Khartoum State, Sudan, during the period from March 2003 to June 2004 to determine the prevalence and incidence rate of milk fever (MF) based on clinical and laboratory diagnosis, and to recognize the risk factors associated with the disease in Khartoum State. The incidence rate was 5.3% among the examined dairy cows. Using Ch-Square cross-tabulation statistics, herd and parity did not represent risk factors for the occurrence of MF. The incidence ranged between 2.2 and 8.0% among the examined herds. The incidence was 2.5%, 4.0% and 9.1% among dairy cows of 1-2, 3-4, and > 5 parities respectively. Milk yield, age and previous history represented high risk factors for the occurrence of MF in this study. Among age groups of 3-4, 5-6 and > 5 years the incidence rates were 3.3%, 2.0% and 10.5% respectively. Cows elder than 6 years constituted 72.7% of the positive cases. Among the milk yield groups, the incidence was significantly higher (19.5%) among dairy cow producing 12-25 liters per day, whereas no milk fever case was reported in dairy cows producing 10 liters or less per day. The disease was studied based on clinical signs and laboratory examinations of serum calcium, phosphorus and magnesium. In all positive cases, body temperature, heart rate and rumen motility were examined. Rectal temperature and rumen motility were significantly low (37.0+ 0.5C° and 0.25+ 0.2 per minute respectively). Heart rate was significantly increased (103.5+ 6.9 beat per minute). Blood calcium and Phosphorus levels were significantly lower (4.6+ 0.5 mg/dl and 2.3+0.4 mg/dl respectively) than the levels measured after treatment and recovery, but magnesium levels remained unaffected before and after treatment. The increased blood calcium levels were followed by immediate recovery of 81.8% of the diseased cows. The recovery delayed in 9.1% of cases. 9.1% of the affected cows died. This study is the first, of its kind, was based on laboratory diagnosis in Sudan with featured results indicating that the parity did not affect the incidence of the disease as the disease occurred in one primi-parous cow (9.1%), and magnesium has no role on the pathogenesis of the disease as the levels remained unchanged before and after treatment.

INCIDENCE ET FACTEURS DE RISQUE DE FIÈVRE VITULAIRE CHEZ DES VACHES LAITIÈRES CROISÉES DANS L'ÉTAT DE KHARTOUM AU SOUDAN

Résumé

Cette étude a porté sur 206 vaches laitières croisées choisies dans différents troupeaux laitiers de l'État de Khartoum au Soudan, au cours de la période de mars 2003 à juin 2004. L'objectif de l'étude était de déterminer les taux de prévalence et d'incidence de la fièvre vitulaire (MF), sur la base du diagnostic clinique et de laboratoire, et de déterminer les facteurs de risque associés à la maladie dans l'État de Khartoum. Le taux d'incidence était de 5,3% chez les vaches laitières examinées. Les statistiques des tableaux croisés du Chi-carré utilisées ont montré que le troupeau et la parité ne représentaient pas des facteurs de risque d'apparition de la MF. Le taux d'incidence variait entre 2,2 et 8,0% chez les troupeaux examinés. Il était de 2,5%, 4,0% et 9,1% respectivement chez les vaches laitières de 1-2, 3-4, et> 5 parités. Dans cette étude, le rendement laitier, l'âge et les antécédents représentaient des facteurs à haut risque d'apparition de la MF. Parmi les groupes d'âge de 3-4, 5-6 et > 5 ans, les taux d'incidence étaient respectivement de 3,3%, 2,0% et 10,5%. Les vaches âgées de plus de 6 ans constituaient 72,7% des cas positifs. Parmi les groupes de vaches laitières, le taux d'incidence était significativement plus élevé (19,5%) chez les vaches produisant 12 à 25 litres par jour, alors qu'aucun cas de fièvre vitulaire n'a été signalé parmi celles produisant 10 litres ou moins par jour. La maladie a été étudiée sur la base des signes cliniques et des examens de laboratoire des taux sériques de calcium, de phosphore et de magnésium. Dans tous les cas positifs, la température du corps, le

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rythme cardiaque et la motilité du rumen ont été examinés. La température rectale et la motilité du rumen étaient très basses (respectivement 37,0± 0,5c et 0,25± 0,2 par minute). Le rythme cardiaque a augmenté de manière significative (103,5± 6,9 battements par minute). Les taux de calcium et de phosphore dans le sang étaient significativement plus faibles (4,6± 0,5 mg / dl et 2,3±0,4 mg / dl respectivement) que les taux notés après le traitement et le rétablissement, mais les taux de magnésium étaient les mêmes avant et après le traitement. L'augmentation des taux de calcium dans le sang a été suivie par le rétablissement immédiat de 81,8% des vaches malades. Le rétablissement a tardé dans 9,1% des cas ; et 9,1% des vaches atteintes sont mortes. Cette étude inédite, menée sur la base du diagnostic de laboratoire au Soudan a noté que la parité n'avait pas affecté l'incidence de la maladie - car celle-ci est apparue chez une vache primipare (9,1%) - et que le magnésium n'avait pas de rôle dans la pathogenèse de la maladie puisque sa teneur est restée la même avant et après le traitement.

Introduction

Metabolic diseases (Production Diseases) are important group of diseases affecting mainly multi-parous dairy cows. The concentration on this group of diseases came from the improvement and advances in breeding programmes which focused on the genetic improvement of the production trait such as milk yield of the selected dairy cows. The increase in milk yield has been accompanied by increasing metabolic problems as milk is produced at the expense of many metabolites.

Milk fever (parturient paresis, parturient or peri-parturient hypocalacemia,) is one of the most important metabolic disorders affecting mainly the lactating dairy cows and less frequently the pregnant cows, ewes and goats worldwide1. 2. The disease is characterized by muscular weakness and unconsciousness3. Most cases of the disease occur during the first two days of calving because milk and clostrum production drain calcium from the blood and some cows are un-able to compensate Sit fast enough, so that the depression of the levels of ionized calcium in tissue fluids is the main biochemical defect in the disease4. Rare cases may occur as paresis in non-pregnant cows after oestrus (Hess, 1969)5 or more than 48 hours post-calving6. The incidence of the clinical form of the disease varies greatly within countries and within areas in the same country, but it generally ranges from 5-10%7, and may exceed 25%8. The prevalence of the subclinical form of the disease is too difficult to be estimated. Age, milk yield, stage of lactation, previous history of the disease, parity, and breed were reported as risk factors associated with the disease4.9..10. Older milking cows are more susceptible to the disease and age was reported to increase the risk of MF by approximately 9% per lactation11. Heifers are rarely affected with milk fever. Among breeds, Jersey breed were reported as susceptible to the disease more than Friesian dairy cows.

The economical importance of the disease arises from its effect on milk yield and reproductive performance of the dairy cows. The occurrence of the disease around calving is a risk factor for many diseases like mastitis, metritis, lameness and abomasal displacement, the diseases which were encountered as major causes of disposal among dairy cows12. The disease was reported as one of the causes of high culling rate among dairy cows in United State13.

The clinical signs of the disease are highly specific and of great importance in diagnosing the disease4.

The objective of this study was to report the incidence of MF among cross-bred dairy cows in Khartoum State, as such study is lacking in the Sudan, and to correlate the reported risk factors with the incidence of the disease.

Material and Methods

Animals and Management:

A number of 206 per-parturient cross-bred (Friesian Zebu cattle) dairy cows in five different herds in Khartoum State were assigned for this study. The five herds (H-1, H-2, H-3, H-4 and H-5) were represented by 45, 46, 50, 40 and 25 cows respectively. H-1 was a governmental herd of the farm of University of Khartoum in Shambat, Khartoum North and the other four herds (H-1 to H-4) were in khartoum North but privately owned. The animal's houses of H-1

to H-4 were constructed from iron poles and corrugated iron sheets with provided sheds. In H-5 the ranch was constructed from local materials with un-adequate shade.

Assessment of Risk Factors and Clinical Examination:

All dairy cows in this study were followed till the date of parturition and then for three weeks post-calving. Information for each cow was reported from the farm record and by direct questions to the animal's owners or attendants. The age, number of parity, milk yield and previous history of milk fever were reported. Cows which developed the clinical signs of milk fever were examined and data were reported. The period of the onset of the clinical signs related to the time of the parturition, response to treatment (immediate response, failure to respond and relapses) were reported. Clinical examination was carried out before the onset of treatment and 24 hours post-treatment and then weekly for three weeks. Rectal body temperature ruminal motility and heart rate were monitored.

Sampling:

Blood Samples were collected from all cows which developed the clinical signs of the disease pre-treatment, 24 hours post-treatment and then weekly for three weeks. Sera were separated and used for laboratory investigation. Blood serum levels of calcium (Ca), phosphorus (Pi) and magnesium (Mg) were determined using the colorimetric method and biochemical kits depending on the absorbance of the sample and standard against the blank.

Treatment:

All MF cases were treated using a solution containing per ml: 175.00 mg calcium gluconate, 100 mg glucose and 00.35 mg magnesium hypophosphite.

Statstical Analysis:

Data were presented as mean + standard deviation of the mean. The data were statistically analyzed using the descriptive Paired T-test to compare means before and after treatment. Data for correlation were analyzed using Chi-Squire crosstabs descriptive statistics.

Results:

Incidence of Milk Fever: During the period of investigation, eleven cows out of 206 developed the conventional and non-conventional signs of MF. The diagnosis was confirmed by determination of pre-treatment blood calcium levels.

Table 1: Percentage of milk fever in dairy cows in five dairy Herds in Khartoum State, 2003-2004.

Herd	No. of Cows	No of Positive Cows	% of MF
H-1	45	1	2.2
H-2	46	3	6.5
H-3	50	3	6.0
H-4	40	2	5.0
H-5	25	2	8.0
Total	**206**	**11**	**5.3**

Chi-Square Value= 2.681[a], P= 0.6

Table 2: Percentage of Milk fever in cows of different ages in Khartoum State, 2003-2004.

Age/ year	No. of Cows	No of Positive Cows for MF	% MF
3-4	30	1	3.3
5-6	100	2	2.0
>6	76	8	10.5
Total	**206**	**11**	**5.3**

Chi-Square value = 10.322[a], P= 0.006

Table 3: Percentage of Milk fever in cows of different parities in Khartoum State, 2003-2004.

Parity	No. of Cows	No of Positive Cows	% MF
1-2	40	1	2.5
3-4	100	4	4.0
>5	66	6	9.1
Total		**11**	**5.3**

Chi-Square value = 2.831[a], P = 0.243

Table 4: Percentage of Milk fever in cows with different milk yield in Khartoum State, 2003-2004.

Milk Yield(lit/d)	No. of Cows	No of Positive Cows	% MF
< 10	60	0	0.0
11-20	100	2	2.0
21-25	46	9	19.5
Total	**206**	**11**	**5.3**

*Chi- Square Value = 24.007, P < 0.0001**

The incidence rate among the examined cows in the five herds during the period of the investigation (2003 -2004) was 5.3% with no significance differences among different herds (Table-1).

Risk factors:

Herd: The incidence of MF among the examined different 5 herds in Khartoum State ranged between 2.2 and 8% with no significant different (P=0.6).

Age:

As shown in table-2, age has a significant effect (P=0.006) on the occurrence of milk fever. According to age groups, most cases of MF (72.7%) occurred in dairy cows more than 6 years old. One case (9.1%) occurred in age group of 3-4 years.

Parity:

The result of the effect of parity on the incidence of MF was shown in table-3. Most cases (54.5%) of the disease occurred in multi-parous dairy cows of more than 5 parities. Four MF cases occurred in cows with 3-4 parities. One case (9.1%) occurred in uni-parous cow. Statistics revealed that the Number of parity has no effect (P=0.243) on the incidence of MF reported in this study.

Milk Yield:

The effect of milk yield on the incidence of MF was reported in table-3. In this study, Milk fever had not been reported in dairy cow yielding 10 liters of milk or less per day. Most cases of the disease occurred in milk yield group producing 21-25 liters per day. Milk yield has a significant effect (P<0.0001) on the incidence of MF and assumed as a great risk factor for the occurrence of the disease in this study.

Previous history of milk fever:

The previous history of milk fever as a risk factor for the disease was reported. Seven MF cases (63.6%) had previous history of the disease. The incidence of the disease is significantly higher in dairy cows with previous history of the disease (Chi-Square Value = 147.548a, P< 0.0001).

Clinical parameters:

Ten positive milk fever cases occurred during the first 24 hours post parturition. One case (9.1%) occurred 48 hours post-calving. The conventional signs of MF were not observed in pregnant cows in this study. The cows which developed the clinical signs of the disease were examined clinically before and after treatment. All cows were in fair body condition except one cow which was in poor body condition. Body temperature, heart rate and rumen motility were as shown in table-4. Rectal body temperature was significantly low (37.0+0.5 C°) before treatment and 24 hours post-treatment and significantly increased at 8

days P.T. and remained unchanged and within the normal range till 21 days. The heart rate was significantly higher before and even 24 hours P.T. The rate decreased significantly at 8 days P.T. and remained unchanged and within the normal limit till 21 days. Rumen motility was significantly reduced before treatment and did not improve till 15 days P.T. Hyperemia of the udders and involuntary letdown of bloody milk were observed in five MF cases and the edema of the udder was observed in one case. Partial uterine prolapsed was diagnosed in one MF case (2.2%).

Biochemical Parameters:

Blood calcium, phosphorus and magnesium levels were measured in all MF cases before the administration of calcium salts, 24 hours, 8 days, 15 days and 21 days P.T. The results were shown in table-5 and figure -1. Calcium and phosphorus levels were significantly lower before treatment, but magnesium level was not affected. The Calcium levels were less than 5.0 mg/dl in all laterally recumbent MF cases. The levels were significantly higher 24 hours P.T. and decreased significantly at 8 days to be 7.4+0.5 mg/dl and remained unchanged till the 21th day P.T.

