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BACTERIAL CONTAMINATION OF STORED TABLE EGGS FROM COMMERCIAL CHICKENS FED GARLIC MEAL ADDITIVE

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Abstract

Table eggs from poultry farms sometimes take weeks before consumption during which period they are either in-transit to consumers or are stored until purchased. Microbial contamination during this period being the cause of spoilage, determines the shelf-life of eggs. Garlic is known to possess antimicrobial activities. Its potential at improving the shelf-life of table eggs was investigated.

Three hundred and fifty-one Isa Brown pullets separated into four groups A, B, C and D of 90, 81, 90 and 90 birds, were placed on garlic-meal feed additive at 0.125%, 0.25%, 0.5% and 0%, respectively. At 53 week-old, sixty eggs/group were kept at room temperature (26-27.5oC), from which 8 eggs/group were selected on the day of lay and weekly for 4 weeks. One ml of vortex mixed albumin and yolk pooled from 4 eggs was diluted 1:10, inoculated on Plate Count Agar-PCA, *Salmonella-Shigella* Agar-SSA, Eosin-Methylene Blue Agar-EMBA and Saborand Dextrose Agar-SDA by pour plate method in duplicates and incubated at 36oC for 72 hours. Discrete colonies were sub-cultured in Nutrient agar and identified using cellular morphology and biochemical characteristics.

Bacterial growths were observed in groups A, C and D (75, 0 and 0 cfu/ml in EMBA, 100, 125 and 225 cfu/ml in PCA and 0, 25 and 25 cfu/ml in SSA, respectively, at 2 weeks of storage. At 3 weeks, all groups had bacterial growth except B, while at 4 weeks, all groups had bacterial growth with B having a load of 25.5 cfu/ml on PCA only. *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumonia*, *Stenotrophomonas maltophilia* and *Citrobacter amalonaticus* were isolated. Garlic-meal in feed of chicken layers at 0.25% delayed bacterial egg contamination, thereby prolonging the shelf-life and reducing the possibility of food poisoning in consumers, as well as, egg wastage with associated economic loss.

Keywords: Bacterial contamination, commercial chicken layers, garlic meal, table eggs, shelf-life.

CONTAMINATION BACTERIENNE DES OEUFS DE TABLE STOCKES PROVENANT DE POULETS COMMERCIAUX SOUMIS A UNE ALIMENTATION ADDITIONNEE DE POUDRE D'AIL

Résumé

Les œufs de table provenant de fermes avicoles prennent parfois des semaines avant d'être consommés, et pendant cette période ils sont soit en transit à destination des consommateurs soit conservés jusqu'à ce qu'ils soient achetés. Comme la contamination microbienne pendant cette période est source de détérioration, elle détermine la durée de conservation des œufs. L'ail est réputé pour ses activités antimicrobiennes, c'est ainsi que son potentiel d'extension de la durée de conservation des œufs de table a fait l'objet d'investigations.

Trois cent cinquante et une poulettes brunes ISA réparties en quatre groupes A, B, C et D de 90, 81, 90 et 90 oiseaux ont reçu une alimentation additionnée de poudre d'ail respectivement aux taux de 0,125%, 0,25%, 0,5% et 0%. À l'âge de 53 semaines, soixante œufs / groupe ont été maintenus à la température ambiante (26-27,5oC), dont 8 œufs / groupe ont été sélectionnés le jour de la ponte et sur une base hebdomadaire pendant 4 semaines. Un ml d'un mélange en vortex d'albumine et de jaune d'œufs provenant de 4 oeufs a été dilué à 1:10, inoculé sur gélose pour numération en plaques-PCA, *Salmonella-*

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Shigella Agar-SSA, Eosin-Methylene Blue Agar-EMBA et Saborand Dextrose Agar-SDA par méthode du milieu coulé en doubles et incubé à 36°C pendant 72 heures. Des colonies discrètes ont été sous-cultivées dans la gélose nutritive et identifiées en utilisant la morphologie cellulaire et les caractéristiques biochimiques.

Des croissances bactériennes ont été observées dans les groupes A, C et D (respectivement 75, 0 et 0 cfu / ml sur EMBA, 100, 125 et 225 CFU / ml sur PCA et 0, 25 et 25 cfu / ml sur SSA à 2 semaines de stockage. À 3 semaines, tous les groupes avaient une croissance bactérienne à l'exception de B, tandis qu'à 4 semaines, tous les groupes avaient une croissance bactérienne, B ayant une charge de 25,5 cfu / ml sur PCA uniquement. Les bactéries *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumonia*, *Stenotrophomonas maltophilia* and *Citrobacter amalonaticus* ont été isolées. La poudre d'ail au taux de 0,25% dans l'alimentation des pondeuses a retardé la contamination des œufs par les bactéries, prolongeant ainsi la durée de conservation et réduisant la possibilité d'intoxication alimentaire chez les consommateurs, ainsi que le gaspillage d'œufs avec les pertes économiques associées.

Mots-clés : Contamination bactérienne, pondeuses commerciales, poudre d'ail, œufs de table, durée de conservation

Introduction

Poultry is the second most widely eaten meat in the world, accounting for about 30% of meat production worldwide, after pork at 38% (Raloff, 2003). The poultry industry in Africa supplies a large proportion of protein in terms of meat and eggs, to the populace. Poultry production is an important aspect of the agricultural economy of developing African countries and an instrument of socio-economic change. In livestock production, poultry next only to ruminants occupies a prominent position in providing animal protein as it accounts for 25% of local meat production in Nigeria (Okunlola and Olofinsawe, 2007).

The importance of poultry production to food security and the economies of developing countries are demonstrated by the fact that they are an important source of nutrition and play an important role in cash flow particularly of resource poor rural populations (Gueye, 2001). In most parts of West Africa, the poultry industry is dominated by chicken which constitute 98% of the poultry population, while other poultry species like turkeys, ducks and quails make up the remaining 2% of the population. Nigeria has an estimated poultry population of 140 million with backyard poultry consisting of 60% (Nnadi and George, 2010). Nigeria is the largest egg producer in Africa recording an average annual growth rate of 4% between 2000 and 2012 when output reached 640,000 tonnes (FAO, 2012). Chicken egg is regarded as a functional food which is easily available and affordable. Eggs are economical source of nutrients for a healthy diet and life, especially important for the mental development of growing children (Zaheer, 2015). In spite of this seemingly massive egg production level in Nigeria and Africa as a continent, protein availability in the diet of most Africans is deficient. According to FAO (2012), annual consumption of eggs which is largely determined by the country's wealth, ranges from 300 g/person in African countries to 19.1 kg/person in Japan. Out of the 43 sub-saharan African countries, only 9 have an average consumption higher than 2 kg while most Asians and Americans eat at least twice that amount. In spite of this level of inadequacy in egg consumption by Africans, egg spoilage due to bacterial contamination consequent upon inappropriate storage condition further reduces availability of chicken eggs and income from egg production. These table eggs from poultry farms sometimes take weeks before consumption during which period they are either in-transit to consumers or are stored until purchased. Microbial contamination during this period being the cause of spoilage, determines the shelf-life of eggs. This is particularly significant in the tropics where the environment provides optimum conditions like high temperature and relative humidity as well as poor biosecurity, for disease agents to thrive. In addition to reducing the amount of wholesome table eggs available for human

consumption, microbial contamination of eggs can cause food poisoning varying from mild symptoms to life threatening situation (Kaneko *et al.,* 1999) constituting a serious public health problem around the world (Safaei, *et al.,* 2011). External and internal quality of eggs defines those characteristics that affect consumer acceptability and preference. The quality of egg once laid cannot be improved but its maintenance is possible. Prompt egg collection and rapid storage in the cool room immediately after lay can minimize or slow down spoilage (Zaheer, 2015). However, cool room facility is not common place in most poultry farms in Africa in spite of the high environmental temperature. An alternative means of prolonging the shelf-life of table eggs is therefore desirable.

Garlic (*Allium sativum L.*) is known to possess antimicrobial activities. It is a well known spice and herbal medicine for the prevention and treatment of a variety of diseases (Adimoradi *et al.,* 2006). It is a member of the onion family Alliceae and has been shown to exhibit antimicrobial, antioxidant, and anti-hypertensive properties (Konjufca *et al.,* 1997; Sivam, 2001). Allicin (diallyl-thiosulfinate) is the major organosulfur compound in garlic which is considered to be biologically active (Raham, 2007). As a natural feed additive, Garlic has been reported to improve broiler growth, feed conversion ratio and decreased mortality (Jagdish and Pandey, 1994; Tollba and Hassan, 2003). Since antibiotic residue had been reported in table eggs by earlier worker (Dipeolu *et al.,* 2000; 2002; 2004), the possibility of the antimicrobial effect of garlic being transovarial, in garlic fed chicken layers was considered. This study was therefore carried out to investigate the impact of garlic as a feed additive in the diet of commercial chicken layers on bacterial contamination of stored eggs with the probability of prolonging the shelf life.

Materials and Methods

The study was carried out with ethical approval from the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/App/2015/065). Three hundred and fifty-one, 15 week-old commercial Isa Brown pullets were kept in battery cages in four separate groups viz, A, B, C and D of 90, 81, 90 and 90 birds, respectively. These pullets had been placed on garlic meal (Patent No. NG/P/2012/285) feed additive at 0.125%, 0.25%, 0.5% and 0% (control), respectively from day-old and were administered Newcastle disease and infectious bursal disease vaccines as appropriate. At 53 week-old, sixty eggs per group were randomly selected and kept at room temperature (26 - 27.5oC), from which 8 eggs each were selected per group on the day of collection i.e. day of lay, and weekly thereafter for 4 weeks, for the purpose of bacteriological examination. The shell of the egg samples was wiped with ethanol (100%) soaked cotton-wool, and further flamed slightly over alcohol lamp to sterilize. A portion of the shell was aseptically opened and 1 ml of egg content (yolk and albumin) was aspirated using sterile 2ml syringe and 19G needle from 10 random spots in each egg. Yolk and albumin taken from four eggs were pooled and mixed thoroughly using vortex mix and 1ml of this mixture was aseptically removed and diluted 1:10 in sterile distilled water. The diluted egg samples were inoculated on Plate Count Agar (PCA), Salmonella-Shigella Agar (SSA), Eosin-Methylene Blue Agar (EMBA) and Saborand Dextrose Agar (SDA) by pour plate method. Plates were inoculated in duplicates per sample and incubated at 36°C for 72 hours before colonies were counted and expressed as number of viable organisms (colony forming unit: cfu) per ml of sample. Average cfu/ml were calculated and discrete colonies from growths observed were sub-cultured in Nutrient agar and identified based on cellular morphology and biochemical characterization using the Microbact 24E bacteriological kit and identification software.

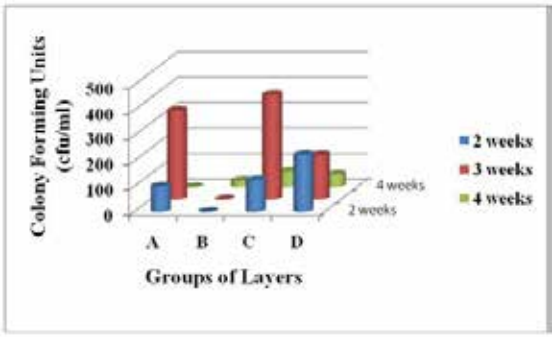

Figure 1: Bacterial load in samples of stored eggs from chicken layers fed graded levels of garlic meal on Plate Count Agar

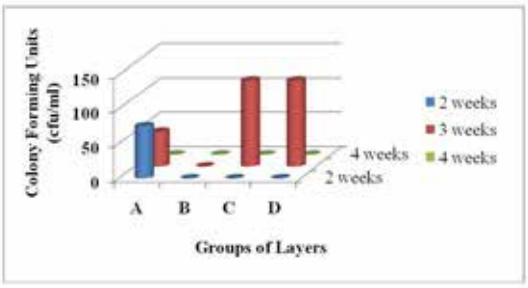

Figure 2: Bacterial load in samples of stored eggs from chicken layers fed graded levels of garlic meal on Eosin-Methylene Blue Agar

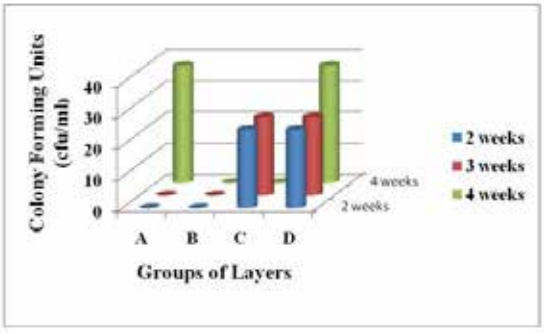

Figure 3: Bacterial load in samples of stored eggs from chicken layers fed graded levels of garlic meal on Salmonella-Shigella Agar

Results

No bacterial growth was observed in samples of eggs produced by hens from all groups on the day of lay, and at 1 week of storage. At 2 weeks of storage, bacterial load in groups A, C and D were 75, 0 and 0 cfu/ml on EMBA; 100, 125 and 225 cfu/ml on PCA and 0, 25 and 25 cfu/ml on SSA (Figures 1, 2 and 3). At 3 weeks of storage, bacterial load in the same groups A, C and D were 50, 125 and 125 cfu/ml in EMBA, 350, 412.5 and 175 cfu/ml in PCA, but remained unchanged at 0, 25 and 25 cfu/ml on SSA. At 4 weeks of storage bacterial load in groups A, C and D were 0, 62.5 and 50 on PCA and 37.5, 0 and 37.5 cfu/ml on SSA. Group B had bacterial growth only at 4 weeks of storage on PCA (25.5 cfu/ml). There was no growth on SDA for the 4 weeks of storage.

Morphological assessment of cells of isolates showed all five isolates to be Gram negative rod with four of them being motile. Other biochemical characteristics are as listed in Table 1. The isolates were biochemically characterized and identified as *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumonia*, *Stenotrophomonas maltophilia* and *Citrobacter amalonaticus*. No growth was observed on SDA throughout the four-week storage period.

Discussion

This study investigated the impact of garlic as a feed additive in the diet of commercial chicken layers, on bacterial contamination of stored eggs with the probability of prolonging the shelf life. The absence of bacterial growth in all eggs sampled on the day of lay and at one week of storage is a confirmation of the wholesomeness of table eggs at the time of lay which extended till one week post storage at room temperature. On PCA, eggs from all the groups had ample bacterial growth except group B which showed no growth at 2 and 3 weeks of storage and only 25.5 cfu/ml load at 4 weeks. This is an indication that contamination of eggs from group B with 0.25% garlic meal in feed, was prolonged till 4 weeks of storage compared with 2 weeks in eggs from the other groups. Eggs from this same group showed no growth on EMBA and SSA during the 4 weeks of storage at room temperature. Thus, none of the isolated and identified bacteria was from eggs laid by layers in group B. *E.coli* contamination of eggs had been reported worldwide (Cortes *et al.,* 2004), and a study by Safaei et al. (2011) reported the contamination of table eggs from

Table 1: Characterization and identification of bacterial isolates observed in table-eggs stored at room temperature for 4 weeks

Characteristics	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
Gram reaction	-ve	-ve	-ve	-ve	-ve
Shape	Rod	Rod	Rod	Rod	Rod
Motility	Motile	Motile	Non-motile	Motile	Motile
Nitrate reduction	+ve	+ve	+ve	+ve	+ve
Lysine	+ve	+ve	+ve	+ve	+ve
Ornithine	+ve	+ve	-ve	+ve	+ve
H2S	-ve	-ve	-ve	-ve	-ve
Glucose	+ve	+ve	+ve	-ve	+ve
Manitol	+ve	+ve	-ve	-ve	+ve
Xylose	+ve	+ve	+ve	-ve	+ve
ONPG	+ve	+ve	+ve	-ve	+ve
Indole	+ve	-ve	-ve	-ve	+ve
Urease	+ve	+ve	+ve	+ve	+ve
V-P	-ve	+ve	+ve	-ve	-ve
Citrate	-ve	+ve	+ve	+ve	+ve
TDA	-ve	-ve	-ve	-ve	-ve
Gelatin	-ve	+ve	-ve	+ve	+ve
Malonate	-ve	+ve	+ve	+ve	-ve
Inositol	-ve	-ve	+ve	-ve	-ve
Sorbitol	-ve	-ve	-ve	-ve	+ve
Rhamnose	+ve	-ve	+ve	-ve	+ve
Sucrose	-ve	+ve	+ve	-ve	-ve
Lactose	+ve	+ve	+ve	-ve	+ve
Arabinose	+ve	+ve	+ve	-ve	+ve
Adonitol	-ve	-ve	-ve	-ve	-ve
Raffinose	-ve	+ve	+ve	-ve	-ve
Salicin	-ve	-ve	-ve	-ve	-ve
Arginine	+ve	+ve	+ve	+ve	+ve
IDENTIFICATION	***Escherichia Coli***	***Enterobacter cloacae***	***Klebsiella pneumonia***	***Stenotrophomonas multiphilia***	***Citrobacter amalonaticus***

+ve = Positive, -ve = Negative

retail markets in Shahrekord, Iran with *E.coli*, Proteus spp. and Klebsiella spp.

According to ICMSF (1986), the whole egg in the shell is self-protective unless abused by excessive exposure to temperature and humidity changes during storage which appears to be the situation in most storage facilities on farms and at retail outlets in Africa. The amount of coliform organisms detected in these eggs i.e. 50 – 125 cfu/ml are more than the limit set by the International Commission on Microbiological Specifications for Foods (ICMSF, 1986) i.e. 10 cfu/ml, with unpleasant implications to public health.

Groups C and D with 0.5% and 0% garlic meal in feed, respectively, altogether had higher bacterial load than groups A and B with group B having the least. Garlic is known to

have antibacterial activity as earlier reported by Palaksha *et al.,* (2010), Nejad et al. (2014) and Eltaweel (2014), especially against *E.coli* and *S.aureus*. Also, Kumar and Sharma (2009) demonstrated antibacterial activity against antibiotic-resistant *E.coli*, *Klebsiella pneumonia*e, Enterococcus spp., Pasteurella aeruginosa and *S.aureus*. In the present study, all five bacteria isolated i.e. *E. coli*, *Enterobacter cloacae*, K. pneumonia, *Stenotrophomonas maltophilia* and *Citrobacter amalonaticus* are potentially pathogenic to humans (Brooke, 2012; Garcia *et al.,* 2016; such that their presence in table eggs is worthy of note. The absence of growth on SDA during the 4 week period of storage shows that there was no fungi contamination.

The least bacterial load which indicates the best antibacterial activity of garlic was obtained in eggs from layers fed 0.25% garlic meal. However, in spite of the known antibiotic activity of garlic, 0.5% garlic meal in feed (Group C) did not produce the best antibacterial activity in egss in this study. This finding concurs with that of Kumar and Sharma (2009) in which the highest concentration of garlic extract (50µg) used in an *in vitro* study did not produce the best bacterial growth inhibition. Thus, it seems that garlic in high doses may not necessarily demonstrate antibacterial activity.

It was therefore concluded that the antibacterial activity of garlic could be transovarial, thereby protecting table eggs against bacterial contamination. This will inevitably prolong the shelf-life of table eggs at room temperature and reduce the risk of human infection by these contaminants, via egg consumption. The inclusion of garlic meal at 0.25% in the feed of commercial chicken layers is therefore recommended.

Acknowledgement

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A RETROSPECTIVE SERO-EPIDEMIOLOGICAL STUDY ON RIFT VALLEY FEVER IN CATTLE IN UGANDA BETWEEN 1997 TO 2009

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Abstract

Surveillance on Rift Valley fever (RVF) in cattle was conducted in Uganda, with a view to ascertaining whether the RVF virus circulated in bovine undetected during the period 1997 to 2009. Four hundred eighty (480) frozen bovine sera collected in 1997, 1998, 2000, 2001 and 2005 were tested using both RVF IgG and IgM ELISA performed in parallel. Additional 685 bovine sera collected in 2006 and 2007 were tested using the Virus Neutralization Test (VNT). A further 232 and 296 bovine sera collected in 2008 and 2009, respectively, were tested using IgM ELISA. The findings revealed a prevalence of anti-RVF virus IgG antibodies in cattle ranging from 0.0 - 2.5% across districts and 0.0 – 2.6% across years (1997 – 2005), without significant differences across districts or years. Meanwhile the prevalence of anti-RVF virus IgM antibodies in cattle ranged from 0.0 – 6.3% across districts and 0.0 – 2.6% across years (1997-2009). Cattle sampled in Kumi district were 7 times (Odds Ratio (OR) 7.0: 95% CL, 1.8-26.9) more likely to be seropositive than those from other districts. Furthermore, cattle sampled in 2008 were 3.4 times (OR 3.4: 95% CL 1.0-10.1) more likely to be seropositive than during other years. Regarding analysis of anti-RVF virus neutralizing antibodies, cattle from Apac district were 3.6 times (OR 3.6: 95% CL 1.9-6.7) more likely to be seropositive than those from other districts. In addition, cattle from large herds (> 100) were 5 times (OR 5.0: 95 CL 2.3 – 10.8) more likely to be seropositive than those from small herds (0-50). Arungi farm in Apac district had a higher likelihood (OR 4.0: 95 CL 1.8-9.3) to have seropositive cattle. The majority of the 685 cattle (90%) had low titres (> 1: 10) of neutralizing antibodies while few (10%) had high titres, ranging from 1:10 to > 1: 80. In conclusion, the study revealed evidence of low level circulation of the RVF virus in cattle in Uganda in certain districts and farms, especially during certain years (2008) when flooding occurred.

Keywords: IgG; IgM; RVFV neutralizing antibodies; Cattle; Uganda

ÉTUDE SERO-ÉPIDÉMIOLOGIQUE RETROSPECTIVE SUR LA FIÈVRE DE LA VALLEE DU RIFT CHEZ LES BOVINS EN OUGANDA, ENTRE 1997 ET 2009

Résumé

La fièvre de la vallée du Rift (RVF) chez les bovins a fait l'objet de surveillance en Ouganda. L'objectif de cette activité était de déterminer si le virus de la RVF avait circulé non détecté chez les bovins au cours de la période 1997 - 2009. Quatre cent quatre-vingt (480) sérums de bovins congelés collectés en 1997, 1998, 2000, 2001 et 2005 ont été examinés en utilisant à la fois l'IgG RVF et l'ELISA IgM réalisés en parallèle. 685 sérums bovins supplémentaires collectés en 2006 et 2007 ont été examinés à l'aide du test de neutralisation du virus (VNT). D'autres 232 et 296 sérums bovins recueillis respectivement en 2008 et 2009 ont été testés à l'aide d'IgM ELISA. Les résultats ont révélé une prévalence d'anticorps anti-RVF IgG chez les bovins allant de 0,0 à 2,5% dans les districts et de 0,0 à 2,6% au fil des années (1997 à 2005), sans différences significatives entre les districts ou les années. Pendant ce temps, la prévalence des anticorps IgM anti-RVF chez les bovins variait de 0,0 à 6,3% dans les districts et de 0,0 à 2,6% par années (1997-2009). Les bovins échantillonnés dans le district de Kumi étaient 7 fois (rapport de probabilités (OR) 7,0: 95% CL, 1,8 - 26,9) plus susceptibles d'être séropositifs par rapport à ceux des autres districts. En outre, les bovins

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échantillonnés en 2008 étaient 3,4 fois (OR 3,4: 95% CL 1,0-10,1) plus susceptibles d'être séropositifs par rapport aux autres années. En ce qui concerne l'analyse des anticorps neutralisants anti-RVF, les bovins du district d'Apac étaient 3,6 fois (OR 3,6: 95% CL 1.9-6.7) plus susceptibles d'être séropositifs que ceux des autres districts. En outre, les bovins provenant de grands troupeaux (> 100) étaient 5 fois (OR 5,0: 95 CL 2,3 - 10,8) plus susceptibles d'être séropositifs que ceux des petits troupeaux (0 à 50). La ferme Arungi dans le district d'Apac avait une plus grande probabilité (OR 4.0: 95 CL 1.8-9.3) d'avoir des bovins séropositifs. La majorité des 685 bovins (90%) avaient des titres faibles (> 1: 10) d'anticorps neutralisants alors que peu (10%) avaient des titres élevés, allant de 1:10 à> 1: 80. En conclusion, l'étude a révélé des preuves d'un faible niveau de circulation du virus RVF chez les bovins en Ouganda dans certains districts et fermes, en particulier pendant certaines années (2008) au cours des périodes d'inondations.

Mots-clés : IgG; IgM; anticorps neutralisant le RVFV ; bovins ; Ouganda

Introduction

Rift Valley fever is an arthropod-borne disease of ruminants, camels and humans caused by the Phlebovirus, and characterized by massive abortions and high mortalities in young animals (Laughlin *et al.,* 1979; Sall *et al.,* 1998). The RVF virus is maintained in the mosquito vector by a low level cycle interaction of hosts during inter-epizootic periods (Lithicum *et al.,* 1985). The Aedes species passes the virus transovarially, from female to its offsprings by infection of eggs in the ovary. Infected eggs can survive through desiccation for months or years. Exceptionally heavy rainfall leading to flooding triggers explosive breeding of mosquitoes and increased amplification of the virus in the mosquito population.

RVF outbreaks have previously been reported in Kenya (2006-2007), Somalia (2006-2007), Tanzania (2007), Sudan (2007), Madagascar (2008) and South Africa (2008 and 2010). Depending on the climate and vegetation of different regions, occurrence of RVF can either be endemic or epidemic. Endemic cycles of RVF are said to occur every 2-3 years in high rainfall forest zones in coastal and central African areas (FAO, 2008). Conditions that favour the explosive multiplication of mosquito vectors periodically occur in extensive areas of Kenya, southern Somalia, southeastern and southern Ethiopia, eastern Uganda, southern Sudan and northern Tanzania (Anon. 2008). Despite the fact that climatic conditions in Uganda favour explosive multiplication of mosquito vectors and occurrence of RVF outbreaks, there been few reports on either RVF outbreaks or evidence of circulation of the RVF virus in cattle. This study was therefore carried out to establish and document the status of RVF infections in cattle, given its location in the endemic zone of RVF.

Materials and Methods

Study area

The study was conducted in fourteen districts in Uganda, namely, Amuria, Arua, Apac, Busia, Dokolo, Kaberamaido, Kayunga, Kiboga, Kumi, Mbarara, Pallisa, Serere, Soroti and Tororo (Figure 1).

Sample collection

A retrospective analysis of cattle samples was conducted starting from 1997 when the RVF outbreak occurred in East Africa during the El Nino-driven flooding (Lithicum *et al.,* 1999). Available 480 bovine sera collected in 1997, 1998, 2000, 2001 and 2005 from Arua, Busia, Kayunga, Kiboga, Mbarara, Pallisa and Tororo districts stored in the serum bank at the National Livestock Resources Research Institute (NaLIRRI) were tested using both RVF IgG and IgM ELISA performed in parallel. In addition, 685 bovine sera collected in 2006 and 2007 from flood-affected districts of Soroti, Rakai and Apac were tested using the Virus Neutralization Test (VNT). A further 232 and 296 bovine sera collected in 2008 and 2009, respectively, from flood-affected districts of Amuria, Dokolo, Kaberamaido, Kumi, Serere and Soroti were tested using IgM ELISA.

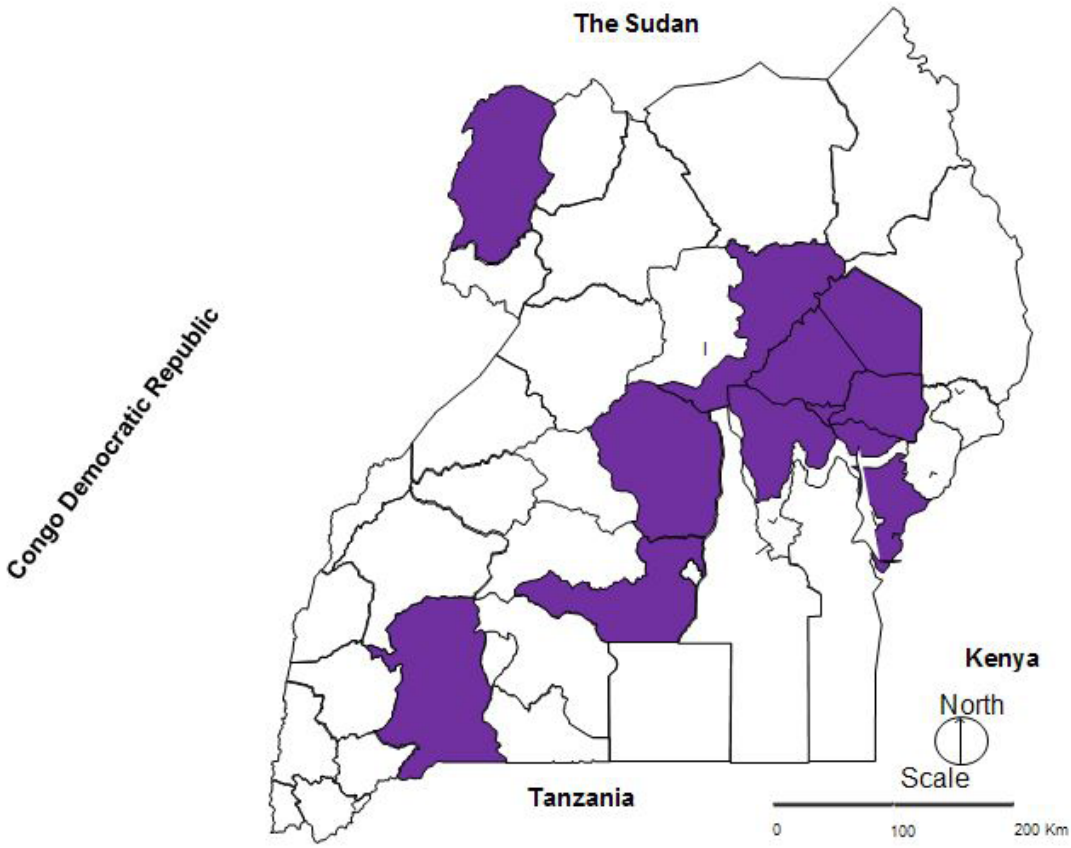

Figure 1: Map of Uganda showing districts where cattle samples (purple) were collected

Serological analysis

The sandwich enzyme-linked immunoassay (ELISA) for detection of anti-RVF IgG antibodies was performed according to the procedure described by Paweska *et al.,* (2003). The enzyme-linked immunoassay for detection of anti-RVFV IgM antibody was performed according to the Manufacturer's protocol. VNT was performed at the National Institute for Communicable Diseases in South Africa according to the method described by the World Health Organisation (2007). For IgG and Ig M–ELISA tests, once the net optical densities (OD) for the strong positive control sera fell between 0.8 and 1.85 and its coefficients of variation were not more than 15% the plates were considered acceptable. Accordingly, any animal that had a percent positivity values (PP) of ≥ 18.1 was considered to be seropositive. For RVF virus neutralizing antibodies, cattle with titres of >1:10 were considered seropositive.

Data analysis

Data analysis was performed using the statistical package Minitab® 16.1.1.0 (Minitab inc., Pennsylvania, USA). Binary logistic regression was performed to establish association between presence of anti-RVFV IgG, anti-RVFV IgM and RVF virus neutralizing antibodies and risk factors: District, Year, Farms, Herd size, Breed, Age and Gender. Risk factor data was applied wherever present since much of the frozen sera had only information on the district of origin and year of collection. However, cattle sera collected progressively, from 2006 to 2009 had information on all risk factors considered. Ideally, a single binary response variable was applied across multi-level risk factor variables. Each of the variables

Table 1: Prevalence of anti-RVFV IgG antibodies in cattle (n = 480) in Uganda, according to district and year

Factor	Level	n	Seroprevalence	OR	95% CL	P-value
District						
	Arua	40	0 (0.0%)	1.0	-	-
	Busia	80	0 (0.0%)	0.0	0.0 – 0.0	0.99
	Kayunga	40	0 (0.0%)	0.0	0.0 – 0.0	0.99
	Kiboga	40	0 (0.0%)	0.0	0.0 – 0.0	0.99
	Mbarara	80	1 (1.3%)	1.2	0.1 - 11.3	0.84
	Pallisa	120	2 (1.7%)	2.0	0.3 - 12.2	0.44
	Tororo	80	2 (2.5%)	3.3	0.5 - 20.6	0.21
Year						
	1997	160	0 (0.0%)	1.0	-	-
	1998	80	0 (0.0%)	0.0	0.0 – 0.0	0.99
	2000	80	1 (1.3%)	1.2	0.1 - 11.3	0.84
	2001	80	5 (6.3%)	3.3	0.5 - 20.6	0.18
	2005	80	0 (0.0%)	0.0	0.0 – 0.0	0.99

Table 2: Prevalence of anti-RVFV IgM antibodies in cattle (n = 1008) in Uganda, according to district and year

Factor	Level	n	Seroprevalence	OR	95% CL	P-value
District						
	Arua	40	0 (0.0%)	1.0	-	-
	Busia	80	0 (0.0%)	0.0	0.0 - 0.0	0.99
	Kayunga	40	0 (0.0%)	0.0	0.0- 0.0	0.99
	Kiboga	80	0 (0.0%)	0.0	0.0 - 0.0	0.99
	Mbarara	80	1 (1.3%)	1.2	0.1 - 11.3	0.84
	Pallisa	80	2 (2.5%)	3.3	0.5 - 20.3	0.19
	Tororo	80	2 (2.5%)	3.3	0.5 - 20.6	0.18
	Soroti	184	3 (1.6%)	1.5	0.4 – 5.6	0.54
	Kumi	48	3 (6.3%)	7.0	1.8 – 26.9	0.01
	Amuria	100	0 (0.0%)	0.0	0.0 – 0.0	0.99
	Dokolo	50	0 (0.0%)	0.0	0.0 – 0.0	0.99
	Kaberamaido	46	1 (2.2%)	1.9	0.2 – 15.2	0.53
	Serere	100	0 (0.0%)	0.0	0.0 – 0.0	0.99
Year	1997	160	2 (1.3%)	1.0	-	-
	1998	80	0 (0.0%)	0.0	0.0 - 0.0	0.99
	2000	80	1 (1.3%)	1.2	0.1 - 11.3	0.84
	2001	80	2 (2.5%)	3.3	0.5 - 20.6	0.18
	2005	80	0 (0.0%)	0.0	0.0 - 0.0	0.99

Factor	Level	n	Seroprevalence	OR	95% CL	P-value
	2008	232	6 (2.6%)	3.4	1.0 – 10.1	0.03
	2009	296	1 (0.3%)	0.2	0.0 – 1.6	0.14

listed as risk factors were applied as classifying variables for logistic regression.