Table 5: Clinical parameters of Positive Milk Fever Cases before and after Treatment in Khartoum State, 2003-2004.

Clinical Parameter	A.T.	24h P.T	8 days P.T	15d P.T	21d P.T.
R.T. (^{0}C)	37.0+0.5*	37.4+0.5*	38.6+0.5**	38.5+0.4*	38.9+0.6*
H.R (Beat/Min)	103.5+6.9**	91.0+14.9**	79.2+5.3**	80.9+7.4*	84.0+6.4*
R.M. (per min.)	0.25+0.2*	0.26+0.2*	0.8+0.2**	1.7+0.4**	2.4+0.5*8

R.T. Rectal temperature, H.R. = Heart Rate, R. M. Rumen Motility, A.T. Before Treatment, P.T. Post-Treatment

** =*Significant, P <0.05,*

* =*Not significant, P< 0.05.*

Table 6: Calcium, phosphors and magnesium levels of MF cases before and after treatment in cross-bred dairy cows in Khartoum State, Sudan (2003-2004).

Biochemical parameter	A.T.	24h P.T	8 days P.T	15d P.T	21d P.T.
Calcium (mg/dl)	04.6+0.5**	08.2+1.5**	07.4+0.5**	7.3+0.3*	07.3.+0.4*
Phosphorus mg/dl)	2.3+0.4**	2.9+0.7**	4.1.+5**	4.0+0.3*	4.5+0.5*
Magnesium (mg/dl)	2.6.2+0.4*	2.7+0.5*	3.4+0.6*	3.0.6+0.4*	3.0+0.4*

** =*Significant, P <0.05,*

* =*Not significant, P< 0.05.*

Treatment:

All recumbent cows were treated by a solution of calcium salts containing calcium gluconate, glucose and magnesium hypophosphite.

Response to treatment:

Nine cases (81.8%) were recovered completely within 24 hours P.T. from a single dose of 500 ml of calcium salts solution. One cow (9.1%) was recovered after a second dose of the solution. One cow (9.1%) died after three hours post-treatment.

Discussion

Milk fever is a life threatening disease of dairy cows and predisposes them to other postpartum problems14.

The study reported that the incidence of milk fever was 5.3% in a total number of 206 cross-bred dairy cows in Khartoum State, Sudan, during the period from January 2003 to June 2004. This study is the first one of its kind to report the incidence of MF based on clinical and laboratory findings. However a report based only on clinical observation reported an incidence of 3.5% among cross-bred dairy cows15.

Ten MF cases out of the 11 MF cases (90.9%) reported in this study occurred within the first 24 hours post-partum, one case (9.1%) occurred 48 hour post-partum. No MF case occurred before calving or more than 48 hours post-calving.

The incidence of MF was reported to be different within countries and within farms in the same areas. But generally the overall incidence of the disease is estimated at 0-10% in USA16, although higher incidence was reported10. In Australia, incidence ranges of 0-30% among different herds with an overall incidence of 3.0% were reported8. In the Sudan, prevention of MF is not a top priority as the disease is not highly prevalent. As proposed, specific control measurement is relevant when the incidence of the disease increases to above 10% among high risk cows17. Because there is a genetic predisposition of cows to milk fever18.19 and this is well defined to certain breeds like Jersey breed, in Sudan, the most dairy cows are cross-bred between Friesian and local breeds, this may decrease the risk for the disease.

Severe hypocalcaemia as a main cause of MF could be attributed due to several risk factors.20.21. The risk factors recognized in this study included age, milk yield, parity and previous history of the disease. Variations in incidence of MF among different dairy herds were reported in many studies22.23. In this study the incidence of MF among 5 dairy herds was not significantly different (P=0.6). The same result was reported in Danish dairy herds 22. This may be due to the same management measures and practices adopted in different dairy farms in Sudan.

Milk yield, parity and breed were reported as important risk factors which contribute to the occurrence of MF4.9.10.22. . Previous history and the age were also reported among the risk factors. Although the age of cows was reported to increase the risk of MF by approximately 9% per lactation11, some authors assigned no age effect on the incidence of MF24. As a result of this study, age represented an important risk factor as 8 out of 11 MF cases (72.7%) occurred in cows more than 6 years old. Increased prevalence rate is highly associated with increased milk yield as 81.8% of MF cases were among the cows producing 21-25 liters of milk per day. The previous occurrence of the disease was highly associated with the higher incidence of MF (P< 0.0001) as 7 cows 63.6%) had milk fever at least once in their production lives. No association between the incidence of MF and the number of parities was found in this study (P=0.24), since 2.5% of MF cases occurred in cows of 1-2 parity. This result is not compatible with many authors who reported parity as a risk factor for the occurrence of MF10.22. Although MF in this study was prevalent in older cows which assumed to have more parities, this can be justified by the inappropriate managemental practices adopted in dairy herds in Khartoum State which results in prolonged calving intervals and thus less parities even in older cows.

The rectal body temperature, heart rate and rumen motility were examined. The pre-treatment rectal body temperature was 37.0+ 0.5 C° and did not change after 24 hours P.T, but elevated to reach the normal range when measured 8 days P.T. and remained steady till 3 weeks later. The relationships between rectal temperature and rumen motility and blood calcium levels were well established25. The heart rate was significantly high before the commencement of treatment followed by significant decrease 24 hours P.T. to settle at normal range thereafter. Rumen motility was significantly slow in affected cows and did not return to normal level even after 8 days P.T. The reported Low rectal temperature, increased heart rate and decreased rumen motility are all due to low level of blood calcium. Response to treatment and recovery from the first dose is related to several clinical characteristics such as the duration of recumbency, body temperature 26 and biochemical parameters24. In this study 81.8% of MF cases responded rapidly from a single treatment. This is due to the immediate onset of treatment and shorter recumbency period. One case (9.1%) responded after the second treatment and one case (9.1%) died after 3 hours of administration of the drug. High recovery rates were reported among cows with phosphorus level > or = 0.9 mmol/l (2.7 mg/dl) and calcium level > or = 1.7 mmol/l (6.0 mg/dl) 24.

Blood Ca and Pi levels were significantly lower in pre-treatment samples of MF cases.

Magnesium was not affected before and after treatment. Ca level elevated significantly P.T. and then significantly decreased to reach 7.4+0.5 mg/dl and remained steady till 3 weeks later. The normal levels of blood metabolites in cross-bred dairy cows in Sudan are still not fully reported, but the levels of Ca, Pi and Mg levels reported in this study 3 weeks P.T. were in accordance with those previously reported levels of these metabolites in healthy pregnant and non-pregnant cross-bred dairy cows in different seasons of the year in Khartoum State, Sudan by27. The authors reported Ca levels ranging from 6.9+ 1.4 to7.9+2.1mg/dl, Pi levels from 4.1+0.7 to 4.6+0.9 mg/dl, Mg levels from 1.7+0.6 to 1.8+0.6mg/dl. The role of Pi and Mg in the pathogenesis of MF is still not fully understood. Hypomagnesaemia and metabolic alkalosis due to high potassium levels (Higher blood pH) was reported in MF cases28. Other authors reported hypermagnesaemia as a risk factor for the occurrence of the disease. In this study, Mg was not affected in all diseased cows and had no role in the incidence of the disease. This finding is compatible with some reports 25. Magnesium levels were within the levels reported in cross-bred dairy cows in Khartoum State in different stage of pregnancies27. Hypophosphataemia was accompanied with MF in this study as serum phosphorus levels were significantly lower in affected cows. This result confirmed that the pathophysiology of milk fever is still not fully understood as different authors reported different findings regarding the levels of Mg and Pi in affected dairy cows 28.23. The disagreements on the effect of blood levels of electrolytes on the pathogenesis and prevalence of MF supported the hypothesis suggested that pathophysiology of the disease MF is still not completely understood as still more arguments are reported, moreover, these disagreements regarding the role of Mg, Ca and K on the occurrence of MF supported the theory of dietary cation anion differences (DCAD) reported by many authors22.29..30.31. The levels of serum calcium which identify the clinical and subclinical hypocalcaemia are not easy to be clearly cut identified. In this study, Serum Ca levels in all cows with clinical MF were less than 5.0 mg/dl (mean = 4.6+0.5 mg/dl), became elevated after treatment and then fell to a level of 7.4+ 0.5 mg/dl which did not change till three weeks after treatment.

In the literature, blood calcium levels in the adult cows are maintained between 8.5 and 10.0 mg/dl and blood calcium levels of 5.5-8.0 are reported as subclinical hypocalcaemia, and levels of <8.0 mg/dl but not < 5.0 mg/dl was encountered for acute hypocalcaemia. In this study, during the acute hypocalcaemia, calcium levels were less than 5.0 mg/dl but more than 4.0 mg/dl. This difference is due to BREED difference since the normal levels of Ca in cross-bred dairy cows is less than those reported in dairy cows of pure-bred highly producing dairy cows. The lower calcium levels in cross-bred dairy cows in Sudan may be due to low milk yield capacity and low metabolic rate. In all recently calved cows, serum calcium falls to as low as 8.0 mg/dl and for this reason approximately all recently calved cows experience a degree of hypocalcaemia32. In this study, Ca levels were raised after injection of calcium salts and fell to reach steady levels which did not exceed 8.0 mg/dl. This level though identified as hypocalcaemia in some countries, but it is the normal level of calcium among healthy dairy cows in Sudan27.

Acknowledgements

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SEROLOGICAL PREVALENCE OF FOOT AND MOUTH DISEASE IN PARTS OF KEFFI LOCAL GOVERNMENT AREA IN NASSARAWA STATE NIGERIA.

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Abstract

About 80% of screened cattle were found to have been infected at least once with one of the seven serotypes of Foot-And- Mouth-Disease virus in areas of Keffi Local Government Area (LGA) in Nassarawa state. A total number of 108 bovine serum samples was collected from Maygaka, Angwa Ninzo and Kofar Hausa areas in Keffi LGA , with age, breed and sex of the animals recorded. Samples were collected based on previous history of foot And Mouth disease in the herd commonly called "Boro" by the herdsmen. Screening procedure was based on antibodies detection for the non structural protein mainly 3ABC protein in bovine serum regardless of the serotype of FMD virus involved using Chekit-FMD-3ABC ELISA (Bommeli Diagnostics, South Africa). Sample was said to be positive when its percentage inhibition (PI) calculated based on the kit manufacturer's recommendation was ≥ 30 %. Categorical variables (sex, age and location) were considered. Out of the total sampled animals, 77.08% was found to have been infected at least once with FMD virus with 33.33% of this infection in Angwa Ninzo, 8.33% in Kofar Hausa and 35.42% in Maygaka. There was no sex or breed predisposition to this disease. Prevalence was calculated by dividing the number of 3ABC ELISA positive animals by the total number of animals tested. Chi-square test was used for comparison of variables and tests were considered as significant at $P < 0.05$. Using double reciprocal simple linear model of analysis, it was observed that there is a significant relationship between and percentage inhibition and age of animal at 95% confidence interval.

Key Words: Seroprevalence, Foot-And-Mouth Disease, Nassarawa.

PREVALENCE SEROLOGIQUE DE LA FIEVRE APHTEUSE DANS DES PARTIES DE LA COLLECTIVITE LOCALE DE KEFFI DANS L'ETAT DE NASSARAWA AU NIGERIA

Resume

Près de 80% du bétail téstés a été infecté au moins une fois par un des sept sérotypes du virus de la fièvre aphteuse dans la région de Keffi Local Government Area (LGA) dans l'état de Nassarawa au Nigeria. Au total 108 échantillons de sérum bovins étaient collectés de Maygaka, Angwa Ninzo et Kofar Hausa, l'age, le sex et l' éspèce prélevé étaient noté. La collecte des échantillon était faite par raport a une précédente infection communement appelée Boro. La procédure de dépistage était basée sur la détection des anticorps contre les protéines non Structural du virus de la fièvre aphteuse plus précisement les anticorps contre la protéine 3ABC, identique pour tous les sept sérotypes connus du virus de la fièvre aphteuse. Le necessaire de diagnostique (Chekit-FMD-3ABC ELISA) utilisé pour cette étude était obtenu de la companie Sud Africaine Bommeli Diagnostics, la procédure fondée sur la technique bien connue du test ELISA (assai chimique immunosorbant à lien enzymatique). Un échantillon était positif de la fièvre aphteuse quant le poucentage d'inibition calculé à base de la recommendation du fabricant éetais ≥30%. Les variables catégoriques (sex, age, location) étaient considérées. Un total de 77,08% de bétail testé avaient été au moins une fois attaint de la fièvre aphteuse. Avec 35,45% de Maygaka, 33,33% de Angwan Ninzo et 8,33% de Kofar Hausa. Utilisant le test Chi- carré la prédisposition à cette endemie par rapport au sex ou à l'espèce bovine n'était pas démontrée dans cette étude $P < 00.5$. La prévalence de cette maladie était obtenue en divisant le nombre total d'animaux séropositive par la technique de 3ABC ELISA par le total d'animaux testés. Utilisant le model d'analyse de regression à double réciproque, il a été observé une relation significative entre l'age de l'animal et le poucentage d'inhibition (PI) à un intervalle de confiance de 95%.

Mot clés: Prevalence sérologique, fièvre aphteuse, Nassarawa.

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Background of the study

Over the years the FMD has caused high losses of cattle in Sub-Saharan Africa (Bastos and Sangare, 2001). The endemic nature of this disease in the sub-region could be attributed to the negligence of both governments and herdsmen since case fatality in adult animals is usually low compared with other diseases such as Trypanosomosis, Rinderpest (Mukhopadhyay, 1999), Contagious Bovine *Pleuropneumonia* and others which have their economic importance through high mortalities. The wide host range, highly contagious nature of the disease has directly and indirectly hindered cattle production (Macpherson, 1995) which represent and important form of financial security and banking beside being an important source of draught power and food, being the major source of protein for human population in the areas concerned. Concurrently, the economic losses incurred from the disease through decreased milk production, poor performance in work animals and wasting have contributed immensely to the poverty level of the rural populations depending mainly on cattle production (Bastos, 1998). The endemicity and common occurrence of FMD among herds of cattle and less in other species (Barnett and Cox, 1999) should be of serious concern with regards to control and eradication.