Results

Prevalence levels of anti-RVFV IgG antibodies in cattle in Uganda from 1997 to 2006 in several districts are shown in Table 1. Seroprevalence levels ranging from 0.0 to 2.5% across districts, and 0.0 to 6.3% across years (1997 to 2006) were observed. No significant difference was observed among districts or years.

Prevalence levels of anti-RVFV IgM antibodies in cattle in Uganda from 1997 to 2009 in several districts are shown in Table 2. Seroprevalence levels ranging from 0.0% to 6.3% across districts and 0.0% to 2.6% across years (1997 to 2009) were observed. Cattle from Kumi district had the highest prevalence of anti-RVFV IgM antibodies (6.3%), and were 7 times (Odds Ratio (OR) 7.0: 95% CL, 1.8-26.9) more likely to be seropositive than those from other districts. In addition, the highest prevalence of anti-RVFV IgM antibodies (2.6%) occurred in 2008 and cattle sampled in 2008 were 3.4 times (OR 3.4: 95% CL 1.0-10.1) more likely to be seropositive than during other years.

Prevalence levels of RVF virus neutralizing antibodies in cattle in Uganda over the period 2006 to 2007 is shown in Table 3. Cattle from Apac district had the highest prevalence of RVF virus neutralizing antibodies (18.4%) and were 3.6 times (OR 3.6: 95% CL 1.9-6.7) more likely to be seropositive than those from other districts. In addition, cattle from large herds (> 100) had the highest prevalence of serum neutralizing antibodies (26.2%) and were 5 times (OR 5.0: 95 CL 2.3 – 10.8) more likely to be seropositive than those from small herds (0-50). Interestingly, prevalence levels among cattle on different farms ranged from 0.0% to 50.0%. Arungi farm in Apac district had a higher likelihood (OR 4.0: 95 CL 1.8-9.3) to have seropositive cattle. No significant difference in prevalence of serum neutralizing antibodies was observed among cattle of different age categories (0-6 months, 7-12 months, 13-24 months and >24 months), with prevalence levels ranging from 5.7% to 10.3% detected. Different cattle breeds (Zebu and Ankole) and gender (male and female) had similar prevalence levels of serum neutralizing antibodies.

The distribution of cattle according to titres of RVFV neutralizing antibodies is shown in Fig 2. The majority of the cattle (90%) had low titres (> 1: 10) of neutralizing antibodies while few (10%) had high titres, ranging from 1:10 to > 1: 80.

Discussion

We present findings of a study conducted in Uganda on Rift Valley fever with a view to ascertaining whether the RVF virus circulated in bovine undetected during the period 1997 to 2009. Bovine sera analyzed had either been collected and stored frozen in 1997, 1998, 2000, 2001 and 2005 from Arua, Busia, Kayunga, Kiboga, Mbarara, Pallisa and Tororo districts during flooding. Or else were progressively collected and tested during flooding in districts of Soroti, Rakai and Apac in 2006 and 2007 and Amuria, Dokolo, Kaberamaido, Kumi, Serere and Soroti in 2008 and 2009.

Seroprevalence levels ranging from 0.0 to 2.5% across districts, and 0.0 to 6.3% across years (1997 to 2006) were observed with no significant difference observed among districts or years. Anti-RVFV IgM antibodies were detected in a few frozen cattle sera collected since 1997. However, these results have to be interpreted with caution since the transitory nature of IgM antibodies in infected animals influences the sensitivity and specificity of the

Table 3: Prevalence of RVF virus neutralizing antibodies in cattle (n = 685) in Uganda, according to districts, herd size, farm, age, breed and gender

Factor	Level	n	Seroprevalence	OR	95% CL	P-value
District	Soroti	430	25 (5.8%)	1.0	-	-
	Rakai	130	12 (9.3%)	1.6	0.8 - 3.3	0.17
	Apac	125	23 (18.4%)	3.6	1.9 - 6.7	0.00
Herd size	Herd size 0-50	513	34 (6.6%)	1.0	-	-
	Herd size 51-100	130	15 (11.5%)	1.8	1.0 - 3.5	0.06
	Herd size > 100	42	11 (26.2%)	5.0	2.3- 10.8	0.00
Farm	Kato	10	1 (10.0%)	1.0	-	-
	Mugisha	40	2 (5.0%)	0.3	0.1 - 1.5	0.17
	Ongom	83	12 (14.5%)	1.2	0.2 – 6.2	0.77
	Arungi	42	11 (26.2%)	4.0	1.8 – 9.3	0.00
	Rwabazaire	20	1 (5.0%)	0.4	0.1 – 2.8	0.33
	Rwakagabo	10	2 (20.0%)	1.7	0.4 – 8.5	0.50
	Kasana	20	3 (15.0%)	1.2	0.3 – 4.4	0.76
	Katerega	20	3 (15.0%)	1.2	0.3 – 4.4	0.76
	Nsinze	10	0 (0.0%)	0.0	0.0 – 0.0	0.99
	Okirror	29	1 (3.4%)	0.2	0.0 – 1.7	0.14
	Olei	10	0 (0.0%)	0.0	0.0 – 0.0	0.99
	Oreeti	10	0 (0.0%)	0.0	0.0 – 0.0	0.99
	Opedun	14	0 (0.0%)	0.0	0.0 – 0.0	0.99
	Egwau	2	1 (50.0%)	6.3	0.4- 102.8	0.19
	OkirrorA	17	1 (5.9%)	0.4	0.1 – 3.0	0.37
	Apuka	21	3 (14.3%)	1.2	0.3 – 3.7	0.94
	Omunyokori	15	1 (6.7%)	0.4	0.1 – 3.5	0.44
	Elumu	2	1 (50.0%)	6.3	0.4-102.8	0.19
Age	0 – 6 months	53	3 (5.7%)	1.0	-	-
	7 – 12 months	97	8 (8.2%)	1.5	0.4 - 5.9	0.56
	13 – 24 months	156	10 (6.4%)	1.1	0.3 - 4.3	0.84
	>24 months	379	39 (10.3%)	1.9	0.6 - 6.4	0.29
Breed	Zebu	391	34 (8.7%)	1.0	-	-
	Ankole	294	26 (8.8%)	1.1	0.6 - 1.7	0.94
Gender	Male	288	22 (7.6%)	1.0	-	-
	Female	397	38 (9.6%)	1.3	0.7 - 2.2	0.37

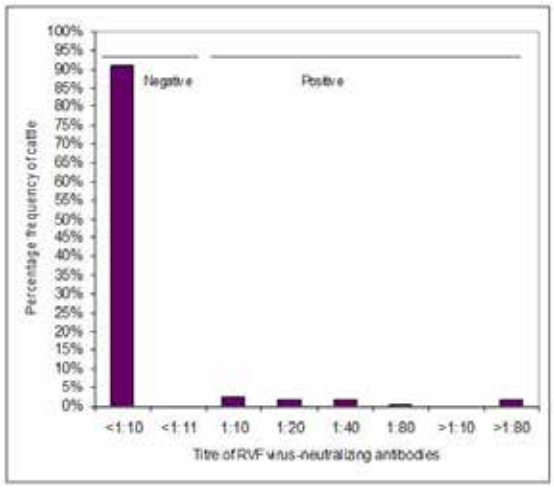

Figure 2: Distribution of cattle (n = 685) according to titre of RVF virus-neutralizing antibodies

IgM detection ELISA (Paweska *et al.,* 2003).

Seroprevalence levels ranging from 0.0% to 6.3% across districts and 0.0% to 2.6% across years (1997 to 2009) were observed. Cattle from Kumi district had the highest prevalence of anti-RVFV IgM antibodies, and were 7 times more likely to be seropositive than those from other districts. In addition, the highest prevalence of anti-RVFV IgM antibodies (2.6%) occurred in 2008 and cattle sampled in 2008 were 3.4 times more likely to be seropositive than during years. FAO's RVF monthly risk map for then (February 2008) based on persistence of NDVI anomalies had placed eastern Uganda in the RVF endemic region (FAO, 2008). Indeed, eastern Uganda experienced exceptionally heavy rainfall during the period October to December 2007 which led to unprecedented episodes of flooding. Indeed, there are several flood-prone spots in the districts of Kumi, Soroti, Amuria, Kaberamaido, Dokolo, Amolatar and Apac in eastern Uganda.

Cattle from Apac district had the highest prevalence of RVF virus neutralizing antibodies and were 3.6 times more likely to be seropositive than those from other districts. Animals in Apac district, especially Arungi farm could be an interepidemic period, when RVFV is believed to depend on transovarial transmission of virus in floodwater Aedes mosquitoes (Lithicum *et al.,* 1985). It must be noted that Apac district borders Lake Kyoga and River Nile that have high rainfall and humidity hence conducive for mosquito multiplication. Such areas with high rainfall and humidity are known to experience RVF outbreaks at irregular intervals of 3-15 years (Anon., 2008).

Cattle from large herds (> 100) had the highest prevalence of serum neutralizing antibodies and were 5 times more likely to be seropositive than those from small herds (0-50). Interestingly, different farms had varying prevalence levels (0.0% to 50.0%). Arungi farm in Apac district had a higher likelihood to have seropositive cattle, suggesting farm as a risk factor influenced the seroprevalence levels.

The majority of the cattle (90%) had low titres (> 1: 10) of neutralizing antibodies while few (10%) had high titres, ranging from 1:10 to > 1: 80. However, prevalence of RVFV-neutralizing titres up to 1:80 in bovine samples confirmed presence RVFV infection. Moreover, detectable RVFV-neutralizing antibodies were observed in samples collected in 2006 and 2007, suggesting there might have been recent virus replication in cattle in these locations.

In conclusion, the study revealed evidence of low level circulation of the RVF virus in cattle in Uganda in certain districts and farms, especially during certain years (2008) when flooding occurred.

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DEVELOPMENT OF TRYPANOSOMOSIS AGGLUTINATION CARD TEST (TACT) UTILIZING FIXED AND STABILIZED PROCYCLIC ANTIGEN FROM CULTURE-DERIVED TRYPANOSOME BRUCEI GAMBIENSE AND ITS EVALUATION IN UGANDA

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Abstract

In a bid to improve field diagnosis of animal trypanosomosis in tsetse-infested African countries, TACT utilizing fixed and stabilized procyclic antigen from culture-derived Trypanosoma brucei gambiense isolate IL2343 was developed and evaluated in Uganda. Its diagnostic sensitivity was evaluated using blood samples from 64 confirmed trypanosome-infected cattle, and its diagnostic specificity using blood samples from 328 trypanosome-free cattle from non-tsetse infested high altitude areas on Mt Elgon. Diagnostic sensitivity and specificity of TACT were also evaluated at varying dilutions of the antigen (1:10, 1:100 and 1:500). Suitability of whole blood and plasma for performing TACT was tested using 50 negative and 133 positive samples. In addition, the detection rate of TACT in comparison to the parasitological test (Haematocrit Centrifugation Technique) was assessed using blood samples from 145 anaemic cattle (PCV≤24), 642 non-anaemic cattle (PCV ≤24), 433 cattle from trypanosomosis-endemic areas, and 500 cattle from a Rhodesiense sleeping sickness focus. Findings revealed that TACT had a diagnostic sensitivity of 94% and a diagnostic specificity of 100%. Its diagnostic sensitivity declined with increasing dilution of antigen while its diagnostic specificity remained constant. Either whole blood or plasma was equally suitable for performance of TACT. Regarding cattle with different pathological status, TACT was three times as sensitive in detecting trypanosome infection in anaemic cattle (clinical cases) but five times as sensitive in detecting *trypanosome* infection in non-anaemic cattle as compared to the parasitological test. Furthermore, 95% of anaemic cattle and 84% of non-anaemic cattle detected positive parasitologically were also detected positive by TACT. Regarding cattle in trypanosomosis-endemic areas, TACT was twenty times as sensitive as the parasitological test in detecting trypanosome infection. Interestingly, 100% of the cattle detected positive parasitologically were also detected positive by TACT. Regarding cattle reservoirs in a Rhodesiense sleeping sickness foci, TACT was three times as sensitive as the parasitological test in the detection of trypanosome infection. 100% of the cattle detected positive parasitologically were also detected positive by TACT. In conclusion, the rapid nature of TACT, affordability, easy of testing and impressive sensitivity for detection of animal trypanosomosis were its major advantages.

Keywords: Animal *trypanosomosis*; Diagnosis; Card Agglutination; Sensitivity; Specificity

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Introduction

Field diagnosis of animal trypanosomosis in tsetse-infested African countries relies largely on parasitological tests such as wet smear, thick smear, Haematocrit Centrifugation Test (HCT) (Woo, 1969) and Buffy Coat Technique (BCT) (Murray *et al.,* 1977). However, these parasitological tests have low sensitivity i.e. can only detect trypanosome infection when the density exceeds 1000 parasites per ml (Paris *et al.,* 1982). Moreover, in many tsetse infested countries in Africa, veterinary diagnostic laboratories are either poorly equipped or understaffed or have fallen into disuse due to underfunding and hence unable to support efficient diagnostic requirements for livestock owners regarding key livestock diseases, including trypanosomosis. Yet, effective control of trypanosomosis requires appropriate diagnosis to ensure correct treatment of individual cases or herds in tsetse-infested areas. Detection of clinical disease could be facilitated by development and dissemination of simple rapid diagnostic tests such as Trypanosomosis Agglutination Card Test or diagnostic decision support systems such as Decision Support Card for field veterinarians and animal health auxiliaries (Magona, 2004; Eisler et al, 2007). Card Agglutination Test for Trypanosomiasis (CATT) (Magnus *et al.,* 1978), a commercially available card agglutination test for detection anti-trypanosomal antibodies has largely been applied for the diagnosis of Human African Trypanosomiasis (HAT) caused by T. b. gambiense. Other serological tests such as Immunofluorescent Antibody Test (IFAT) (Katende *et al.,* 1987) and Enzyme-linked Immunosorbent Assay (ELISA) (Van Knapeu *et al.,* 1977) have low specificity, require well-trained manpower and are more suitable for laboratory-based analysis of samples for large-scale epidemiological studies. Pearson et al. (1986), Olaho-Mukani *et al.* (1991) and Ngaira et al. (1992) developed and evaluated methods for use of live procyclic trypanosomes in card agglutination tests. However, maintaining a constant supply of antigen under field conditions remained an obstacle. Moreover, living procyclic trypanosomes have the tendency to auto-agglutinate under ambient temperature if the test is not read quickly, thereby increasing the level of false positivity. Akol *et al.* (1999) used fixed and stabilized procyclic antigen derived from T.b. rhodesiense in a card agglutination test which was successful evaluated for serodiagnosis of T.b. rhodesiense sleeping sickness. Given the demand for improved field diagnosis for effective control of animal trypanosomosis in rural areas of Africa, TACT utilizing fixed and stabilized procyclic antigen from culture-derived *Trypanosoma brucei gambiense* isolate IL2343 was produced and evaluated for its sensitivity and specificity in detecting anti-trypanosomal antibodies in infected cattle.

Materials and Methods

Preparation of stabilized procyclic antigen

Propagation of Trypanosoma brucei gambiense IL2343 procyclic forms in culture

Trypanosoma brucei gambiense IL2343 procyclic forms were propagated in TVM culture media at 27 °C at Hokkaido University Research Center for Zoonosis Control. Parasites were then washed twice in PSG buffer and centrifuged at 2000 rpm for 10 minutes. This was followed by five times of freezing and thawing to lyse the parasites. Parasite lysis was followed by sonication to further mince the parasite lysate. After which the lysate was centrifuged at 14,000 rpm, 4°C for 30 min. The supernatant was then harvested, subjected to molecular sieving and protein concentration determined.

Western blot analysis

Western blot analysis was performed to assess the level of antigenicity and cross-reaction between *T. brucei gambiense* and *T. evansi* crude antigen with the Variant Soluble Glycoprotein (VSG) using llama anti-VSG polyclonal antiserum. Analysis was performed on the whole parasite and on the soluble VSG of *T. brucei gambiense* and *T. evansi* isolates.

SDS PAGE analysis of purified T. brucei gambiense soluble VSG

SDS PAGE analysis was performed on the whole parasite, crude homogenate and purified *T. brucei gambiense* soluble VSG.

Separation of Trypanosoma brucei gambiense PCF proteins by mass spectrometry and testing their antigenicity for serodiagnosis

Separation of *Trypanosoma brucei gambiense* Procyclic form proteins was undertaken using mass spectrometry. The products consisted of forward phase protein fractions that had a higher protein concentration followed by the reverse phase protein fractions that had lower protein concentration. Both sets of protein fractions were then tested for antigenicity using ELISA against bovine sera.

Preservation and enzyme stabilization of procyclic antigen

Purified procyclic form antigen composed of largely the Soluble VSG amounting to 500 ml was mixed with enzyme stabilizers and stored at either room temperature (25°C) or 4°C in laboratory at Hokkaido University Research Centre for Zoonosis Control in Sapporo, Japan until transported to NaLIRRI, Tororo Uganda for field testing.

Final card agglutination antigen for routine use

Before use, the procyclic antigen was fixed with acetone and mixed with a dye. For each test, fifty micro-litres of antigen kept at room temperature (25°C) were placed on a spot of the test card (Fig. 4 a and b) and fifty micro-litres of each test sera added. The antigen and sample were mixed by rotating the card slowly, observing for agglutination over a period of 3-5 minutes.

Evaluation of the diagnostic sensitivity and specificity under field conditions in Uganda using bovine samples

The diagnostic sensitivity and specificity of TACT, and their variation with different dilutions of the Trypanosoma antigen were evaluated. Assessment of the diagnostic sensitivity was performed using 64 blood samples from proved trypanosome-infected cattle from endemic areas. While the assessment of the diagnostic specificity was performed using 328 blood samples from trypanosome-free cattle from non-tsetse infested high altitude areas in Mt Elgon. The diagnostic sensitivity and specificity of TACT were assessed at varying dilutions of the antigen, ranging from 1: 10 to 1:100 and 1:500.

Evaluation of the detection rate of TACT using either blood or plasma samples

The detection rate of TACT using either blood or plasma was assessed in order to understand the form of samples, either whole blood or plasma for performance of TACT.

Field evaluation of TACT in comparison to parasitological tests

The detection rate of TACT in comparison to parasitological tests for trypanosome infection was assessed using blood samples from cattle of different pathological status i.e. as anaemic (n = 145) and non-anaemic (n = 642). In addition, the detection rate of TACT in comparison to parasitological tests for trypanosome infection was assessed using blood samples (n = 433) from cattle from trypanosomosis-endemic areas of Tororo district and Kamuli district and as well using blood samples (n = 500) from cattle suspected to be reservoirs for *T. brucei Rhodesiense* in a sleeping sickness foci in Soroti district.

Statistical analysis

Sensitivity and specificity for TACT utilizing the fixed and stabilized *T. brucei gambiense* procyclic antigen were calculated using the computer program EPI INFO™ 6 (CDC, Atlanta, Georgia, USA). Test sensitivity was defined as the proportion of blood samples detected positive by TACT that were truly positive with confirmed presence of trypanosomes. Test specificity was defined as the proportion of blood samples detected negative by TACT that was truly negative in that were collected from trypanosome-free cattle from high-altitude tsetse-free areas in Mt. Elgon.

Results

Up to 500 ml of procyclic antigen was produced through laboratory culture at Hokkaido University. Testing of protein concentration of *T. brucei gambiense* crude and soluble VSG antigen showed that crude antigen had a protein concentration of 650.75 µg and soluble VSG antigen had a protein concentration of 390.50 µg (Table 1).

Western blot analysis of *T. brucei gambiense* viz-a-vis *T. evansi* whole parasites against llama anti-VSG polyclonal antiserum demonstrated a conspicuous protein band at 60 kDa (indicative of the VSG) and another protein band at 41kDa in both *T. brucei gambiense* and *T. evansi*, suggesting strong antigenicity of procyclic forms and strong cross-reaction between *T. brucei gambiense* and *T. evansi* (Fig. 1a). Furthermore, western blot analysis of *T. brucei gambiense* and *T. evansi* crude antigen and soluble VSG against llama anti-VSG polyclonal antiserum revealed strong protein bands at 60 kDa and a light protein band at 55 kDa, suggesting existence of cross-reaction between *T. brucei gambiense* and *T. evansi* crude antigen (Fig. 1b). The strong protein band at 60 kDa within the soluble VSG antigen suggested that antigen from Trypanosoma brucei species exhibit strong cross-reaction with other trypanosome species, hence provide good antigen for serodiagnosis. Further still, SDS PAGE analysis performed on the *T. brucei gambiense* whole parasite, crude homogenate and purified soluble VSG demonstrated a strong protein band at 60 kDa (Fig. 2). Separation of *T. brucei gambiense* procyclic form (PCF) antigen into different protein fractions by mass spectroscopy is shown in Fig. 3. Up to eight different protein fractions were separated. The fractions had protein concentrations ranging from 3.4 µg/ml to 53.7 µg/ml.

ELISA optical density (OD) values for testing of selected *T. brucei gambiense* procyclic form forward phase protein fractions against positive and negative anti-trypanosomal bovine sera are shown in Table 2. There was a substantial and distinctive difference in OD values of positive (0.580 – 1.701) and negative bovine sera (0.237 – 0.521), indicating that the protein fractions had good antigenicity.

ELISA optical density (OD) values for testing of selected *T. brucei gambiense* procyclic form reverse phase protein fractions against bovine sera for antigenicity is shown in Table 3. Initial procyclic form reverse phase protein fractions (PCF 26C2 F1 – PCF 26C2 F5) had low antigenicity, given that there was no clear distinction between OD values for positive bovine sera (0.771 – 0.916) and for negative bovine sera (0.770 – 0.912). However, the later procyclic form reverse phase protein fractions (PCF 26C2 F6 – PCF 29C5 F8) had slightly better antigenicity, given a distinction between OD values for positive bovine sera (0.820 – 1.385) and OD values for negative bovine sera (0.590 – 0.965) despite minor overlap.

The reaction on the TACT test card (before agglutination) following placement of the blood sample together with a drop of fixed and stabilized dyed procyclic antigen is shown in Fig. 4a and the reaction on the TACT test card following agglutination after 3-5 minutes, indicative of a positive sample is shown in Fig. 4b.

Table 1: Protein concentration of *T. brucei gambiense* crude and soluble VSG antigen

Antigen type	Protein concentration	Amount	Total Protein
T.brucei gambiense crude antigen	2603.0µg/ml	0.25ml	650.75µg
T. brucei gambiense soluble VSG antigen	1562.0µg/ml	0.25ml	390.50µg

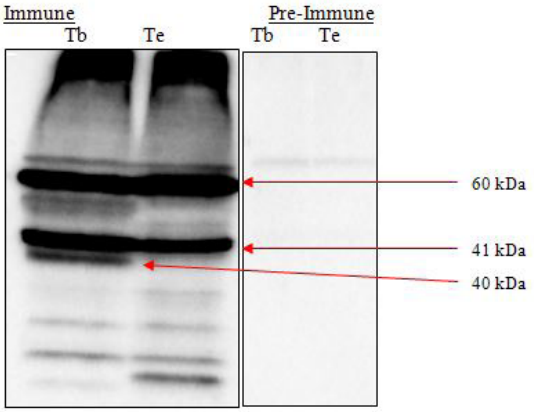
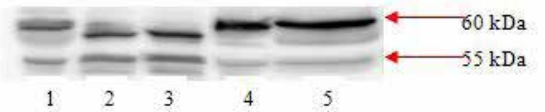
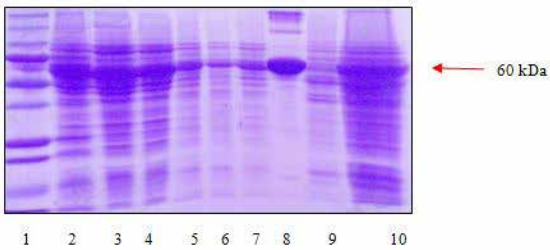


Fig. 1a: Protein bands as a result of Western blot analysis of *T. brucei gambiense* viz-a-vis *T. evansi* whole parasites against llama anti-VSG polyclonal antiserum



1-Tb whole parasite, 2-Te crude antigen, 3-Tb crude antigen, 4-Te soluble VSG, 5-Tb soluble VSG

Fig. 1b: Protein bands resulting from western blot analysis of *T. brucei gambiense* and *T. evansi* crude antigen and soluble VSG against llama anti-VSG polyclonal antiserum



1-wt band
2-Tb whole parasite
3&4-Tb sVSG
5, 6 & 7-Tb crude homogenate
8-BSA 1mg/ml
9 Tb sVSG
10 Tb whole parasite

Fig. 2: SDS PAGE analysis of *T. brucei gambiense* whole parasite, crude homogenate and purified soluble VSG

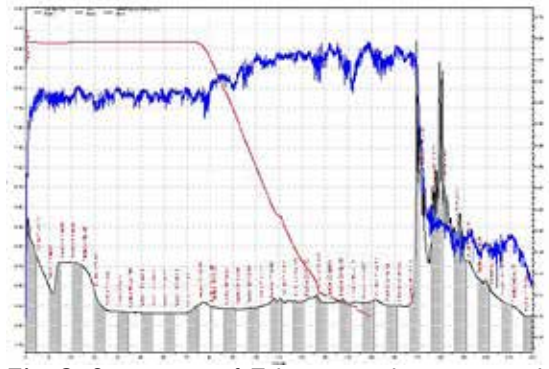


Fig. 3: Separation of *T. brucei gambiense* procyclic form proteins fractions using mass spectrometry

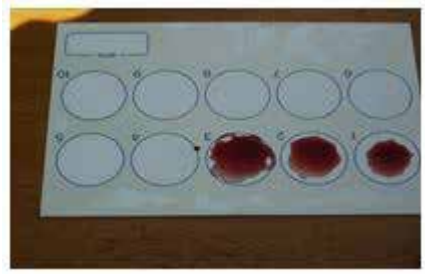


Fig. 4a: TACT Test card (before agglutination)

Fig. 4b: TACT Test card (presence of agglutination in the well indication positive)

The findings of evaluation of the diagnostic sensitivity and specificity under field conditions in Uganda using bovine samples are shown in Table 4. TACT achieved a diagnostic sensitivity of 94% and a diagnostic specificity of 100%. In addition, findings of the evaluation of the status of the diagnostic sensitivity and diagnostic specificity with changing dilution of the TACT antigen: 1:10, 1:100 and 1:500 are shown in Fig. 5. TACT diagnostic sensitivity declined from 94% to 20% with increasing dilution of the TACT antigen from 1:10 to 1:100 and 1:500. On the contrary, the TACT

Table 2: ELISA testing of selected *T. brucei gambiense* procyclic form forward phase protein fractions against bovine sera for antigenicity

Antigen	Protein conc. µg/ml	Coating dilution	OD Positive sera	OD Negative sera
PCF B5	3.4	1:20	0.858	0.345
PCF B2	1.7	1:20	0.818	0.352
PCF C2	21.8	1:20	0.781	0.368
		1:40	0.705	0.286
PCF C3	16.8	1:20	0.837	0.336
		1:40	0.687	0.262
PCF C4	8.4	1:20	0.830	0.369
PCF C5	3.4	1:20	0.812	0.360
PCF C8	8.4	1:20	0.808	0.403
PCF C11	53.7	1:20	1.011	0.475
		1:40	0.724	0.243
		1:80	0.638	0.237
PCF crude	3989	1:20	1.701	0.521
		1:40	1.362	0.413
		1:80	0.580	0.256
		1:160	0.666	0.242

Table 2: ELISA testing of selected *T. brucei gambiense* procyclic form forward phase protein fractions against bovine sera for antigenicity

Antigen	Protein conc. µg/ml	Coating dilution	OD Positive sera	OD Negative sera
PCF 26C2 F1	15.0	1:20	0.912	0.834
PCF 26C2 F2	1.0	1:20	0.886	0.698
PCF 26C2 F3	3.0	1:20	0.770	0.902
PCF 26C2 F4	4.0	1:20	0.928	0.916
PCF 26C2 F5	5.0	1:20	0.885	0.771
PCF 26C2 F6	2.0	1:20	1.048	0.670
PCF 26C2 F7	10.0	1:20	0.820	0.662
PCF 26C2 F8	3.0	1:20	0.970	0.590
PCF 27C3 F1	3.0	1:20	1.120	0.705
PCF 27C3 F2	2.0	1:20	1.307	0.841
PCF 27C3 F3	1.0	1:20	1.173	0.800
PCF 27C3 F4	2.0	1:20	1.187	0.806
PCF 27C3 F5	8.0	1:20	1.106	0.786
PCF 28C4 F1	4.0	1:20	1.165	0.829
PCF 28C4 F2	5.0	1:20	1.333	0.819
PCF 28C4 F3	3.0	1:20	0.926	0.693
PCF 28C4 F4	6.0	1:20	1.372	0.806
PCF 28C4 F5	5.0	1:20	1.366	0.795

Antigen	Protein conc. µg/ml	Coating dilution	OD Positive sera	OD Negative sera
PCF 28C4 F6	5.0	1:20	1.371	0.863
PCF 28C4 F7	2.0	1:20	1.385	0.783
PCF 29C5 F1	14.0	1:20	1.377	0.751
PCF 29C5 F2	1.0	1:20	1.359	0.760
PCF 29C5 F3	5.0	1:20	1.303	0.739
PCF 29C5 F4	22.0	1:20	1.306	0.844
PCF 29C5 F5	68.0	1:20	1.332	0.965
PCF 29C5 F8	4.0	1:20	1.284	0.829

Table 4: Diagnostic sensitivity and specificity of TACT

	Trypanosome-infected cattle	*Trypanosome*-free cattle	Total
TACT positives	60	0	60
TACT negatives	4	328	332
Total	64	328	392

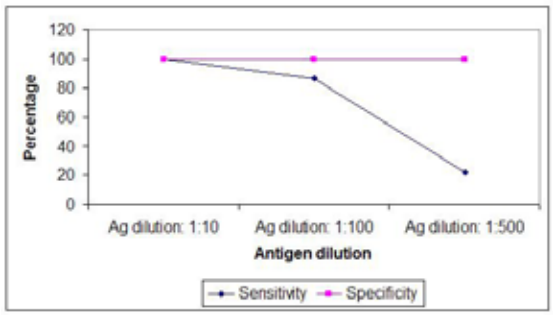


Fig. 5: Variation in the diagnostic sensitivity and specificity of TACT according to different antigen dilution

diagnostic specificity remained constant at 100% despite increasing the dilution of the TACT antigen from 1:10 to 1:100 and 1:500.

The findings of the evaluation of the detection rate of TACT using either blood or plasma are shown in Table 5. The TACT detection rate for trypanosome infection using 50 negative blood or 50 negative plasma samples was equally 0%. Likewise, the TACT detection rate for trypanosome infection using 133 positive blood or 133 positive plasma samples was equally 100%. This suggested that either whole blood or plasma was suitable for performance of TACT.

The findings for the detection rate of TACT in comparison to parasitological tests for trypanosome infection using 145 blood samples from anaemic cattle (PCV≤24) and 642 blood samples from non-anaemic cattle (PCV ☐24) is shown in Table 6. TACT had a detection rate of 44.1% for trypanosome infection in anaemic cattle as compared to 15.2% by the parasitological test: hence TACT was three times as sensitive. In addition, TACT had a detection rate of 16.7% for trypanosome infection in non-anaemic cattle as compared to 3.0% by the parasitological test: hence TACT was five times as sensitive. Similarly, 21 out of 22 (95%) anaemic cattle detected by the parasitological test as infected with trypanosomes were also detected positive by TACT. While 16 out of 19 (84%) non-anaemic cattle detected by the parasitological test as infected with trypanosomes were as well detected positive by TACT. Hence a huge majority of the positive samples detected by the parasitological test were also detected positive by TACT.

Findings for the detection rate of TACT in comparison to parasitological tests for trypanosome infection in cattle from trypanosomosis-endemic areas of Tororo district and Kamuli district are shown in Table 7. 115 out 433 (26.6%) cattle were detected

Table 5: Evaluation of the detection rate of TACT using either blood or plasma samples

Category	TACT detection rate		
	No. cattle	Blood	Plasma
Negative samples	50	0%	0%
Positive samples	133	100%	100%

Table 6: Comparison of detection rates of TACT and parasitological test (Hematocrit Centrifugation Technique) for trypanosome infection in either anaemic or non-anaemic cattle

	Detection rate			
Category	No. cattle	TACT positives	HCT positives	Both tests
Anaemic (PCV ≤ 24.0)	145	64 (44.1%)	22 (15.2%)	21 (14.5%)
Non-anaemic (PCV □ 24.0)	642	107 (16.7%)	19 (3.0%)	16 (2.5%)
Total	787	171 (21.7%)	41 (5.2%)	37 (4.7%)

PCV = Packed Cell Volume

Table 7: Comparison of detection rates of TACT and parasitological test (Hematocrit Centrifugation Technique) for trypanosome infection in cattle in trypanosomosis-endemic areas in Uganda

Trypanosomosis-endemic areas		No. cattle	TACT positives	HCT positives	Both tests
District	Village				
Kamuli	Namwenda	132	4 (3.0%)	3 (2.3%)	3 (2.3%)
Kamuli	Kasolwe	168	5 (3.0%)	1 (0.6%)	1 (0.6%)
Tororo	Kayoro	133	106 (79.7%)	1 (0.8%)	1 (0.8%)
Total		433	115 (26.6%)	5 (1.2%)	5 (1.2%)

Table 8: Comparison of detection rates of TACT and parasitological test (Hematocrit Centrifugation Technique) for trypanosome infection in cattle reservoirs in Rhodesiense sleeping sickness foci in Uganda

Rhodesiense endemic areas		No. cattle	TACT positives	HCT positives	Both tests
District	Village				
Iganga	Busowobi	100	15 (15.0%)	7 (7.0%)	7 (7.0%)
Pallisa	Nabiswa	100	9 (9.0%)	3 (3.0%)	3 (3.0%)
Pallisa	Kataka	100	11 (11.0%)	8 (8.0%)	8 (8.0%)
Soroti	Omagara	100	21 (21.0%)	3 (3.0%)	3 (3.0%)
Soroti	Adoku	100	13 (13.0%)	2 (2.0%)	2 (2.0%)
Total		500	69 (13.8%)	23 (4.6%)	23 (4.6%)

infected with trypanosomes by TACT as compared to only 5 out of 433 (1.2%) by the parasitological test: hence TACT was twenty times as sensitive as the parasitological test in trypanosomosis-endemic areas. Interestingly, 5 out 5 (100%) detected by the parasitological test as infected with trypanosomes were also detected positive by TACT, suggesting TACT did not miss any of the positives detected by the parasitological test.

Findings of the detection rate of TACT as compared to the parasitological test for trypanosome infections in cattle suspected to be reservoirs for *T. brucei rhodesiense* in

a sleeping sickness foci in Soroti district are shown in Table 8. 69 out of 500 (13.8%) of the cattle were detected infected with trypanosomes by TACT as compared to only 23 out 500 (4.6%) by the parasitological test: hence TACT was three times as sensitive under such conditions. Moreover, 23 out of 23 (100%) of the cattle detected by the parasitological test were detected positive for trypanosome infection by TACT. This likewise indicated that TACT did not miss any trypanosome infection detected by the parasitological test among cattle largely suspected to be reservoirs of *T. brucei rhodesiense* infection in a sleeping sickness foci.

Discussion

TACT, a penside test for detection of animal trypanosomosis utilizing fixed and stabilized procyclic antigens from culture-derived *Trypanosoma brucei gambiense* IL2343 was developed at Hokkaido University Centre for Zoonosis Control in Japan and evaluated in Uganda using sera from non-infected and trypanosome-infected indigenous cattle. Although use of parasitological tests to determine the status of negative and positive cattle was undoubtedly a limitation in the test evaluation due to unavailability of better gold standards, additional criteria such as use of positive samples originating from cattle in trypanosomosis-endemic areas and negative samples from cattle in tsetse-free high-altitude areas on Mt. Elgon had to be devised. Thrusfield (1995) recommended determination of test agreement to ascertain test validity when there is no clear gold standard. Despite this limitation, diagnostic sensitivity and specificity obtained with TACT utilizing *T. brucei gambiense* procyclic antigen were quite comparable to those reported by Pearson *et al.,* (1986) and Olaho-Mukani *et al.,* (1991).

Viability of the antigen over the entire period of the field evaluation at room temperature (25oC) evidently showed that stability of the antigen is not affected by ambient temperature. Implying that fixing the procyclic trypanosome antigen adequately preserved the antigenicity of the surface epitopes. This was in line with findings of similar previous studies (Akol *et al.,* 1999).

CATT/T.evansi, a card agglutination test for anti-trypanosomal antibodies against *T. evansi* has been used successfully in camels. Like TACT is a quick and easy-to-use test and can be performed in the field. CATT/T.evansi test is reported to have a sensitivity of 86-100% and a specificity of 96-98% (Gutierrez *et al.,* 2000). Similar to previous studies on CATT/*T. evansi* (Gutierrez *et al.,* 2000), TACT had a diagnostic sensitivity of 94% and a diagnostic specificity of 100%. It was noted that TACT diagnostic sensitivity declined from 94% to 20% with increasing dilution of the TACT antigen from 1:10 to 1:100 and 1:500, suggesting the best TACT antigen dilution was 1:10 for optimal diagnostic sensitivity. Otherwise, TACT diagnostic specificity was not affected by the dilution of the antigen. In addition, it was proved that either whole blood or plasma was suitable for performance of TACT.

Regarding cattle with different pathological status, TACT was three times as sensitive as the parasitological test in detecting trypanosome infection if performed on anaemic cattle i.e. clinical cases of trypanosomosis. Better still, TACT was five times as sensitive as parasitological tests in detecting trypanosome infection if performed on non-anaemic cattle. It was further noted that 95% of anaemic cattle detected by the parasitological test as infected with trypanosomes were also detected positive by TACT. While 84% of non-anaemic cattle detected by the parasitological test as infected with trypanosomes were as well detected positive by TACT. Hence TACT could improve overall detection of trypanosomosis if performed in series with the parasitological test combined with clinical examination.

Regarding cattle in trypanosomosis-endemic areas, TACT was twenty times as sensitive as the parasitological test in detecting trypanosome infection. Interestingly, 100% of the cattle detected positive parasitologically were also detected positive by TACT

For detection of trypanosome infections in cattle suspected to be reservoirs

for *T. brucei rhodesiense* in a sleeping sickness foci like in Eastern Uganda, TACT proved to be three times as sensitive as the parasitological test. Moreover, 100% of the cattle detected by the parasitological test to have trypanosome infection were detected positive by TACT, suggesting TACT did not miss any trypanosome infection among cattle largely suspected to be reservoirs of *T. brucei rhodesiense* infection in a sleeping sickness foci. This was largely because of the high level of cross-reaction among trypanosome antigen among the Trypanozoon group to which *T. brucei gambiense*, *T. brucei rhodesiense*, *T. brucei brucei* and *T. evansi* belong. In the absence of a quick field antibody detection test for tsetse-transmitted animal trypanosomosis, TACT could be a promising alternative test. However, further large-scale evaluation of the test is required before it can be used for routine field application.

In conclusion, the rapid nature of TACT, affordability, easy of testing and impressive sensitivity for detection of animal trypanosomosis are its major advantages. Results are ready within 3-5 minutes, and a drop of blood is mixed with a drop of test antigen on a card. The test is much cheaper regarding reagents and equipment than conventional parasitological tests for trypanosomosis. However, diagnostic antigen requires back-up tissue culture facilities for production of culture-derived trypanosomes for mass production of antigen for TACT and for ready supply for routine diagnosis of animal trypanosomosis.

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EFFECT OF ORAL LEVAMISOLE TREATMENT OF COCKERELS ON THEIR RESPONSES TO EXPERIMENTAL INTRAOCULAR INFECTION WITH VELOGENIC NEWCASTLE DISEASE VIRUS

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Abstract

This study was conducted to evaluate the effect of oral levamisole treatment of cockerels on their responses to experimental intraocular infection with velogenic Newcastle disease virus (NDV) and to assess whether the treatment would affect the course of the disease process by altering the immune response. There were 3 infected groups of 20 cockerels each (4 weeks of age) with 10 as non-infected controls. The infected groups were orally treated with levamisole at 5 mg/kg daily for 3 consecutive days either before infection (TBI) or from days 4 after infection (TAI), or were not treated (NTI). Morbidity, antibody responses, mortality and pathological changes were monitored in the infected groups during the period post-infection (pi). Clinical morbidity was not affected by treatment, but mortality period was significantly ($P<0.05$) shorter in TBI and TAI than in NTI groups. Aggregate score of clinical signs was significantly ($p<0.05$) lower (56.3%) in TBI than in TAI (87.5%) and NTI (100%) groups. All infected birds showed weight loss and stunting. Hyperthermia was more severe in TBI than TAI and NTI on day 2 post-infection (pi). Antibody response was lower ($P<0.05$) in TBI and TAI than NTI on days 7-14 pi, but was higher ($P<0.05$) in TBI than TAI. Thereafter, TBI exhibited higher ($P<0.05$) antibody response than TAI and NTI on day 21 pi. Neuronal damage by the virus in all infected groups was evident histopathologically but only TAI and NTI showed torticollis. Levamisole treatment of infected birds did not reduce the morbidity and mortality, but suppressed antibody response at the early phase followed by a delayed strong antibody response in TBI probably due to earlier activated T-helper cell function. Therefore, immunological alterations attributable to levamisole treatment did not seem to have rescued the chickens from the outcome of the disease to warrant its use to manage Newcastle disease outbreaks.

Keywords: Clinical signs, cockerels, haemagglutination inhibition test, intraocular infection, levamisole treatment, velogenic Newcastle disease virus.

EFFET DU TRAITEMENT ORAL AU LEVAMISOL DES COQS SUR LEURS RÉACTIONS À L'INFECTION INTRAOCULAIRE EXPÉRIMENTALE AVEC LE VIRUS VELOGÈNIQUE DE LA MALADIE DE NEWCASTLE

Résumé

Cette étude a été menée dans le but d'évaluer l'effet du traitement oral au levamisol des coqs sur leurs réactions à une infection intraoculaire expérimentale par le virus vélogénique de la maladie de Newcastle (NDV) et pour déterminer si le traitement affecterait le processus morbide en modifiant la réponse immunitaire. L'étude a utilisé 3 groupes infectés de 20 coqs chacun (4 semaines d'âge) avec 10 témoins non infectés. Les groupes infectés ont été traités par voie orale avec du levamisol à raison de 5 mg / kg par jour pendant 3 jours consécutifs avant l'infection (TBI) ou à partir du jour 4 après l'infection (TAI) ou n'ont pas reçu de traitement (NTI). La morbidité, les réactions aux anticorps, la mortalité et les changements pathologiques ont été surveillés dans les groupes infectés au cours de la période post-infection (pi). La morbidité clinique n'a pas été affectée par le traitement, mais la mortalité était significativement

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plus élevée (P <0,05) dans les TBI et TAI par rapport aux groupes NTI. Le score agrégé des signes cliniques était significativement plus faible (56,3%) dans les TBI par rapport aux groupes TAI (87,5%) et NTI (100%). Tous les oiseaux infectés ont montré une perte de poids et un retard de croissance. L'hyperthermie était plus sévère dans les TBI que les TAI et les NTI au jour 2 post-infection (pi). La réponse des anticorps était plus faible (P <0,05) dans les TBI et les TAI par rapport aux NTI aux jours 7-14 pi, mais était plus élevée (P <0,05) chez les TBI que chez les TAI. Par la suite, les TBI ont présenté une réponse d'anticorps plus élevée (P <0,05) que les TAI et les NTI au jour 21 post-infection. Les dommages neuronaux causés par le virus dans tous les groupes infectés étaient évidents d'un point de vue histopathologique, mais seulement les TAI et les NTI présentaient des torticolis. Le traitement au levamisole des oiseaux infectés n'a pas réduit la morbidité et la mortalité, mais a supprimé la réponse des anticorps à la phase initiale suivie d'une réponse d'anticorps forte retardée chez les TBI probablement en raison de la fonction cellulaire T-helper activée plus tôt. Par conséquent, les altérations immunologiques attribuables au traitement au levamisole ne semblent pas avoir sauvé les poulets de l'issue de la maladie pour justifier son utilisation dans la prise en charge des épidémies de la maladie de Newcastle.

Mots-clés : signes cliniques, coqs, test d'inhibition de l'hémagglutination, infection intraoculaire, traitement au lévamisol, virus velogène de la maladie de Newcastle.

Introduction

Infection of chickens with Newcastle disease virus (NDV), a paramyxovirus serotype 1 has been reported to cause induction of nitric oxide in heterophils and peripheral mononuclear cells leading to impaired phagocytic activity of heterophils and increased susceptibility to secondary infections due to immunosuppression (Lam *et al.,* 1996; Ahmed *et al.,* 2007). Levamisole has been reported to enhance both humoural and cellular immune responses (Soppi *et al.,* 1979; Yin *et al.,* 2006) in immunosuppressed chickens (Sigh and Dhawedkar, 1993). Oladele *et al.* (2012) reported that levamisole had an anti-inflammatory property with delayed hypersensitivity and the capacity to boost productivity in broilers especially during immunosuppression. The effect of levamisole on antibody production following vaccination against NDV in chicken is ambiguous, because it may or may not enhance antibody production (Sanda *et al.,* 2008; Emikpe *et al.,* 2010; Habibi *et al.,* 2012). In this study, we evaluated the effects of oral levamisole treatment of cockerels on their responses to experimental intraocular infection with velogenic Newcastle disease virus, intending to ascertain whether the immuno-stimulatory effect of levamisole would influence the course of the disease process with the ultimate aim of defining the utility of levamisole in Newcastle disease control during outbreaks.

Materials and Methods

Experimental Chickens

Seventy unvaccinated day-old cockerels used for this experiment were obtained from Obasanjo Farms in Ota, Ogun State, Nigeria, and were raised to 4 weeks of age for the experiment by standard procedure. The cockerels were kept in locally constructed cages (1.5m^2) in an animal house and were fed commercial chick feeds (Vital feeds®, GCOML, Jos, Nigeria), with ad-libitum water supply.

Newcastle Disease Virus

A vial of lyophilized NDV was obtained from Virology Unit of the National Veterinary Research Institute (N.V.R.I.), Jos, Nigeria. The strain was of chicken origin and was characterized as viscerotropic and velogenic. The lyophilized virus was reconstituted with 1.5ml sterile phosphate buffered saline (pH 7.2) to obtain virus egg infective dose (EID) EID50 of $10^{8.5}$/ml and subsequently diluted to $10^{6.5}$/ml to be used to infect birds intraocularly with 0.05ml in each eye (Badau *et al.,* 2015).

Experimental Design

At 4 weeks of age, 3 groups of 20 cockerels each were infected with NDV while 10 served as non-infected (NI) controls. The infected cockerels were treated orally with

levamisole (Anglian nutrition product company UK) at 5 mg/ kg daily for 3 consecutive days, either from 3 days before infection (TBI) or from day 4 post-infection (TAI); or were not treated (NTI). The infected and non-infected birds were housed in different locations to eliminate contact among groups and biosecurity measures were ensured to avoid extraneous contaminant infections. The clinical signs, body weights, rectal temperatures, haemagglutination inhibition (HI) antibody titres and lesions were monitored in the birds.

Scoring of Clinical Signs

The clinical signs were grouped into signs of malaise (depression and droopiness), disturbances of the digestive system (inappetence and diarrhoea), respiratory signs (sneezing and rales) and nervous signs (paralysis) and, in sequence, were scored 1, 2, 2 and 3 respectively, based on their association with severity of the disease and mortality as proposed by Badau (M.V. Sc. Dissertation, 2014). The aggregate scores of the infected groups were used to compare the clinical severity of the manifested disease among the groups.

Body Weight Change

The body weights of the birds were estimated by the use of a weighing balance (Camry Premium, Zhongshan, China) on days 0, 7, 14 and 21 post-infection (pi).

Rectal Temperature

The rectal temperature (RT) of each bird was measured with a digital thermometer (Hartmann® Digital Thermometer, Heidenheim, Germany) on days 0, 2, 4, 7, 14 and 21 pi.

Determination of Haemagglutination Inhibition (HI) Antibody Titre

Blood (3 ml) was collected from each bird through the jugular vein, allowed to clot and the serum was harvested into cryotubes. One percent red blood cell (RBC) working suspension was prepared using blood from NDV-free chickens for the HI test and lyophilized ND vaccine LaSota virus was used as the antigen. Haemagglutination test was carried out to determine the titre and a working dilution of the antigen.

Antibody to NDV was determined using a modification of HI test, as described by El-Yuguda *et al*. (2009). HI test was carried out after thawing the frozen sera to room temperature. A two-fold serial dilution of 25µl of each neat test serum with physiologic buffered saline (PBS) was made in a microtitre plate and 25µl of 4 haemagglutination units (HAU) of NDV antigen was added to each well except the last well (serum control). Finally, 25µl of 1% suspension of chicken RBC was added to all the wells and the plate was incubated at room temperature (35 - 38 oC) for 45 minutes. Positive samples were identified by the formation of button at the bottom of each well.

Necropsy

Carcasses were subjected to detailed postmortem examination following the procedure described by Majó and Dolz (2011).

Histopathology

The formalin fixed tissues were processed, after which the paraffin embedded tissues were sectioned at about 4µm thickness. The sections were stained with haematoxylin and eosin (H&E) stain according to standard procedure (Akpavie, 2014).

Statistical Analysis

The HI titres were summarized as frequencies of titres in infected and non-infected groups. Geometric mean titre (GMT) of HI antibody and standard deviation (SD) were calculated as previously reported (Badau *et al.,* 2015). Other data were summarized as mean ± SD. Differences between mean values of infected and non-infected groups were assessed by ANOVA and Turkey post-test. Chi-square statistic with Yate's continuity correction was used to test for the differences in occurrences of clinical signs, and gross lesions in infected groups. Analyses were carried out using computer software (GraphPad Instat, 2003 version, http://www.graphpad.com/apps/index.cfm).

Table 1: Description of clinical signs and frequencies of occurrence in cockerels after experimental intraocular infection with velogenic Newcastle disease and treated with levamisole before (TBI) or after (TAI) viral infection, or without levamisole treatment (NTI).

Clinical signs	Description	Groups	No. (%) affected
Inappetence	Lack of desire for feed	TBI	16 (80)
		TAI	14 (70)
		NTI	17 (85)
Depression	Reduction in activity characterized by reluctance to move	TBI	16 (80)
		TAI	14 (70)
		NTI	17 (85)
Droopiness	Bending or hanging down of the head or wing	TBI	16 (80)
		TAI	14 (70)
		NTI	17 (85)
Sneezing	Forceful expulsion of air from the nose and mouth by an involuntary spasmodic contraction of the muscle of expiration	TBI	0 (0)
		TAI	0 (0)
		NTI	1 (5)
Wings and legs paralysis	Loss of power of voluntary movement in the muscle of the wing and leg	TBI	1 (5)
		TAI	2 (10)
		NTI	1 (5)
Greenish diarrhea	Watery stool stained with bile	TBI	16 (80)
		TAI	14 (70)
		NTI	17 (85)
Rales	Loud breath due to air passing through narrowed bronchi during inspiration or expiration	TBI	0 (0)
		TAI	3 (15)
		NTI	2 (10)
Torticollis	Twisting of the neck characterized by turning the head inward or sideways	TBI	0 (0)
		TAI	3 (15)
		NTI	2 (10)
Stunted growth	Failure to grow by adding body weight	TBI	5(100)
		TAI	9(100)
		NTI	4(100)

No significant ($P<0.05$) difference in the frequencies of occurrence of the clinical signs

Table 2: Aggregate score of clinical signs in groups of cockerels after experimental intraocular infection with velogenic Newcastle disease virus and treated with levamisole before (TBI) or after (TAI) viral infection, or without levamisole treatment (NTI)

Clinical signs	Rank score	Score of infected groups		
		TBI	TAI	NTI
Depression	1	1	1	1
Droopiness	1	1	1	1
Inappetence	2	2	2	2

Table 2 continued

Clinical signs	Rank score	Score of infected groups		
		TBI	TAI	NTI
Sneezing	2	0	0	2
Wing and leg paralysis	3	3	3	3
Greenish diarrhoea	2	2	2	2
Rales	2	0	2	2
Torticollis	3	0	3	3
Aggregate score (%)	16	9 (56.3)[a]	14 (87.5)[b]	16 (100)[b]

[a, b] Scores with different superscripts are significantly (P<0.05) different.

Table 3: Time and rate of mortality of cockerels infected with velogenic Newcastle disease virus without levamisole treatment (NTI) or with levamisole treatment before (TBI) or after (TAI) infection.

Group	Time of mortality	Mortality, n (%)	P-value		Chi-square with Yates correction	
			One sided	Two sided	One sided	Two sided
NTI	8.4±2.9[a]	16 (80%)				
TBI	6.4±1.4[b]	15 (75%)*	0.18	0.71	1.82	0.14
TAI	6.5±1.3[b]	11 (55%)*	0.35	0.18	0.14	1.82

**Not significant compared with NTI by chi-square statistics*

[a, b] Mean ± standard deviation with different superscripts are significantly (P<0.05) different from the control group NTI by one-way ANOVA and Dunnett post-hoc test.

Results

Clinical Signs

Onset of apparent clinical signs in all infected (TBI, TAI, NTI) groups was on days 3-4 pi, but rectal temperature was recorded on days 2-4 pi. The clinical signs of infected birds are summarized in Table 1 with frequencies of occurrence in the treatment groups. No significant (P>0.05) variation occurred in the frequencies of the clinical signs due to levamisole treatment. The aggregate scores of the clinical signs in the various infected groups are presented in Table 2. The aggregate score of the clinical signs was significantly (P<0.05) reduced in TBI groups, but not in TAI group, when compared with NTI group, because this group did not manifest rales nor torticollis like other groups did.

Period and Rate of Mortality

The time and rate of mortality in the infected groups are presented in Table 3. The period of mortality was significantly (P<0.05) shorter in the TBI and TAI groups compared with NTI group, but there was no significant (P>0.05) difference in the period between TBI and TAI groups. There was no significant (P>0.05) variation in mortality rates in TBI and TAI compared to NTI groups.

Variation in Live Body Weights

The mean live body weights (MBWs) of cockerels in the NDV infected and non-infected groups are presented in Table 4. Birds in NI group had a significant (P<0.05) increase in MBWs on days 7 (59.6%), 14 (147%) and 21 (181.9%) pi when compared to MBWs on day 0. However, MBWs in the infected groups did not show any significant (P>0.05) increase from MBWs on day 0. Instead, the infected birds in TAI group significantly (P<0.05) lost MBWs on days 14 (67.7%) and 21 (22.5%) pi, while those in NTI group significantly (P<0.05) lost MBWs only on day 7 (7.4%) pi compared to MBWs on day 0. TBI group did not lose weight during infection like TAI and NTI groups, but failed to gain weight after day 0 pi. The MBWs of non-infected birds (NI group) was 112-236% higher than those of the infected (TBI, TAI, NTI)

Table 4: Body weight (g) of cockerels after experimental intraocular infection with velogenic Newcastle disease virus and treated with levamisole before (TBI) or after (TAI) viral infection, or without levamisole treatment (NTI) compared to the non-infected (NI) group

	Days Post Infection			
Group	**0**	**7**	**14**	**21**
NI	188.0±16.9[a]	300.0±55.4[b]	465.0±74.1[c]	530.0±25.8[d]
	(n=10)	(n=10)	(n=10)	(n=10)
TBI	172.0±16.4[a]	191.4±103.2[a]	190.0±26.5[a]	206.0±39.1[a]
	(n=20)	(n=7)	(n=5)	(n=5)
TAI	203.5±16.9[a]	215.0±63.1[a]	137.8±33.1[b]	157.8±27.7[b]
	(n=20)	(n=10)	(n=9)	(n=9)
NTI	216.0±24.8[a]	179.1±37.3[b]	200.0±38.1[ab]	250.0±24.5[a]
	(n=20)	(n=11)	(n=5)	(n=4)

[a-d] *Mean±standard deviation with different superscripts along the same rows are significantly (p<0.05) different.*

Table 5: Rectal temperature (oC) of cockerels after experimental intraocular infection with velogenic Newcastle disease virus and treated with levamisole before (TBI) or after (TAI) viral infection, or without levamisole treatment (NTI) compared to the non-infected (NI) group

	Days Post Infection					
Group	**0**	**2**	**4**	**7**	**14**	**21**
NI	41.5±0.1[a]	41.5±0.1[a]	41.5±0.1[a]	41.6±0.1[a]	41.51±0.07[a]	41.5±0.1[a]
	(41.3-41.6)	(41.4-41.6)	(41.4-41.6)	(41.5-41.8)	(41.40-41.60)	(41.4-41.6)
	(n=10)	(n=10)	(n=10)	(n=10)	(n=10)	(n=10)
TBI	41.5±0.1[a]	42.1±0.3[b]	42.6±0.5[b]	40.6±2.8[a]	41.58±0.20[a]	41.6±0.2[a]
	(41.4-41.7)	(41.8-42.6)	(41.5-43.0)	(34.6-43.0)	(41.40-41.90)	(41.3-41.8)
	(n=20)	(n=20)	(n=20)	(n=7)	(n=5)	(n=5)
TAI	41.5±0.1[a]	41.7±0.2[c]	42.5±0.6[b]	40.8±3.2[a]	41.52±0.74[a]	41.7±0.2[a]
	(41.2-41.6)	(41.5-42.1)	(41.4-43.0)	(32.0-42.5)	(39.60-42.0)	(41.4-42.1)
	(n=20)	(n=20)	(n=20)	(n=10)	(n=9)	(n=9)
NTI	41.5±0.1[a]	41.8±0.1[c]	42.6±0.4[b]	38.9±4.8[a]	40.1±3.6[a]	41.7±0.2[a]
	(41.4-41.6)	(41.5-42.0)	(41.7-43.0)	(32.0-42.5)	(32.0-41.9)	(41.5-41.9)
	(n=20)	(n=20)	(n=20)	(n=10)	(n=7)	(n=4)

[a, b, c,] *Mean±standard deviation with different superscripts on the same row are significantly (p<0.05) different.*
Range in parenthesis
n, Number of birds

groups on day 21 pi. The birds that survived the infection up to day 21 pi in all infected groups remained stunted having failed to significantly, gain weight as compared to the NI group.

Rectal Temperature

The rectal temperatures (RT) of all the groups are presented in Table 5. The mean RT was significantly (P<0.05) higher in the infected groups (TBI, TAI, NTI) than the control (NI) means on days 2 to 4 pi. RT in TBI group was significantly (P<0.05) higher than in TAI and NTI groups on day 2 pi. On day 4 pi, all the infected groups had comparable mean RTs. Some birds in TBI (7/20; 35%), TAI (7/20; 35%) and NTI (5/20; 25%) groups had RT up to 43.0°C on day 4 pi. Thereafter, mean RTs were within control values on days 7, 14 and

21 pi. On day 7 pi, some birds in TBI group (2/7; 28.6%) were hypothermic (34.6°C, 39.5°C) and one of them in the group (14.3%) had RT of 43.0°C. Some birds in TAI group (2/10; 20%) were also hypothermic (39.3°C, 32.0°C) and in NTI group, a few (3/10; 30%) had RT of 32.0°C. On day 14 pi, hypothermia was recorded in TAI (1/9; 11.1%) and NTI (2/7; 28.6%) groups, but not in TBI group. On day 21 pi, no bird was hypothermic in all the infected groups, RT of 42.0°C was encountered in some birds (2/9; 22.2%) in only TAI group which was above the upper limit of the control values (41.8°C).

Serum Antibody Response

The geometric mean titres (GMTs) of HI antibody in NDV-infected and NI groups of birds are presented in Table 6. On day 0 pi, GMTs of all the groups were comparable. The NI birds had significant (P<0.05) increase in GMTs on days 14 and 21 of the experiment when compared to day 0. GMTs were significantly (p<0.05) higher in all the infected (TBI, TAI, NTI) than in NI birds on days 7-21 pi. The GMTs of NTI group on days 7 and 14 pi were significantly (P<0.05) higher than GMTs of TBI and TAI groups, but on day 21 pi, the GMT of NTI was significantly (P<0.05) higher than GMT of TAI. The GMT was significantly (P<0.05) higher in TBI than TAI groups on day 7 pi (1.95 fold) and 14 pi (1.07 fold), respectively. On day 21 pi, GMT of TBI was significantly (P<0.05) higher than those of TAI and NTI by 6.96 and 5.85 folds, respectively. In the transition from day 14 to 21 pi, GMTs of TAI and NTI groups significantly (P<0.05) decreased by 1.32 and 1.23 folds, respectively; whereas GMT of TBI group significantly increased by 4.92 fold.

Gross Pathology

The gross lesions observed in the infected birds and their frequencies of occurrence are summarized in Table 7. Lungs in TBI and NTI groups were congested and oedematous, while TAI group had only congestion. Spleen lesions were mild congestion with multifocal grayish areas in TBI birds, mild swelling and ecchymoses in TAI birds, and swelling and paleness with multifocal petechiae in NTI birds. Kidney lesions were moderate to marked swelling in TBI and TAI birds, and marked congestion with urates deposit in the ureters of NTI birds. Liver lesions in TBI birds were moderate swelling and congestion with pale streaked areas, while in TAI and NTI birds were marked congestion. Proventricular lesions in all the infected groups were moderate to marked glandular petechiae and ecchymoses. Intestinal lesions in all the infected groups were marked ecchymoses on the mucosae and foci of ulcers covered by diphtheritic membrane. Caecal tonsils in TBI and TAI birds were raised and

Table 6: Geometric mean titre (GMT) of haemagglutination inhibition antibody in cockerels after experimental intraocular infection with velogenic Newcastle disease virus and treated with levamisole before (TBI) or after (TAI) viral infection, or without levamisole treatment (NTI) compared to the non-infected (NI)

	Days Post Infection			
Group	**0**	**7**	**14**	**21**
NI	2.4±1.6[a]	2.0±1.3[a]	6.5±2.1[b]	32.0±1.9[c]
	(n=10)	(n=9)	(n=10)	(n=7)
TBI	1.5±1.1[a]	57.0±2.1[b]	1,448.0±12.8[c]	7,131.0±2.2[d]
	(n=13)	(n=6)	(n=4)	(n=5)
TAI	1.7±1.2[a]	29.3±2.4[c]	1,351.0±1.7[d]	1,024.0±1.7[e]
	(n=15)	(n=8)	(n=5)	(n=7)
NTI	1.7±1.2[a]	75.0±2.1[d]	1,504.0±3.2[e]	1,218.0±3.2[f]
	(n=15)	(n=9)	(n=4)	(n=4)

[a-f] *Mean±standard deviation with different superscripts along the same columns and rows are significantly (P<0.05) different*

Table 7: Frequencies of occurrence of gross lesions in cockerels experimentally infected with velogenic Newcastle virus and treated with levamisole before (TBI) or after (TAI) viral infection, or without levamisole treatment (NTI) in the population involved

Organs Affected	Lesions	Groups	Number (%) Affected in the Population Involved
Lung	Congestion, oedema	TBI	1/15 (6.7)
		TAI	2/11 (18.2)
		NTI	2/16 (12.5)
Spleen	Congestion, petechiae and enlargement	TBI	2/15 (13.3)
		TAI	1/11 (9.1)
		NTI	1/16 (6.3)
Caecal tonsils	Ecchymoses and necrosis	TBI	9/15 (60.0)
		TAI	6/11 (54.5)
		NTI	9/16 (56.3)
Proventriculus	Ecchymotic and petechial haemorrhages on glands	TBI	11/15 (73.3)
		TAI	5/11 (45.5)
		NTI	9/16 (56.3)
Intestine	Haemorrhages	TBI	4/15 (26.7)
		TAI	1/11 (9.1)
		NTI	5/16 (31.3)
Liver	Congestion, pale streaked areas	TBI	6/15 (40.0)
		TAI	5/11 (45.5)
		NTI	3/16 (18.8)
Kidney	Congestion, enlargement, urate deposit in ureters	TBI	3/15 (20.0)
		TAI	1/11 (9.1)
		NTI	5/16 (31.3)

No significant (P>0.05) variations in the frequencies of occurrences of lesions in organs among the groups.

haemorrhagic, while necroses were observed in NTI birds. The frequencies of occurrence of gross lesions in the proventriculus (25/47; 53.2%) and caecal tonsils (24/47; 51.1%) were significantly (P<0.05) higher than in liver (14/47; 29.8%), intestine (10/47; 21.3%), lung (5/47; 10.6%) and spleen (4/47; 8.5%), but occurrences of intestinal lesions were more frequent (P<0.05) than the splenic lesions. Occurrences of liver lesions were also significantly (P<0.05) higher than lesions in lungs and spleen. There was no significant (P<0.05) effect of levamisole treatment on the frequencies of occurrence of the lesions in any of the organs.