Foot and mouth disease (FMD) is a highly contagious viral disease affecting over 70 species of domestic and wild cloven-hoofed animals (Hedger, 1981). It affects cattle, sheep, goats, pigs and wild ruminants, causing significant production losses in adult animals and death in young stock. It is the single most important disease influencing global trade in live animals and animal products (Brooskby *et al.,* 1964) and is on the List A of the Office International des Epizooties.

FMDV is the type species of the Aphthovirus genus of the Picornaviridae family. There are seven serotypes of FMD virus namely A, O, C, SAT 1, SAT 2, SAT 3, Asian 1 that have been identified serologically, and multiple subtypes occur within each serotype (Bachrach, 1968, Belsham, 1993). Infection with one serotype does not confer immunity against another.

Non-structural protein which are identical in all the seven serotypes of FMDV have been characterized and are used for development of serological tests (Mackay *et al.,* 1998) that have been very useful in the epidemiological studies for differentiation between vaccinated and infected animals with FMDV. In many Sub- Saharan African countries where vaccination is not carried on, the test for Non-Structural FMD virus proteins is very useful for rapid diagnosis and confirmation of the presence of the disease (Diego *et al.,* 1997) in the areas of study. After an animal is infected a number of FMDV non-structural protein are produced during the replication cycle of the virus in the infected cell. Some of which are been shown to be highly immunogenic especially the 2C, 3A, 3D and the poly protein, 3ABC which are specific protein of the non structural protein complex of the FMDV. Most Serological test such as the liquid phase blocking ELISA measure antibodies produced against structural capsid protein of the FMDV which are produced in both infected and vaccinated animals and are serotype specific.

Vaccine are being formulated by inactivation of FMDV in tissue culture some level of purification of the inactivated virus from cellular and NSP components residuals in the cell and addition of adjuvant to increase antigenicity (Mowat *et al.,* 1978). Vaccination is greatly complicated by existence of seven immunologically distinct types of virus (Brown, 1992; Ekboir, 1999), many widely different subtypes and the continuous emergence of new types. Vaccination programmes require man power to vaccinate very large numbers of animals two or three times a year and for post vaccinal surveillance. Again there is disease implications for administrative (administration of multiple or cummulative doses to achieve prophylactic protection) and surveillance farm visits. Localization of highly dynamic (foci) spots of infection perpetuation will limit the risk of disease propagation and as such reduced need of repeated vaccination exercises (Rweyemanu, 1984; Hunter, 1998) and of course the cost attached especially in countries where test and slaughter is unthinkable.

The onset of clinical disease is heralded by precipitate fall in milk yield and high fever, severe depression and anorexia, followed by

appearance of an acute painful stomatitis. There is salivation smacking of the lips (Fig: 1), formation of vesicles/bullae on the buccal mucosae and the dental pad. This rupture within 24hrs leaving raw painful surfaces which heals in about a week (Fig: 1). Concurrently with the oral lesion vesicles appear on the feet, particularly on the cleft and the coronets. Rupture cause acute discomfort and gross lameness, animal often remain recumbent (Fig: 2). Secondary bacterial invasion may interfere with healing and lead to involvement of deep structures of the feet. The vesicles may appear on the teat, severe mastitis may ensue. Abortion and infertility are common sequels of the disease. In outbreaks heavy mortalities are recorded in young calves as a result of severe myocardial damage while typical vesicular lesions in the mouth and feet may be absent.

Materials and Methods

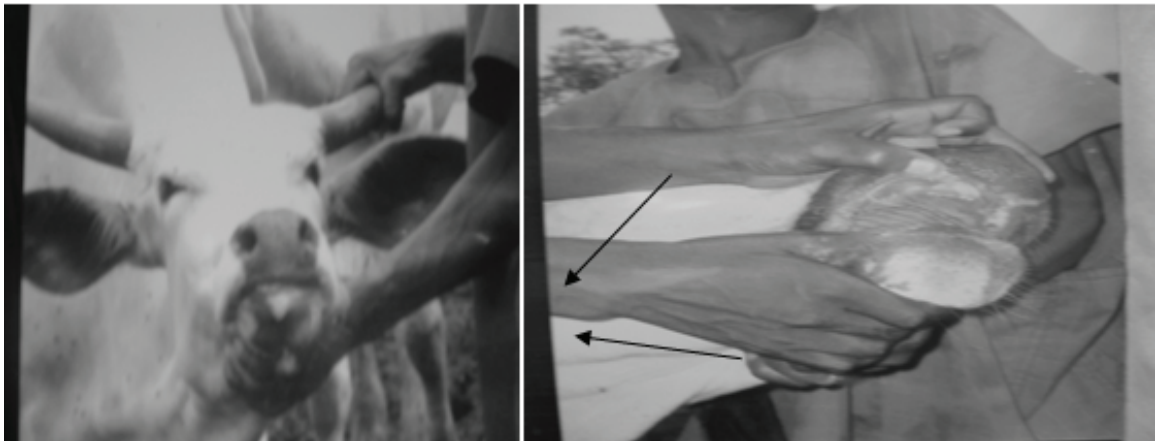


Figure 1: Oral lesions involving the dental part in Foot-And-Mouth Disease

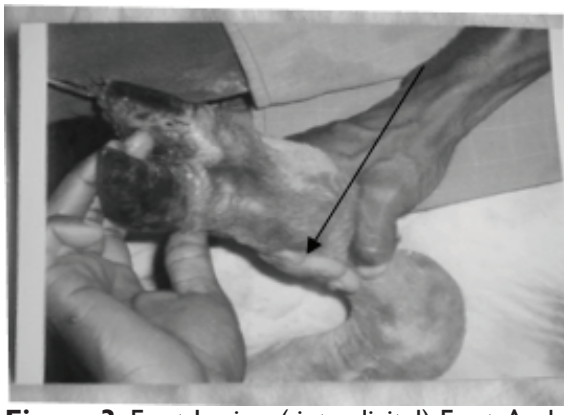


Figure 3: Foot Lesion (interdigital) Foot-And-Mouth Disease

Blood Samples were collected by jugular puncture without anti coagulant using vacuutainer needle and tubes. The blood was then spung within 12 hrs of collection at 1500 rpm in a centrifuge and serum sample harvest from each specimen. The serum was then preserved at -30^{0}C till the time it was used. The CHEKIT-FMD 3ABC bovine ELISA kit (Bommeli Diagnostics, South Africa) was used and this was indicated to be rapid, simple, sensitive and specific method for detecting antibodies against pathogen of FMDV (Roeder *et al.,* 1987) in serum samples of bovine origin. The test detects antibodies against 3ABC protein independent of the serotype of FMD virus (De Deigo *et al.,* 1997). In the kit, the entire necessary reagents for the standard indirect ELISA technique were included with polystyrene microtiter plate pre-coated recombinant FMD 3ABC protein. Dilutions of samples to be tested were incubated in the wells. Any antibody specific for 3ABC protein binds to the antigen in the wells. A peroxidase labeled anti-IgG-conjugate was added which binds to antibody of sample complexes with antigen. The TMB-containing substrate was added to the wells. In this assay, adequate washing procedure were undertaken in order to remove unbound reagent at each step of the testing procedure. The degree of color development measured by spectrophotometer is directly proportional to the amount of antibody in the sample serum specific to the antigen. The result was read by microplate spectrophotometer, where the optical density (OD) was measured at 405 nm within 15 min after addition of stop solution. The OD in wells coated with non structural proteins (NSP) 3ABC were corrected by subtraction of the corresponding wells containing the control antigens. This value called Percentage Inhibition (PI) was obtained base on the following

formula (given by manufacturer)

$PI = [OD_{sample} - OD_{negative}]/[OD_{positive} - OD_{negative}] \times 100$.
OD_{sample} = Optical Density of the test serum
$OD_{negative}$ = Optical Density of the negative control
$OD_{positive}$ = Optical Density of the positive control

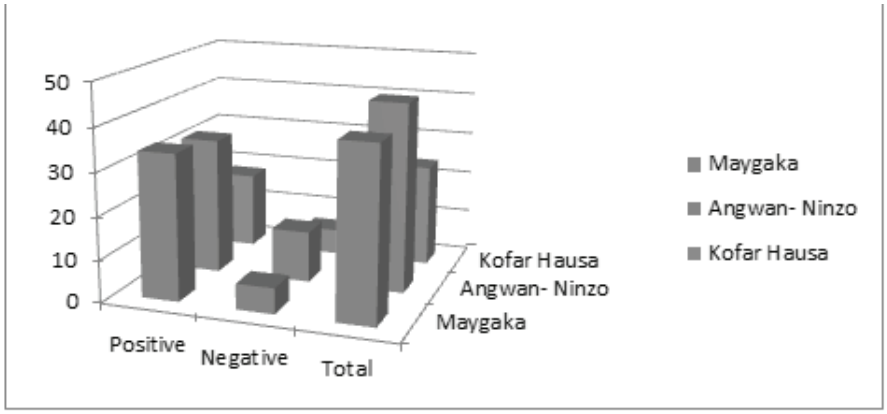
A test sample was then said to be positive of FMD when its PI ≥ 30 %. Positive Sera were then grouped into three different categories using graded anti body response into low (30-50%), medium (51-70%) and highly (71-90%) positive sera and the significance of multiple infections on the response was estimated using multivariate logistic regression.

Table 1 shows the analysis of the data collected with respect to origin, breed and sex. it reveals that 40.7% of the data were collected in Angwan Ninzo, follow by Maygaka (37.0%) and the remaining (22.2%) were collected in Kofar Hausa. it also shows that the majority (87.0%) of the breed selected were WF follow by very small portion of SG, RB and Mixed breed with 9.3%, 2.8% and 0.9% respectively. the analysis with regard to sex shows that 51.9% are female while 48.1% are male.

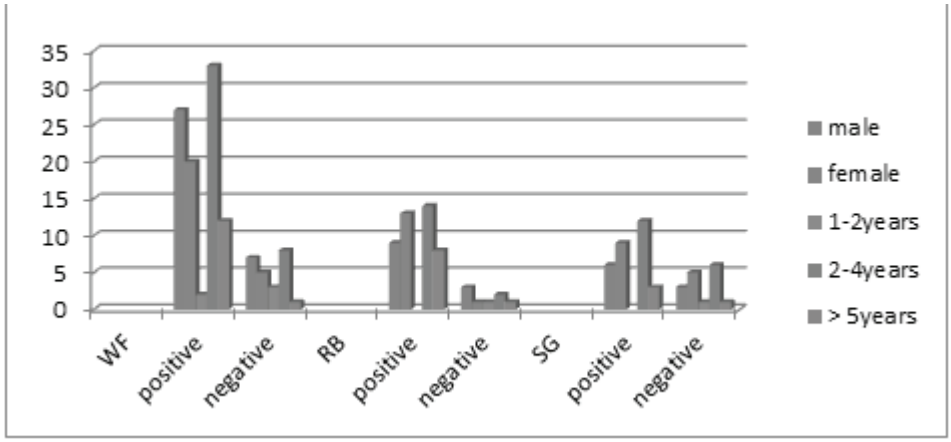
Table 2 shows chi-square statistics for examining

Table 1: Results and Interpretation

Origin	Frequency	Percent (%)
Angwan Ninzo	44	40.7
Kofar Hausa	24	22.2
Maygaka	40	37.0
Total	108	100.0
Breed		
Mixed	1	.9
RB	3	2.8
SG	10	9.3
WF	94	87.0
Total	108	100.0
Sex		
F	56	51.9
M	52	48.1
Total	**108**	**100.0**



Total number of positive animals in localities sampled



Positive animals amongs breed and age group

Hypothesis: There is no predisposition of FMD with breed and sex.

Table 2: Chi-Square Tests analysis on predisposition of FMD with Breed and Sex

Sex		Value	df	P-value
F	Pearson Chi-Square	1.253	2	.535
	Likelihood Ratio	1.903	2	.386
M	Pearson Chi-Square	1.484	2	.476
	Likelihood Ratio	2.516	2	.284

the hypothesis: there is no predisposition of FMD with breed and sex. Since X2 (0.535) for sex female and the different types of breed and X2 (0.476) for sex male and the different type of breed are all greater than 0.05 we do not reject the Null hypothesis at 5% significance level and therefore conclude that there is not predisposition of FMD with breed and sex

Simple Regression - PI vs. Age
Dependent variable: PI (Percentage Inhibition)
Independent variable: Age
Double reciprocal model: Y = 1/(a + b/X)

The output shows the results of fitting a double reciprocal model to describe the relationship between PI and Age. The equation of the fitted model is

A.PI = 1/(0.00871559 + 0.0698559/A.Age)

Since the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between PI and Age at the 95.0% confidence level.

Discussion

Construction of a two-way 2 by 3 contingency table was made to show the frequency of occurrence of Foot and Mouth Disease unique pairs of values for Results base on origin, breed, sex and age of the animals. From Chi-square results there was neither sex, breed, or age predisposition to FMD in this area.

This study shows that FMD revolves in this area given that more than 70% of screened animals had experienced a clinical infection. The endemic nature of FMD in this region is well known but the complexity of its control remains an important challenge if this has to be successful. The multivariate logistic regression in this study was used to evaluate the effect of multiple infection on percentage inhibition on assumption that older animal in endemic area

will have had more chances of exposure to the field virus than a younger one. The test for antibodies against non structural protein is not a direct quantitative test but the color change is based on the proportion of antigen/antibody complexes formed in test serum. Percentage Inhibition (PI) is a proportion of the optical density of test serum measured. There was a strong positive correlation with the magnitude of antibody response and the age of animals. FMD has multiple serotypes that circulate indiscriminately, and various types and subtypes that does not confer protective immunity. In this study it is considered that antibodies against NSP will be higher after several exposures since these proteins are identical in all the seven serotype.