Histopathology

Brains sections in all the infected groups showed multifocal neuronal degeneration with satellitosis. Lungs in TBI and TAI birds showed mild congestion and oedema, while NTI birds had congestion with interstitial thickening by oedema fluid. Spleens were characterized by mild to marked lymphocyte depopulation in the white pulp. kidneys of TAI and TBI birds had marked hydropic degeneration of the renal tubules with deposition of proteinaceous materials in the intratubular lumen, while NTI birds showed marked renal tubular necrosis and deposition of proteinaceous materials in the glomerular basement membrane. Proventricular lesions in all the infected groups

showed multifocal vascular congestion at the mucosae and lamina propria. Intestinal lesions in TBI and TAI birds showed lymphoid aggregates (MALT) infiltrating into the mucosae, whereas NTI had diffuse haemorrhages into the mucosae, which was absent in TBI and TAI birds. TBI and TAI birds had multifocal hepatocellular necrosis with congestion of the sinusoids, whereas NTI birds had severe peri-portal hepatocellular necrosis. Caecal tonsils in TBI, TAI and NTI groups had mild, moderate and marked lymphoid tissue depletion, respectively. Therefore, microscopic lesions consistently occurred in all groups (TBI, TAI, NTI).

Discussion

Levamisole treatment of the NDV-infected chickens was expected to alter their immune profiles to the extent that innate and adaptive immune responses would limit the onset and severity of the disease process, but the result of this study showed that the incubation period, rates of morbidity and mortality and the frequencies of clinical signs and lesions were not significantly affected by the treatment. These findings seemed to suggest that the levamisole treatment did not remarkably alter the course of the disease except in a few measurable ways such as eliciting more severe transient and early hyperthermia, reduced aggregate score of clinical signs, reduced mortality period, and abetting of respiratory distress and torticollis in those treated before infection. Furthermore, levamisole treatment reduced antibody response during the early phase of infection, but the antibody response increased later to titres exceeding those of the untreated chickens. This pattern of antibody response in NDV infection was unique and directed attention to the nuances of the modulation of immune response by levamisole in Newcastle disease, which had not been previously reported in literature, but such differential responses had been reported in other experimental models (AbdaAlla *et al.,* 1995; Holcombe *et al.,* 2006; Li *et al.,* 2006;).

Antibody titres on day 0 pi in naive chickens were probably as a result of residual maternal antibodies or low-level seroconversion from environmental exposure to endemic NDV antigens (Badau *et al.,* 2015). Thereafter, levamisole treatment of NDV-infected chickens suppressed antibody responses on days 7-14 pi and the immunosuppression was more severe when treatment was done during active infection (TAI) than treatment before infection (TBI). It indicated that levamisole undermined more intensely the immunogenic effect of NDV if administered during a subsisting infection. Antibody response was reported to occur after inoculation of chickens with live NDV with production of IgM and IgG commencing from day 4 and 7 post-inoculation, respectively (Al-Garib *et al.,* 2003).

There was a report of enhanced mitogenic responses in chickens treated with levamisole at 0.25mg/kg associated with increased IgM and IgG antibodies (Soppi *et al.,* 1979). Other reports showed that IgG stimulated antibody production (Kühlmann-Raben *et al.,* 1987; Zhang *et al.,* 2009). However, Holcombe *et al.* (2006) suggested that levamisole may be immunosuppressive in individuals with normal immune function, whereas high doses of levamisole in feed was immunosuppressive in fish (Li *et al.,* 2006). The suppression of antibody responses by levamisole may arise from an immune response phenotype to immuno-stimulation, which tips the balance against humoral immunity with a bias for cell-mediated immunity when T-helper cells have been activated. T-helper cell functions have been divided into type 1 and 2, which predominantly produce interferon-gama (IFN-ϒ) and interleukin-4 (IL-4) from Th-1 and Th-2 respectively (Mosmann *et al.,* 1989). Levamisole enhances T-cell response, facilitates Th-1 differentiation and makes Th-1 responses to dominate those of Th-2 (Zhang *et al.,* 2009; Fu *et al.,* 2016). The cytokine responses after levamisole treatment have been shown to shift immune response away from type 2 and this was considered capable of blunting type 2-biased immune response by boosting type 1 compartment with enhanced production of IFN-ϒ (Szeto *et al.,* 2000; Chen *et al.,* 2007). Th-1 activity was reported to suppress B-cell responses by producing immunosuppressive

IFN-ϒ (Reynolds *et al.,* 1987), being directly cytotoxic for activated B-cells (Janeway *et al.,* 1988) and suppressing optimally stimulated Th-2 cells (Asano and Hodes, 1983; Mosmann *et al.,* 1989). Therefore, the levamisole-treated NDV-infected chickens had decreases in antibody titres during the early phase of infection, most probably, mediated by stimulated T-helper cell functions with suppressed seroconversion.

The antibody response of NDV-infected chickens rebounded on day 21 pi in those treated with levamisole before infection and was about 7 folds of the titre in untreated-infected chickens. The suppression of the humoral immune response might have been alleviated by the elimination of levamisole from the body system of the chickens, after which the blunting of the Th-2 and antibody responses were abrogated. By 21 days after oral administration of a single dose of levamisole (40mg/kg) to chickens, no levamisole residue was detected in the plasma and tissues (El-Kholy and Kemppainen, 2005), implying that the immunomodulatory effect of levamisole dissipated in the infected chickens at the time of rebound of antibody titres, but the primed T-helper cells and B-cells were most likely responsible for intense antibody production superceding the titre in untreated-infected chickens. It is noteworthy that those treated during infection did not exhibit the rebounding of antibody titre after levamisole clearance; and this might be due to the depletion of activated B-cells by direct cytotoxic functions of T-helper activation, in the scenario (TAI) where the preceding humoral immunosuppression was more severe than the former (TBI).

Levamisole treatment before infection prevented the manifestation of rales and torticollis, thereby reducing the aggregate score on clinical signs for the group. This group of chickens had little pulmonary edema to preclude rales. The secretion of IFN-ϒ and IL-1 before lung injury makes the lung tissue less likely to develop early edema and respiratory distress (Torre *et al.,* 1994; Heremans *et al.,* 2000). The levamisole-induced IFN-ϒ and interleukin production (Redondo *et al.,* 1987; Kimball *et al.,* 1991; Chen *et al.,* 2007) before NDV infection might have protected the lungs from remarkable viral injury, and the lack of hypoxic stress possibly prevented torticollis by reducing hypoxic nervous tissue injury (Badau *et al.,* 2015). The infected groups that were treated had shorter mortality period than the untreated, suggesting a more acute course of the disease in the treated than untreated groups. The mortalities in the treated groups occurred when the humoral immunity was suppressed and the stimulated cell-mediated immunity was expected to produce pro-inflammatory cytokines causing the lesions (Fu *et al.,* 2016). The infective virus was a virulent strain and could elicit a stronger and more persistent cell-mediated immunity than a less virulent strain (Rauw *et al.,* 2009), making ample contribution to the pro-inflammatory and cytotoxic cytokines responsible for the lesions. The study had shown that the levamisole treatment did not ameliorate the lesions, but had exacerbated the development of lesions where they occurred to reduce the mortality period. Similar lesions occurred in treated and untreated chickens and their frequencies of occurrence did not differ, indicating the pathogenic trend was maintained irrespective of treatment. Thus, hepatorenal lesions and metabolic disturbances associated with catabolic acute-phase and cytokine responses contributing to stunting and lack of weight gain in the disease were not affected by levamisole treatment (Kotler, 2000; Badau *et al.,* 2015). The transient cytokine stimulated hyperthermia in the disease (Kapczynski *et al.,* 2013; Badau *et al.,* 2015) was briefly aggravated by levamisole treatment before infection and it might have been connected to the enhanced stimulation of IFN-ϒ secretion (Chen *et al.,* 2007), which is associated with induction of fever (Ackerman *et al.,* 2012; Rasoli *et al.,* 2014).

Conclusion

It could be concluded from this study that levamisole treatment before or after infection appeared not to have significantly modified the morbidity and mortality rates of the disease. However, mortality period was

shorter in TBI and TAI than in NTI and aggregate score of clinical signs was lower in TBI than TAI and NTI. Although mortality period was shorter in TAI than NTI, both groups had no difference in aggregate scores of clinical signs. Hyperthermia was more severe in TBI than TAI and NTI at the early acute phase. The humoral immunosuppression during the early phase in the treated chickens was probably mediated by stimulation of T-cell function with blunting of B-cell activation. Levamisole treatment before infection had the capacity to elicit a delayed strong antibody response, which was not associated with protection of the birds against the effect of the virus as the morphological changes in the organs did not seem to be influenced by levamisole treatment, and this humoral response was perhaps due to revamping of B-cell activity after systemic clearance of levamisole. Therefore, the utility of levamisole treatment to control the outcome of Newcastle disease outbreak was not affirmed by this study.

Conflict of Interest Statement

The authors declared that there was no any conflict of interest.

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EFFECT OF RUMINAL PLASTIC BAGS ON WELLBEING OF GOATS

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Abstract

Clinical manifestations due to presence of plastic bags in the rumen of goats were studied. Sixteen (16) one year old male (castrate) small East African goats with an average weight of 24.5kg were used for the study. The animals were divided into 4 groups of 4 animals each (n=4). Three of the groups had, 129g, 258g and 387g of plastic bags, respectively, introduced into the rumen through rumenotomy, while the fourth group without implants served as control. All animals in both test and control groups were observed daily for changes in vital parameters and clinical manifestations for a period of 6 weeks following implantation. Presence of plastic bags in the rumen of the goats was clinically characterized by anorexia, severe depression, discomfort (grunting sounds), dehydration, firmness and asymmetrical distention of the abdomen, reduced ruminal movements, diarrhoea with intermittent constipation, recumbency and death. Severity of the observed clinical signs increased with the quantity and duration of the plastic bags in the rumen. The animals lost varying degrees of body weight proportional to the quantity of plastic bags in the rumen. Generally, presence of plastic bags in the rumen imposed a serious health burden on the goats affecting their overall wellbeing and weight gain subsequently leading to mortality of some of the animals. Presence of the plastic bags in the rumen could affect the overall productivity and production of goats. The significance of this research is in its contribution to understanding the effects of ingestion and accumulation of plastic bags in the rumen of goats.

Key words: Clinical signs, goats, plastic bags, rumen, wellbeing.

EFFET DE LA PRESENCE DE SACS PLASTIQUES DANS LE RUMEN SUR LE BIEN-ETRE DES CHEVRES

Résumé

Les manifestations cliniques dues à la présence de sacs plastiques dans le rumen des chèvres ont été étudiées. Seize (16) chèvres mâles âgés d'un an (castrés) d'Afrique de l'Est pesant en moyenne 24,5 kg ont été utilisées pour l'étude. Les animaux ont été divisés en 4 groupes de 4 animaux chacun (n = 4). Trois des groupes avaient 129g, 258g et 387g de sacs en plastique respectivement introduits dans le rumen par rumenotomie, tandis que le quatrième groupe sans implants a servi de témoin. Tous les animaux des groupes expérimentaux et témoins ont été observés quotidiennement afin de détecter les changements dans les paramètres vitaux et les manifestations cliniques pendant une période de 6 semaines après l'implantation. La présence de sacs en plastique dans le rumen des chèvres a été caractérisée cliniquement par l'anorexie, la dépression sévère, l'inconfort (grognements), la déshydratation, la fermeté et la distension asymétrique de l'abdomen, la réduction des mouvements ruminaux, la diarrhée avec constipation intermittente, l'immobilisation et la mort. La gravité des signes cliniques observés a augmenté avec la quantité des sacs en plastique et la durée de leur séjour dans le rumen. Les animaux ont perdu un degré de poids variable proportionnel à la quantité de sacs plastiques présents dans le rumen. D'une manière générale, la présence de sacs en plastique dans le rumen a imposé une charge morbide des chèvres, a affecté leur bien-être

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total et leur gain de poids entraînant la mortalité de certains animaux. La présence des sacs en plastique dans le rumen pourrait affecter la productivité globale et la production de chèvres. L'importance de cette recherche réside dans sa contribution à la compréhension des effets de l'ingestion et de l'accumulation de sacs plastiques dans le rumen des chèvres.

Mots-clés : Signes cliniques, chèvres, sacs en plastique, rumen, bien-être

Introduction

Goats contribute to food security, significant reduction in poverty, improved soil fertility for crop production and overall improvement of livelihoods in low income countries in Africa and Asia (Lebbie, 2004). Poor health, however, is a major constraint to increased goat production under the traditional small-holder production systems, whereby animals mainly roam and seek their own feed (Devendra, 1999). Roaming and scavenging for feed expose the animals to many health hazards including ingestion and accumulation of indigestible foreign materials in the rumen (Remi-Adewunmi *et al.,* 2004). Over 80% of the energy supply for goats is obtained through fermentation of feed materials in the rumen (Randall *et al.,* 2002). When the process of fermentation and absorption of volatile fatty acids is interfered with as a result of accumulation of indigestible foreign bodies in the rumen, the animal is deprived of valuable nutrients for its survival (Igbokwe *et al.,* 2003).

Presence of indigestible materials in the rumen leads to many complications in sheep (Igbokwe *et al*, 2013) and probably in goats. Clinical manifestations of impaction of the rumen of small ruminants with indigestible foreign bodies have been widely speculated to vary from mild indigestion to life threatening systemic signs (Gyang, 1991). The actual clinical effects due to known types of indigestible materials, quantities of these materials, degree of obstruction, as well as the duration of their presence in the rumen that caused the observed clinical manifestations have not been reported. This is due to the fact that rumen foreign bodies are found only at slaughter or during necropsy.

Previous studies on waste generation has shown that plastic bags are the largest component of solid waste generated, especially in urban and peri-urban settings and hence the most prevalent in polluting pastures and grazing areas (NEMA, 2003; Mangizvo, 2012). Thus, they are the most commonly ingested foreign bodies in goats (Omidi *et al.,* 2012; Sileshi *et al.,* 2013; Otsyina *et al.,* 2015). This study therefore reports on the clinical manifestations and wellbeing of goats experimentally implanted with plastic bags into their rumen for a period of 42 days.

Materials and Methods

Ethical approval

Animal use was approved by the Biosafety, Animal use and Ethics Committee (BAEC) of the Faculty of Veterinary Medicine, University of Nairobi, Kenya, according to international standards of animal use in research; clearance certificate number: 11250313.

Experimental animals

Sixteen (16) castrated small East African goats with a mean body weight of 24.5kg (± 0.1kg) and body condition score of 3.0 ± 0.5 (on a scale of 1-5) were used in the study. The animals were housed in groups of four (4) for the whole period of study and handled as previously described by Otsyina *et al*. (2016). Briefly, the goats were allowed 6 weeks to acclimatize to the environment and the feed. Feed and drinking water were provided *ad libitum*. They were treated against endo-parasites and ecto-parasites. All goats were subjected to routine physical examination over the acclimatization period.

The animals were assigned to 4 experimental groups using stratified random sampling on the basis of weight of the animals, such that the mean weight of animals in each of the experimental groups was not statistically different. The groups were designated GE1, GE2, GE3 and GC4. Three of the groups (GE1,

GE2 and GE3) had 129g, 285g and 387g of plastic bags, respectively, implanted into the rumen through rumenotomy as previously described by Hendrickson (2007). The fourth group (GC4) served as control on which rumenotomy was done but no plastic bags were implanted. The plastic bags implanted were the non-perforated small soft polythene bags (KEBS Industries Ltd, Nairobi, Kenya). Each polythene bag measured 167 mm x 290 mm in size, 30 micrometers thick and a packet of 100 pieces weighed 129 g. These were the most common type of plastic bags found in the rumen of sheep and goats during an abattoir study carried out prior to this study (Otsyina *et al.,* 2015).

Clinical Examination of test and control animals

Both test and control animals were monitored daily for 6 weeks (42 days) and all vital parameters as well as clinical manifestations were noted and recorded. The clinical parameters evaluated in the goats implanted with plastic bags as well as the control included: rectal temperature, heart rate, respiratory rate, colour of mucous membranes, ruminal motility, defaecation and level of appetite. These parameters were monitored and recorded daily for a period of 6 weeks (42 days) post-operatively. The rectal temperature was measured using a digital clinical thermometer (Hartman® - United Kingdom). Each animal was weighed once a week to the nearest 0.20 kg using a spring balance (Salter® - England). Feeding was monitored continuously to determine appetite and changes in feed consumption. Ruminal motility and level of gas accumulation in the rumen were monitored daily by visual observation and using a stethoscope. Defaecation was monitored daily to check for presence or absence of faeces and character of faeces (colour, consistency and odour). All data generated were recorded on clinical data forms.

Results

Clinical findings in goats with 129g, 258g and 387g of plastic bags implanted in the rumen are presented in Tables 1 - 3. In both the test and control animals, temperature, respiratory and pulse rates were within normal range.

Goats with 129g of plastic bags in the rumen did not show distension or firmness of the abdomen. They had inappetence initially but their appetite significantly improved 2 weeks after implantation, with feed and water intake being restored to pre-implantation levels from the third week onwards. Animals in this group as well as the control were not depressed or dehydrated and their ruminal movements were 2-3 per minute, and they had normal pelleted faeces throughout the 6-week study period (Table 1).

Goats with 258g of plastic bags showed a very firm abdomen that was not distended. They were anorexic and water intake also reduced. Severe depression, dehydration, rough hair coat and reduced ruminal movements (1-2 per minute) were observed in all the goats implanted with 258g of plastic bags. They also showed persistent grinding of teeth, severe weight loss and pale mucous membranes (Table 2).

Goats with 387g of plastic bags implanted in the rumen showed a very firm abdomen without distension. They were anorexic with reduced water intake. Feed intake was markedly reduced and this trend continued over the entire period of the study. Severe depression, dehydration, rough hair coat and reduced ruminal movements (1-2 per minute) were observed in all the goats in this group. Diarrhoea with intermittent constipation, persistent grinding of the teeth, increased foamy salivation, severe weight loss and pale mucous membranes were also observed in the goats with 387g of plastic bags implanted in the rumen (Table 3).

Mean body weights of all the goats with different quantities of plastic bags implanted in the rumen reduced compared to the control over the period. The reduction in body weights was proportional to the level or quantity of plastic bags present in the rumen.

Table 1: Clinical findings in goats implanted with 129g of plastic bags into the rumen for 6 weeks

Parameters	Clinical observations / Duration of implantation					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
General body condition	1	1	1	1	1	1
Abdominal distension	0	0	0	0	0	0
Mucous membrane colour	0	0	0	0	0	0
Demeanor	0	0	0	0	0	0
Conformation	0	0	0	0	0	0
Gait	0	0	0	0	0	0
Appetite	1	1	0	0	0	0
Water intake	1	1	0	0	0	0
Dehydration	0	0	0	0	0	0
Rumen motility/min	2-3	2-3	2-3	2-3	2-3	2-3
Rumen consistency	0	0	0	0	0	0
Defaecation	1	1	1	1	1	1
Character of faeces	0	0	0	0	0	0
Appearance of hair coat	1	1	1	1	1	1
Grinding of teeth	0	0	0	0	0	0
Foamy saliva	0	0	0	0	0	0
Regurgitation of plastic bags	0	0	0	0	0	0
Weight loss	0	0	0	0	0	0
Mortality	0	0	0	0	0	0

Key:

General body condition: Good =1, fair =2, poor=3, very poor =4;Abdominal distension: none = 0, moderate = 1, Severe – 2; Mucous membrane colour: normal = 0, mildly pale = 2, pale = 3; Demeanor: bright/alert= 0, slightly depressed = 1, depressed = 2, severely depressed = 3; Conformation: normal =0, slightly ached back = 1, ached back = 2; Gait: normal = 0, ataxia = 1;Appetite: normal = 0, moderately reduced =1, reduced = 2, markedly reduced = 3, anorexia = 4;Water intake: normal = 0, reduced =1, markedly reduced = 2; Dehydration: none = 0, slight =1, moderate = 2, marked =3; Rumen consistency: doughy = 0, firm =1, very firm = 2; Defaecation: present =1, occasional =2, absent =3; Character of feaces: normal pellets = 0, hard pellets =1, loose =2, pasty = 3, diarrhoea = 4, no faeces = 5;Appearance of hair coat: glossy =1, rough = 2; Grinding of teeth: absent = 0, occasional =1, continuous = 2; Foamy saliva: absent = 0, present = 1; Regurgitation of plastics bags: absent = 0, present = 1;Weight loss: absent = 0, present = 1

Table 2: Clinical findings in goats implanted with 258g of plastic bags into the rumen for 6 weeks

Parameters	Clinical observations / Duration of implantation					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
General body condition	1	2	3	4	4	4
Abdominal distension	0	0	0	0	0	0
Mucous membrane colour	1	1	2	2	3	3
Demeanor	1	2	3	3	3	3
Conformation	1	2	2	2	2	2
Gait	1	1	1	1	1	1
Appetite	3	4	4	4	4	4
Water intake	1	2	2	2	2	2
Dehydration	3	3	3	3	3	3

Parameters	Clinical observations / Duration of implantation					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Rumen motility/min	1-2	1-2	1-2	1-2	1-2	1-2
Rumen consistency	1	2	2	2	2	2
Defaecation	1	1	2	2	2	3
Character of faeces	3	4	4	4	4	-
Appearance of hair coat	1	2	2	2	2	2
Grinding of teeth	1	2	2	2	2	2
Foamy saliva	1	1	1	1	1	1
Regurgitation of plastic bags	0	0	0	0	0	0
Weight loss	0	0	1	1	1	1
Mortality	0	0	1	0	0	0

Key:

General body condition: Good =1, fair =2, poor=3, very poor =4; abdominal distension: none = 0, moderate = 1, Severe – 2; Mucous membrane colour: normal = 0, mildly pale = 2, pale = 3; Demeanor: bright/alert= 0, slightly depressed = 1, depressed = 2, severely depressed = 3; Conformation: normal =0, slightly ached back = 1, ached back = 2; Gait: normal = 0, ataxia = 1; Appetite: normal = 0, moderately reduced =1, reduced = 2, markedly reduced = 3, anorexia = 4; Water intake: normal = 0, reduced =1, markedly reduced = 2; Dehydration: none = 0, slight =1, moderate = 2, marked =3; Rumen consistency: doughy = 0, firm =1, very firm = 2; Defaecation: present =1, occasional =2, absent =3; Character of feaces: normal pellets = 0, hard pellets =1, loose =2, pasty = 3, diarrhoea = 4, no faeces = 5; Appearance of hair coat: glossy =1, rough = 2; Grinding of teeth: absent = 0, occasional =1, continuous = 2; Foamy salivation: absent = 0, present = 1; Regurgitation of plastics bags: absent = 0, present = 1 Weight loss: absent = 0, present = 1

Table 3: Clinical findings in goats implanted with 387g of plastic bags into the rumen for 6 weeks

Parameters	Clinical observations / Duration of implantation					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
General body condition	2	3	3	4	4	4
Abdominal distension	0	0	0	0	0	0
Mucous membrane colour	1	2	2	2	2	2
Demeanor	3	3	3	3	3	3
Conformation	2	2	2	2	2	2
Gait	1	1	1	1	1	1
Appetite	3	3	4	4	4	4
Water intake	1	2	2	2	2	2
Dehydration	1	2	3	3	3	3
Rumen motility/min	1-2	1-2	1-2	1-2	1-2	1-2
Rumen consistency	2	2	2	2	2	2
Defaecation	2	2	2	2	2	2
Character of faeces	4	4	4	4	4	4
Appearance of hair coat	2	2	2	2	2	2
Grinding of teeth	2	2	2	2	2	2
Foamy saliva	1	1	1	1	1	1
Regurgitation of plastic bags	0	0	0	0	0	0
Weight loss	1	1	1	1	1	1
Mortality	0	0	0	0	0	1

Key:
General body condition: Good =1, fair =2, poor=3, very poor =4; Abdominal distension: none = 0, moderate = 1, Severe – 2; Mucous membrane colour: normal = 0, mildly pale = 2, pale = 3; Demeanor: bright/alert= 0, slightly depressed = 1, depressed = 2, severely depressed = 3; Conformation: normal =0, slightly ached back = 1, ached back = 2; Gait: normal = 0, ataxia = 1; Appetite: normal = 0, moderately reduced =1, reduced = 2, markedly reduced = 3, anorexia = 4; Water intake: normal = 0, reduced =1, markedly reduced = 2; Dehydration: none = 0, slight =1, moderate = 2, marked =3; Rumen consistency: doughy = 0, firm =1, very firm = 2; Defaecation: present =1, occasional =2, absent =3; Character of feaces: normal pellets = 0, hard pellets =1, loose =2, pasty = 3, diarrhoea = 4, no faeces = 5; Appearance of hair coat: glossy =1, rough = 2; Grinding of teeth: absent = 0, occasional =1, continuous = 2; Foamy saliva: absent = 0, present = 1; Regurgitation of plastics bags: absent = 0, present = 1; Weight loss: absent = 0, present = 1

Discussion

Normal body temperature, respiratory and heart rates of goats with various quantities of plastic bags implanted in the rumen observed in the current study was consistent with previous findings in goats impacted with ingested indigestible rumen foreign bodies (Ghurashi *et al.,* 2009; Raoofi *et al.,* 2012) and sheep with plastic bags in the rumen (Otsyina *et al.,* 2016). Presence of foreign bodies in the rumen does not appear to exert significant pressure on the diaphragm to interfere with respiratory and heart rates, as observed in the current study.

The firmness on the left side of the abdomen without any distension observed in the goats, may be due to the limited expansion capacity of the rumen (Tan, 1988). This observation however, is contrary to what has been reported in sheep impacted with the same quantities of plastic bags in the rumen, in which asymmetrical distension of the abdomen with firmness on the left side was observed (Otsyina *et al.,* 2016). This finding in goats also disagrees with previous reports by Igbokwe *et al*. (2003), Mozaffari *et al*. (2009) and Debaris and Mousami (2010) in sheep, although the quantities and nature of offending foreign bodies were not stated in their studies.

The sheep regurgitated some of the plastic bags implanted in the rumen (Otsyina *et al.,* 2016), possibly creating room for ruminal gasses to accumulate and distend the rumen, this was not observed in goats. This finding could be a significant clinical observation that differentiates goats from sheep with plastic bag impaction. The pale mucous membrane observed in goats with higher quantities of plastic bags may be due to interference of rumen microbial activity, poor feed intake and mineral imbalance as a result of presence of the plastics bags in the rumen.

Reduced feed intake and inappetence observed in goats implanted with 258 g and 387 g of plastic bags is similar to previous findings in sheep (Igbokwe *et al.,* 2003), goats (Baillie and Anzuino, 2006) and cattle (Debaris and Mousami, 2010) with indigestible foreign bodies in the rumen. Factors that determine feed intake by ruminants are related to the capacity of the gastro-intestinal tract, digestibility of the feed and the rate of passage through the gastro-intestinal tract (Conrad, 1966). Intake of roughages is also reported to be related to the amount of fatty acid production in the rumen in ruminants (Blaxter *et al.,* 1961). The presence of plastic bags in the rumen may have hindered effective fermentation and functioning of the rumen microflora (Radostitis *et al.,* 2009), thus, resulting in reduced feed intake. In addition, the extent of continuous stretching of the ruminal wall and reduced ruminal motility due to the presence of 258 g and 387 g of plastic bags in the rumen may have stimulated the hypothalamus and satiety centre leading to loss of appetite or inappetence (Mozaffari *et al.,* 2009).

The dehydration observed in the current study concurs with previous findings in goats (Debaris and Mousami, 2010) whose rumens were impacted with indigestible materials. The dehydration may have resulted from reduced water intake and stress as previously reported by Rossi and Scharrer (1994). Therefore, reduction in feed intake will lead to decreased water intake, hence dehydration. A similar finding has been reported in sheep with foreign materials in the rumen (Abdullahi *et al.,* 1984; Igbokwe *et al.,* 2003) but not in goats.

Severe depression, persistent grinding of teeth, arching of the back, rigidity of the limbs

and reluctance to move suggested discomfort which could possibly have been due to pain in the abdomen. Such observations have been reported in ruminants with rumen impaction (Reddy *et al.,* 2004; Debaris and Mousami, 2010). It is possible that distension and impaction of the rumen with the plastic bags resulted in stretching of the ruminal wall causing pain, hence the signs of discomfort. The persistent grinding of teeth seen in these animals supports this position. The foamy salivation observed in animals implanted with 258g and 387g of plastic bags may be a consequence of the persistent grinding of teeth as a result of the pain in the rumen. This has not been previously reported in goats or other animals.

The change in character of faecal materials with intermittent diarrhoea and constipation has been reported previously in sheep and goats with indigestible foreign rumen bodies (Igbokwe *et al.,* 2003; Debaris and Mousami, 2010). These findings may have resulted from irritation and enteritis probably caused by chemical irritants released from the implanted plastic bags or due to hepatic complications leading to a syndrome that caused both diarrhoea and constipation (Radostitis *et al.,* 2009). Constipation and dry pelleted faeces could also be attributed to the reduced or lack of water intake, hence dry contents in the colon (Otsyina *et al.,* 2016).

The hypomotility of the rumen of 1-2 weak contractions per minute which were also weak is consistent with ruminants having rumen impaction as reported previously for sheep (Igbokwe *et al.,* 2003). The hypomotility of the rumen may have been caused by either a reduction of excitable drive to the gastric centres or an increase in inhibitory inputs (Grunberg and Constable, 2009). Inhibitory inputs to the gastric centres can be caused by excessive distension of the rumen as well as some degree of hypocalcaemia. Hypomotility of the rumen in hypocalcaemia is due to the general effect of reduced levels of ionized calcium on smooth muscle contractility (Grunberg and Constable, 2009). However, hypocalcaemia was not observed in sheep implanted with the same quantities of plastic bags in a previous study (Otsyina *et al.,* 2016).

Loss of body weight in goats implanted with quantities of plastic bags in the current study is corroborated by reports of Ghurashi *et al.,* (2009) and Debaris and Mousami (2010) for sheep. However, these authors did not report on the specific quantities of foreign bodies or the duration of their presence in the rumen. Goats with as little as 129g of plastic bags in the rumen lost weight within two weeks after implantation and did not recover the weight loss in the 6 weeks of observation. The weight loss in the current study is due to drastic reduction in feed and water intake leading to reduction of fermentable ingesta in the rumen and possible disturbances in microbial fermentation and reduced fatty acid production and absorption (Randall *et al.,* 2002). Loss of body water as well as reduction in feed intake is associated with weight loss (Shkolnik *et al.,* 1972; Abdalla *et al.,* 2010). The higher weight loss with increased quantities of plastic bags in the rumen, over time suggests greater interference with fermentative and physiological activities in the rumen.

Conclusion

Results of the study shows that presence of plastic bags in the rumen of goats is characterized by anorexia, depression, reduced feed and water intake, abdominal distension, reduced ruminal motility and reduced rumination. Furthermore these animals presented with dehydration, altered faecal characteristics with some animals having dry hard and pelleted faeces, or loose pasty or watery faeces. Other signs included discomfort mainly manifested by grinding of teeth and arched back, recumbency and death. Severity of the clinical symptoms however, depended on the degree of impaction, quantity and duration of plastic bags in the rumen. Goats seem to be more severely affected than sheep. The animals consequently lost weight depending on the quantity of plastic bags in the rumen.

Presence of plastic bags in the rumen imposed a serious health burden on the goats affecting their total wellbeing, weight gain and

leading to mortality of some of the animals subsequently affecting their overall productivity and production. It is recommended that indigestible rumen foreign bodies be considered as a differential diagnosis in goats presenting with the aforementioned clinical signs.

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Conflict of Interest

There is no conflict of interest whatsoever regarding this manuscript.