This area can be identified as a region of priority in vaccination procedure whenever is started. This will reduce persistent distribution of the disease to other localities. It will equally help reduction of disease occurrence.

Acknowledgement

My sincere appreciations go to Prof. C.A.O Adeyefa Department of veterinary Medicine, University of Ibadan, Nigeria. He made arrangement of field contacts for the collection of samples and storage, also storage of the ELISA Kit. This was a serious challenge in this part of the world where power supply is very epileptic.

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HOUSING: PLASTERED VERSUS UN-PLASTERED BROODER WALLS IN POULTRY PRODUCTION

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Abstract

An investigation was carried out to study the health impact of inadequate brooder house with poor make-up on chicks. Two brooder houses were constructed with a mixture of sand and cementitious materials consisting of lime and/or gypsum to form concrete blocks. Brooder 1 was plastered with cement (cemented) and served as control while brooder 2 was not plastered with cement (un-cemented) and served as the treatment. One hundred (100) day old broilers of mixed sexes and also 100 cockerels were used in the experiment that lasted for 28 days. Fifty broilers (50) and fifty (50) cockerels were randomly assigned to the plastered and un- plastered brooder houses. The results obtained showed that broilers placed in plastered building suffered 6 mortalities out of 50 representing 12% and also that 4 cockerels representing 8% died in the cemented brooder. Results obtained in the un- plastered brooder walls showed that 31 broilers representing 62% died while 22 cockerels equivalent to 44% suffered mortality. The high incidence of mortalities in the un- plastered brooder house could be due to the ingesting of mixture of cement/sand particles used in moulding the blocks which became too toxic for the chicks at their tender age. Conclusion was reached that keeping in view with the hazards of cement, it is advisable therefore, to plaster the walls of the poultry houses to prevent the birds from pecking and swallowing cement particles.

Keywords: Broilers, brooder, cemented, cockerels, un-cemented

LOGEMENT : POUSSINIERE AUX MURS CREPIS OU NON CREPIS DANS LA PRODUCTION DE VOLAILLES

Resume

Une enquête a été menée dans le but d'évaluer l'impact d'une poussinière inadéquate avec une mauvaise finition sur la santé des poussins. Deux poussinières ont été construites en utilisant un mélange de sable et de matériaux de ciment constitués de chaux et / ou gypse destinés à fabriquer des blocs de béton. La poussinière 1 a été crépie au ciment (cimentée) et a servi de témoin tandis que la poussinière 2 n'a pas été crépie (non cimentée) et a servi de traitement. Cent (100) poussins de chair âgés d'un jour, des deux sexes, et 100 jeunes coqs ont été utilisés dans l'expérience qui a duré 28 jours. Cinquante poussins de chair (50) et cinquante (50) jeunes coqs ont été répartis de manière aléatoire à la poussinière crépie, et cinquante poussins de chair (50) et cinquante (50) jeunes coqs à la poussinière non crépie. Les résultats obtenus ont montré que les poussins de chair placés dans un logement crépi ont connu 6 mortalités sur 50 (soit 12%) et que 4 jeunes coqs représentant 8% sont morts dans la poussinière cimentée. Les résultats obtenus dans la poussinière aux murs non crépis ont montré que 31 poussins de chair et 22 jeunes coqs sont morts, représentant respectivement 62% et 44%. Le taux de mortalité élevé dans la poussinière non cimentée pourrait être dû à l'ingestion d'un mélange de particules de ciment / sable utilisés dans le moulage des blocs, devenu trop toxique pour les poussins compte tenu de leur jeune âge. La conclusion est donc que compte tenu des dangers du ciment, il est conseillé de crépir les murs des poulaillers pour empêcher les oiseaux de picorer et d'avaler des particules de ciment.

Mots-clés : poussins de chair, poussinière, cimenté, jeunes coqs, non cimenté

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Introduction

Animal production especially in developing countries are faced with problems of climate change, feed shortage, disease prevalence and high cost of feed ingredients. Genetic and environmental factors have been identified to contribute to effective performance and survival of livestock species (Preston and Willis, 1988). Just like humans, housing, food (feed) and water are considered to be the basic requirements in poultry production. Unfortunately, inadequate housing with poor make-up threatens the lives and health of chickens.

Poultry houses have often been constructed with a mixture of sand and cementitious materials consisting of lime and/ or gypsum to form concrete blocks. Most studies have been conducted on the effect of cement dust (Alakija *et al.,* 1990; Noor *et al.,* 2000; Laraqui *et al.,* 2001; Al-Neaimi *et al.,* 2001; Meo *et al.,* 2002; Mwaiselage *et al.,* 2005) in humans, plants and animals.

Cement is manufactured from clay and limestone mixture that is calcined in klin (Poornajaf *et al.,* 2010) It is a mixture of calcium oxide (CaO) (62% - 66%), silicon oxide (SiO_2) (19% - 22%), , aluminum tri-oxide (Al_2O_3) (4% - 8%), ferric oxide (Fe_2O_3) (2% - 5%), magnesium oxide (MgO) (1% - 2%) (Oleru, 1984) and also selenium (Hogue *et al.,* 1981), thallium (Brochaus *et al.,* 1981) and other impurities (Short and Petsonk, 1996). Cement may be defined as a gray powder -like adhesive substance (Yang *et al.,* 1996). It may also be defined as mineral dust which when mixed with water form a plaster-like adhesive mass (Bazas, 1980).

There is dearth of information on the consequence of placing birds in a brooder house constructed with un-plastered concrete blocks. This paper will therefore focus on the health impact of brooding chicks in un- plastered walls in a brooder house.

Materials and Methods

The study was conducted at No. 4 Divine Avenue off Oro-Ekpo Road in Obio-Akpor Local Government Area of Rivers State, South-South of Nigeria. The walls of the brooder houses were made of concrete blocks. Brooder 1 was plastered with cement and served as control while brooder 2 was not plastered with cement and served as the treatment. The brooder houses were washed and disinfected before the arrival of the birds. The feeding and drinking equipment were also cleaned and disinfected before placing them in the pens. The chicks were raised on deep liter system. Polythene was utilized in the covering of the wire gauzed sides of the building to conserve environmental temperature within the building.

One hundred (100) day old broilers of mixed sexes and also 100 cockerels were used in the experiment. Fifty broilers (50) and fifty (50) cockerels each were randomly assigned to the plastered and to un- plastered brooder houses. Brooding was done using 200 watts electric bulb and substituted with kerosene stoves for maintenance of heat in both houses.

Routine vaccinations and medications were strictly adhered to in the course of this study; standard sanitary management was also followed while feed and water were administered *ad libitum*. Feed with protein content of 22% and energy content of 2800ME/ kcal/kg was used in a brooding experiment that lasted for 28 days (4 weeks).

The measured parameter was only mortality. Daily mortality was recorded and the sum total was done every 4 days. The crop of the dead birds were visually examined.

The data collected were subjected to analysis using simple percentage. Line graph was employed to further interpret the results.

Results and Discussion

The data on mortality due to the type of wall employed are presented in Table 1. The results obtained showed that broilers placed in plastered building suffered 6 mortalities out of 50 representing 12%. The results also showed that 4 cockerels representing 8% died in the plastered brooder.

Results obtained in the un- plastered brooder walls showed that 31 broilers representing 62% died while 22 cockerels equivalent to 44% died. From the results obtained in both brooder houses it could be inferred that cockerel had higher survivability

Table 1: Mortality rates in different types of walls in chick brooder

	Cemented		Un-cemented	
Days	**Broilers**	**Cockerels**	**Broilers**	**Cockerels**
4	1	0	2	0
8	2	1	5	3
12	0	0	8	4
16	0	2	6	6
20	2	1	5	4
24	1	0	3	3
28	0	0	2	2
Total	**6**	**4**	**31**	**2**
% Mortality	**12%**	**8%**	**62%**	**44%**

rate.

Figs. 1 and 2 shows that the mortality rates in birds (both broilers and cockerels) in the un- plastered brooder increased with increase in days. The mortality rate in broilers and cockerels peaked on the 12th and 16th day, respectively, and was followed with a decline.

It has been established that the main route of entry of cement dust particles in the body is the respiratory tract and/or the gastrointestinal tract by inhalation or swallowing ,respectively (Meo *et al.,* 2002). Pecking on the un- plastered walls by the birds resulted to swallowing the mixture of cement and sand particles used in moulding the concrete block.

The high incidence of mortalities in the un- plastered brooder house could be due to the ingesting of mixture of cement/sand particles used in moulding the blocks which became too toxic for the chicks to handle at their tender age. This was evident from the ruptured crops in some of the dead birds as observed visually. It was also observed that dead birds whose crops were not ruptured were bloated. This observation is in line with the findings of Meo *et al.,* (2002). They asserted that cement dust causes lung function impairment, chronic obstructive lung disease, restrictive lung disease, pneumoconiosis and carcinoma of the lungs, stomach and colon. Other studies have shown that cement dust may enter into the systemic circulation and thereby reach essentially all the organs of body and affects the different tissues including heart, liver, spleen, bone, muscles and

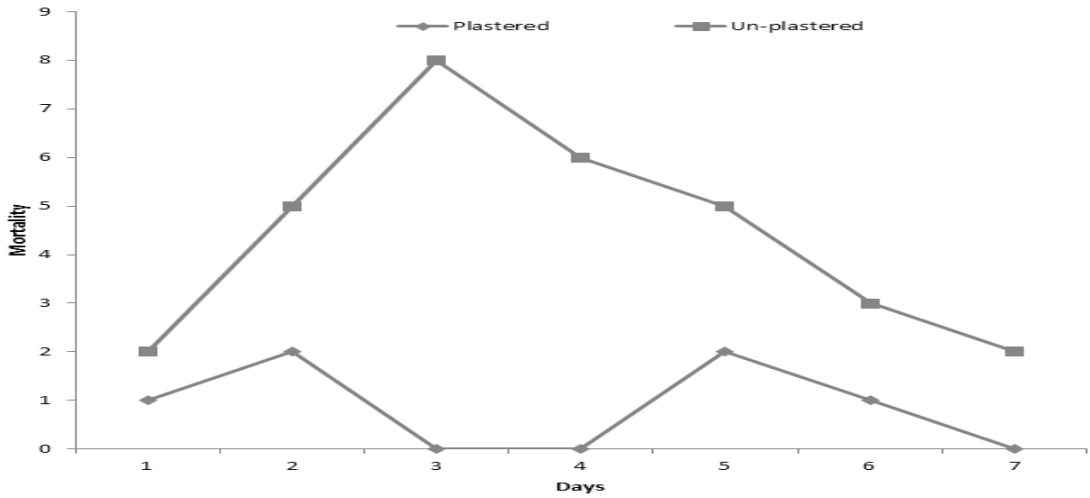


Figure 1: Mortality of rates in broilers brooded in plastered and un- plastered walled house

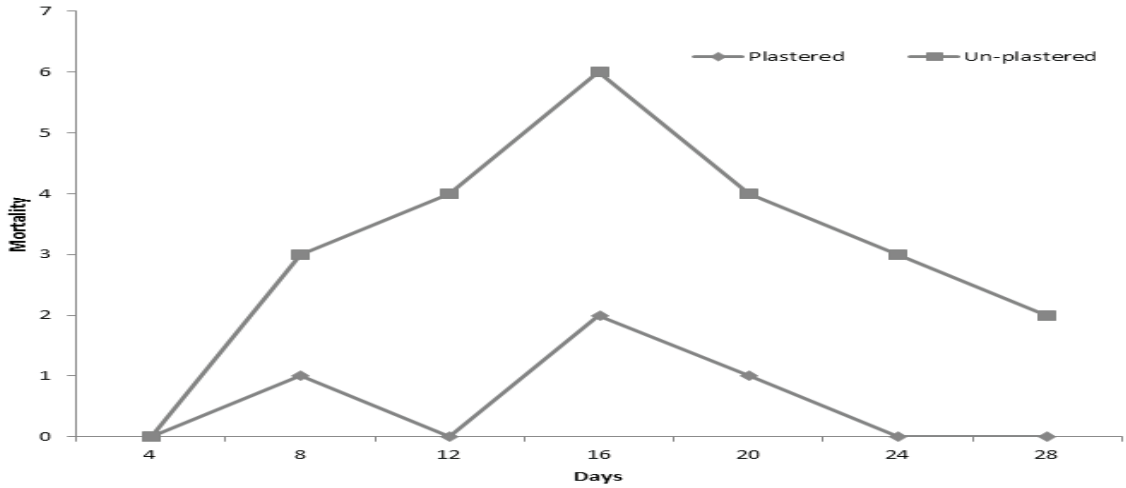


Figure 2: Mortality rates in cockerels brooded in plastered and un- plastered walled house

hairs and ultimately affecting the microstructure and physiological performance (Alakija *et al.,* 1990; Noor *et al.,* 2000; Laraqui *et al.,* 2001; Al – Neaini *et al.,* 2001; Meo *et al.,* 2002, Mwaiselage *et al.,* 2005).

Conclusion

Mortality was generally higher in the un-plastered brooder wall which was constructed using concrete blocks without plastering to provide coating. Keeping in view the hazards of cement, it is advisable therefore, to plaster the walls of the poultry houses to prevent the birds from pecking and swallowing cement particles.

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DETERMINATION OF RISK FACTORS AND LEVEL OF AWARENESS OF CAPRINE BRUCELLOSIS AMONGST GOAT OWNERS IN OJU LGA, BENUE STATE, NIGERIA.

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Abstract

In Oju, goats are the major livestock kept and there is a well established goat market held every five days. This study was aimed at determining the risk factors associated with caprine brucellosis and assessing level of awareness amongst goat owners in Oju Local Government Area (LGA) of Benue State using a structured questionnaire. The questionnaire sought information on biodata of goat owners, goat data, health indicators and risk factors for both goats and goat owners. The management system and ways of handling the animals posed risks to the goats, owners and other handlers. This study established risk factors such as lack of vaccination, introduction of newly purchased goats into the herd without isolation or examination by a professional, semi-intensive system of management, improper disposal of after-birth materials, lack of use of protective clothing when handling goats and lack of washing and disinfection of pens. The fact that none of the goat owners could recognize brucellosis as a disease affecting their goats and did not vaccinate against the disease shows their unawareness of the disease and as a zoonosis. An awareness campaign on brucellosis should be carried out in Oju LGA and Benue State in general to enlighten them on this important zoonosis.

Keywords: Risk factors, awareness, questionnaire, caprine brucellosis, Oju LGA, zoonosis

DÉTERMINATION DES FACTEURS DE RISQUE ET NIVEAU DE SENSIBILISATION À LA BRUCELLOSE CAPRINE PARMI LES PROPRIÉTAIRES DE CHÈVRES À OJU LGA, DANS L'ETAT DE BENUE AU NIGERIA

Résumé

A Oju, la chèvre est le principal animal élevé, qui a un marché bien établi ouvert tous les cinq jours. Cette étude visait à déterminer les facteurs de risque associés à la brucellose caprine et à évaluer le degré de sensibilisation des propriétaires de chèvres dans la collectivité locale d'Oju (LGA) de l'Etat de Benue, à l'aide d'un questionnaire structuré. Le but du questionnaire était de recueillir des informations sur les données biographiques des propriétaires de chèvres, les données sur les chèvres, les indicateurs de santé et les facteurs de risque tant pour les chèvres que les propriétaires de chèvres. Le système d'élevage et les méthodes de manipulation des animaux posent des risques pour les chèvres, les propriétaires et les autres personnes qui s'occupent des chèvres. Le questionnaire structuré utilisé dans cette étude a déterminé les facteurs de risque, à savoir le manque de vaccination, l'introduction de chèvres nouvellement acquises dans le troupeau sans isolement ou examen par un professionnel, le système d'élevage semi-intensif, l'élimination inadéquate des matériaux après la mise-bas, le manque d'utilisation de vêtements de protection lors de la manipulation de chèvres et le fait de ne pas nettoyer et de désinfecter les enclos. Le fait qu'aucun des propriétaires de chèvres n'ait pu reconnaître la brucellose comme étant une maladie affectant leurs chèvres et le fait de ne pas faire vacciner ces animaux contre cette maladie est un une indication de leur méconnaissance de la maladie et de son caractère zoonotique. En conclusion, une campagne de sensibilisation sur la brucellose devrait être effectuée à Oju LGA et l'État de Benue en général pour donner aux propriétaires de chèvres des informations sur cette importante zoonose.

Mots-clés : facteurs de risque, sensibilisation, questionnaire, brucellose caprine, Oju LGA, zoonose

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Introduction

Brucellosis is a zoonotic and contagious systemic bacterial disease primarily of ruminants but also affecting man and other livestock, characterized by inflammation of the genital organs and foetal membranes, abortion, sterility and formation of localized lesions in the lymphatic system and joints (1, 2). *Caprine brucellosis* is primarily caused by *Brucella melitensis* although infection by *B. suis* (3) and *B. abortus* (4) has occasionally been found in goats. The sources of infection for goats are foetuses, foetal membranes, vaginal secretions and the environment and the most common route of transmission is via the gastrointestinal tract following ingestion of contaminated pasture, forage and water. Humans get infected by consuming unpasteurized milk and milk products of infected animals and by direct contact when handling Brucella infected products with bruised skin. Unhygienic attitude of animal handlers also lead to spread of the disease (5). Infection by inhalation of airborne agents is also possible. The clinical signs in goats are similar to those observed in other species of animals and the main sign is abortion, which occurs most frequently in the third or fourth month of pregnancy. Infection of the mammary gland is common and in chronically infected herds, the signs of the disease are generally not very apparent (6).

There are serological reports of brucellosis in small ruminants in various parts of Nigeria (7, 8, 9, 10, 11). It has also been reported as affecting humans (12, 13) in Nigeria and the greatest prevalence of the infection is found in those countries with a high incidence of *B. melitensis* infection in goats and sheep. Brucellosis in humans is hardly diagnosed in hospitals in Nigeria despite suggestions that the magnitude of infections may be greater than appreciated (14, 15).

Caprine brucellosis being a zoonosis par excellence has a direct impact on public health and livestock production and therefore is a major threat to humans. It adversely influences international trade of animals and animal products. It is of particular importance among the zoonoses not only because of the heavy economic losses but also of its direct impact on human health in terms of human illness, physical incapacity and loss of manpower in addition to economic losses in medical care and reduction in productivity which remain a matter of concern in many parts of the world (16).

A very large proportion of families in the LGA keep goats and sheep as a major source of animal protein and also as a source of income. The animals are kept in the semi-intensive management system where the small ruminants fend for themselves during the day in the dry season but return to pens in the evenings. In the rainy season, they graze or are tethered on common grazing grounds in the day time but return to pens in the evenings. This management system and goat handling procedures were carefully examined to determine risk factors and assess the level of awareness amongst the goat owners/handlers.

Materials and methods

The study area Oju LGA, Benue State, Nigeria. Oju is located in the South Senatorial zone of Benue State and in the southern guinea savannah of Nigeria. It lies within latitude 6' 50' 43N and longitude 8' 25' 3E with an altitude of 180 metres (593 feet). Total annual rainfall range is 1500mm – 1750mm and the monthly mean temperature is 16 ^{0}C – 37 ^{0}C. Oju has an estimated population of 180,000 (17). The inhabitants are the Igede speaking people of Benue State. Agriculture is the mainstay of the economy and livestock production, which is mainly goat, is kept on semi-intensive.

A structured questionnaire was designed and administered by face to face interview to thirty three goat owners with the study area between September – October 2013. The goat owners were randomly selected at the goat market held every five days and also from the randomly selected communities of Ikachi, Ogengeng, Ikachi Road, Egga, Umoda and Zion Hill. Three goat owners were selected from each of the mentioned communities while fifteen were selected at the market. Some of the questions had "yes" or "no" answer options and some had specific answers as options. The questionnaire had five sections A-E. The biodata of the goat owner was contained in section A

and had questions on sex, age, marital status and level of education. Section B contained information on herd data and included questions on age of goat, sex of goat, breed of goat, source of goat, average number of goats sold per week and other markets where the goats are sold. Section C contained information on health indicators for goats and had questions on diseases that commonly affect goats, what is done when diseases occur and if abortion, retained placenta, epididymitis or repeat breeding had occurred in the goats. Section D contained information on health indicators for goat owners and had questions on some symptoms experienced by goat owners and the actions taken when the symptoms occurred. Information on risk factors for goats and goat owners was contained in section E and had questions on management system, vaccination, how aborted foetuses and retained placenta were handled, disposal of after-birth materials, cleaning of goat pens, if newly purchased goats are isolated, drinking of goat milk and use of protective clothing. The questions were explained to the respondents by the researcher using Igede and English languages and their responses were written down.

Data obtained were analysed using Microsoft Excel Spreadsheet and results are presented using bar and pie charts.

Results

A total of 33 questionnaires were administered to 33 goat owners in Oju Local Government Area, of which 27 were male and 6 were female. Sixteen of the owners were between the ages of 20 and 40 years while 17 were above 40 years. All goat owners practiced semi-intensive management system.

None of the owners recognized brucellosis as a disease affecting their goats. However, 18 (55%) and 15 (45%) said goat catarrh (PPR) and helminthosis, respectively ,affected their goats.

None of the goat owners interviewed in Oju LGA vaccinated his/her goats with Brucella vaccine.

Twelve (36%) of the owners said abortion and retained placenta had occurred in their goats. One (3%) each, said epididymitis and repeat breeding had occurred while 19 (58%) did not notice abortion, retained placenta, epididymitis or repeat breeding.

All owners introduced newly purchased goats to the herd without isolation or examination by an animal health professional.

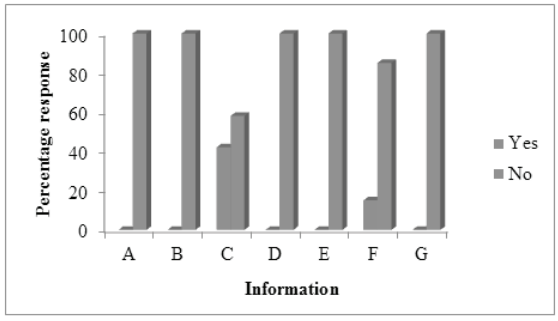
All owners interviewed use bare hands when handling goats; none uses coverall, laboratory coats, boots, hand gloves or face masks.

One (3%) each, of the goat owners have had persistent fever and profuse sweating at night, 3 (9%) admitted having joint pains, while 28 (85%) did not have any of the mentioned symptoms.

None of the goat owners interviewed in Oju LGA consumed goat milk. Figure 1 summarises the analysis of the questionnaire administered to goat owners in Oju.

Fourteen (42%) of goat owners improperly disposed after-birth materials in nearby bushes and refuse dumps while 19 (58%) buried them in a pit as shown by Figure 2.

All goat owners cleaned the goat pens by sweeping only. They neither washed with detergents nor applied disinfectants (as shown by Figure 3).



Key

A: Occurrence of brucellosis in the goats

B:Vaccination of goats with Brucella vaccine

C: Observation of abortion, retained placenta, epididymitis and repeat breeding

D: Isolation of newly purchased goats

E: Use of protective clothing during goat handling

F: Occurrence of persistent fever, profuse night sweating and joint pains in goat owners

G: Consumption of goat milk

Figure 1: Summary of analysis of the questionnaire administered to goat owners in Oju

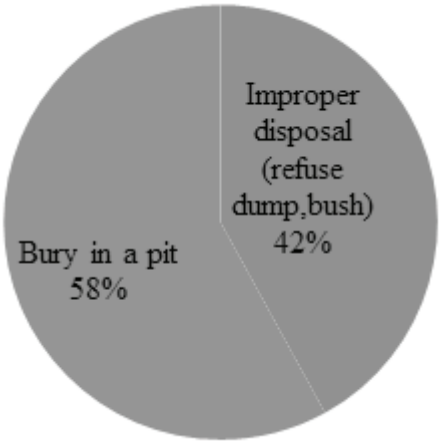


Figure 2: Disposal of after-birth materials by goat owners in Oju LGA

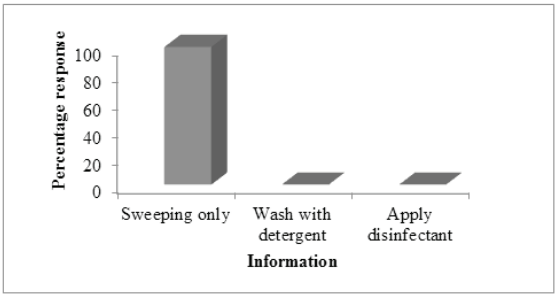


Figure 3: Cleaning of goat pens in Oju LGA

Discussion

The questionnaire revealed that all the goat owners in Oju practiced semi-intensive system of management and introduced newly purchased goats into the herd without isolation and examination by an animal health professional. The semi-intensive system of management greatly influence the spread of the infection as the spread among herds generally follows the movement or gathering of infected animals and the main risk for introducing the disease into a previously non-infected area is by purchase of infected animals. Intermingling of herds may occur under semi-intensive or extensive system of management and also in static village herds where animals are taken daily for grazing on common pastures (18).

Forty two percent of the respondents improperly disposed after-birth materials in refuse dumps and nearby bushes while 58% buried them in a pit. The primary route of dissemination of Brucella to other animals and man is the placenta, foetal fluids and vaginal discharges expelled by infected nanny goats after abortion or full-term parturition (19). The after-birth materials improperly disposed of could be picked up by stray dogs and if infective, can serve as a source of infection to healthy animals and humans as dogs have been shown to be mechanical and biological vectors of brucellosis (20).

None of goat owners vaccinated their goats against brucellosis and this shows pointer to their unawareness of the disease and it poses a risk to both the animal and human population. Vaccination when used exhaustively greatly decreases the prevalence of brucellosis in both animal and human population (21) and prevention of human brucellosis is dependent on the control of the disease in domestic livestock mainly by test and slaughter and mass vaccination (22).

All goat owners interviewed cleaned their goat pens by sweeping only. No detergents or disinfectants were used. Also, owners used bare hands when handling goats with no protective clothing such as coverall, laboratory coats, boots, hand gloves or face masks used. This puts them at risk of the disease as Brucellae can gain entry through intact skin or abrasions (23). Use of bare hands can serve as a means of spread among animals and humans through direct contact if the organism is retained on the hands as Brucella may retain infectivity for several months in water, aborted foetuses and foetal membranes, faeces and liquid manure, wool, hay, on buildings, equipment and clothes (24).

Goat milk is not consumed in Oju LGA according to this survey and this eliminates the potential risk of transmission of Brucellae to humans through consumption of goat milk.

This study has established risk factors such as lack of vaccination, introduction of newly purchased goats into the herd without isolation or examination by a professional, semi-intensive system of management, improper disposal of after-birth materials, lack of use of protective clothing when handling goats and cleaning of pens by sweeping without disinfection. There have been clamours for the Federal Government to provide veterinarians in each of the Local Government Areas of Nigeria and this

is justified. It is recommended that goat handlers wear protective clothing during handling and aborted materials and placenta should be properly disposed of by deep burial or burning. An awareness campaign on brucellosis should be carried out in Oju LGA and Benue State in general to enlighten them on this important zoonosis. A National policy on the control of brucellosis is indicated.