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EFFECTS OF SUPERLIV® SUPPLEMENTATION IN FEED ON SOME BIOCHEMICAL PARAMETERS AND EGG QUALITY INDICES OF POST PEAK SHIKA BROWN LAYERS

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Abstract

To explore the mechanisms for production performance enhancement effects of Superliv®(Superliv), an Ayurvedic proprietary herbal supplement for livestock and poultry, post peak Shika Brown layers were exposed to the herbal mixture in feed and monitored over 12 weeks for effects on some blood chemistry and egg quality parameters. Fifty post peak Shika Brown layer chickens that have been in lay for over 38 weeks were randomly assigned to treatment groups A-E of 10 birds each. Group A hens received plain feed and served as control while groups B, C, D or E were fed diets supplemented with Superliv at the rate of 250g, 500g, 750g or 1000g per ton respectively, during a study period of 12 weeks. They were assessed before supplementation and subsequently, weekly; for biochemical parameters such as whole blood glucose, total serum protein, serum albumin and globulin as well as egg quality factors including: egg weight, albumen index, percentage shell, shell thickness, specific gravity and Haugh unit scores. From the results obtained, Superliv supplementation in feed caused significant decreases in overall mean total serum proteins ($p<0.010$) and serum albumin ($p<0.018$), increases in overall mean whole blood glucose concentration ($p<0.020$) but no significant changes in serum globulin as well as all egg quality factors evaluated. It is suggested that Superliv may promote production performance in post peak Shika Brown hens by enhancing the mobilization of plasma proteins into tissue sites for egg and body weight building. No adverse changes in egg quality traits may be associated with Superliv feed supplementation in layers.

Key words: Superliv, Blood chemistry, Egg quality, Feed supplements, Layers

EFFETS DE LA SUPPLÉMENTATION SUPERLIV® DANS LES ALIMENTS SUR CERTAINS PARAMÈTRES BIOCHIMIQUES ET INDICES DE LA QUALITÉ DES OEUFS DES PONDEUSES SHIKA BROWN EN PERIODE POST-PIC

Résumé

Dans le souci d'explorer les mécanismes des effets de Superliv® (Superliv) sur l'amélioration de la performance à la production - un supplément à base de plantes ayurvédiques pour bétail et volailles, les pondeuses Shika Brown en période post-pic ont été exposées au mélange d'herbes dans les aliments et ont été surveillées pendant 12 semaines afin de détecter les effets sur les paramètres de la chimie du sang et de la qualité des oeufs. Cinquante pondeuses Shika Brown en période post-pic pendant plus de 38 semaines ont été réparties de manière aléatoire aux groupes de traitement A-E de 10 oiseaux chacun. Les poules du groupe A ont reçu une alimentation ordinaire et ont servi de témoins tandis que les groupes B, C, D ou E ont reçu des régimes alimentaires additionnés de Superliv respectivement aux taux de 250 g, 500 g, 750 g ou 1000 g par tonne, pendant une période d'étude de 12 semaines. Elles ont été évaluées avant la supplémentation et après celle-ci, chaque semaine, en analysant les paramètres biochimiques tels que la glycémie totale, la protéine sérique totale, l'albumine sérique et la globuline, ainsi que les facteurs de qualité des œufs, notamment le poids des œufs, l'indice d'albumine, le pourcentage de coquille, l'épaisseur de la coquille, la gravité spécifique et les scores de l'unité Haugh. Il ressort des résultats obtenus que la supplémentation des aliments avec Superliv a provoqué des diminutions significatives des protéines

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sériques totales moyennes globales (p <0,010) et de l'albumine sérique (p <0,018), des augmentations de la concentration globale moyenne de glucose dans le sang total (p <0,020), mais aucun changement significatif dans la globuline de sérum et tous les facteurs de qualité des œufs évalués. Ceci porte à croire que Superliv peut favoriser les performances de production chez les poules Shika Brown en période post-pic en améliorant la mobilisation des protéines plasmatiques dans les sites tissulaires pour la construction des poids des œufs et du corps. Aucun changement défavorable dans les traits de qualité des œufs ne peut être associé à la supplémentation alimentaire avec Superliv chez les pondeuses.

Mots-clés : Superliv, chimie du sang, qualité des œufs, compléments alimentaires, pondeuses

Introduction

Commercial poultry production outfits, under intensive management, rely on feed supplements or additives to attain and sustain production targets, product quality and profitability (Scott,2009). Since the late 1940s when vat culture medium of Streptomyces aureofaciens,a mold that elaborates an antibiotic known as chlortetracycline, was shown to elicit growth responses in exposed pigs and poultry, antibiotic or antibacterial based supplements have remained dominant production performance enhancement supplements in the poultry industry (Mathews, 2001). For this purpose, antibacterials are included in poultry feed or water at sub-therapeutic levels with the ultimate of aim of increasing production performance of the birds (Scott, 2009; Ewing and Cole, 1994). This practice has, however, lead to widespread public health concerns due to increasing cases of drug resistance by bacteria organisms; traceable to antibacterial prophylaxis for growth in food animals, including poultry (Aarestrup, 2012; BSAS, 2009; Butaye *et al*, 1999). There is now concerted efforts to reduce prophylactic use of antibacterials as feed additives in food animals to the barest minimum while the search for safer alternative agents for boosting productive performance of food animals continues (Aarestrup, 2012; WHO, 2012; FDA, 2012). Interest in phytogenic feed additives as alternatives to antibacterials is currently on the increase mostly because of their safety attributes (Windisch *et al*, 2008; Craig, 1999).

Superliv is an Ayurvedic commercial herbal preparation comprising a mixture of five related herbs namely; *Picorrhiza kuroa* (Picorrhiza), *Andrographis paniculata* (Creat), Boerhauvia diffusa (Horse-Purslane), Solanium nigrum (Night shade) and Swertia chirata (Chirata). Recent phytochemical and elemental analyses of Superliv commercial powder mixture demonstrated the presence of carbohydrates, tannins, cardiac glycosides, saponins, flavonoids and alkaloids as well as key elements such as Fe, Ca, Mg, Na and K (Jibike *et al*, 2011). The mixture is reported to act as a liver stimulant when applied through feed or water to various farm animals including poultry. In poultry, it is reported to enhance productivity, reduce morbidity and mortality in supplemented flocks without toxic effects (Shivakumar *et al*, 2005). Recently, Jibike *et al* (2011), demonstrated that Superliv supplementation in feed, enhanced egg production and live weight gains in post peak Shika Brown layers under harsh sahelian environment. The mechanism(s) of production enhancement associated with Superliv supplementation in layers are not yet clearly understood though the capacity for the herbal mixture to stimulate increased egg and live weight gains in post peak layers has been clearly demonstrated (Jibike *et al*, 2011). The present study attempts to explore the mechanism(s) of production enhancement attributes of Superliv by assessing the effects of the herbal additive on some blood chemistry parameters and egg quality indices of post peak Shika Brown layers.

Materials and Methods

Experimental Chicken

Fifty actively laying post peak Shika Brown layer chicken (Gallus gallus) were purchased from a commercial farm in sahelian North Eastern Nigeria and used for the

experiments. The hens were all under the same management with standard up to date vaccination records. On arrival, they were allowed to acclimatize for 2 weeks during which they were stabilized, routinely switched from source farm feed to experimental feed and observed for morbidities and mortalities. Subsequently, they were randomly divided into five treatment groups A-E of 10 birds each and housed in a battery cage under the same roof at the rate of 2 birds per cubicle. Group A birds received plain feed and served as control while groups B, C, D and E hens were fed feed supplemented with Superliv at the rate of 250g, 500g, 750g or 1000g per ton, respectively, during the experimentation period of 12 weeks. Feed and water were provided ad libitum throughout the study period.

Experimental Feed

Commercial layers' mash (Livestock Feeds Nig. PLC) was used as the basal feed. The feed was purchased in bulk, stored and used during the study period. Each group of 10 layers received 1.5kg of feed daily. The component ingredients and proximate analysis of the feed have been reported (Jibike *et al*, 2011).

Test Drug

Superliv was purchased from Animal Care Konsult Nig. Ltd as a greenish amorphous powder for inclusion in feed at a recommended rate of 500g/ton. The preparation is an Ayurvedic herbal mixture containing 5 different herbs. To achieve the various inclusion rates for the respective treatment groups, the drug was added to 25kg of feed, partially mixed in a plastic bowl and then transferred to an on farm mixing device for thorough mixing. For 250g, 500g, 750g and 1000g per ton inclusion levels, 6.25g, 12.5g, 18.75g or 25g of Superliv was, respectively, added to 25g of basal feed and thoroughly blended. Phytochemical and elemental analyses of Superliv have been reported (Jibike *et al*, 2011).

Blood Sample Collection

Five birds in each group were bled on alternate weeks. About 5ml of blood was collected from each bird through the wing vein using 5ml syringe and 21 gauge sterile hypodermic needle, for blood chemistry evaluation. 2ml of the blood was immediately discharged into heparinized sample bottle for whole blood glucose content determination while the rest was discharged into plain sample bottles and allowed to clot and produce serum. The serum was subsequently harvested, following centrifugation of clotted blood sample at 30rpm for 15mins, then decanted into plastic bijou bottle and stored at -20oC until analyzed. The serum was used for assay of total serum protein, serum albumin and globulin concentrations. Blood samples were collected before supplementation commenced (week 0) and subsequently, weekly, during the study period.

Egg Collection

Three representative eggs from the last collection for the week per group were appropriately identified and pooled for evaluation of egg quality indicators such as: egg weight, albumen index, specific gravity, percentage shell, shell thickness and Haugh unit scores. Eggs were collected before Superliv supplementation commenced and subsequently on weeks 2,3,4,5,6, and 9 of treatments.

Experimental Protocol

Determination of Whole Blood Glucose

The ONE TOUCH® test strips for testing glucose levels in whole blood were used to evaluate glucose concentrations in heparinized blood samples within 30mins of collection. The test strip was first put in place on the customized digital meter (Lifescan Inc., California, USA). After thorough mixing, enough of the heparinized blood sample was dropped to cover a designate area (test spot) on the test strip using a dropping pipette. The blood was allowed to stand for 45 seconds, after which the test results display digitally on a monitor screen on the glucose meter. Values are expressed in mg/dl and reported as weekly group mean (± SD) from week 2 of supplementation.

Determination of Total Serum Protein

The Biuret method for the quantitative *in vitro* assay of total protein in serum was employed. This is a manual procedure that measures total serum protein based on the principle that cupric ions in alkaline medium interact with protein peptide bonds resulting in formation of a coloured complex. The intensity of the colour is proportional to the amount of protein present in the test sample and can be measured using appropriate colorimetric or spectrophotometric approaches (Tietz, 1995). For the purpose of the present evaluations, Randox® test kit (Randox Laboratories Ltd, Crumlin Co., Aritrim UK) with total protein concentration of 58.48g/L in the standard was purchased and used while colour matching was carried out colorimetrically using Corning's Colorimeter 252 (Corning's Ltd, England). Each serum sample was subjected to appropriate titrimetric procedures using component reagents in the Randox test kit (Tietz, 1995). Colour intensity measurement was carried out colorimetrically at a wave length of 540nm. Reagent blank was used to calibrate the colorimeter scale. Total protein concentration (g/L) in the serum sample was finally determined through standard calculations (Tietz, 1987).

Determination of Serum Albumin and Globulin

The Bromocresol green manual method (Tietz, 1987) for measuring serum or plasma albumin content was used to assay albumin concentration of each serum sample. Randox albumin test kit (Randox Laboratories Ltd, Crumlin Co., UK) containing ready to use reagents and standard with albumin concentration of 29g/L was employed as prescribed (Tietz, 1987). The optical densities of resultant solutions following titration of test sera with reagents were measured colorimetrically as for total protein but at a wavelength of 600nm. Following determination of colour density values of serum samples, equivalent albumin concentrations were obtained by standard calculations and expressed in g/L. The globulin concentration of each test serum sample was determined as the difference between total serum protein and serum albumin concentrations expressed in g/L Values are reported as mean ±SD of 5 serum samples per group per week.

Determination of Egg Quality Indices

Three representative eggs per group, collected at weekly intervals were subjected to standard procedures for the determination of egg quality factors. Egg weight, albumen index, percentage shell, shell thickness, specific gravity and Haugh unit score were evaluated by standard methods (Thompson and Hamilton, 1982; Haugh, 1937; Heiman and Carter, 1936).

Statistical Analysis

Data collected were organized into tables as weekly mean values ± SD. Two way ANOVA was used to compare overall group means with significant differences detected at 95% confidence limits. Tukey-Kramer multiple comparison test was applied to separate overall means using appropriate statistical soft ware (GraphPad, 2000).

Results

Findings on Whole Blood Glucose

The results of the weekly and overall whole blood glucose assessments are presented in Table 1. There is significant ($p<0.02$) difference in the overall mean whole blood glucose values of groups D and E birds while all the other supplemented groups demonstrated values comparable to that of control plain feed fed hens.

Findings on Total Serum Protein

Table 2 shows the results of weekly and overall evaluations of total serum protein of control and supplemented hens. There are significant ($p<0.010$) decreases in overall mean total serum protein of Superliv supplemented groups when compared with that of plain feed fed control group with the exception of group D that recorded similar overall mean value as the control.

Table 1: MEAN (+SD) WEEKLY WHOLE BLOOD GLUCOSE (mg/dl) OF GROUPS OF SHIKA BROWN LAYERS FED PLAIN FEED(A), FEED CONTAINING 250g/ton(B), 500g/ton(C), 750g/ton(D) OR 1000g/ton(E) OF SUPERLIV® AS FEED ADDITIVE DURING A STUDY PERIOD OF 12 WEEKS

WEEKS OF FEEDING	GROUPS				
	A	B	C	D	E
2	175.00 +11.14	194.33 +8.96	198.00 +5.00	204.00 + 9.54	197.33 + 26.50
3	193.33 +10 .69	196.75+20.09	188.60+76.60	170.00 + 43.86	187.80 + 46.74
4	166.50 + 11.15	189.75+13.50	166.50+19.23	152.75 + 13.88	192.60 + 6.07
5	182.60 + 18.81	192.80 +28.35	180.80+4.87	184.50 + 10.34	198.67 + 16.05
6	183.00 + 13.91	199.60+ 26.48	162.20+43.65	161.40 + 32.94	204.00 + 19.34
8	227.67 + 17.62	161.00 +34.07	207.25+39.09	203.75 + 49.44	254.25 + 32.12
9	181.80 + 20.86	204.20 +19.28	200.60 +9.02	190.80 + 35.93	202.80 + 9.52
10	189.20 + 22.79	162.60+33.77	187.40+10.57	189.80 + 39.14	210.80 + 17.02
11	207.80 + 18.73	219.60+19.37	201.40+25.77	192.40 + 28.38	225.20 + 6.53
*OVERALL	189.66^{a} +18.35	191.18^{a}+18.78	188.08^{a} +15.77	183.27^{ab}+18.09	208.16^{ac}+ 20.41

** Overall means with different superscript(s) differ significantly (P < 0.020)*

Table 2: M EAN (+SD) WEEKLY TOTAL SERUM PROTEIN (g/L) OF GROUPS OF SHIKA BROWN LAYERS FED PLAIN FEED(A) OR FEED CONTAINING 250g/ton(B), 500g/ton(C), 750g/ton(D) OR 1000g/ton(E) OF SUPERLIV® AS FEED ADDITIVE DURING A STUDY PERIOD OF 12 WEEKS

WEEKS OF FEEDING	GROUPS				
	A	B	C	D	E
0	58.26 + 6.68	50.26 + 9.18	66.93 + 18.93	56.58 + 11.09	57.85 + 16.07
1	77.73 + 7.09	71.11 + 40.18	71.11 + 10.18	66.67 + 13.34	44.44 + 26.94
2	61.39 + 6.84	51.87 + 8.74	59.19 + 3.77	63.63 + 22.98	48.30 + 1.80
3	35.78 + 12.86	47.41 + 16.07	46.37 + 17.12	45.22 + 17.86	56.06 + 32.25
4	89.80 + 35.37	54.00 + 35.78	57.60 + 17.36	65.60 + 13.48	67.00 + 23.35
5	76.43 + 10.75	69.15 + 12.42	63.30 + 8.33	63.74 + 11.81	69.01 + 18.65
6	85.89 + 20.10	76.03 + 9.46	95.27 + 19.69	97.22 + 16.67	95.76 + 21.06
8	48.87 + 19.53	42.61 + 6.20	28.24 + 4.65	55.56 + 19.06	48.91 + 3.72
9	94.64 + 12.81	78.00 + 8.69	71.33 + 8.69	93.33 + 14.14	66.67 +15.63
10	97.44 + 17.70	73.10 + 14.06	59.33 + 20.97	95.74 + 14.62	66.97 + 9.53
11	73.31 + 18.00	52.00 + 15.32	53.84 + 14.43	51.17 + 10.95	38.43 + 5.24
*OVERALL	72.69^{a} + 19.66	60.50^{b} + 12.96	61.14^{b} + 16.67	68.59^{ab}+ 18.42	59.95^{b} + 15.78

** Overall means with different superscript(s) differ significantly (P < 0.010)*

Findings on Serum Albumin and Globulin

Superliv supplementation of Shika Brown layers' diets as employed in the present study caused significant ($p<0.018$) decreases in overall mean serum albumin levels relative to plain feed fed control value as shown in Table 3. The observed effects seem most obvious in groups B and E hens while groups C and D hens recorded comparable values with control group. Table 4 demonstrates observations with respect to serum globulin. There are no significant differences between overall

Table 3: MEAN (+SD) WEEKLY SERUM ALBUMIN (g/L) OF GROUPS OF SHIKA BROWN LAYERS FED PLAIN FEED(A) OR FEED CONTAINING 250g/ton(B), 500g/ton(C), 750g/ton(D) OR 1000g/ton(E) OF SUPERLIV® AS FEED ADDITIVE DURING A STUDY PERIOD OF 12 WEEKS

WEEKS OF FEEDING	GROUPS				
	A	B	C	D	E
0	51.45 + 8.17	40.15 + 10.34	57.83 + 12.40	41.94 + 12.05	37.92 + 9.60
1	46.01 + 7.79	32.46 + 16.23	33.74 + 3.56	31.43 + 19.04	16.42 + 18.28
2	39.82 + 10.19	29.00 + 9.86	32.87 + 9.67	31.76 + 11.46	30.24 + 13.56
3	26.29 + 10.51	32.71 + 13.93	35.72 + 17.54	24.08 + 2.75	36.29 + 10 84
4	53.40 + 15.98	43.92 + 28.94	38.74 + 8.21	43.48 + 9.84	33.47 + 11.24
5	24.12 + 6.58	21.30 + 12 57	24.52 + 3.91	30.06 + 9.57	31.64 + 13.02
6	42.79 + 15.23	24.65 + 4.36	25.74 + 12.72	28.64 + 1.98	23.20 + 3.49
8	27.32 + 1.34	23.86 + 4.68	14.45 + 4.74	25.50 + 8.26	29.33 + 10.93
9	42.72 + 14.11	25.02 + 6.00	29.50 + 5.04	28.35 + 4.52	35.60 + 7.40
10	34.14 + 1.18	37.02 + 6.42	41.00 + 3.97	40.00 + 3.97	37.83 + 7.55
11	20.28 + 3.12	21.70 + 3.67	22.14 + 2.43	21.94 + 2.70	20.88 + 1.97
*OVERALL	37.14^{a} + 14.26	30.09^{b} + 13.59	32.39^{ab} + 11.47	31.56^{ab} + 10.69	30.79^{b} + 10.21

** Overall means with different superscript(s) differ significantly (P < 0.010)*

Table 4: MEAN (+SD) WEEKLY SERUM GLOBULIN (g/L) OF GROUPS OF SHIKA BROWN LAYERS FED PLAIN FEED(A) OR FEED CONTAINING 250g/ton(B), 500g/ton(C), 750g/ton(D) OR 1000g/ton(E) OF SUPERLIV® AS FEED ADDITIVE DURING A STUDY PERIOD OF 12 WEEKS

WEEKS OF FEEDING	GROUPS				
	A	B	C	D	E
0	9.15 + 4.24	10.11 + 7.57	9.11 + 10.06	14.64 + 11.22	23.08 + 11.56
1	31.72 + 9.65	38.65 + 24.73	37.37 + 6.68	32.01 + 8.70	28.03 + 20.73
2	21.57 + 11.90	22.87 + 12.70	26.33 + 10.72	20.16 + 7.18	17.95 + 14.95
3	9.31 + 3.00	14.70 + 6.43	10.65 + 10.43	21.24 + 16.46	20.61 + 31.13
4	36.40 + 19.45	10.08 + 12.41	18.86 + 10.97	22.12 + 6.26	33.53 + 12.79
5	52.30 + 12.83	47.85 + 20.05	38.79 + 10.65	33.69 + 15.37	37.37 + 24.49
6	43.10 + 29.57	51.38 + 15.52	69.53 + 16.96	68.59 + 16.02	72.56 + 21.09
8	21.55 + 18.88	18.75 + 7.04	13.79 + 1.59	30.06 + 12.45	19.58 + 9.56
9	51.92 + 15.01	52.99 + 5.42	41.83 + 12.33	64.98 + 10.04	31.07 + 19.95
10	63.30 + 18.28	36.12 + 14.36	18.33 + 12.39	55.74 + 14.06	29.14 + 15.34
11	53.05 + 17.14	30.31 + 12.28	31.71 + 15.22	29.23 + 9.47	17.55 + 6.09
OVERALL	35.76 + 18.64	30.35 + 16.18	28.75 + 17.80	35.68 + 18.74	30.04 + 15.58

** Overall means with different superscript(s) differ significantly (P < 0.010)*

Table 5: OVERALL MEAN (+SD) EGG QUALITY FACTORS OF EGGS FROM GROUPS OF SHIKA BROWN LAYERS FED PLAIN FEED (A) OR FEED CONTAINING 250g/ton (B), 500g/ton (C), 750g/ton (D) OR 1000g/ton (E) OF SUPERLIV AS FEED ADDITIVE DURING A STUDY PERIOD OF 12 WEEKS

Egg quality Factors	GROUPS					P-values
	A	B	C	D	E	
Egg weight (g)	57.35±3.28	57.47±3.34	60.59±2.61	61.11±5.97	56.84±4.76	0.082
Albumen index (mm/g)	0.012±0.002	0.012±0.002	0.012±0.002	0.013±0.002	0.013±0.002	0.653
Percentage Shell (%)	9.29±0.47	9.27±1.45	9.82±1.01	9.46±0.97	9.65±1.20	0.567
Specific gravity (d)	1.067±0.003	1.068±0.004	1.071±0.006	1.071±0.004	1.069±0.005	0.186
Shell thickness(mm)	0.17±0.04	0.16±0.03	0.17±0.13	0.17±0.03	0.17±0.04	0.920
Haugh unit Score (%)	87.94±1.96	88.35±0.40	88.27±0.50	88.18±0.67	88.94±1.12	0.435

mean values of supplemented and plain feed fed control groups. The overall mean serum globulin concentration of the control hens at 36.90g/L is only marginally higher than those of supplemented hens ranging from 28.75 to 35.68 g/L.

Findings on Egg Quality Indices

None of the egg quality indices assessed was significantly affected by Superliv supplementation in diets as employed. Table 5 demonstrates the overall mean values of the various egg quality indicators evaluated for control and supplemented hens, over the period of experimentation. Though Superliv inclusions in diets seemed to cause consistently higher overall mean indices values, these are largely marginal. Both plain feed fed control and supplemented hens produced normal eggs of acceptable qualities as depicted by comparable Haugh unit scores.

Discussion

The results of the present studies indicate that Superliv supplementation in layers' diets as employed may significantly increase whole blood glucose concentration, decrease total serum protein and serum albumin with insignificant decreases in serum globulin concentrations. These observations are in contrast to those reported earlier by Oyagbemi *et al* (2008), who demonstrated increases in total plasma protein, plasma albumin, globulin and fibrinogen concentrations following exposure of cockerels to Stressroak, a phytogenic Ayurvedic supplement of the same herbal origin as Superliv. In their study, Oyagbemi *et al* (2008) included Stressroak in drinking water of the cockerels during a period of 30-60 days. In another previous report Vidyarthi *et al* (2008), observed lower serum glucose levels and no changes in serum protein of broilers supplemented with Superliv. The significant increase in whole blood glucose concentration recorded in the present study was due largely to a sharp difference between group D and E hens that received 750g/ton or 1000g/ton Superliv inclusion in diets, respectively. Whereas all supplemented groups demonstrated comparable overall mean whole blood glucose concentrations with control hens, Superliv supplementation in Shika Brown layers may actually cause little or no effects in whole blood glucose levels.

The decreases in overall mean total serum protein concentrations as demonstrated in the present study did not fit a typical dose dependent trend as decreases of 16.77%, 15.8%, 6.4% or 17.53% with reference to control value were recorded for 250g, 500g, 750g or 1000g per ton, Superliv inclusion rates in feed, respectively. Similar response pattern was observed for overall mean serum albumin with values of 18.9%, 12.95%, 15.02% or 17.10% lower than that of control were observed for treatment groups B, C, D or E respectively. It is possible that beyond the inclusion rate of 250g/ton, an elimination or deactivation mechanism for the active principle in Superliv was triggered but got saturated as inclusion rate of 1000g/ton was reached. From the current observations, it is apparent that the decreases in overall mean total serum protein of the Shika Brown hens were driven mainly by decreases in overall mean serum albumin. Manwar *et al* (2005), reported no significant changes in total serum protein and serum albumin of Bellicharana broilers fed Azadiracta indica leaves powder supplemented diets, though, the supplement caused significant increases in weight gain. More recent reports (Jibike *et al*, 2011) had shown that Superliv supplementation induced significant increases in hen day egg production and live weight of Shika Brown layers associated with marginally lower feed consumption rate. It appears that Superliv supplementation caused intense protein demand, arising from stepped up egg and tissue building; which was met by enhanced mobilization of albumin from the plasma into tissue sites. This view is supported by the observations, in earlier report (Jibike *et al*, 2011) that the supplemented layers with marginally higher overall mean total serum protein and serum albumin values also demonstrated lower egg production and live weight values.

Superliv supplementation in the feed of the layers as employed in the current study did not produce any significant changes in the egg quality indicators evaluated. The overall mean shell thickness values for both control and supplemented groups appear to be on the lower side of normal. This is attributable to the age of the layers, having been in lay for over 35weeks at the time of commencing the experiments. It has been established that absorption and mobilization of calcium, a critical component of egg shell; by layers decreases to less than 50% of normal after 40 weeks of age (Gupta, 2008). Hence, the general relative thinness of the eggs laid by the Shika Brown hens in the current study may be a reflection of the age effect on egg shell thickness. Other workers who used herbal products as feed additive in layers have reported little or no effects on egg quality traits as has been observed in the present study (Wang *et al*, 2009; Tyagi *et al*, 2005; Fasuyi *et al*, 2005). In general, though Superliv supplementation did not significantly enhance egg quality indices, the herbal feed additive ensured that eggs from the supplemented hens fell within good commercial quality limits as reflected in acceptable Haugh unit scores.

In conclusion, this study has revealed that Superliv may promote egg production and live weight gains in aging layers by increasing the mobilization of albumin from blood into egg and tissue sites. Though details of how this mobilization takes place remain unclear, it is likely that the herbal preparation inclusion in feed, over time; creates a demand pull for protein deployed at tissue and egg sites which is met not by increased feed intake but mobilization of plasma protein store. This phenomenon depicts Superliv as a typical performance enhancer most suitable for post peak layer flocks whose egg production machinery have began to weaken. Further, the herbal preparation did not cause any adverse effects on egg quality. We therefore, recommend Superliv as alternative feed additive for such older flocks to extend their productive life span and profitability with no ill effects on the birds' welfare and consumer.

Impact Factor

This study demonstrates that Superliv may enhance egg production post peak layers by promoting movement of albumin from plasma into tissue sites. Though, the mechanism of this effect remain unclear, the process does not seem to affect egg quality traits in any negative way. Thus Superliv supplementation in

ration is a safe way to boost egg production in aging hens.

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MAPPING THE DISTRIBUTION OF TSETSE AND ANIMAL *TRYPANOSOMOSIS* IN SELECTED DISTRICTS OF UGANDA TO FACILITATE TARGETING CONTROL MEASURES

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Abstract

Declining Government resources and donor aid for tsetse and *trypanosomosis* control have compelled countries to delegate control activities to farm-level veterinary extension personnel. While effective control measures such as chemotherapy, tsetse-trapping and restricted insecticide-treatment of livestock exist, implementation of such measures en masse has become increasingly impractical. Therefore mapping the distribution of tsetse and *trypanosomosis* hotspots was undertaken to ease targeting control measures by district administrative units in Amuria, Dokolo and Kaberamaido districts of Uganda. The districts were divided into grid sites using the program Arcview GIS 3.2. Villages representing the grid sites were physically identified through ground-truthing. Parasitological and tsetse surveys were then conducted in each village to establish the prevalence of *trypanosomosis* in cattle and apparent tsetse density. A total of 2430, 1304 and 1392 cattle were tested in Amuria, Dokolo and Kaberamaido districts, of which 230 (9.5%), 144 (11.0%) and 106 (7.6%) were detected positive, respectively. At village level, the prevalence of *trypanosomosis* ranged from 1-24%, 1-28% and 2-18% in Amuria, Dokolo and Kaberamaido districts, respectively. Tsetse surveys conducted concurrently in the respective villages revealed apparent tsetse densities ranging from 0.0-2.4, 0.6-13.2 and 0.6-9.0 flies/trap/day (F/T/D) in Amuria, Dokolo and Kaberamaido districts, respectively. Glossina fuscipes fuscipes was the sole tsetse species caught. Matching apparent tsetse densities at village level with their corresponding prevalences of *trypanosomosis* revealed that Amuria district had low tsetse densities with high corresponding prevalences of *trypanosomosis*, suggesting that cattle were encountering a high proportion of infected tsetse. In contrast, Dokolo district had high tsetse densities with low to medium corresponding prevalences of *trypanosomosis*, suggesting cattle were encountering a low proportion of infected tsetse. Kaberamaido district had low tsetse densities with corresponding low to medium prevalences of *trypanosomosis*, suggesting that cattle were equally encountering a low proportion of infected tsetse. Mapping to highlight hotspots was anticipated to facilitate planning and prioritization of resources for targeting tsetse and *trypanosomosis* control by district departments of veterinary services.

Keywords: Animal *trypanosomosis*; Control; mapping; prioritization of resources; Tsetse flies; Uganda

CARTOGRAPHIE DE LA DISTRIBUTION DES GLOSSINES ET DE LA TRYPANOSOMOSE ANIMALE DANS CERTAINS DISTRICTS DE L'OUGANDA POUR FACILITER LE CIBLAGE DES MESURES DE CONTRÔLE

Résumé

La diminution des ressources gouvernementales et l'aide des bailleurs de fonds en faveur de la lutte contre les glossines et la trypanosomose ont obligé les pays à déléguer les activités de contrôle au personnel de vulgarisation vétérinaire au niveau de l'exploitation agricole. Bien que des mesures de contrôle efficaces telles que la chimiothérapie, le piégeage des mouches tsé-tsé et le traitement insecticide restreint des animaux existent, la mise en œuvre de telles mesures en masse est devenu de plus en plus impraticable. Par conséquent, la cartographie de la distribution des points névralgiques de la mouche tsé-tsé et de la trypanosomose a été effectuée pour faciliter le ciblage des mesures de contrôle par les unités

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administratives dans les districts d'Amuria, Dokolo et Kaberamaido en Ouganda. Les districts ont été divisés en grid sites en utilisant le programme Arcview GIS 3.2. Les villages représentant les grid sites ont été physiquement identifiés par la vérification sur terrain. Des enquêtes parasitologiques et sur les glossines ont ensuite été menées dans chaque village pour déterminer la prévalence de la trypanosomose chez les bovins et la densité apparente des mouches tsé-tsé. Au total, 2430, 1304 et 1392 bovins ont été testés respectivement dans les districts d'Amuria, Dokolo et Kaberamaido, avec respectivement 230 (9,5%), 144 (11,0%) et 106 (7,6%) aux résultats positifs. Au niveau des villages, la prévalence de la trypanosomose variait de 1 à 24%, de 1 à 28% et de 2 à 18% respectivement dans les districts d'Amuria, Dokolo et Kaberamaido. Les études sur les tsé-tsé menés simultanément dans les divers villages ont révélé des densités apparentes de glossines allant de 0,0-2,4 ; 0,6-13,2 ; et 0,6-9,0 mouches / piège / jour (F / T / D) respectivement dans les districts d'Amuria, Dokolo et Kaberamaido. Glossina fuscipes fuscipes était la seule espèce de glossine capturée. La comparaison des densités apparentes des tsé-tsé au niveau des villages avec leurs prévalences correspondantes de trypanosomose a révélé que le district d'Amuria avait de faibles densités de glossines avec une forte prévalence correspondante de trypanosomose, ce qui fait penser que les bovins ont parmi eux une forte proportion infectée par la mouche tsé-tsé. En revanche, le district de Dokolo avait des densités élevées de tsé-tsé avec des prévalences de trypanosomose correspondantes variant de faible à moyenne, ce qui porte à croire que les bovins ont parmi eux une faible proportion infectée par les glossines. Le district de Kaberamaido avait de faibles densités de glossines avec des prévalences de trypanosomose correspondantes variant de faible à moyenne, ce qui fait penser que les bovins ont également parmi eux une faible proportion infectée par les mouches tsé-tsé. La cartographie visant à mettre en évidence les points névralgiques a été envisagée en vue de faciliter la planification et la priorisation des ressources pour le ciblage du contrôle des tsé-tsé et de la trypanosomose par les services vétérinaires des départements de santé des districts.

Mots-clés : trypanosomose animale ; contrôle ; cartographie ; priorisation des ressources ; mouches tsé-tsé ; Ouganda

Introduction

Originally, tsetse flies (Glossina spp.) infested 106400 km^2, 50% of the entire landmass of Uganda (Chizyuka, 1998). However, declining resources for large-scale control programmes in Uganda led to unprecedented resurgence of tsetse and *trypanosomosis* in areas where it had been controlled and as well as emergence occurred in new areas.

Both human and animal *trypanosomosis* are a problem in several places in Southeastern and Northeastern Uganda, transmitted by the tsetse species G. fuscipes fuscipes (Lancien *et al.,* 1990; Okoth *et al.,* 1991) and to a lesser extent G. pallidipes (Magona *et al.,* 1997; Magona *et al.,* 2005). Tsetse is estimated to infest approximately 7,000km^2 in this area, where a human population of 2.1 million lives of which 50% earn less than 1 US$ per day. Crop-livestock smallholdings are the dominant farm type. There are approximately 500,000 indigenous Zebu cattle used for milk, meat, traction and savings. A series of sleeping sickness epidemics have been recorded and over one million people died in a devastating epidemic (Abaru, 1985). In previous epidemics (1976-82 and 1984-93), over 50,000 sleeping sickness cases were confirmed. While the geographical distribution of rhodesiense and gambiense sleeping sickness is believed to be separate in Uganda, there are concerns that an overlap might occur (Hutchinson *et al.,* 2003).

The prevalence of animal *trypanosomosis* was estimated at 11.9% under the intensive dairy system and 25% under the communal grazing systems (Magona and Mayende, 2002). Of all the confirmed cases of *trypanosomosis*, 64% are due to *Trypanosoma vivax*, 30% due to *T. congolense* and 6% due to *T. brucei* (Anon., 1996). Outbreaks of haemorrhagic *T. vivax* infection have been reported too and prevalences of *T. vivax* infection of 26.5-34.8% were recorded (Magona *et al.,* 1997). In an outbreak of haemorrhagic *T. vivax* in Tororo, a prevalence of 12.2% among cattle herds and an associated

mortality rate of 35% were reported (Magona *et al.,* 2008).

Control of tsetse and both human and animal *trypanosomosis* in Uganda was attempted in the past by way of donor-funded large-scale deployment of insecticide-impregnated pyramidal traps (Lancien *et al.,* 1990) integrated with limited application of pour-on and chemotherapy against animal *trypanosomosis* (Magona *et al.,* 1998). This led to successful reduction of the tsetse apparent density by 95-99.5% and the detected prevalence of animal *trypanosomosis* by 79-94% (Magona *et al.,* 1998), and drastic suppression of human *trypanosomosis* in some areas over a 7-year period. However, due to lack of a sustained supply of control materials and drugs because of lack of investment for sustaining disease management programs there was an up-surge of both animal and human *trypanosomosis* in the aftermath of the project.

In a similar initiative that started in 2001 under the project: Farming in tsetse controlled areas (FITCA), attempts were made to control tsetse through tsetse trapping and pour-on application, and *trypanosomosis* by means of block treatment of cattle in high-risk subcounties (over 5% prevalence) with isometamidium chloride and diminazene aceturate in 12 districts in Uganda. Furthermore, 5 communal spraying crush groups were introduced in the project districts.

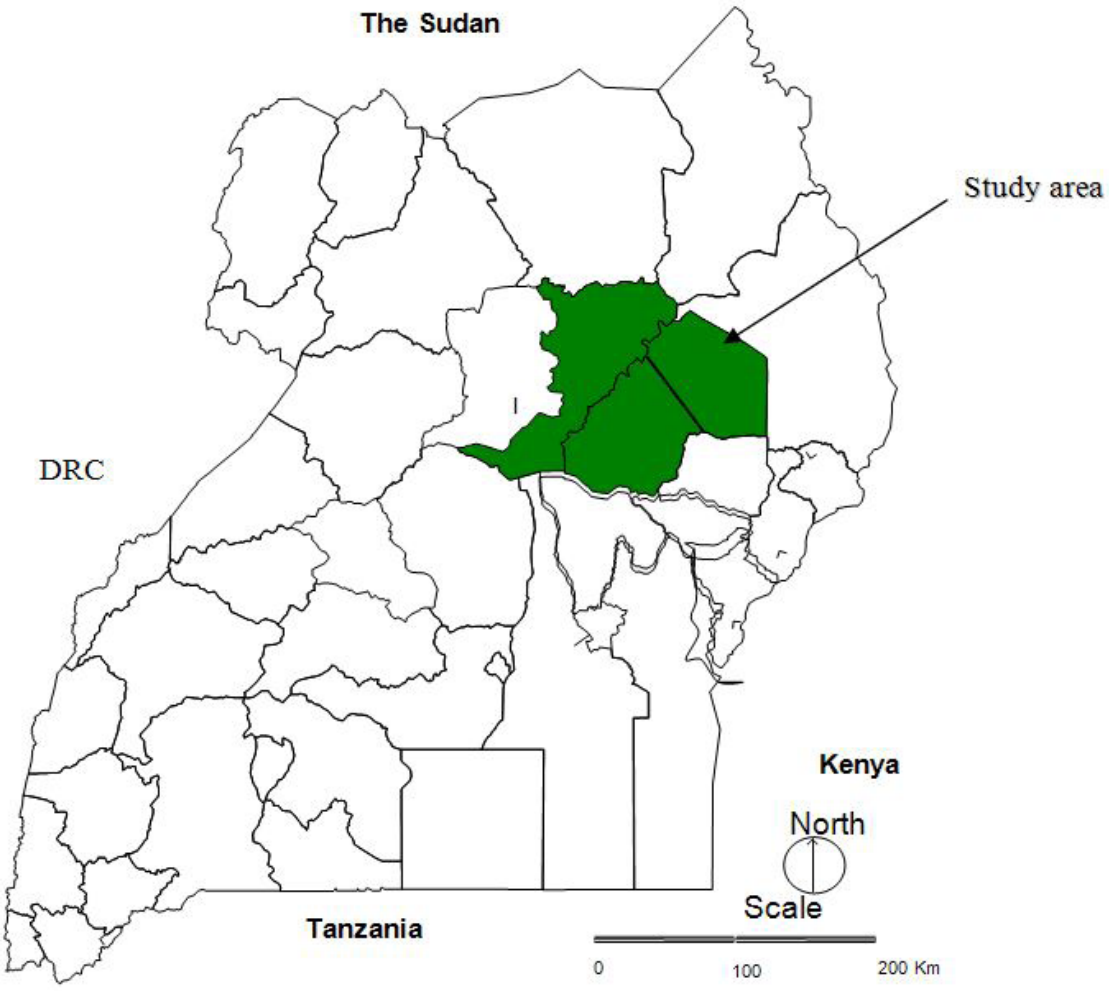

Figure 1: Map of Uganda showing the location of Amuria, Dokolo and Kaberamaido districts

Unfortunately, the problem of *trypanosomosis* was suppressed to a limited degree (Magona, personal communication) due to insufficient coverage and unsustained control efforts.

Despite existence of effective control measures for tsetse and *trypanosomosis* such as chemotherapy, tsetse trapping and restricted insecticide-treated of livestock, implementation of such measures en masse in Uganda is difficult because government has decentralized veterinary services to districts. This was particularly so because local governments in districts do not have sufficient resources to finance and implement emergency disease control. In this paper, we report a strategy of mapping tsetse and *trypanosomosis* distribution in order to highlight hotspots for targeting control measures with the merge district resources.

Materials and Methods

Development of geographical grids

The study was conducted in Amuria, Dokolo and Kaberamaido districts (Figure 1) where outbreaks of both human and animal *trypanosomosis* had been reported in Uganda. Using the program Arcview GIS 3.2 (Eseri, USA), each district map was divided into grid squares of 27 by 27 km. With the help of a Geographical Positioning System (GPS), field teams visited individual districts and conducted ground truthing in order to identify grid sites. Fifty (50) grid sites in total were identified in selected districts: Amuria (22), Dokolo (13) and Kaberamaido (15).

Following identification of the location of the grid sites, existing administrative village units (2-3 km2) representing the grid sites were identified and recorded. In each village, a tsetse survey was carried out to establish the apparent tsetse density. A tsetse team from the National Livestock Resources Research Institute (NaLIRRI) in Uganda in collaboration with entomology personnel in respective districts conducted the surveys. Tsetse trapping was carried out using 10 biconical traps deployed in each village for 72 hours. Traps were checked daily and tsetse flies were harvested, counted, sexed and the apparent tsetse density (F/T/D) was determined per village and recorded.

Further to the tsetse surveys, animal *trypanosomosis* surveys were concurrently carried out in identified village sites. A veterinary team from the National Livestock Resources Research Institute in collaboration with veterinary personnel from respective districts conducted the surveys. Up to 100 cattle were randomly selected using the systematic sampling technique from 150-200 cattle presented by several farmers in each village site. They were then bled and blood samples examined for trypanosomes using both the Haematocrit centrifugation technique (Woo, 1969) and Buffy coat technique (Murray *et al.,* 1977).

Production of distribution maps

Datasets constituting prevalence of *trypanosomosis* and apparent tsetse density for the different village sites in a given district were incorporated into a map using the program ArcView GIS 3.2. Prevalence and tsetse density datasets were overlaid to produce complete maps depicting the distribution and magnitude of animal *trypanosomosis* and tsetse infestation in each pilot district.

Dissemination of distribution maps to respective districts

Dissemination workshops were held with policymakers and extension personnel in respective pilot districts of Amuria, Dokolo and Kaberamaido. Routine use of the maps for planning, monitoring and managing tsetse and *trypanosomosis* control was emphasized. Hotspots with high density of tsetse infestation and high prevalence of animal *trypanosomosis* were highlighted on the maps for district extension personnel in the three pilot districts to prioritize control efforts. Control strategies recommended included chemotherapy, tsetse-trapping and restricted insecticide-treatment of livestock whether alone or in combination.

Results

Of the 2430 cattle tested for *trypanosomosis* in Amuria district, 230 (9.5%) were detected positive. Village-level prevalence of *trypanosomosis* ranged from 1% to 24%, while apparent tsetse density ranged from 0.0 to 2.4 F/T/D (Table 1).

For Dokolo district, out of 1304 cattle tested for *trypanosomosis* 144 (11.0%) were detected positive. Village-level prevalence of *trypanosomosis* ranged from 1% to 28%, and the apparent tsetse density ranged from 0.6 to 13.2 F/T/D (Table 2).

Of the 1392 cattle tested for *trypanosomosis* in Kaberamaido district, 106 (7.6%) were detected positive. Village-level prevalence of *trypanosomosis* ranged from 2% to 18%, and the apparent tsetse density ranged from 0.6 to 9.0 F/T/D (Table 3). G. fuscipes fuscipes was the sole tsetse species caught in all the districts, suggesting that it was the main vector for *trypanosomosis*.

In Figure 1, the prevalences of *trypanosomosis* in the respective villages in Amuria district were matched with their corresponding apparent tsetse densities. Most villages had low tsetse densities but correspondingly high prevalences of *trypanosomosis*. However, in few villages *trypanosomosis* was detected in cattle but the trapping team failed to catch tsetse flies. By and large, there was a substantial correlation (58.5%) between the prevalences of *trypanosomosis* in respective villages in Amuria district to the corresponding apparent tsetse densities.

Likewise, the prevalences of *trypanosomosis* in the respective villages in Dokolo district were matched with their corresponding apparent tsetse densities (Figure 2). Most villages had high tsetse densities with corresponding low to medium prevalences of *trypanosomosis*. A few villages had high prevalences of *trypanosomosis*. *Trypanosomosis* was detected in cattle in all villages and the trapping team caught tsetse flies in all villages in Dokolo district. Generally, there was a low correlation (29.6%) between the prevalence of *trypanosomosis* in respective villages in Dokolo district to the corresponding apparent tsetse densities.

Furthermore, the prevalences of *trypanosomosis* in the respective villages in Kaberamaido district were matched with their corresponding apparent tsetse densities (Figure 3). Most villages had low tsetse densities with corresponding low to medium prevalences of *trypanosomosis*. *Trypanosomosis* was detected in cattle in all villages and the trapping team caught tsetse flies in all villages in Kaberamaido district. Generally, there was a low correlation (30.0%) between the prevalence of *trypanosomosis* in respective villages in Dokolo district to the corresponding apparent tsetse densities.

Distribution maps depicting varying level of prevalence of *trypanosomosis* and apparent tsetse density in Amuria, Dokolo and Kaberamaido districts are shown in Figures 4 to 6, respectively. Red, yellow and green colours on the map depict areas with high prevalence of *trypanosomosis* (prevalence above 10%), then medium prevalence of *trypanosomosis* (prevalence 6-10%) and low prevalence of *trypanosomosis* (below 5%), respectively. Unmarked areas were generally free of *trypanosomosis*. Tsetse fly symbols of different sizes on the maps corresponded to levels of tsetse infestation.

Distribution maps were ultimately disseminated to 6 policymakers and 42 veterinary and entomology extension workers responsible for implementation of tsetse and trypanosomsis control in the districts of Amuria, Dokolo and Kaberamaido. The agreed principle was that resources for tsetse and *trypanosomosis* control were to directed areas with in high prevalence of *trypanosomosis* and high tsetse density as a matter of top priority. The likely course of action in such areas would preferably be mass chemotherapy coupled with regular insecticide-treatment of livestock and frequent surveillance. Areas with Medium prevalence of *trypanosomosis* and medium tsetse density took second priority. For such areas regular tsetse trapping for vector control coupled with frequent testing of livestock in order to identify and treat only infected animals would suffice. Areas with low prevalence of

Table 1: Prevalence of *trypanosomosis* in cattle and tsetse apparent density in sampling sites in Amuria district in Uganda

District	Subcounty	Village	No. of cattle	Tb	Tc	Tv	Mixed	Prevalence (%)	Total Tsetse	F	M	F/T/D
Amuria	Kujju	Abule	150	0	2	9	12	15	4	2	2	2.4
	Kujju	Abia	130	0	2	4	3	7	0	0	0	0.0
	Obalanga	Aridai	132	0	0	1	1	1	3	0	3	1.8
	Obalanga	Iyalakwe	100	0	1	2	2	5	1	1	0	0.6
	Asamuk	Obur	150	0	3	1	1	3	0	0	0	0.0
	Asamuk	Apeduru	131	0	1	6	3	8	4	2	2	2.4
	Asamuk	Asamuk	110	0	4	4	3	10	4	3	1	2.4
	Wera	Iningo-otomei	102	0	6	13	4	22	3	2	1	1.8
	Abarilela	Katine	110	0	3	4	4	10	4	3	1	2.4
	Abarilela	Ododoi	100	0	4	2	6	12	3	1	2	1.8
	Abarilela	Ongutoi	105	0	6	13	4	22	3	3	0	1.8
	Morugatuny	Odekere	100	0	2	9	4	15	4	3	1	2.4
	Morugatuny	Aita	100	0	0	2	2	4	1	0	1	0.6
	Orungo	Owangai	100	0	1	2	4	7	0	0	0	0.0
	Orungo	Oelai	100	0	2	1	4	7	0	0	0	0.0
	Acowa	Atarukot	110	0	6	14	4	22	4	2	2	2.4
	Acowa	Ocito	100	0	2	6	7	15	4	3	1	2.4
	Acowa	Adepari	100	0	1	6	1	8	4	1	3	2.4
	Kapelebyong	Acegerekuma	100	0	1	2	1	4	1	1	0	0.6
	Kapelebyong	Acumet	100	0	1	4	2	7	0	0	0	0.0
	Kapelebyong	Oditel	100	0	1	3	0	4	1	0	1	0.6
	Kapelebyong	Olobai	100	0	0	1	0	1	1	1	0	0.6

Table 2: Prevalence of *trypanosomosis* in cattle and apparent tsetse density in sampling sites in Dokolo district in Uganda

District	Subcounty	Village	No. of cattle	Tb	Tc	Tv	Mixed	Prevalence (%)	Total Tsetse	F	M	F/T/D
Dokolo	Agwata	Ocama oyam	100	0	1	8	0	9	1	0	1	0.6
	Agwata	Acungapenyi	100	0	2	5	2	10	2	2	0	1.2
	Agwata	Odeye	100	0	2	2	1	5	2	0	2	1.2
	Agwata	Ayito	80	0	0	1	0	1	3	1	0	1.8
	Dokolo	Araki	100	0	5	13	1	19	8	8	0	4.8
	Batta	Alapata-ocero	100	0	3	0	2	5	19	15	4	11.4
	Batta	Omanabunga	111	0	4	5	0	8	22	11	11	13.2
	Dokolo	Anyac	111	0	9	14	8	28	18	10	8	10.8
	Dokolo	Agwenonywal	110	0	3	20	2	23	20	19	1	12.0
	Kangai	Adita	92	0	2	2	2	6	18	8	10	10.8
	Kangai	Abalang	100	0	2	4	0	6	18	10	8	10.8
	Kwara	Ageni	100	0	2	5	3	10	18	12	7	10.8
	Agwata	Aminamini	100	0	1	7	1	9	2	1	1	1.2

Table 3: Prevalence of *trypanosomosis* in cattle and apparent tsetse density in sampling sites in Kaberamaido district in Uganda

District	Subcounty	Village	No. of cattle	Tb	Tc	Tv	Mixed	Prevalence (%)	Total Tsetse	F	M	F/T/D
Kaberamaido	Otuboi	Omotot	100	0	2	1	2	5	1	1	0	0.6
	Otuboi	Arapapai	100	0	2	0	0	2	3	2	1	1.8
	Ochero	Awelu	100	0	6	2	3	11	2	2	0	1.2
	Kobulubulu	Kakado	130	0	7	11	5	18	3	2	1	1.8
	Kobulubulu	Asenyi	130	0	2	8	7	13	3	2	1	1.8
	Bululu	Agule	62	0	1	3	1	8	2	1	1	1.2
	Kaberamaido	Gwetom	70	0	0	2	1	4	1	0	1	0.6
	Kaberamaido	Omakotomiti	100	0	1	0	1	2	5	4	1	3.3
	Anyara	Amoru	100	0	0	1	1	2	2	1	1	1.2
	Kalaki	Okongo	100	0	2	0	1	3	15	11	4	9.0
	Kalaki	Kasere	100	0	2	2	2	6	3	2	1	1.8
	Alwa	Oyama	100	0	0	2	1	3	5	3	2	3.3
	Kaberamaido	Abirabira	100	0	7	7	4	14	2	1	1	1.2
	Ochero	Odubai	100	0	6	2	2	10	2	1	1	1.2

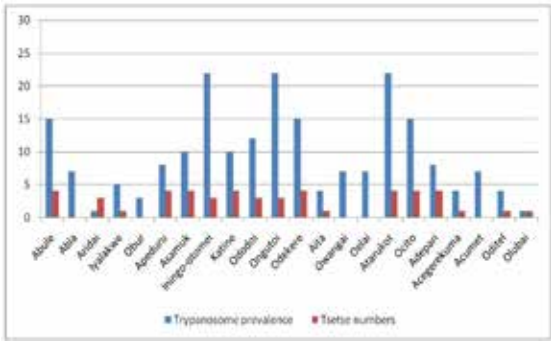

Figure 1: Matched distribution of total tsetse caught vis-a-viz the prevalence of *trypanosomosis* in cattle in villages visited in Amuria district, Uganda

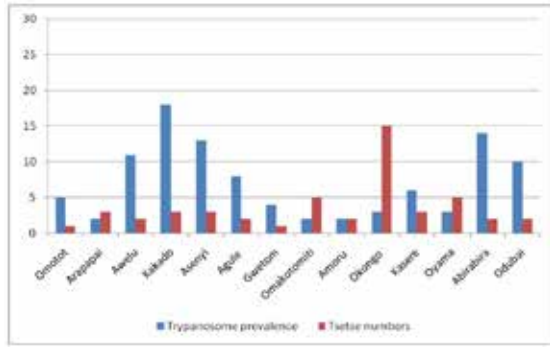

Figure 3: Matched distribution of total tsetse caught vis-a-viz the prevalence of *trypanosomosis* in cattle in villages visited in Kaberamaido district, Uganda

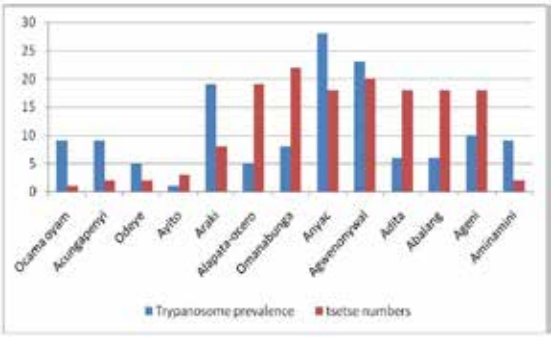

Figure 2: Matched distribution of total tsetse caught vis-a-viz the prevalence of *trypanosomosis* in cattle in villages visited in Dokolo district, Uganda

trypanosomosis and low tsetse density took the third priority. For such areas only periodic testing of livestock and tsetse trapping to monitor the magnitude of *trypanosomosis* and tsetse infestation was adequate.

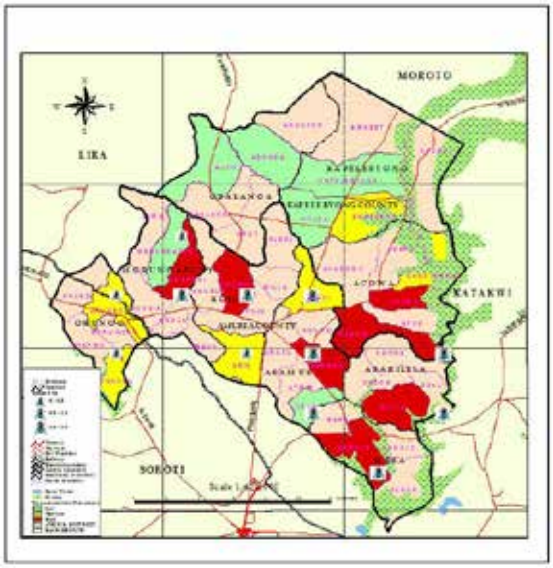

Figure 4: Map showing distribution of tsetse and *trypanosomosis* in Amuria district in Uganda

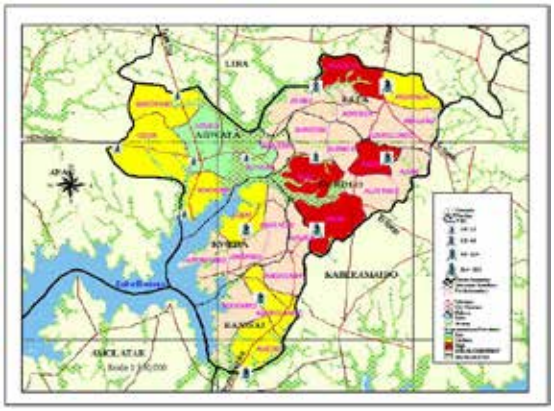

Figure 5: Map showing distribution of tsetse and *trypanosomosis* in Dokolo district in Uganda

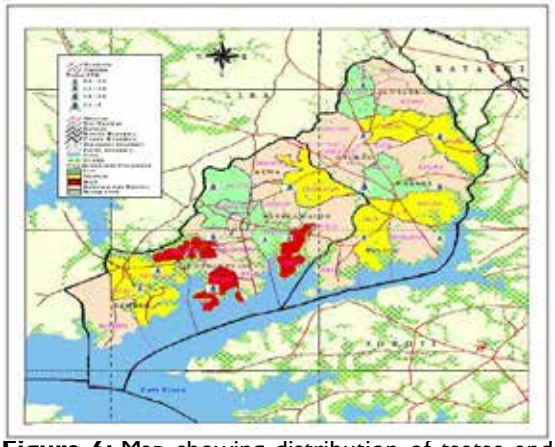

Figure 6: Map showing distribution of tsetse and *trypanosomosis* in Kaberamaido district in Uganda

Discussion

We report on the production of maps for the distribution of tsetse and animal *trypanosomosis* for three pilot districts of Amuria, Dokolo and Kaberamaido in Uganda to facilitate targeted farm-level implementation of control by veterinary extension personnel. Disease maps have been recommended for guiding planning, managing and monitoring interventions across the whole spectrum of neglected tropical diseases, including Human African Trypanosomiasis (HAT) (Simarro *et al.,* 2010). For effective implementation of control measures, a HAT Atlas initiative was launched by WHO and FAO to enable mapping of HAT in time and space (Simarro *et al.,* 2010). Other initiatives regarding mapping tsetse and *trypanosomosis* implemented, include, spatial analysis of trypanosome patterns in Suba and Teso districts in Kenya (Thumbi *et al.,* 2010), mapping of the spatial and temporal distribution of sleeping sickness in western Kenya (Rutto and Karuga, 2009) and the FAO initiative on mapping land cover and tsetse fly distribution in sub-Saharan Africa (Cecchi *et al.,* 2008).

Eastern Uganda where the pilot districts are located still remains the major focus of T. b. rhodesiense sleeping sickness with G. fuscipes fuscipes as the major vector (Welburn *et al.,* 2001). The area affected by T. b. rhodesiense sleeping sickness was reported to have increased 2.5-fold since 1985 (Picozzi *et al.,* 2005), extending further north into Soroti, Kaberamaido and Lira districts (Fevre *et al.,* 2005). Outbreaks of animal *trypanosomosis* too continue to occur in Uganda (Magona *et al.,* 2008) with resultant impairment of agricultural development and livestock productivity. As the situation of tsetse and *trypanosomosis* worsens day by day, so do funds for tsetse and *trypanosomosis* control increasingly decline. Like in many African countries, the structural adjustment program (SAP) was introduced in Uganda in the 1990s. This was followed by implementation of the policy on decentralization of veterinary services and subsequent retrenchment of tsetse and *trypanosomosis* control employees. The remaining skeletal

personnel were not delegated to districts that had sufficient resources nor do considered tsetse and *trypanosomosis* control a priority. This eventually led to the increase in tsetse population and AAT outbreaks countrywide. With this state of affairs, making of diagnosis and treatment decisions for *trypanosomosis* in such rural settings in Africa has increasingly become the domain of farmers, extension workers and agro-veterinary traders (Machila et al, 2003). Given that such groups, including district extension workers operate under limited funding, decision support tools are an absolute necessity to guide planning and prioritization of tsetse and *trypanosomosis* control. For this reason, piloting the production of distribution maps for districts to facilitate targeted tsetse and *trypanosomosis* control was initiated in Uganda.

Distribution maps have been recommended by other workers for provision of a direction for surveillance and control efforts or evaluation of actual or potential effectiveness of interventions (Kitron, 2000). GIS has also been used to combine data such as livestock distribution, agriculture and arable potential in order to identify areas where tsetse flies constrain agricultural developments (Robinson *et al.,* 2002). Spatial distribution of prevalence would illustrate specific locations at risk from an epidemiological viewpoint (Michel *et al.,* 2002).

The prevalence of *trypanosomosis* and apparent tsetse density were the key parameters to depict the level of risk in specific villages afflicted by tsetse and *trypanosomosis*. The prevalence of *trypanosomosis* and apparent tsetse densities at village ranged from low to high in all the pilot districts.

Most villages in Amuria had low tsetse densities but correspondingly high prevalences of *trypanosomosis* with a substantial correlation between the prevalence of *trypanosomosis* and apparent tsetse density at village level. This suggested that the risk of cattle in Amuria encountering infected tsetse was substantial despite villages having low densities of tsetse, at times being too low to enable catches during trapping. In contrast, in Dokolo district most villages had high tsetse densities with corresponding low to medium prevalences of *trypanosomosis* with low correlation between the prevalence of *trypanosomosis* and apparent tsetse densities. This suggests that Dokolo district had a conducive riverine environment (given the proximity shores of Lake Kyoga) that allowed the thriving of the riverine tsetse species Glossina fuscipes fuscipes, hence the large number of tsetse caught. However, the low to medium prevalence of *trypanosomosis* suggested that cattle encountered a high number of tsetse but which were largely not infected with trypanosomes. In-turn, a large number of cattle on which tsetse took blood meal were not carrying trypanosome parasites hence tsetse not being able to get infected. For Kaberamaido, most villages had low tsetse densities with corresponding low to medium prevalences of *trypanosomosis* with low correlation between the prevalence of *trypanosomosis* and apparent tsetse densities suggesting the area had a low proportion of infected tsetse. It is reported that the risk of transmission is primarily linked to the intensity of the encounters between infected vectors and hosts (Michel *et al.,* 2002).

As opposed to other previous studies that relied on remote sensing to model tsetse distribution and *trypanosomosis* risk (Robinson *et al.,* 2002; Bouyer *et al.,* 2006), this study illustrates how tsetse and *trypanosomosis* surveys were extensively conducted in villages around grid sites in order to map tsetse and *trypanosomosis* distribution. Unfortunately, production of tsetse distribution maps through such ground-based vector surveys is increasingly is considered to be expensive (Thumbi *et al.,* 2010).

Equipping policymakers and extension workers with distribution maps of tsetse and *trypanosomosis* was anticipated to have an immense impact in improving tsetse and *trypanosomosis* control at farm-level. Robinson et al (2002) contends that there is an increasing need to identify areas where intervention is most likely to be technically, economically, socially and environmentally sustainable, given the declining Government resources and donor

aid for the control of tsetse and *trypanosomosis* in most African countries.

The distribution maps developed highlighted high, medium and low risk areas. However, regular application of chemotherapy and insecticide-treatment of livestock was prescribed only for high and medium risk areas. According to Robinson et al (2002), areas where AAT constrains agricultural development directly or prevents expansion from areas of highland pressure into adjacent areas need to be prioritized for tsetse control. This applied to a few sampled sites in Amuria, Dokolo and Kaberamaido districts as highlighted by the findings. The areas were mainly infested by G. fuscipes fuscipes species of tsetse.

Conclusion

In conclusion, mapping hotspots was anticipated to facilitate effective planning and prioritization of resources for targeting tsetse and *trypanosomosis* control by district departments of Veterinary Services.

Acknowledgement

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VALIDATION OF SITE-SPECIFIC SPRAYING OF CATTLE FOR *TRYPANOSOMOSIS* CONTROL IN AREAS INFESTED WITH GLOSSINA FUSCIPES FUSCIPES IN UGANDA

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Abstract

In a bid to enhance bovine *trypanosomosis* control in tsetse-infested rural areas in Uganda by livestock keepers, restricted application of deltamethrin to legs and ventral abdomen of cattle was assessed for its effectiveness in reducing the prevalence of *trypanosomosis* and tsetse apparent density in areas infested with Glossina fuscipes fuscipes over a 10-months period. The study was conducted in 9 villages in Amuria, Dokolo and Kaberamaido districts from December 2008 to September 2009. A total of 600 cattle were sprayed and tested for *trypanosomosis* every four weeks in six experimental (sprayed) villages, two per district. In addition, 300 cattle were tested for *trypanosomosis* every four weeks in three control (non-sprayed) villages, one per district. Simultaneously, tsetse-trapping was conducted to assess reduction in tsetse apparent density in both experimental and control villages. A decline in the prevalence of *trypanosomosis* from 10% to 0%, 12% to 1% and 11% to 0% was achieved in experimental villages in Amuria, Dokolo and Kaberamaido, respectively, between December 2008 and July 2009. Correspondingly in the same villages, a decline in the tsetse apparent density from 3.0 to 0.0, 2.7 to 0.0 and 1.5 to 0.0 was achieved in Amuria, Dokolo and Kaberamaido, respectively, between December 2008 and July 2009. In contrast in control villages, the prevalence of *trypanosomosis* in cattle varied from 7% to 12%, 3% to 18%, and 3% to 10% in Amuria, Dokolo and Kaberamaido, respectively, during the same period. In same villages, the apparent tsetse density varied from 0.8 to 1.2, 0.8 to 3.0, and 0.2 and 2.4 in Amuria, Dokolo and Kaberamaido, respectively, over the same period. This study revealed a 100% decline in prevalence of *trypanosomosis* over a period of 8 months; and a 100% decline of tsetse apparent density over a period of 7 months. Cattle in experimental (sprayed) villages had a significantly higher ($P < 0.05$) mean PCV than those in control (non-sprayed) villages. The low-cost property of this method associated with reduced amount of insecticide used; its simplicity of application and quick effect in improving the health of livestock; and its ability to simultaneously control tsetse and ticks, tremendously attracted farmers' participation.