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AVIAN INFLUENZA, NEWCASTLE AND GUMBORO DISEASE ANTIBODIES AND ANTIGENS IN APPARENTLY HEALTHY WILD BIRDS IN KADUNA STATE, NIGERIA

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Abstract

Studies on avian influenza and Newcastle disease focus on waterfowls, considered natural reservoirs of these viruses. This study surveyed avian influenza (AI), Gumboro and Newcastle disease antibodies and antigens in birds in live wild bird markets (LWBMs), live poultry markets (LPMs) and free flying in Kaduna State from March to June, 2012. Enzyme linked immunoabsorbent assay (ELISA) and hemagglutination inhibition (HI) tests were used to detect AI viral nucleoprotein and H5- and H7- subtype antibody while Newcastle disease (ND) antibody were detected using HI test. Gumboro disease (GD) antibody was detected by agar gel immunodiffusion test (AGID). Polymerase chain reaction (PCR) was used in the detection of ND and AI viral antigens. Of the 157 birds tested by ELISA, representing 35 species of 27 families 9.2 % had influenza A antibody. About 23.1 % families and 17.6 % species had Influenza A antibody. The families with influenza A antibodies were Anatidae, Ardeidae, Ciconiidae, Columbidae, Numididae and Pynonotidae; and the species were Pychonotus barbatus, Ardea cinerea, Numida meleagris, Streptopelia senegalensis, Anas platyrhynchos and Ciconia ciconia. The AI antibody prevalence was 4.4 % in free flying birds; 17.1 % in LPM and 20 % in LWBM. The AI antibodies prevalence in Kaduna was 14.3 % while carnivorous bird's prevalence was 16.7 %. All samples positive for influenza A antibodies were negative for H5- and H7- subtype antibodies. None of the 98 birds tested for GD antibodies, comprising 20 families and 26 species had GD antibodies. Of the 196 birds tested for ND antibodies comprising 31 families and 50 species the prevalence was 20.4 % with a mean titre of 8.03 ± 0.27 log2 and 75.0 % of sero-positive birds having antibody titre ≥ 7 log2. Family and species ND antibody prevalence was 45.2 % and 34 %, respectively. Newcastle disease antibodies sero-positive families were Anatidae, Ciconiidae, Cisticolidae, Columbidae, Coraciidae, Jacanidae, Numididae, Passeridae, Phasianidae, Pyenonotidae, Rallidae and Sturdidae. Among the ND sero-positive families, 85.7 % (12/13) had ND antibody titre ≥7 log2. The species with ND antibodies were *Coracias abyssinicus, Amaurornis flavirostra, Actophilornis africanus, Porphyrio alleni, Pychonotus barbatus, Francolinus bicalcaratus, Camaroptera brachyura, Numida meleagris, Streptopelia senegalensis, Anas platyrhynchos, Passer griseus, Ciconia ciconia, Torgos tracheliotus, Lamprotornis chloropterus, Buphagus africanus*. Newcastle disease antibody prevalence was 14.6 % in free flying birds, 9.8 % in LPM and 27.8 % in LWBMs. Of the 300 birds tested for AI and ND antigen comprising 35 families and 62 species neither antigen was detected. This is the first report of AI antibody in *Pychonotus barbatus, Ardea cinerea, Streptopelia senegalensis* and *Ciconia ciconia* in Nigeria. The study concludes that wild birds in Kaduna State were exposed and responded to AI and ND antigens with Numida meleagris, Pychonotus barbatus and Streptopelia senegalensis likely to act as bridge species. *Ciconia ciconia* and *Ardea cinerea* are likely to play a role in the introduction of AI into Nigeria. Wild birds are likely to play a role in epidemiology of ND in Nigeria. Surveillance programs for monitoring and identification of AIV in wild birds in Nigeria should thus not focus solely on waterfowls, commercial farms and LPM but LWBMs and free flying birds should be included.

Key Words: Avian influenza, bridge species, Free flying wild birds, Gumboro disease, Live wild bird markets, Newcastle disease, Nigeria.

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ANTICORPS ET ANTIGENES DE L'INFLUENZA AVIAIRE, DE LA MALADIE DE NEWCASTLE ET DE LA MALADIE DE GUMBORO CHEZ DES OISEAUX SAUVAGES APPAREMMENT EN BONNE SANTE DANS L'ÉTAT DE KADUNA AU NIGERIA

Resume

Les études sur l'influenza aviaire et la maladie de Newcastle se concentrent sur les oiseaux aquatiques, considérés comme des réservoirs naturels de ces virus. La présente étude a examiné les anticorps et antigènes de l'influenza aviaire (IA), de la maladie de Gumboro (MG) et de la maladie de Newcastle (MN) chez des oiseaux se trouvant sur les marchés d'oiseaux sauvages vivants (MOSV), les marchés de volailles vivantes (MVV) et les oiseaux en vol libre dans l'Etat de Kaduna, de mars à juin 2012. Le test de dosage immunoenzymatique (ELISA) et le test d'inhibition de l'hémagglutination (IH) ont été utilisés pour détecter la nucléoprotéine virale de l'IA et l'anticorps des sous-types H5 et H7, tandis que l'anticorps de la maladie de Newcastle (MN) a été détecté en utilisant le test d'IH. L'anticorps de la maladie de Gumboro (MG) a été détecté au moyen de l'épreuve d'immunodiffusion en gélose (IDG). La réaction en chaîne de la polymérase (PCR) a été utilisée pour la détection des antigènes viraux de la MN et de l'IA. Des 157 oiseaux examinés avec ELISA, représentant 35 espèces de 27 familles, 9,2% avaient l'anticorps de l'influenza A. Près de 23,1% des familles et 17,6% des espèces avaient l'anticorps de l'influenza A. Les familles ayant des anticorps de l'influenza A étaient des : anatidés, ardéidés, ciconiidae, columbidés, numididés et pynonotidae. Les espèces étaient : Pychonotus barbatus, Ardea cinerea, Numida meleagris, Streptopelia senegalensis, Anas platyrhynchos et Ciconia ciconia. Le taux de prévalence des anticorps de l'IA était de 4,4% chez les oiseaux en vol libre ; 17,1% chez les oiseaux des MVV et 20% pour les MOSV. Le taux de prévalence des anticorps de l'IA à Kaduna était de 14,3%, tandis que celui des oiseaux carnivores était de 16,7%. Tous les échantillons positifs pour les anticorps de l'influenza A étaient négatifs pour les anticorps des sous-types H5 et H7. Aucun des 98 oiseaux testés pour les anticorps de la MG, comprenant 20 familles et 26 espèces avaient des anticorps anti MG. Des 196 oiseaux examinés pour les anticorps anti MN, comprenant 31 familles et 50 espèces, le taux de prévalence était de 20,4%, avec un titre moyen de 8,03 ± 0,27 log2, et 75,0% des oiseaux séropositifs avaient un titre d'anticorps ≥ 7 log2. Le taux de prévalence des anticorps anti MN dans les familles et les espèces était respectivement de 45,2% et 34%. Les familles séropositives pour les anticorps de la maladie de Newcastle étaient les : anatidés, ciconiidae, cisticolidés, columbidés, coraciidés, jacanidés, numididés, passeridae, phasianidae, pyenonotidae, rallidés et sturdidae. Parmi les familles séropositives pour la MN, 85,7% (12/13) avait des titres d'anticorps anti MN ≥7 log2. Les espèces ayant des anticorps anti MN étaient : Coracias abyssinicus, Amaurornis flavirostra, Actophilornis africanus, Porphyrio alleni, Pychonotus barbatus, Francolinus bicalcaratus, Camaroptera brachyura, Numida meleagris, Streptopelia senegalensis, Anas platyrhynchos, Passer griseus, Ciconia ciconia, Torgos tracheliotus, Lamprotornis chloropterus, Buphagus africanus. Le taux de prévalence des anticorps de la maladie de Newcastle était de 14,6% chez les oiseaux en vol libre, 9,8% pour les MVV et 27,8% pour les MOSV. Des 300 oiseaux examinés pour rechercher l'antigène de l'IA et de la MN, comprenant 35 familles et 62 espèces, aucun antigène n'a été détecté. C'est la première fois que des anticorps anti IA sont signalés chez Pychonotus barbatus, Ardea cinerea, Streptopelia senegalensis et Ciconia ciconia au Nigeria. L'étude a conclu que les oiseaux sauvages dans l'État de Kaduna ont été exposés et ont répondu aux antigènes IA et ND, Numida meleagris, Pychonotus barbatus et Streptopelia senegalensis étant susceptibles de servir d'espèces-relais. Ciconia ciconia et Ardea cinerea sont susceptibles de jouer un rôle dans l'introduction de l'influenza aviaire au Nigeria. Les oiseaux sauvages sont susceptibles de jouer un rôle dans l'épidémiologie de la MN au Nigeria. Les programmes de surveillance conçus pour la surveillance et l'identification du virus de l'IA chez les oiseaux sauvages au Nigeria ne devraient donc pas se concentrer uniquement sur les oiseaux aquatiques, les fermes commerciales et les MVV ; ils devraient également inclure les MOSV et les oiseaux en vol libre.

Mots-clés : influenza aviaire, espèces-relais, oiseaux sauvages en vol libre, maladie de Gumboro, marchés d'oiseaux sauvages vivants, maladie de Newcastle, Nigeria.

Introduction

Surveillance and monitoring are essential for identifying the activity of pathogens in wild bird populations. With the increasing recognition that wildlife diseases have a considerable impact on wildlife, human, and domestic animal health, there is the need to understand the pattern of interaction at the wild bird -livestock interface of which LWBMs and LPMs are link for free flying bird and poultry. Live wild bird markets can be a valuable resource for the surveillance and monitoring of the activity of pathogen of free living wild birds.

The emergence and world wide spread of HPAI H5N1 over the last decade led to enhanced surveillance for avian influenza viruses (AIV) and the study of influenza ecology (Karesh *et al.,* 2007). Wild birds, especially members of the orders, Anseriformes and Charadriiformes are considered AIV reservoirs, harboring the low-pathogenic strains with most AIV surveillance targeting aquatic birds (Swayne and Suarez, 2000; DeLiberto *et al.,* 2009; Hesterberg *et al.,* 2009). In spite of the AIV-aquatic bird relationship, AIV have generally been reported across avian species although prevalence of 0 % have been reported in non-aquatic birds (Munster *et al.,* 2007; Cumming *et al.,* 2011; Thinh *et al.,* 2012). Hosts of HPAI H5N1 are varied and non-aquatic birds may be important in AIV transmission although currently their role is not properly understood (Kou *et al.,* 2009).

Wild birds have also been considered as potential reservoirs for Newcastle disease virus (NDV), but they have rarely been infected and reported to spread NDV (Alexander, 1995). Most virulent NDV isolates have been reported from dead wild birds (Alexander, 2011). There are reports of low virulent NDV from wild birds mutating to virulent ND in poultry (Alexander, 2001). Similarly, a significant number of NDVs virulent for chickens has been isolated from wild birds in Europe over the last decade (Alexander, 2011).

Due to limited disease surveillance in wild birds in Africa and Nigeria, information on NDV and AIV ecology, host distribution and interaction are scarce though highly pathogenic influenza virus (HPAI) has been detected in free flying birds in Nigeria (Gadnet *et al.,* 2008).

The study was conducted to determine AI, ND and GD prevalence in wild birds within LWBMs, free flying birds and LPM to identify wild birds involved in the possible transmission of AIV, NDV and GDV in Kaduna State, Nigeria. The study also generated baseline data for understanding AIV, NDV and GDV within these units.

Materials and Methods

Study Area

The study was carried out in Kaduna State, located in North Western Nigeria between latitude 8° 45''- 11o30'' North and longitude 6°11'' – 9°East (RIM, 1993). It shares boundary with Kastina, Kano, Plateau, Niger, Zamfara, Bauchi, Nassarawa States and FCT. It has 23 local government areas with a population of 6 million people and 2,821,092 poultry of which 90% is local poultry raised extensively (RIM, 1993).

The annual temperature is 34°C with hottest months being March-April (40°C) and the coolest period (13.2°C) being December during severe harmattan with rainfall varying between 1,000 mm and 1,500 mm (RIM, 1993). The vegetation varies from the Guinea Savannah in the south to the Sudan Savannah in the North (RIM, 1993).

Sampling

Wild bird in LWBMs, free flying and semi-domesticated birds from live poultry markets (LPMs) were sampled during the study. Five sampling locations – Anchau, Kaduna, Karoye, Samaru and Sabon Gari were chosen based on poultry density, presence of LWBMs and LPMs; water bodies.

Sample size for the study was not pre-determined due to lack of information on the prevalence rate of AI/ND and the inability to estimate the population of wild birds in Kaduna State so a targeted sampling was done. All birds sampled (except roosting birds) were marked using a permanent marker to avoid multiple sampling of the same bird.

Live wild birds in Kaduna LWBMs were sampled after live wild bird sellers in Kaduna LWBMs were approached and consent

obtained for participation in the study. Free flying birds were captured by mist nets, hunting and use of other traps. Hunters gave consent for hunted birds to be sampled. For free flying wild birds roasting on trees, faecal samples were collected by the use of a white paper. Two semi domesticated species – guinea fowls and mallard ducks were selected due to their arboreal nature and likelihood of interacting with wild birds especially migratory birds and local poultry. Live mallards and guinea fowls were sampled from Anchau LPM after obtaining consent from sellers.

All birds were visually identified with the aid of a field guide by Borrow and Demey (2004) except roosting birds which were identified using a binoculars with magnification 7x 50.