Keywords: Site-specific; spraying; cattle; *trypanosomosis* control; Glossinna f. fuscipes; Uganda

VALIDATION DE LA PULVÉRISATION DU BÉTAIL SPÉCIFIQUE AU SITE POUR LE CONTRÔLE DE LA TRYPANOSOMOSE DANS LES ZONES INFESTÉES PAR GLOSSINA FUSCIPES FUSCIPES EN OUGANDA

Résumé

Dans la perspective d'améliorer le contrôle, par les éleveurs, de la trypanosomose bovine dans les zones rurales infestées de mouches tsé-tsé en Ouganda, l'application limitée de la deltaméthrine aux pattes et à l'abdomen ventral des bovins a été évaluée pour déterminer son efficacité dans la réduction de la prévalence de la trypanosomose et de la densité apparente des glossines dans les zones infestées par Glossina fuscipes fuscipes, sur une période de 10 mois. L'étude a été menée dans 9 villages des districts d'Amuria, Dokolo et Kaberamaido, de décembre 2008 à septembre 2009. Au total, 600 bovins ont été aspergés et testés pour rechercher la trypanosomose toutes les quatre semaines dans six villages expérimentaux (soumis à la pulvérisation), deux par district. En outre, 300 bovins ont été examinés pour

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rechercher la trypanosomose toutes les quatre semaines dans trois villages témoins (non soumis à la pulvérisation), à raison d'un par district. Simultanément, le piégeage des mouches tsé-tsé a été réalisé pour évaluer la réduction de la densité apparente dans les villages expérimentaux et témoins. Une diminution de la prévalence de la trypanosomose de 10% à 0%, de 12% à 1% et de 11% à 0% a été enregistrée respectivement dans les villages expérimentaux d'Amuria, Dokolo et Kaberamaido, entre décembre 2008 et juillet 2009. Parallèlement dans les mêmes villages, une diminution de la densité apparente des glossines de 3,0 à 0,0, de 2,7 à 0,0 et de 1,5 à 0,0 a été notée respectivement dans Amuria, Dokolo et Kaberamaido, entre décembre 2008 et juillet 2009. Par contre, dans les villages témoins, la prévalence de la trypanosomose chez les bovins variait de 7% à 12%, de 3% à 18% et de 3% à 10% respectivement à Amuria, Dokolo et Kaberamaido pendant la même période. Dans les mêmes villages, la densité apparente des mouches tsé-tsé variait de 0,8 à 1,2, 0,8 à 3,0 et 0,2 et 2,4 respectivement à Amuria, Dokolo et Kaberamaido, au cours de la même période. Cette étude a révélé une baisse de 100% de la prévenance de la trypanosomose sur une période de 8 mois, et une baisse de 100% de la densité apparente des glossines sur une période de 7 mois. Les bovins des villages expérimentaux (soumis à la pulvérisation) avait un hématocrite moyen significativement plus élevé (P <0,05) que celui des villages témoins (non soumis à la pulvérisation). Le faible coût de cette méthode, associé à une quantité réduite d'insecticides utilisés, la simplicité de son application, son effet rapide dans l'amélioration de la santé animale, et sa capacité à contrôler simultanément les glossines et les tiques, a attiré énormément la participation des éleveurs.

Mots-clés : spécifique au site ; pulvérisation ; bovins ; contrôle de la trypanosomose ; Glossinna f. fuscipes; Ouganda

Introduction

The use of insecticide-treated cattle (ITC) is widely regarded as an attractive and usually low-cost method of controlling tsetse. This technique offers the possibility of dealing with tick and tsetse problems simultaneously and can thus be integrated into farmers' existing tick control regimes. It involves livestock keepers and is regarded by them as conferring a 'private' benefit to their own treated cattle, rather than conferring a public benefit to livestock in the area (Eisler *et al.,* 2003; Shaw et al. 2007).

Treating cattle with insecticide is an increasingly important means of controlling tsetse flies as livestock keepers, rather than government or donor agencies, are now largely responsible for funding and implementing interventions against trypanosomiasis (Eisler *et al.,* 2003). Consequently, cheap methods of control that can be applied by farmers themselves are more likely to be sustainable than expensive and complex alternatives such as aerial spraying or the sterile insect technique. Moreover, farmers will tend to select interventions that control several diseases rather than just one; treating cattle with insecticide to control tsetse may also control tick-borne diseases, whereas deploying insecticide-treated targets will not (Vale & Torr, 2004).

Studies on means of reducing costs suggest tsetse the vectors of trypanosomiasis, feed largely on the belly and lower legs of older and larger cattle (Torr & Hargrove, 1998; Vale *et al.,* 1999; Torr *et al.,* 2001), hence treating only the feeding sites of tsetse on older and larger cattle, dubbed site-specific spraying, could reduce the cost of control of these vectors by 90% as compared to the current cost of whole body spraying. It is also reported that this selective approach also reduces the risks to dung fauna (Vale & Grant, 2002) and the enzootic stability of tick-borne diseases (Eisler *et al.,* 2003).

This 90% reduction in the insecticide treatment makes it much more economical and convenient for poor farmers, hence enhancing adoption of the spraying method. This paper reports the results of a validation study conducted in Amuria, Dokolo and Kaberamaido districts of Uganda to assess the effectiveness of site-specific spraying of cattle over a 10-months period in reducing the prevalence of *trypanosomosis* and tsetse apparent density in areas infested with Glossina fuscipes fuscipes.

Materials and Methods

Experimental design

A multidisciplinary team visited 9 villages composed of 6 experimental and 3 control villages in three districts of Amuria (3), Dokolo (3) and Kaberamaido (3). In each village, the team together with the district entomology and veterinary personnel conducted tsetse trapping, testing of cattle blood for trypanosome infection, and assessment of the health status of cattle by measure of their packed cell volume (PCV). Site-specific spraying of cattle was carried out only in experimental villages.

Trapping was carried out over 72 hours after which, tsetse flies caught in each study village were counted and sexed. The apparent tsetse density measured as number of flies per trap per day was then determined for each study village and recorded.

At each site cattle were bled from the jugular vein using heparinized vacutainers. Blood samples were then examined for trypanosome infection using both the Haematocrit centrifugation technique (Woo, 1969) and Buffy coat technique (Murray *et al.,* 1977) to establish the prevalence of *trypanosomosis*. In addition, PCV was measured for onward assessment of the animal health status. The breed, sex and age of each animal examined were recorded. All animals positive for *trypanosomosis* were treated as an incentive to attract high farmer turn-up. Ultimately, the prevalence of bovine *trypanosomosis* was determined for each study village and recorded.

Site-specific spraying of cattle

All animals brought for screening in experimental villages were sprayed on the legs, ventral abdomen and dewlap with Deltamethrin every four weeks.

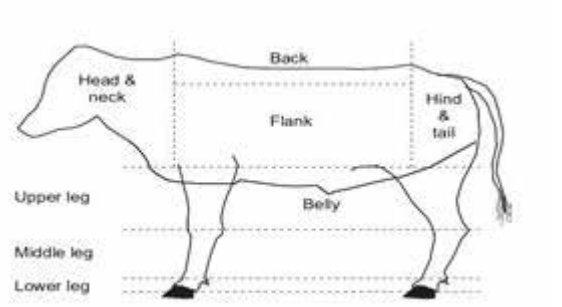

Figure 1: Body parts of a cow to be sprayed. Legs (Entire leg-Upper, Middle & lower leg) (Torr *et al.,* 2007)

Figure 2: Demonstration on site-specific spraying of cattle in the field in Dokolo district, Uganda

Results

The effect of site-specific spraying of cattle on the prevalence and apparent tsetse density over the period December 2008 to September 2009 in project districts of Amuria, Dokolo and Kaberamaido is shown in Table 4. The prevalence of *trypanosomosis* in cattle in villages with spraying in Amuria district dropped from 10% in December 2008 to 0% by July 2009. While the prevalence of *trypanosomosis* in cattle in control villages varied from 7% to 12% during the same period. The apparent tsetse density in the same villages dropped from 3.0 to 0.0 over the same period but in the control villages it varied between 1.2 and 0.8.

In Dokolo district, the prevalence of *trypanosomosis* in cattle in villages with spraying dropped from 12% in December 2008 to 1%

by July 2009, while the apparent tsetse density dropped from 2.7 in December 2008 to 0.0 by June 2009. In the control villages, the prevalence of *trypanosomosis* in cattle varied from 3 % to 18%, while the apparent tsetse density varied from 0.8 to 3.0 over the same period.

In Kaberamaido district, the prevalence

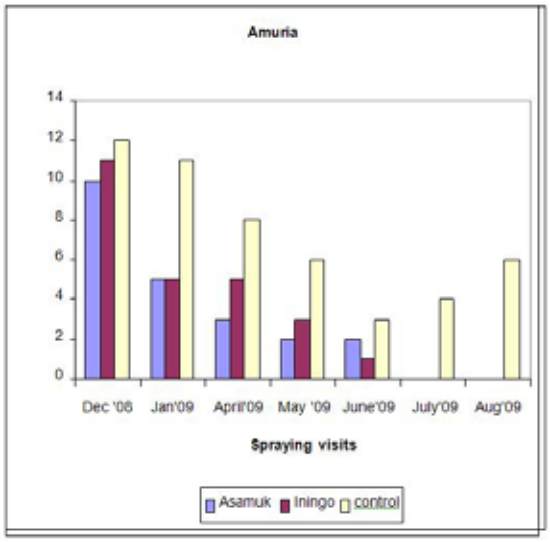

Figure 3: Effect of site-specific spraying of cattle on the prevalence of *trypanosomosis* in experimental villages as compared to the control village in Amuria district

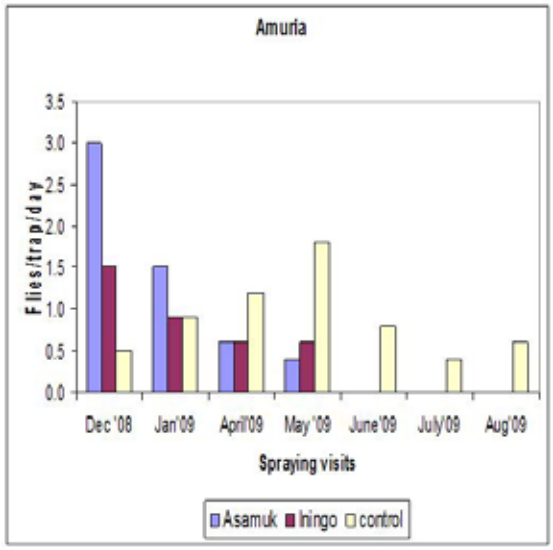

Figure 4: Effect of site-specific spraying of cattle on apparent tsetse density in experimental villages as compared to the control village in Amuria district

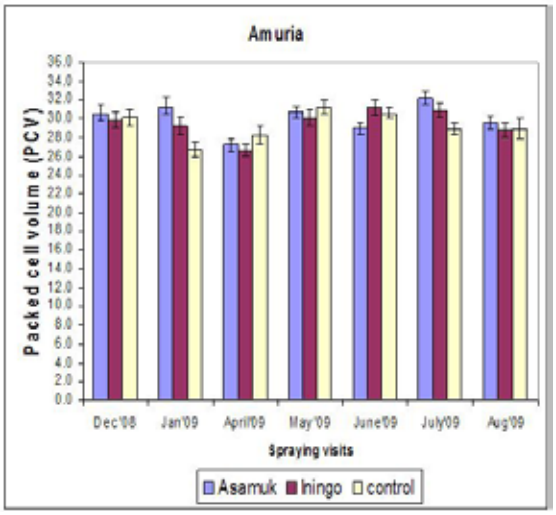

Figure 5: Effect of site-specific spraying of cattle on their mean PCV in experimental villages as compared to the control village in Amuria district

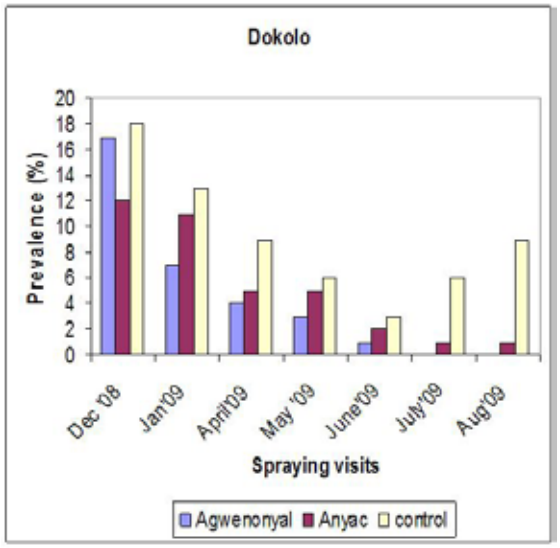

Figure 6: Effect of site-specific spraying of cattle on the prevalence of *trypanosomosis* in experimental villages as compared to the control village in Dokolo district

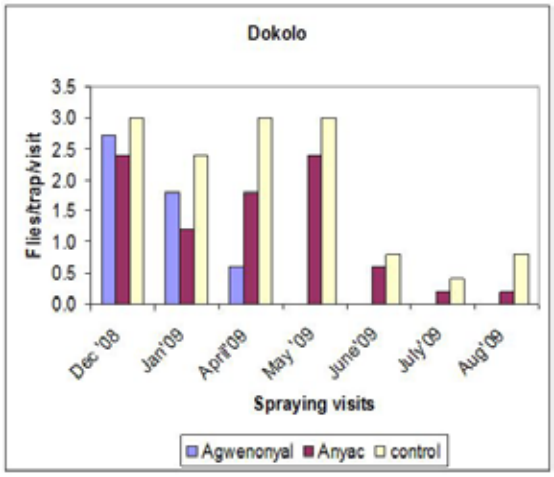

Figure 7: Effect of site-specific spraying of cattle on apparent tsetse density in experimental villages as compared to the control village in Dokolo district

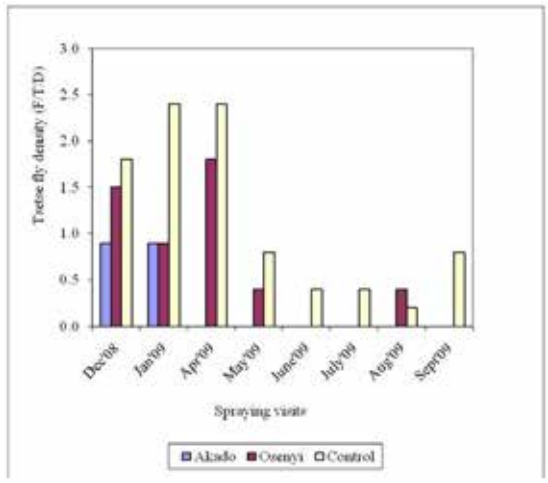

Figure 10: Effect of site-specific spraying of cattle on apparent tsetse density in experimental villages as compared to the control village in Kaberamaido district

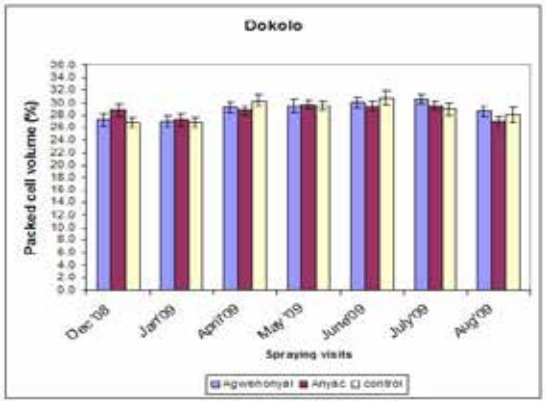

Figure 8: Effect of site-specific spraying of cattle on their mean PCV in experimental villages as compared to the control villages in Dokolo district

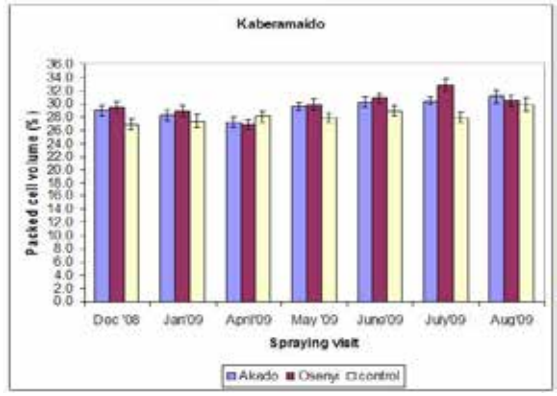

Figure 11: Effect of site-specific spraying of cattle on their mean PCV in experimental villages as compared to the control village in Kaberamaido district

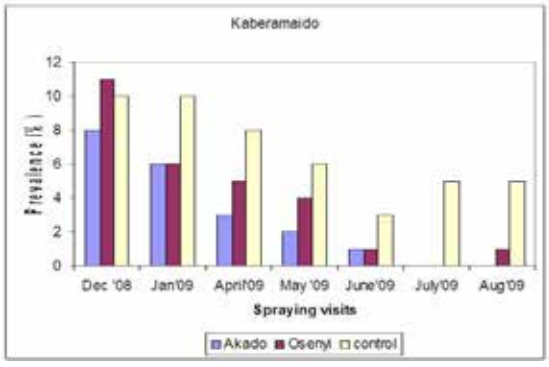

Figure 9: Effect of site-specific spraying of cattle on the prevalence of *trypanosomosis* in experimental villages as compared to the control village in Kaberamaido district

Table 1: Effect of site-specific spraying of cattle on the prevalence of *trypanosomosis* and apparent tsetse density in Amuria, Dokolo and Kaberamaido districts

Visit	Parameter		Amuria			Dokolo		Kaberamaido		
	Village	Asamuk	Iningo-otomei	Atubakinei	Anyac	Agwenonywal	Iguli	Akado	Osenyi	Awelu
	Experimental status	Spraying	Spraying	No spraying	Spraying	Spraying	No spraying	Spraying	Spraying	No spraying
December 08	Cattle sprayed	100	100		100	100		100	100	
	Cattle sampled	100	100	100	100	100	100	100	100	100
	Prevalence (%)	10	11	12	12	17	18	8	11	10
	Tsetse Density (F/T/D)	3	1.5	0.5	2.4	2.7	3	0.9	1.5	1.8
January 09	Cattle sprayed	150	130		136	182		162	145	
	Cattle sampled	100	100	100	100	100	100	100	100	100
	Prevalence (%)	5	5	11	11	7	13	6	6	10
	Tsetse Density (F/T/D)	1.5	0.9	0.5	1.2	1.8	2.4	0.9	0.9	2.4
April 09	Cattle sprayed	186	197		207	134		215	128	
	Cattle sampled	100	100	100	100	100	100	100	100	100
	Prevalence (%)	3	5	8	5	4	9	3	5	8
	Tsetse Density (F/T/D)	0.6	0.6	1.2	1.8	0.6	3	0	1.8	2.4
May 09	Cattle sprayed	162	122		121	139	0	168	164	0
	Cattle sampled	100	100	100	100	100	100	100	100	100
	Prevalence (%)	1	4	6	5	3	6	2	4	6
	Tsetse Density (F/T/D)	0.4	0.2	0.6	0.8	0	1	0	0.4	0.8
June 09	Cattle sprayed	119	151		129	132		139	128	0
	Cattle sampled	100	100	100	100	100	100	100	100	100
	Prevalence (%)	2	1	3	2	1	3	1	1	3
	Tsetse Density (F/T/D)	0	0	0.8	0.6	0	0.8	0	0	0.4
July 09	Cattle sprayed	104	181		109	126	0	142	131	0
	Cattle sampled	100	100	100	100	100	100	100	100	100
	Prevalence (%)	0	0	4	1	0	6	0	0	5
	Tsetse Density (F/T/D)	0	0	0.4	0.2	0	0.4	0	0	0.4
August 09	Cattle sprayed	116	169		100	118		120	130	
	Cattle sampled	100	100	100	100	100	100	100	100	100

Visit	Parameter		Amuria			Dokolo			Kaberamaido		
	Village	Asamuk	Iningo-otomei	Atubakinei	Anyac	Agwenonywal	Iguli	Akado	Osenyi	Awelu	
	Experimental status	Spraying	Spraying	No spraying	Spraying	Spraying	No spraying	Spraying	Spraying	No spraying	
	Prevalence (%)	0	0	6	1	0	9	0	1	5	
	Tsetse Density (F/T/D)	0	0	0.6	0.2	0	0.8	0	0.4	0.2	
September 09	Cattle sprayed	120	180		150	120		135	140		
	Cattle sampled	100	100	100	100	100	100	100	100	100	
	Prevalence (%)	0	0	7	0	0	7	0	0	6	
	Tsetse Density (F/T/D)	0	0	0.8	0	0	0.9	0	0	0.8	

of *trypanosomosis* in cattle in villages with spraying in Kaberamaido declined from 11% in December 2008 to 0% by July 2009, while the apparent tsetse density declined from 1.5 in December 2008 to 0.0 by June 2009. In the control villages the prevalence of trypanosomosis varied between 3% and 10%, while apparent tsetse density varied from 0.2 to 2.4 over the same period.

The effect of site-specific spraying of cattle on the prevalence of *trypanosomosis*, apparent tsetse density and mean PCV in Amuria district is shown in Figures 3, 4 and 5, respectively. A 100% decline in prevalence of *trypanosomosis* was achieved within a period of 8 months, while a 100% decline in apparent tsetse density was achieved in 7 months. In addition, it was observed that cattle in experimental villages had a significantly higher ($P < 0.05$) mean PCV than those in control villages.

The effect of site-specific spraying of cattle on the prevalence of *trypanosomosis*, apparent tsetse density and mean PCV in Dokolo district is displayed in Figures 6, 7 and 8, respectively. Unlike Amuria district, a maximum decline of 92% in the prevalence of *trypanosomosis* and 100% in apparent tsetse density was achieved within a period of 8 and 7 months, respectively.The mean PCV of cattle in experimental villages was higher than for those in the control villages but not significantly so.

The effect of site-specific spraying of cattle on the prevalence of *trypanosomosis*, apparent tsetse density and mean PCV in Kaberamaido district is shown in Figures 9, 10 and 11, respectively. Results obtained in Kaberamaido were similar to those obtained in Amuria district.A 100% decline in prevalence of *trypanosomosis* and apparent tsetse density was achieved within a period of 8 and 7 months, respectively. In addition, cattle in experimental villages had a significantly higher ($P < 0.05$) mean PCV than those in control villages.

Discussion

In the present study we report findings of the assessment of the effectiveness of site-specific spraying of cattle over a 10-months period in reducing the prevalence of *trypanosomosis* and tsetse apparent density in areas infested with Glossina fuscipes fuscipes in Uganda. The findings revealed that site-specific spraying was effective, given the fact that in villages where cattle were sprayed in Amuria district, the prevalence of *trypanosomosis* dropped from 10% to 0% and apparent tsetse density also dropped 3.0 to 0.0 over the period of 10 months, representing 100% decline. While in villages where cattle were not sprayed in the same district the prevalence of *trypanosomosis* remained static between 7% and 12% and apparent tsetse density remained between 0.8 and 1.2 over the same period. Equally in Dokolo district, the prevalence of *trypanosomosis* in cattle in villages where cattle were sprayed declined by 92% while the apparent tsetse density declined by 100%. Contrastingly, in villages where cattle were not sprayed, the prevalence of *trypanosomosis* in cattle remained between 3 % and 18% and apparent tsetse density remained between 0.8 and 3.0 over the 10-month period. Likewise, in Kaberamaido district, in villages where cattle were sprayed, both the prevalence of *trypanosomosis* in cattle and apparent tsetse density declined by 100% over a 10-month period. Contrastingly, in villages where cattle were not sprayed, the prevalence of *trypanosomosis* remained between 3% and 10%, while apparent tsetse density remained between 0.2 and 2.4 over the 10-months period. Similar studies previously conducted on Glossina morsitans morsitans and Glossina Pallidipes in Zimbabwe revealed that restricting the application of insecticide on cattle reduces the seasonal persistence periods to 10 – 15 days if only the legs and belly were treated, 5 – 15 days if only the legs were treated and < 5 days for the more restricted treatments., cutting down the cost of insecticide by 40%, improve efficacy by 27% (Torr *et al.,* 2007). It is encouraging to note that restricted treatment approach, which uses about 20% of the insecticide required for the whole body treatment, is only about 21% less effective per animal treated (Kajunguri, 2014).

Apart from the decline in the prevalence of *trypanosomosis* and apparent tsetse density in villages where cattle were sprayed, cattle in such villages had a significantly higher ($P < 0.05$) mean PCV than their counterparts in villages where cattle where not sprayed. This implies site-specific spraying of cattle equally still offers the possibility of dealing with tick and tsetse problems simultaneously, hence leading to improved health of cattle as indicated by improved PCV values. It still confers the 'private' benefit to treated cattle as previously reported in other studies (Eisler *et al.,* 2003; Shaw et al. 2007).

These findings are in line with reports by other workers (Muhanguzi et al, 2014) that revealed that restricted insecticide application on as low as 25% of a village cattle herd in stable African trypanosomiasis transmission area is sufficient in the control of *T. brucei* s.l. and 50–75% village herd coverage being sufficient to control *T. vivax* and *T. congolense*.

As regards farmer participation, site-specific spraying of cattle attracted farmers' participation right from the beginning of the trial, given the immediate improvement of cattle condition upon spraying.

In conclusion, site-specific spraying of cattle every 4 weeks led to a 100% decline in prevalence of *trypanosomosis* over a period of 8 months; and a 100% decline of tsetse apparent density over a period of 7 months. The low-cost property of this method associated with reduced amount of insecticide used; its simplicity of application and quick effect in improving the health of livestock; and its ability to simultaneously control tsetse and ticks, tremendously attracted farmers' participation. Ideally, site-specific spraying of cattle leads to effective control of *trypanosomosis*, anaplasmosis, babesiosis, cowdriosis and theileriosis (East Coast fever), simultaneously.

This study revealed Cattle in experimental (sprayed) villages had a significantly higher ($P < 0.05$) mean PCV than those in control (non-sprayed) villages.

Acknowledgement

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INOCULATION OF *MYCOPLASMA MYCOIDES* MYCOIDES BY ENDOTRACHEAL INTUBATION PRODUCES A MILDER DISEASE THAN BY CONTACT TRANSMISSION

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Abstract

Cattle infected by endotracheal intubation with *Mycoplasma mycoides* subspecies mycoides (Mmm) have been used in trials of Contagious Bovine Pleuropneumonia (CBPP) pathogenesis. Its advantage is that the precise moment of infection is known, unlike with the in-contact transmission when the exact time of inoculation in each animal is not known. In this study, the two methods of inoculation were directly compared, by analysing clinical signs and pathological outcome in three controlled trials. Intubation produced smaller lung lesions and a much milder disease, mainly the chronic form of CBPP, than contact transmission. The mode of inoculation may influence disease outcome and this should be taken into consideration when studying the pathogenesis of the disease and vaccine efficacy.

Keywords: CBPP; *Mycoplasma mycoides*; intubation; in-contact transmission

L'INOCULATION DE *MYCOPLASMA MYCOIDES* MYCOIDES PAR INTUBATION ENDOTRACHEALE PRODUIT UNE FORME PLUS LEGERE DE LA MALADIE PAR RAPPORT A LA TRANSMISSION PAR CONTACT

Résumé

Les bovins infectés par intubation endotrachéale avec *Mycoplasma mycoides* sous-espèce mycoides (Mmm) ont été utilisés dans les essais de pathogenèse de la pleuropneumonie contagieuse bovine (PPCB). Son avantage réside dans le fait que le moment précis de l'infection est connu, contrairement à la transmission par contact où l'heure exacte d'inoculation dans chaque animal n'est pas connue. Dans la présente étude, les deux méthodes d'inoculation ont été directement comparées, en analysant les signes cliniques et les résultats pathologiques dans trois essais contrôlés. L'intubation a produit des lésions pulmonaires plus petites et une forme beaucoup plus légère de la maladie, principalement la forme chronique de la PPCB par rapport à la transmission par contact. Le mode d'inoculation peut donc influencer l'issue de la maladie, et ceci devrait être pris en considération lors de l'étude de la pathogenèse de la maladie et de l'efficacité du vaccin.

Mots-clés : PPCB; *Mycoplasma mycoides* ; intubation ; transmission par contact

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Introduction

Contagious bovine pleuropneumonia (CBPP), a respiratory disease that affects cattle, water buffalo, bison and yak is caused by *Mycoplasma mycoides* subspecies mycoides (Mmm). The disease is of economic importance throughout most of Sub-Saharan Africa. This is due to high mortality in outbreaks and productivity losses that make it a risk to food security. Furthermore, it contributes to a reduction in access to international markets on a continent where approximately 30-50% of those keeping livestock in sub-Saharan Africa live below the poverty line (Tambi *et al.,* 2006); Thornton *et al.,* 2002).

The disease has been shown to manifest clinical forms that include per acute, acute and chronic forms. The peracute form is characterised by sudden death while the acute form manifests as cough, fever and laboured breathing of prolonged duration. The chronic form manifests loss of weight, and cough on exertion (Mariner *et al.,* 2006; Provost, 1987). There is no laboratory animal model to conduct studies on CBPP. Previously, challenge of vaccinated cattle in experimental vaccination trials used the subcutaneous route of inoculation (Piercy and Knight, 1957) but because this model of infection was unreliable, in vivo studies on the disease since then have been conducted on cattle by infecting them by contact transmission and/or endotracheal intubation

The contact transmission method, in which CBPP-infected cattle are mixed with naïve animals in a ratio of one to two respectively, has the advantage of simulating a natural infection. However, it is costly because it utilises an extra group of donor cattle during trials. In addition, contact transmission has unpredictable transmission rates (Dedieu *et al.,* 2005; Provost, 1987) and transmission of mycoplasma occurs at different times in different individual animals. In contrast, with the intubation method the experimenter knows the exact infection dose and the precise time of inoculation in all animals (Hudson and Turner, 1963; Nkando *et al.,* 2010) allowing for better comparison of clinical and pathological signs in individual animals. The only shortcoming of endotracheal intubation is in the use of sedatives reported to cause death in some cattle due to inspiration of ruminal contents while recuperating from anaesthesia. A few studies mentioned that clinical and pathological outcome of CBPP was higher in animals exposed by contact than by intubation (Huebschle *et al.,* 2003; Scacchia *et al.,* 2011), however, these studies compared few animals. The objective of this study is to test and establish a suitable method of infecting cattle to be used in trials involving vaccines and antimicrobials studies (Jores *et al.,* 2013). This paper compares disease severity, clinical and pathological events between endotracheal intubation and the in-contact method, in a large number of cattle from several different experiments during controlled field trials conducted at Kenya Agricultural and Livestock Research Organization (KALRO), formerly Kenya Agricultural Research Institute (KARI)-Veterinary Science Research Institute (VSRI), formerly Veterinary Research Centre (VRC).

Materials and methods

Cattle

The study reports on experiments performed at different times in various years. All the cattle used in the studies were males of the zebu breed aged 2 to 3 years, purchased from Kakamega, a CBPP free zone. Each time, the animals were tested for CBPP using slide agglutination serum test (SAST) before purchase. They were then transported to the KALRO-Kakamega station where they were ear-tagged, drenched and castrated for ease of management. Serum collected from each of the animals was tested by complement fixation test (CFT) and on finding the samples negative the animals were transported from Kakamega to KALRO-VSRI-Muguga. The cattle were randomly assigned into groups for intubation and contact transmission. Thereafter, the animals were examined daily for development of CBPP clinical signs such as fever, cough, nasal discharge and laboured breathing. Animals were killed in extremis or at end of trial and lesions

measured at post-mortem.

All experiments were carried out under the permission of VSRI-Muguga Institutional Animal Care and Use Committee (IACUC), reference numbers: KARI/VRC/IACUC/1/29092009 and KARI/VRC/IACUC/2/00122010. Endpoint of experiments was either 10 days of fever or an animal recumbent for 48 hours and not eating or at the end of experiment after minimum 8 weeks post challenge.

Acclimatization

On arrival at Veterinary Science Research Institute-Muguga, the cattle were grazed for one month before commencement of experiment. During this period, they were vaccinated against foot and mouth disease, black quarter (blackleg), anthrax and lumpy skin disease.

Preparation of inoculum for challenge

Preparations for inoculum commenced one and a half month before intubation. At this time, a culture of pathogenic Mmm (B237 strain) from Thika, Kenya (Nkando *et al.,* 2010) stored at passage 3 was revived from the freeze-dried material. Mmm culture for infection (inoculum) was prepared using modified newings tryptose broth containing tryptose (20 g; Sigma-Aldrich (UK), glucose (5 g), sodium chloride (5 g), thallous acetate (0.5 g; Sigma), disodium hydrogen phosphate (2.5 g), glycerol (5 ml), with the following additives: penicillin (0.03%), 0.5% phenol red (4 ml), fresh yeast extract (10 ml; Sigma), 25% sodium pyruvate (2 g) and pig serum (150 ml; obtained from local slaughterhouse). Five ml of broth was added to a 5 ml aliquot of freeze-dried culture shaken and allowed to stand on the bench for 30 min before dispensing 0.3 ml into bijou bottles containing 2.7 ml of broth to make 1 in 10 dilutions. This was placed in the incubator at 37 0C and upon growth was up scaled to make quantities sufficient for animals at 60 ml per animal. Confirmation of growth was through observation of change of phenol red in the medium and filaments in the broth. At every stage of up scaling, the cultures were incubated for 48 hrs at 37 0C. Growth was monitored daily based on colour change and appearance of filaments. Prior to use and after use, a sample of the culture was collected for titration to estimate the number of mycoplasmas in the culture used for inoculation. The titration was carried out in a 10-fold dilution series from 10-1 to 10-10 liquid medium.