Sample Collection

Cloacal, faecal and oropharyngeal swabs

Oropharyngeal area of wild bird was swabbed by inserting a dry cotton swab into the oropharynx and gently swabbing the wall. The cloacae of wild birds were swabbed by inserting a cotton swab deeply into the vent and vigorously swabbing the wall until the swab was stained with faecal material. Faecal droppings of roasting wild birds were also collected.

The swabs and faeces from each bird were pooled placed in viral transport medium. Samples were labeled and immediately chilled in a flask with ice packs and transported to the laboratory to be stored in liquid nitrogen until used for viral antigen detection.

Blood

About 0.5-2 ml of blood was collected from wild birds through venepuncture, using sterile hypodermic needles and syringes. The blood was collected and allowed to clot at room temperature from which sera were obtained and stored for – 20^{0}C until used for serology.

Detection of Avian Influenza Antibodies

Enzyme linked immunoabsorbent assay for avian influenza antibodies

Antibodies to the nucleoprotein of influenza A virus was detected using a commercially available blocking enzyme-linked immunosorbent assay (AniGen AIV Ab ELISA) test kit following manufacturer's recommendation.

Haemagglutination inhibition test for avian influenza H5 and H7 subtype antibodies

An alpha haemagglutination inhibition (HI) test was carry out on all sera positive for influenza A nucleoprotein using standard procedures recommended by OIE (OIE, 2010). The test antigen used were an inactivated H5 and H7 subtype – antigens while the positive sera were H5N2 and H7N9 both prepared by Istituto Zooprofilattico OIE/FAO Laboratory For AI and NDV delle Venezie.

Newcastle Disease Virus Antibodies by Haemaglutination Inhibition (HI) Test

An alpha haemagglutination inhibition (HI) test was carry out on all sera for ND antibodies using standard procedures recommended by OIE (OIE, 2010). Newcastle disease antigen and a positive sera used as control were obtained from the Avian Virology Laboratory, NVRI -Vom.

Detection of Gumboro Disease Antibodies by Agar Gel Immunodiffusion Test

Agar gel immunodiffusion (AGID) test was performed as described by (Harai *et al.,* 1972).

Detection of Avian Influenza and Newcastle Disease Virus Antigen

Processing of swabs and viral extraction

The swabs samples were thawed, vigorously agitated and allowed to settle for 30 minutes at room temperature, then filtered into 1.5 ml tube. The filtrates were stored at – 700C until required for RNA extraction.

Five hundred micro litre of filtrate was dispensed into a 1.5 ml tube for viral ribo-nucleic acid (RNA) extraction. Viral RNA was extracted using Qiagen QI AampR viral RNA mini kit according to manufacturer's instructions. The extracted RNA was used for rT-PCR.

Reverse transcription of RNA

The extracted RNA samples were reverse transcribed into cDNA using

Superscript IIIR. Each sample of purified RNA was denatured for 10 minutes at 72^0 C by mixing 5 µl of sample RNA with Mix 1 comprising 5 µl of random primer (0.03 ug/ul; 1:100), 2 µl of a 10 mM dNTP Mix and 1 µl of sterile, distilled water. The denatured RNA was then chilled on ice and 7 µl of Mix 2 (comprising 4 µl of 5X first-strand buffer, 1 µl of 0.1 M DTT, 1 µl RNase OUT (40 U/ml) and 1 µl of Superscript III (200 U/ml)) was added. The mixture was incubated for 80 minutes at 500 C and later inactivated for 15 minutes at 70^0 C. The resultant product is the cDNA from which a 1: 5 dilution was made by diluting 2.5 µl of cDNA into 10 µl of Rnase free water. The RNA was reverse transcribed using G-StormR thermo cycler PCR system (Model- GS0001). The 1:5 dilution cDNA was then used for PCR detection of AI and ND.

Polymerase chain reaction for avian influenza antigen detection

The polymerase chain reaction for AI detection was performed using Forward and Reverse primers sequence for the matrix (M) gene – ChenF (M52C) : 5' - TTCTAACCGAGGTCGAAACG-3' and ChenR (M253): 5'-AGGGCATTTTGGACAAAKCGTCTA - 3' as primers respectively.

To 2.5 µl of the 1:5 cDNA template from each sample was added in a 22.5 µl of PCR mix comprising 15.9 µl Rnase free water, 2.5 µl PCR Buffer (10X), 1 µl MgCl2 (25 mM), 0.5 µl dNTPs (10 mM), 1.25 µl ChenF (M52C) and ChenR (M253) primers, 0.1 µl Platinum Taq. The matrix gene segment was amplified using G-StormR thermo cycler PCR system (Model-GS0001) which amplified a 250 bp fragment of avian influenza genome.

Polymerase chain reaction was performed by denaturing cDNA at 950C for 30 seconds, annealing at 580C for 30 seconds and extending the fragment at 720C for 1 minute. The above program was repeated for 40 cycles with a final extension at 720C for 10 minutes and PCR products maintained at 40C. The PCR products (amplicons) were then detected using gel electrophoresis.

Newcastle disease virus antigen detection

The polymerase chain reaction for ND antigen detection was performed using two rounds of PCR with the 2nd round being a Nested PCR.

In the first round PCR, the primers used were FOP1: 5' TACACCTCATCCCAGACAGGGTC 3' and FOP2:5'AGGCAGGGGAAGTGATTTGTGGC 3'. It involved adding 2.5 µl of the 1:5 cDNA template from each sample to 22.5 µl of PCR mix comprising 17.2 µl Rnase free water, 2.5 µl PCR Buffer (10X), 2 µl MgCl2 (25 mM), 0.5 µl dNTPs (10 mM), 0.1 µl FOP1 and FOP2 primers, 0.1 µl Platinum Taq. The primers used for the nested PCR were FIP1: 5' TACTTTGCTCACCCCCCTT 3' and FIP2: 5' CATCTTCCCAACTGCCACT 3'.

The nested PCR involved adding 2.5 µl of the 1:5 cDNA template from each sample to 22.5 µl of PCR mix comprising 16.3 µl Rnase free water, 2.5 µl PCR Buffer (10X), 2 µl MgCl2 (25 mM), 0.5 µl dNTPs (10 mM), 0.5 µl FIP1 and FIP2 primers, 0.2 µl Platinum Taq. The 1st round and nested PCR was performed by denaturing cDNA and 1st round PCR product respectively at 950 C for 30 seconds, annealing at 58^0C for 30 seconds and extending the fragment at 72^0C for 1 minute. The above program was repeated for 40 cycles with a final extension at 72^0C for 10 minutes and PCR products maintained at 100 C. The PCR product expected after 1st round is 532 bp and was used for the nested PCR (2nd round PCR). The expected PCR product of the nested PCR was 280 bp. The PCR products were then detected using gel electrophoresis.

Agarose gel electrophoresis of amplicons

1.5% agarose (Roche) gel prepared in 1X TAE Buffer (Roche) to which ethidium bromide (Promega) was poured into a gel frame and placed into the electrophoresis chamber (BioradR electrophoresis tank) with 1xTBE covering the gel.

Five micro litres of amplicons from each reaction tube was transferred to a well in a microtitre plate which was mixed with 3 µL gel loading buffer (Promega) and loaded into wells of the agarose gel separately. The molecular weight marker was loaded into the first well while the positive control was loaded in the last well of the gel.

The chamber was closed and electrodes attached. The gel was allowed to run at 150 V for 23 minutes using BioradR power pack. The gel was viewed over a UV light source and documented by photographing with a KodakR camera. The sizes of amplicons – fragments were compared with the marker.

Data Analysis

The data obtained from questionnaires and serology were analysed by descriptive statistics using SPSS version 17.0 (SPSS Inc. Chicago, IL, USA). The frequency, mean, standard error of mean and chi square values of cross tabulations were calculated. Values of $p \leq 0.05$ were considered significant.

Results

The study revealed avian influenza antibody prevalence of 9.2 % (14/153). Of the 26 families and 34 species tested 23.1 % (6/26) families and 17.6 % (6/34) species had influenza A antibodies. The families and species prevalence were as shown in Table 1. However, among species with AI antibodies, Ciconia ciconia had highest prevalence (Figure 1).

The AI prevalence in free flying birds was 4.4 % (4/90) (Table 2). Among the birds possessing AI antibodies, 26.7 % (4/15) were in LWBM and free flying birds and 40 % (6/15) LPMs birds ($p = 0.05$; $df = 3$; $X2 = 7.6$).

The AI prevalence in Anchau was 12 % (6/50), 14.3 % (5/35) for Kaduna, 5.8 % (4/69) for Samaru and 0 % (0/3) for Koraye. Among the AI positive birds, 40 % (6/15) were from Anchau, 33.3 % (5/15) were from Kaduna and 26.7 % (4/15) were from Samaru.

Based on the feeding habits of the birds, AI prevalence were 16.7 % (1/6) for carnivorous birds, 3.7 % (1/27) for grainvores, 12.7 % (13/102) for omnivores and 0% for insectivores (0/19), fruguvore (0/2) and nectarivore (0/1). None of the birds having influenza A nucleoprotein antibodies possessed antibodies to H5- and H7- subtype.

Of all the birds tested for IBD (0/98), comprising of 20 families and 26 species, none had antibodies against infectious bursa disease virus. The species tested for GD antibodies were African black crake (0/2); African thrush (0/1); Allen gallinule (0/1); Black crown crane (0/1); Black winged stilt (0/2); Brown babler (0/1); Cattle egret (0/1); Common bulbul (0/3); Common kestrel (0/1); Ethiopian swallow (0/1); Francolin (0/14); Grey backed (0/1); Grey heron (0/1); Helmeted guinea fowl (0/26); Honey guide (0/1); Lappet-faced vulture (0/1); Laughing dove (0/10); Mallard duck (0/18); Pigeon (0/1); Quail (0/1); Red eyed dove (0/1); Scarlet chested sun bird (0/1); Spur winged lapwing (0/1); Squalco heron (0/1); White stork (0/3).

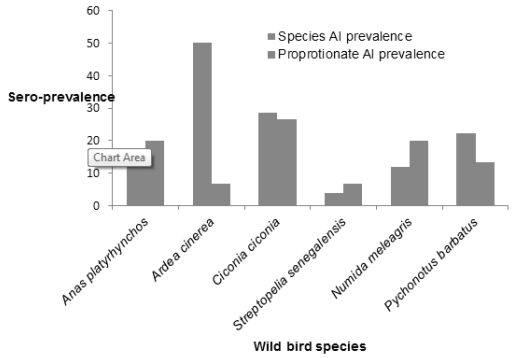


Figure 1: Avian influenza sero-prevalence among wild bird species in Kaduna State, Nigeria.

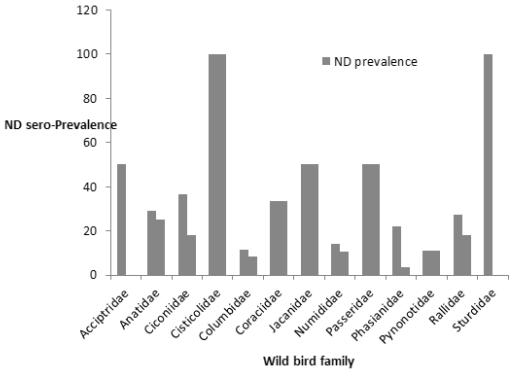


Figure 2: Newcastle disease prevalence among wild birds families in Kaduna State

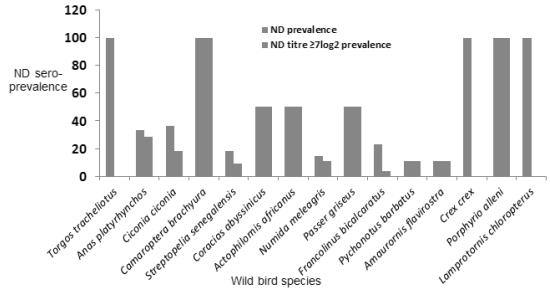


Figure 3: Newcastle disease prevalence among wild birds' species in Kaduna State, Nigeria.

Table 1: Avian influenza sero-prevalence among wild bird families and species in Kaduna State, Nigeria.

Family/Species	Avian influenza sero-prevalence
ANATIDAE	**16.7 % (3/18)**
Anas platyrhynchos	17.6 % (3/17)
ARDEIDAE	**25 % (1/4)**
Ardea cinerea	50 % (1/2)
CICONIIDAE	**28.6 % (4/14)**
Ciconia ciconia	28.6 % (4/14)
COLUMBIDAE	**3.8 % (1/26)**
Streptopelia senegalensis	4 % (1/25)
NUMIDIDAE	**12 % (3/25)**
Numida meleagris	12 % (3/25)
PYNONOTIDAE	**22.2 % (2/9)**
Pychonotus barbatus	22.2 % (2/9)

Table 2: Avian influenza sero-prevalence among wild bird within different sampling units in Kaduna State, Nigeria.

Sampling unit	Avian influenza sero-prevalence	Sero-positive wild bird species
Free flying wild bird	4.4 % (4/90)	*Streptopelia senegalensis* (2/90)
		Ardea cinerea (1/90)
		Pychonotus barbatus (1/90)
Live wild bird market	20% (4/20)	*Ciconia ciconia* (4/20)
Live poultry market	17.1 % (6/35)	*Numida meleagris* (3/35)
		Anas platyrhynchos (3/35)

The ND sero-prevalence was 20.4 % (40/196) with a mean ND titre of 8.03 ± 0.27 log2 and 75.0 % (30/40) of the birds had ND antibody titre of ≥ 7log2. About 45.2 % (14/31) families had antibodies to ND with 85.7 % (12/14) having titre ≥ 7log2. The prevalence within families ranged from 11.1 % for Pyenonotidae to 100% for Cisticolidae (Figure 2). The ND species sero-prevalence was 34 % (17/50) with 76.5 % (13/17) having ND antibody titre ≥7 log2 (Fig. 3).

The ND antibody prevalence was 14.6 % (18/123) in free flying birds, 9.8 % (4/41) in LPM and 27.8 % (5/18) in LWBMs ($p = 0.00$; $df = 6$; $x2 = 61.46$). However, 72.2 % (13/18) of free flying birds with ND antibody had titre ≥ 7 log2. Among the LPM and LWBM birds with ND antibodies, 50 % (2/4) and 40 % (2/5) respectively had ND antibody titre of ≥ 7 log2 ($p = 0.03$; $df = 3$; $X2 = 9.01$).

The ND prevalence in Anchau and Kaduna were 7.5 % (4/53) and 47.4 % (18/48) respectively. However, Samaru had a prevalence of 17.6 % (18/102) while Koraye and Zaria ND prevalence were 0% (0/1) and 0% (0/2) respectively ($p = 0.00$; $df = 10$; $x2 = 37.41$). Fifty per cent (2/4) of birds from Anchau which had ND antibodies had titre ≥ 7 log2 and 83.3 % (15/18) of the birds from Kaduna and 72.2 % (13/18) from Samaru had ND titre ≥ 7 log2 ($p = 0.02$; $df = 3$; $x2 = 9.01$).

The families of birds without ND antibodies were African thrush (0/3); Barnyard geese (0/2); Black billed wood dove (0/1); Black crown crane (0/2); Black headed lapwing (0/1); Black winged stilt (0/2); Blue billed roller (0/1); Brown babler (0/2); Cattle egret (0/4); Common kestrel (0/1); Ethiopian swallow (0/1); Greater painted snipe (0/2); Grey heron (0/1); Honey guide (0/1); Long tailed shrike (0/1); Osprey (0/1); Parrot (0/1); Pia piac (0/1); Pigeon (0/2); Quail (0/1); Red eyed dove (0/2); Rock dove

(0/1); Scarlet chested sun bird (0/1); Senegal coucal (0/1); Spur winged lapwing (0/1): Squalco heron (0/2); Village weaver (0/1); Vinaceous dove, (0/2); Western grey plantain eater (0/3); White stork (0/3); white-faced whistling duck (0/1); Wood dove, (0/1); Wood sand piper (0/1) and Yellow crowned goneleck (0/3).

All 35 families and species tested for AI and ND antigen were negative. The species of birds tested for presence of AI and ND antigen were *Coracias abyssinicus* (0/1), *Amaurornis flavirostra* (0/7), *Actophilornis africanus* (0/19), *Barnyard geese* (0/8); *Turdus pelios* (0/2), *Porphyrio alleni* (0/2), *Pychonotus barbatus* (1/1), *Francolinus bicalcaratus* (0/36), *Camaroptera brachyura* (0/2), *Numida meleagris* (0/31), *Streptopelia senegalensis* (0/28), *Anas platyrhynchos* (0/17), *Passer griseus* (0/2), *Ciconia ciconia* (0/16); *Turtur abyssinicus* (0/1); *Balearica pavonina* (0/10); *Vanellus tectus* (0/2); *Himantopus himantopus* (0/3); *Coracias cyanogaster* (0/1); *Turdoides plebejus* (0/2); *Bubulcus ibis* (0/5); *Falco tinnunculus* (0/1); *Crex crex* (0/1); *Pterocles coronatus* (0/3); *Cucoo finch* (0/1); *Hirundo aethiopica* (0/1); *Egretta alba* (0/3); *Rostratula benghalensis* (0/2); *Honey guide* (0/1); *Torgos tracheliotus* (0/1); *Lamprotornis chloropterus* (0/2); *Long-tailed shrike* (0/1); *Lamprotornis caudatus* (0/1); *Pandion haliaetus* (0/1); *Pavo cristatus* (0/3); *Ptilostomus afer* (0/1); *Corvus albus* (0/1); *Pigeon* (0/1); *Hedydipna platura* (0/1); *Pelecanus rufescens* (0/1); *Porphyrio porphyrio* (0/4); *Coturnix coturnix* (0/5); *Streptopelia semitorquata* (0/3); *Lanius collurio* (0/1); *Streptopelia capicola* (0/1); *Columba guinea* (0/9); *Chalcomitra senegalensis* (0/5); *Centropus senegalensis* (0/2); *Speckled pigeon* (0/2); *Vanellus spinosus* (0/4): *Ardeola ralloides* (0/4); *Cinnyris cupreus* (0/1); *Turkey* (0/11); *Streptopelia vinacea* (0/6); *Crinifer piscator* (0/3); *Dendrocygna viduata* (0/1); Wood dove, (0/1); *Tringa glareola* (0/2); *Serinus mozambicus* (0/3) and *Laniarius barbarous* (0/3).

Discussion

This is the first reported extensive survey for AIV distributions in wild birds in Kaduna State, Nigeria. Though influenza A antigen has been reported in *Anas platyrhynchos* in Kaduna State, literature search on previous detection of AIV antibodies in *Pychonotus barbatus, Ardea cinerea, Streptopelia senegalensis* and *Ciconia ciconia* in Nigeria was negative (Assam *et al.,* 2011).

The study revealed that these birds were exposed and responded to influenza A antigen. However, the energy need for other body function of wild bird such as reproduction and feather development can negatively affect immune response resulting in adaptive suppression of antibody production via mechanisms such as immune trade-offs (Deerenberg *et al.,* 1997; Hanssen *et al.,* 2004).

The presence of AI antibodies in mallard duck and guinea fowl confirms that poultry purchased from LPM are likely to have been exposed to AIV thereby increasing the likelihood of exposing free range poultry to AIV as local poultry farms source breeding stock from LPM (Kambai, 2011; Ameji *et al.,* 2012). The exposure of *P. barbatus* and *S. senegalensis* to AI antigen are likely to be at watering points where they are likely to interact with waterfowls. *P. barbatus* and *S. senegalensis* are possible AI bridge species to poultry as they regularly interact with local poultry and usually visit commercial poultry farms.

The study highlights the importance of LWBMs in the epidemiology of AI in Nigeria. The LWBMs are not incorporated in the routine national surveillance for AI in Nigeria and the current study revealed high AI antibody prevalence in LWBMs which are sources of waterfowl and other exotic birds in Nigeria. The study revealed that birds from LPMs, LWBMs and free flying wild birds have been exposed to AIV. It also highlights the possibility of AIV transmission between these epidemiologic units as wild birds purchased from LWBMs and LPMs are raised by free range management during which they interact with local poultry and free flying birds scavenging for food. The LWBMs birds are more likely to be exposed to AIV as most of the birds sold in LWBMs are waterfowls which are reservoirs of AI (Olsen *et al.,* 2005).

The high AI prevalence reported in Kaduna was a reflection of the high prevalence in LWBMs which are located in Kaduna though the prevalence in Samaru revealed that backyard poultry with minimal biosecurity poses a risk to free flying birds. The study further revealed high

AI antibody prevalence in carnivorous birds highlighting the increased risk of predation and scavenging on other birds to AIV (Stallknecht and Brown, 2007).

Contrary to previous reports of GDV antibodies in cattle egret and pigeons in Ibadan, Nigeria, none of the birds tested in this study had GDV antibodies (Fagbohun *et al.,* 2000a). The lack of sero-conversion might be due to decay of antibodies if birds were exposed to GDV since virus is stable and endemic in Nigeria or immune trade-off exhibited by the birds (Whitaker and Fair, 2002; Martin *et al.,* 2003; Hanssen *et al.,* 2004). The study revealed that GDV may not be a limiting factor in the survival of wild birds as these birds interact with local poultry in which GD has been reported and is endemic (Nawathe *et al.,* 1979; Assam *et al.,* 2011).

The study confirms exposure of wild birds to NDV though the prevalence in this study was lower than previous reports in central and western Africa (Molla and Maina, 2008). The difference might be due to the high population of local poultry (75 %) sampled in the previous study.

The ND sero-prevalence for Streptopelia senegalensis and N. meleagris in Kaduna State is similar to the prevalence in Ibadan and Plateau State, Nigeria respectively (Fagbohun *et al.,* 2000b; Mai *et al.,* 2004). However, the *Anas platyrhynchos* ND sero-prevalence in this study (33.3 %) was significantly higher than that reported for the same species in Plateau State, Nigeria (6.7 %) (Mai *et al.,* 2004). Nonetheless, this is the first report of exposure of T*orgos tracheliotus, Ciconia ciconia, Camaroptera brachyuran, Coracias abyssinicus, Actophilornis africanus, Pychonotus barbatus, Amaurornis flavirostra, Crex crex, Porphyrio alleni, Lamprotornis chloropterus* and *Buphagus africanus* to NDV in Nigeria. These wild bird species may play a role in the epidemiology of ND in poultry.

The presence of ND antibodies in birds may be from exposure from to a field NDV or ND vaccine virus ingested when scavenging in poultry farms or poultry manure spread on crop farms. Similarly, scavengers such as Torgos tracheliotus are likely to have been exposed to NDV through ingestion of contaminated or infected poultry carcass.

The inability of birds to develop ND antibodies for protection would result in an epidemic following exposure to a virulent field virus. The epidemic could be misdiagnosed as HPAI and the mortality that ensues would lead to loss of a rare gene pool within the wild bird population.

The high mean ND antibody titre indicates that the birds would be protected from mortality and reduction in egg production (Gutierrez-Ruiz *et al.,* 2000). The high antibody titre of ND with 75 % of ND sero-positive birds having a titre ≥ 7 log2 indicates recent natural infection with NDV with possible excretion of NDV (Gutierrez-Ruiz *et al.,* 2000). However, only *Gallus gallus* and *Porphyrio allenis* would be protected from NDV transmission due to attainment of flock immunity (Boven *et al.,* 2008). The study indicate the possibility of the circulation of field NDV in *Anatidae, Ciconiidae, Cisticolidae, Columbidae, Coraciidae, Jacanidae, Numididae, Passeridae, Phasianidae, Pyenonotidae* and families. The species of birds in these families could serve as reservoirs of NDV for poultry.

Though the species ND antibody prevalence was low, with over three-quarter of the seropositive birds indicating active circulation of ND virus, *Pychonotus barbatus, Streptopelia senegalensis* and *Passer griseus* could be involved in ND transmission and spread between poultry farms which they regularly visit. However, *Anas platyrhynchos, Ciconia ciconia* and *Porphyrio alleni* could be liable for the spread and maintenance of NDV within LWBMs while *Coracias abyssinicus, Amaurornis flavirostra, Actophilornis africanus, Numida meleagris* and *Anas platyrhynchos* are likely to spread NDV to free range poultry.

The study revealed that ND antibody prevalence in birds from LWBMs was higher than in birds from LPMs and free flying birds. This is likely due to high concentration and interaction of a wide variety of wild birds held in close confinement and the resultant stress might enhance the shedding, mixing and dissemination NDV (Warwick *et al.,* 2012a). In addition, the poor biosecurity within the LWBMs is likely to have contributed to the difference in sero-prevalence.

Despite the high prevalence in LWBMs,

the study revealed active NDV challenge among free flying wild birds indicating that they are likely to be important in the spread and transmission of NDV to poultry in Nigeria contrary to reports in western countries with improved poultry biosecurity (Fagbohun *et al.,* 2000b; Oladele *et al.,* 2012).

The high NDV antibody titre in birds from Kaduna and Samaru implies birds are protected from ND with high flock immunity although they are likely to be excreting NDV. Therefore, if new birds are introduced into these locations they are at risk of ND infection (Boven *et al.,* 2007). Therefore, migratory birds transiting through these locations without previous exposure to NDV are likely to experience an epidemic which might be misdiagnosed as HPAI (Assam *et al.,* 2011).

This is the first Nigerian study to report absence of antibodies against NDV in over 50 species of Nigerian birds. However, absence of antibodies does not indicate lack of susceptibility of these species of birds to NDV, but might rather be due to lack of exposure to the virus or inability of birds to mount an immune response due to diversion of resource to other needs such as reproduction (Hanssen *et al.,* 2004).

The study revealed that guinea fowls and mallard ducks raised on free range have been exposed to both influenza A and ND viruses thereby exposing the free flying wild birds to both viruses as they scavenge for feed through contact with chicken faeces and secreation. Guinea fowls and mallard ducks interacts with other waterfowls and migratory birds at flood lands.

No AI or ND antigens were detected though detection of low pathogenic avian influenza and ND antigen was expected in waterfowls which are natural reservoirs for AI and ND (Stallknecht and Brown, 2007; Soliman *et al.,* 2012). This is probably due to absence of viruses because sampling was done in out of breeding season when there are few susceptible hosts (Easterday *et al.,* 2007). However, the viruses may be present but were not detected because of short period of excretion, very low prevalence and small sample size. Similarly, contamination of cloacal swabs by PCR-inhibitors and non-specific primers are likely to contribute to non detection of AI and ND antigens (Molla and Maina, 2008). Equally, the presence of AI and ND antibodies without detection of antigens might be an indication that infections were not recent (Molla and Maina, 2008).

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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
January 2014

Aims and scope
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.

The Bulletin of Animal Health and Production publishes articles on original research on all aspects of animal health and production, biotechnology and socio-economic disciplines that may lead to the improvement animal resources. Readers can expect a range of papers covering well-structured field studies, manipulative experiments, analytical and modeling studies of the animal resources industry in Africa and to better utilization of animal resources.

The BAHPA encourages submission of papers on all major themes of animal health and production, wildlife management and conservation, including:

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- Marketing, economics
- Infectious and non infectious disease
- Parasitology
- Genetic improvement and biotechnology
- Animal production, nutrition and welfare
- Science and policy in animal health and production
- Beekeeping and honey bees
- Ecology and climate change impacts on animal resources in Africa
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- Socio economics and economics of animal resources development

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The language of submission should be either in U.K. English or Standard French. The abstract is translated to the other three languages of the African Union (Arabic, English, French and Portuguese), by the editors, after acceptance. Full articles submitted in French will also be published in English.

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Full papers of original research

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- *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.
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