Intubation

Intubation was carried out by introducing a tube through the nostril to the larynx and down to the trachea of cattle. Each animal was inoculated with 60 ml of culture with an Mmm concentration of 108 cfu/ml, followed by 30 ml of 1.5% agar boiled in distilled water and 30 ml of phosphate buffered saline (PBS) to flush down all the material. The addition of agar was suggested to improve the infection rate as discussed by (Nkando *et al.,* 2010).

Contact transmission

For contact transmission, intubated animals were observed for clinical signs, including fever before introducing them to naive ones in a ratio of one to two respectively, from day 14 on-wards. An animal was considered to have fever when it showed a rectal temperature of 39.5 0C and above. An animal was considered to show clinical signs of CBPP if it showed fever for three or more consecutive days.

Clinical examination

Rectal temperatures of each animal were recorded daily between 8.00 am and 10.00 am for the period of study following intubation and contact transmission. Clinical responses in both groups included nasal discharge, cough and laboured breathing.

Post-mortem

Post-mortem was carried out for a selection of reasons including: not eating for 48 hours, end of trial, weight loss and 10 days of fever. Criteria for killing animals were the same in all three experiments and for intubated and in-contact animals. Animals for necropsy were killed by captive bolt and exsanguinations. A record of gross pathological change in all

organs was made, with particular attention to the lungs. Lesion scoring was carried out to determine severity of the disease in diseased animals using the pathology score described by (Hudson and Turner, 1963). Briefly, using this method, the pathology score is calculated based on the size and duration of lesion (whether adhesions are fibrous or fibrinous). Using the diameter, the presence of encapsulated, resolving or fibrous lesions or the presence of pleural fibrous adhesions only were allocated a score of 1 regardless of the size. Other types of lesions including consolidation (hepatisation) due to fibrinous pneumonia or sequestration of necrotic mass were scored 2. In addition, if Mmm was isolated a value of 2 was added to the lesion score. The lesion score was then multiplied by an arbitrarily selected factor depending on average diameter of the lesion. A lesion size under 5 cm was rated 1; that over 5 cm and under 20 cm was rated 2 while a lesion over 20 cm was rated 3. Hence, the maximum pathology score was (2+2) *3=12.

Statistical analysis

Data was entered into Microsoft excel 2010 and exported to SPSS 21.0 for analysis. Chi-square and ANOVA were used to measure association between clinical signs, deaths, lesions and analysis of in-group variations between intubates and in-contacts in different trials. General linear model was used to test for the differences in pathology scores. R-statistical was used to plot medians for pathology scores between intubates and in-contacts in all trials.

Results

Tables 1 and 2, and figures 1, 2 and 3 show details of findings in different groups of experiments, including the cattle that showed fever, those that were killed in extremis after showing severe clinical signs and pathology of CBPP in cattle infected by either intubation or contact transmission. More in-contacts developed lesions than intubates in trial I, II, III and total of all trials as shown in figure 2. Although the median of intubates and contacts in trial I are comparable, there are significant variations in medians between intubates and in-contacts in trial II, trial III and total of all trials as shown in figure 3.

Experiment I

In this trial (Wesonga and Thiaucourt, 2000), a total of 40 intubates and 41 in-contacts were used. No unwanted reactions were observed in cattle following manipulation related to intubation. The 22 (55%) intubates showed fever with 10 (25%) cattle being killed in extremis after showing severe clinical CBPP and 36 (90%) intubates showing chronic lesions of pleural fibrosis and encapsulated parenchymal necrosis (sequestration) at post-mortem. Similarly, 30 (73%) in-contact cattle showed fever with 13 (31%) of them being killed in extremis after showing severe clinical CBPP and 40 (97.6%) with pathology characteristic of CBPP at post-mortem. The first acute infections occurred on day 7 after intubation, while the first acute cases occurred on day 45 in the in-contact animals, suggesting faster manifestation of clinical disease in intubates than in-contacts. The last post mortem was carried out 133 days after intubation, or 127 days after contact. There was significant difference ($p<0.05$) in pathology scores between contacts and intubates, although medians are comparable (Figure 3). However, in this experiment, there was no significant association ($p>0.05$) between mode of transmission and number of animals with clinical signs, killed in extremis or number of animals with lesions.

Experiment II

In this study (Nkando *et al.,* 2010), a total of 15 intubates and 10 in-contacts were used. No unwanted reactions were witnessed in cattle following manipulation related to intubation. Ten (66.7%) and eight (80%) animals showed fever in intubates and in-contacts respectively. The first acute infections occurred on day 16 after intubation, while the first acute cases occurred on day 42 in the in-contact animals, suggesting faster manifestation of clinical disease in intubates than in-contacts. One intubate (6.7%) was killed in extremis after showing severe clinical CBPP and 12 (80%)

intubates showed lesions at post-mortem with 3 developing acute lesions of fibrinous pneumonia and 9 showing chronic lesions of pleural fibrosis and encapsulated parenchymal necrosis (sequestration). In the group of in-contact cattle, 5 (50%) cattle were killed in extremis after showing severe clinical CBPP and all others developed lesions, 5 with acute and 5 with chronic lesions. Three (3) of the in-contact cattle had pleural fluid in their thoracic cavity. There was no significant association ($p>0.05$) between mode of transmission to development of clinical signs and lesions, although the magnitude of the lesions was higher in the in-contact animals (Figure 1). There was significant difference between contacts and intubates in pathology scores ($p<0.05$) and in the number of animals killed in extremis ($p<0.05$).

Experiment III

In this trial, a total of 17 intubates and 10 in-contact cattle were used. No unwanted reactions were witnessed in cattle following manipulation related to intubation. None of the animal was killed in extremis in all the 17 intubates within the observation period. The last animals were killed on day 144 after intubation or on day 128 days in-contact. In the in-contact infection trial, 5 (50%) of the cattle survived until end of trial. Two (20%) animals were killed in extremis while three (30%) were killed for not eating for at least three days due to CBPP. There was no significant association ($p>0.05$) between mode of transmission to development of clinical signs and lesions. However, there was significant association ($p<0.05$) between mode of transmission, mortality and pathology scores between contacts and intubates

Clinical response and necropsy in intubates

The cattle started showing clinical CBPP signs on day 2 post-intubation, including fever, cough and mucus secretion. In the 17 intubates, 12 (66.7%) exhibited fever for at least two days and up to 10 days in the experimental period. One of the animals showed fever intermittently for ten days, starting from the second day post inoculation. Three animals showed fever for only two days. Fever was generally intermittent in all animals and ranged from 39.5 0C to 41.1^{0}C. Five (29.4%) animals did not show fever. Chronic lesions were observed in the twelve (70.6%) cattle that experienced fever at slaughter at the end of the trial while 5 (29.4%) cattle did not develop lesions. A culture for isolation of Mmm from the lung tissues was made in all intubates and was found positive.

Clinical response and necropsy in in-contact cattle

Eight (80%) out of ten animals showed a temperature rise above 39.5 0C for a period between 2 and 10 days. Temperature above 39.5 0C was first recorded on day 54 post exposure. One animal showed fever for 10 days. Seven animals showed fever for at least 3 days. Fever was not recorded in two animals. On post-mortem examination, the pleural cavity in 5 animals contained copious amounts of yellowish coloured clear fluid. Other lesions included consolidation of the lung tissue, marbling with heavy deposits of fibrin flocculates. In 3 of cattle, the lesions were well developed sequestra. Two animals did not develop lesions. Mmm was isolated in all the 10 cattle.

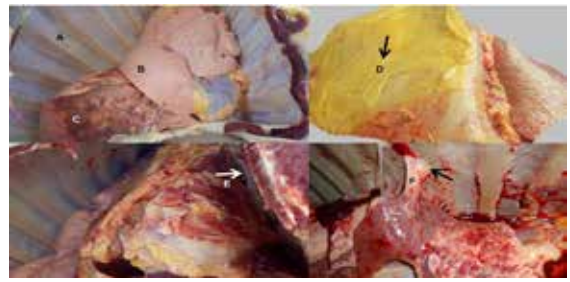

Figure 1: Representative post-mortem findings of cattle challenged by intubation or contact: A) Normal thoracic cavity of an animal killed on day 28 post-intubation; B) Typical normal lung of an animal killed on day 28 post-intubation; C) Less severe lesion of an animal killed on day 28 post-intubation; D) Contrast of enlarged lung lobes on the left with marbling (black arrow) and less severe reaction on the right in an animal killed in extremis on day 42 post-contact; E) Fibrous adhesions on thoracic cavity (white arrow) of an animal killed in extremis on day 42 post-contact and F) Thoracic adhesions, less severe lesion of intercostal adhesions between parietal and visceral pleurae (black arrow) in an animal killed at termination of trial post-intubation.

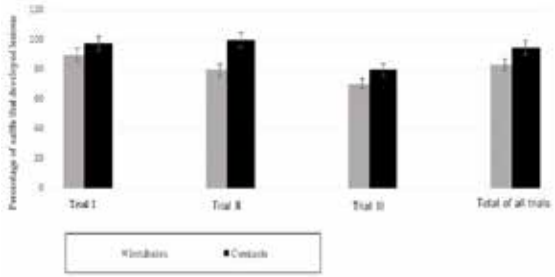

Figure 2: Differences in percentages of animals with lesions between intubates and in-contacts in different trials

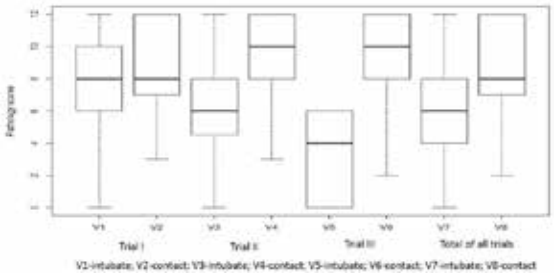

Figure 3: Box plots of pathology scores (with median shown) between intubates and in-contacts in different trials

Combined results

Overall, combined results for the three experiments indicated that there was no significant association ($p>0.05$) between mode of transmission to development of clinical signs such as fever but there was significant association ($p<0.05$) between mode of transmission to development of lesions. In addition, there was significant association ($p<0.05$) between mode of transmission and mortality. Similarly, there was significant difference ($p<0.05$) in pathology scores between contacts and intubates. Tables 1 & 2 show the total cattle used in each group, details of clinical signs, the proportion of animals euthanized in extremis, the number of animals with lesions and the average pathology scores of cattle infected by intubation or contact transmission in different trials.

Table 1: Details of cattle showing clinical signs and average scores in intubates and contacts in all trials

Year	**1999**		**2004**		**2013**		**Total of 3 trials**	
Trial	**Trial I**		**Trial II**		**Trial III**		**All trials**	
	Intubates	**Contacts**	**Intubates**	**Contacts**	**Intubates**	**Contacts**	**Intubates**	**Contacts**
Total cattle used	40	41	15	10	17	10	72	61
Number that showed fever	22	30	10	8	12	8	44	46
Average scores:								
• Coughing	1	2	2	2	2	2	2	2
• Nasal discharge	2	2	1	2	2	2	2	2
• Respiratory effort	1	2	2	2	2	2	2	2
• General appearance	1	2	1	2	2	2	1	2
Total of all scores	**1**	**2**	**2**	**2**	**2**	**2**	**2**	**2**

The scores in table 1 were rounded off to a whole number i.e. ≥1.5 ≈ 2. The clinical scores were assigned based on averages of number of animals showing clinical signs, time of appearance and severity as follows: 0: Coughing, nasal discharge, respiratory effort were absent and general appearance was normal; 1: Cough and nasal discharge were mild; respiratory effort was hyperpnoea; general appearance was subdued; 2: Severe coughing; significant nasal discharge; respiratory effort-obvious dyspnoea; appearance-reluctant to rise; 3: Distressed respiratory effort; general appearance-unresponsive to external stimuli.

Table 2: Number of animals and p-value of cattle killed in extremis, developed lesions and pathology scores

Year	1999			2004			2013			Total of all trials		
	Trial I			Trial II			Trial III			Total trials		
	intub.	cont.	p	intub.	cont.	p	intub.	cont.	p	intub.	cont.	p
Total cattle used	40	41		15	10		17	10		72	61	
Number killed in extremis	10	13	0.337	1	5	0.023	0	5	0.003	11	23	0.003
Number with lung lesions	36	40	0.172	12	10	0.198	12	8	0.475	60	58	0.029
Average pathology scores	7.3	8.9	0.025	6.2	9.1	<0.001	3.3	8.8	<0.001	6.1	8.9	<0.001

Data used in these experiments in table 2 was as described by (Wesonga and Thiaucourt, 2000). Whereas in table above, intub. ≈ intubates; cont. ≈ contacts.

Discussion

The results of the study suggest that infection by intubation produced a milder form of the disease than transmission by contact. Significantly more animals presented with clinical signs and more animals had to be killed in extremis in the in-contact groups. There were no significant differences in the number of animals with lesions, but that is likely to be because those percentages were high in all experiments. But the severity of lesions was significantly higher in the contact groups. Other reports also suggested differences between the two methods (Huebschle *et al.,* 2003; Scacchia *et al.,* 2011), however, the suggestions were based on few numbers of animals.

Although it is impossible to compare the infective doses between the two groups, largely because it is not possible to correctly estimate the number of mycoplasma that are transmitted between animals by aerosol, it seems unlikely to be the cause of the difference. Cultures contain a very high number of mycoplasma, easily 108 organisms per ml, and animals get 60 ml of such suspension poured straight into the lungs. It is difficult to see how an animal could inhale such numbers in a natural situation. The reason for the difference must lie elsewhere, such as the precise location in the lungs where the pathogens establish colonies or the lack of optimal conditions for adhering to the lung epithelial cells. A large suspension of mycoplasma may not provide the ideal environment for colonization and growth. Alternatively, the large number of mycoplasma in one location may provide a stimulus for innate host responses that may rapidly prevent pathogen expansion, while smaller numbers of pathogens in different locations may delay the initiation of inflammatory responses until the pathogens have multiplied to a sizeable colony.

When comparing the three experiments, it was noticed that in-contact animals develop a similar degree of pathology, unlike intubates which differ between the three experiments. This could be due to various factors, such as passage of mycoplasma culture, culture conditions or the intubation procedure. Conditions of in-contact infections are more homogeneous, because in these cases two naïve animals are put in contact with an infected animal showing clinical signs. It is likely that repeated intubations would also produce higher infection rates and more reproducible results, however, that has never been tested.

Finally, a potential major contributory factor is differential gene expression between mycoplasma originating from the lung and mycoplasma cultivated *in vitro*. A number of such differences have been described recently (Weldearegay *et al.,* 2015), as well as differences between *in vitro* and biofilm-grown mycoplasma, the latter possibly being the natural infective state of mycoplasma and expressing proteins that may be involved in adhesion (McAuliffe *et al.,* 2008). Because of this, cultured mycoplasma may be much less effective in establishing a lung infection than mycoplasma originating in vivo. A lower expression of adhesion molecules or

other virulence factors in cultured mycoplasma may make them less infectious after transmission. In this scenario, it is important that data obtained from intubated animals are confirmed by the in-contact method.

Conclusion

The mode of infection appears to have an effect on the outcome of the disease with contact transmission producing a more severe disease compared to intubation. This should be taken into consideration when planning for studies on the pathogenesis of CBPP. In addition to reproducing a more severe disease, contact transmission is a suitable method in trials because it simulates a natural infection, and is therefore recommended, especially where finances are not a limiting factor. However, intubation assures that animals are infected at the same time, while the exact time of infection of each animal after contact is not known and can vary widely (Dedieu *et al.,* 2005).

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ISOLATION OF LUMPY SKIN DISEASE VIRUS FROM CATTLE IN AND AROUND KOMBOLCHA AND DESSIE, NORTH EASTERN ETHIOPIA

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Abstract

In November there were reports on Lumpy Skin Disease from 2 different areas of Kombolcha (one intensive dairy farm) and Hayke (small holders). Therefore, Lumpy Skin Disease Outbreak Investigation was conducted from November 2015 to April 2016 on a total 194 local zebu and cross breeds of cattle. Thus, we observed as animals were asymptomatically diseased. The sampling method was purposive and a skin nodular tissue samples were taken from 11 from both breeds in order to isolate the field strain of Lumpy Skin Disease Virus circulating in and around Kombolcha and Dessie and accessing outbreaks of Lumpy Skin Disease in the area. The commonly observed clinical signs in the outbreak during the study period were fever, circumscribed nodules on the skin with different size, necrotic nodules and deep scab formation, edematous swelling of legs, enlargement of superficial lymph nodes, Lameness and enlargement of superficial lymph node. The questionnaire survey indicated as there was high incidence of the disease in wet seasons than dry seasons and protective level of the vaccine became low in the study area. Tissue samples were collected and virus was isolated on Vero cells. Furthermore, the isolated virus was identified by conventional Polymerase Chain Reaction and Real time Polymerase Chain Reaction technique which is more sensitive molecular advanced technique for diagnosis of Capripox virus. In conclusion as complained by the owners and during field examination, Lumpy Skin Disease was found to be a serious disease in the study area. So, further investigation is needed on identification of the causative agents and Molecular characterization of Lumpy Skin Disease Virus and risk factors of the disease in South Wollo Zone.

Key words: Cattle, Dessie and Kombolcha, LSD, LSDV, PCR

ISOLEMENT DU VIRUS DE LA DERMATOSE NODULAIRE PROVENANT DE BOVINS DANS ET AUTOUR DE KOMBOLCHA ET DESSIE, DANS LE NORD-EST DE L'ÉTHIOPIE

Resume

En novembre, des informations faisant état de la présence de la dermatose nodulaire contagieuse dans 2 zones différentes de Kombolcha (une ferme laitière intensive) et Hayke (petits exploitants) ont été communiquées. Par conséquent, une enquête sur les foyers de dermatose nodulaire a été menée de novembre 2015 à avril 2016 sur un total de 194 zébus locaux et de races croisées de bovins. Ainsi, nous avons constaté que les animaux étaient malades mais ne présentaient de symptômes. La méthode d'échantillonnage était raisonnée, et des échantillons de tissus nodulaires cutanés ont été prélevés sur 11 choisis dans les deux races, afin d'isoler la souche de terrain du virus de la dermatose nodulaire contagieuse qui circulait dans et autour de Kombolcha et Dessie et accédant aux foyers de dermatose nodulaire contagieuse dans la région. Les signes cliniques fréquemment observés dans le foyer pendant la période d'étude étaient la fièvre, les nodules circonscrits de différentes tailles sur la peau, les nodules nécrotiques et la formation de gale profonde, l'enflure œdémateuse des pattes, l'élargissement des ganglions lymphatiques superficiels et les boiteries. L'enquête par questionnaire a indiqué qu'il y avait une forte incidence de la maladie pendant les saisons humides par rapport aux saisons sèches et que le niveau de protection fourni par le vaccin était faible dans la zone d'étude. Des échantillons de tissus ont été prélevés et le virus a été isolé sur des cellules Vero. En outre, le virus isolé a été identifié par une réaction classique en chaîne par

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polymérase et une réaction en chaîne par polymérase en temps réel qui est une technique moléculaire de pointe plus sensible pour le diagnostic du virus Capripox. En conclusion, comme l'ont indiqué les propriétaires et lors de l'examen sur le terrain, la dermatose nodulaire contagieuse s'est révélé être une maladie grave dans la zone d'étude. Ainsi, d'autres recherches sont nécessaires pour l'identification des agents étiologiques et la caractérisation moléculaire du virus de la dermatose nodulaire contagieuse et des facteurs de risque de la maladie dans la zone de South Wollo.

Mots-clés : bovins, Dessie et Kombolcha, DNC, VDNC, PCR

Introduction

Ethiopia has the most abundant livestock population in Africa (FAO, 2005) and the cattle population is estimated to be 56.71million, out of this total cattle population, the female cattle constitute about 55.45% and the remaining 44.55%are male cattle Regarding age groups, the majority of the cattle population (that is about 63.52percent) is in the 3 years and less than 10 years age category, with about 27.59 % male and about 35.92 % female. Moreover, about 16.72% are between age one and three years and those with age category 10 years and over took small portion i.e. 2.07 percent of the total estimated number of cattle population (CSA, 2014/2015).

The livestock sub sector accounts for 40% of the agricultural Gross Domestic Product (GDP) and 20% of the total GDP without considering other contributions, e.g. traction power, fertilization and transportation (Aklilu, 2002). In 2004 the livestock sector contributed around 12% of total foreign currency earnings (Anon, 2009).about 99% of cattle populations are of local Zebu breed. Genetically and geographically the main breed classifications in Ethiopia are Arsi, Fogera, Horo, Borana, Nuwer, Sheko and Afar breeds. The remaining 1% of exotic breeds is kept mainly for dairy production in and around urban areas (Gari *et al.,* 2010). Traditional cattle management in the rural part of the country is extensive. Animals are free-ranging in communal grazing fields and different age groups are herded together. Natural grass post-harvest crop residuals and straw are the main source of feed. Concentrate feeds and feed additives are seldom used (Alemayehu, 2006).

Lumpy skin disease (LSD) is an acute to sub acute viral disease of cattle that can cause mild to severe symptoms including fever, nodules in the skin, in the mucous membranes and in the internal organs, skin oedema, lymphadenitis and sometimes death. The disease can result in economic losses due to decreased milk production, traction power loss, weight loss, poor growth, abortion, infertility and skin damage (Ali *et al.,* 1990; OIE, 2004). Milk production ceases and pneumonia is a common sequel in animals with lesions in the mouth and respiratory tract (Davies, 1991; OIE, 2008). Severe and permanent damage to hides from the skin lesions could also bring a considerable loss to the tannery industries and loss of foreign currency since hides are one of the export commodities in Ethiopia (Bayou *et al.,* 1998). Moreover, it is a disease that hinders improvement of the livestock development and also international livestock trade movement due to phyto-sanitary regulations by many live livestock and livestock products importing countries (Gari *et al.,* 2008).

LSD was first identified in sub-Saharan Africa in Zambia in 1929. Currently its distribution has extended from Sub-Saharan countries to Egypt and Western Africa (Davies, 1991). Epidemiological distribution trend of LSD suggests that there could be a considerable potential risk of the disease spreading further into North Africa, the eastern ward of Egypt to the Middle East countries and to Mediterranean regions because of global climatic changes and trade movement in animal and animal products (Davies, 1991; Babiuk *et al.,* 2008).

Morbidity and mortality of the disease vary considerably depending on the breed of cattle, susceptibility levels of the population, insect vectors involved in the transmission and

isolates of the virus. In endemic areas, morbidity is usually around 10% and mortality ranges between 1% and 3% (Davies, 1991; Babiuk *et al.,* 2008). The mechanical transmission by the biting flies is the most effective method (Chihota *et al.,* 2001). The incidence of LSD occurrence is high during wet seasons when biting-fly populations are abundant and it declines or ceases during the dry season (Anon, 2009).

In Ethiopia, LSD was first observed in the western part (southwest of Lake Tana) in 1983 (Mebratu *et al.,* 1984). Studies based on clinical disease observation around Nekemt town have reported a prevalence of 7.02% (Regassa, 2003). Another study based on seroprevalence in Southern Ethiopia reported a prevalence of 6% (Gari *et al.,* 2008). Targeted sampling from outbreak areas around southern range-lands, Wolliso town and North Ethiopia reported prevalence of 11.6%, 27.9% and 28%, respectively (Asegid B Addis Ababa University DVM Thesis; Beshahwured, 1991; Gari *et al.,* 2008). But most document or reports were shown prevalence of LSDV, there is a recent report on the isolation and molecular characterization of LSDV on outbreak samples collected from different areas of the country (Gelaye *et al.,* 2015); but the previous study did not include samples originated from Kombolcha and Dessie surrounding areas. Therefore the objectives of this research were to assess the LSD outbreak occurred in Kombolcha and Dessie towns and their peri-urban areas and to isolate field strains of LSD viruses circulating in the study areas.

Materials and Methods

Study area

The study was conducted starting from November 2015 to April 2016 in and around Kombolcha and Dessie towns which are found in Amhara Region South Wollo zone, North eastern Ethiopia.

Dessie town is situated at the north-east part of country at a distance of about 401 km away from Addis Ababa, located at 11 08' North latitude and 39 38' East longitudes and has an elevation of 2600 meter above sea level. The area gets 936 to 1070 mm Hg rainfall annually. The mean monthly minimum and maximum temperatures were 12.37oC and 26.27oC respectively (National Meteorology Service Agency 2010 Dessie Branch, Dessie, Ethiopia). The livestock population of the area comprises of 18,724 cattle, 22,248 sheep, 2572 goats, 1879 horses, 833 mules, 3762 donkeys and 37,557 heads of chickens (Dessie Agricultural office (2015): Dessie, Ethiopia personal communication).

Kombolcha town is found North East of Ethiopia at 375km from Addis Abeba in Amhara regional state. The town is located in a range of altitudes between 1500 and 1840m above sea level with average rainfall of 750 to 900 ml during the study period. Its annual temperature ranges from 25 to 30°C and the relative humidity of the region varies from 23.9 to 79% (National Meteorology Service Agency 2010 Kombolcha Branch, Kombolcha, Ethiopia). The population of livestock in the town includes 19687 Cattle, 6905 sheep, 11133 Goats, 774 Horses, 2629 Donkeys, 77 mules, 29915 poultry, 624 bee colony and 331 male camels (Kombolcha Agricultural office (2015): Kombolcha, Ethiopia personal communication).

Study animals

The study populations were Cross HF-Zebu and pure Zebu cattle included in the study. The origins of those animals are from different kebele of in and around Dessie and Kombolcha. The cattle which are found in this area are kept under semi intensive and intensive management system. The farms included in the study were categorized as smallholder and big dairy farms based on herd size and management system and farmers who had ox for traction power. Smallholder dairy farms were urban type dairy farms having 2 to 6 cattle under semi-intensive management system. However, each big dairy farm held more than 100 cattle and young and adult animals were managed in a separate barn.

Study design and sampling strategy

The study was conducted from November 2015 to April 2016 for the isolation of Lumpy Skin Disease virus from purposively

selected cattle which show the clinical signs of the disease. The reason for the purposive sampling was since strictly random sampling procedure might not be possible due to Active outbreaks were assessed for conducting the field survey, questionnaire data and sample collection. When an active outbreak of LSD was encountered or reported, field investigations were conducted and information was gathered by interviewing cattle owners and district animal health workers.

Methodology

Questionnaire survey

A total of eleven animal owners from kombolcha and hayke were interviewed using a semi-structured questionnaire about the outbreak occurred to collect data on the number of sick and dead animals and vaccination history of the affected animals as indicated in (annex 1) to supplement the work.

Observation

Observation of individual animals was also conducted on a total of 194 cattle. These exposed animals were inspected for developing typical LSD clinical signs such as fever, circumscribed nodules on the skin with different sizes, necrotic nodules and deep scab formation, edematous swelling of legs, enlargement of superficial lymph nodes.

Virus isolation and PCR assay

During clinical examination samples for virus isolation and antigen detection were collected from clinically sick animals according to the procedures of OIE (2010), about 1 grams of nodular skin tissue were collected from 11selected cases which showed suspected lumpy skin disease lesions (at different stages of development) and kept in sterile universal bottle containing glycerol buffer put in icebox for transportation and kept under deep freeze until processing. The collected scabs were processed at National Veterinary Institute (NVI) according to procedure described by OIE (2010), (indicated at Annex 2) and cultured on kidney cells of Africa Green Monkey (Vero) cells for virus isolation.

Viral DNA was extracted from those cell cultures showing cytopathic effects to do Polymerase Chain Reaction (PCR) for confirmation by amplification of RPO30 gene of LSD virus using capripoxvirus-specific primer: Forward and reverse primers had the sequences (SpGpRNAPol-F and SpGpRNAPol-R with sequences 5'TCTATGTCTTGATATGTGGTGGATAG-3 and 5'AGTGATTAGGTGGTGTATTATTTTCC-3', respectively) that encodes for the test gene. A general PCR (convectional and real-time) described by Lamien et al. (2011) and Gelaye et al. (2013) respectively (indicated at Annex 2) was followed.

Data analysis

Descriptive analysis was used for summarizing the data. Morbidity and mortality were determined at district level.

Results

Questionnaire survey

Each of the respondents were asked about total number of animals, number of sick animals, number of dead animals (indicated at table 1), seasonal occurrence of the disease, source of outbreaks and vaccination history. The cattle owners indicated the incidence of LSD to be high during wet seasons than dry season. The owners also added that the protective level of the vaccine has decreased and the source of out breaks was contact at communal point during communal grazing in semi-intensive farms and introduction of infected animals in intensive farms. As the respondents said the local name of the disease is "gurbrb".

Observation

During the study period, one outbreak was encountered and investigated. The outbreak occurred on November, 2015 in Kombolcha and Hayk. The common clinical signs observed in cattle affected by LSD virus

Table 2: The number of animals examined for LSD infection and proportion of sick and dead animals are presented

Town	Total number of animals examined	Total number of animals sick	Total number of animals dead	Morbidity	Mortality
Kombolcha	168	7	3	4.17	1.78
Hayk	26	7	1	27	3.8
Total	194	14	4	7.22	2.8

were fever, circumscribed nodules on the skin with different size, necrotic nodules and deep scab formation, edematous swelling of legs, enlargement of superficial lymph nodes, Lameness and superficial lymph node enlargement (Fig 2).

Virus isolation

The suspensions obtained from processed skin tissue were inoculated into African Green Monkey Kidney Cells (Vero) cells after filtration through 0.45 µm filter. Out of the 11inoculated samples 11of them showed cytopathic effect characterized by

Figure 2: Characteristic of LSD with generalized circumscribed skin nodules over the entire body

Figure 3: None infected Vero cell (Negative control)

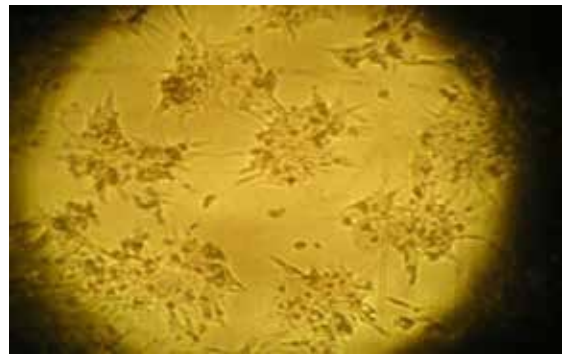

Figure 4: CPE formation on day ten.

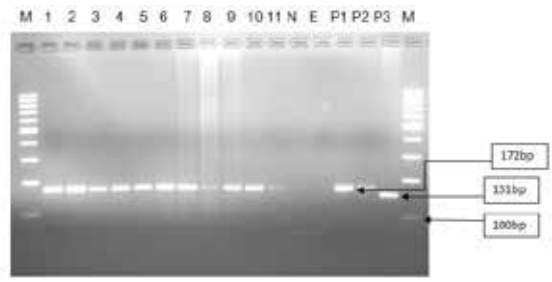

Figure 5: LSD suspected skin nodule samples classical PCR test result gel picture. Where;

LSDV=172bp, GTPV=172bp, SPPV=151bp.
M- Molecular marker started 100bp
1- MB1030/15-Code 2 Positive around 172bp
2- MB1030/15-Code 3 Positive around 172bp
3- MB1030/15-Code 4 Positive around 172bp
4- MB1030/15-Code 6 Positive around 172bp
5- MB1030/15-Code A Positive around 172bp
6- MB1030/15-Code D positive around 172bp
7- MB1030/15-Code C Positive around 172bp
8- MB1030/15-Code H Positive around 172bp
9-MB1030/15-Code B- positive around 172bp
10-MB1030/15-Code F- Positive around 172bp
11- MB1030/15-Code 7 -Positive around 172bp
E-Extraction control template-No amplification
N-Negative control without template-No amplification
P1-Positive control for LSD -Positive around 172bp
P2- Positive control for GTPV - Positive around 172bp
P3-Positive control for SPPV-Positive around 151bp

small syncytia, small round on Vero cell cultures starting from the 4th day up to 14th day post-inoculation. CPEs were seen as destruction of monolayer, infected cells were rounded formed singly and aggregation of died cells. None of the negative controls produced any CPE (Figure 3).

Conventional polymerase chain reaction

Amplification of extracted DNA was done using the primer pair: 5'TCTATGTCTTGATATGTGGTGGATAG-3 and 5'AGTGATTAGGTGGTGTATTATTTTCC-3', was amplified having the gene sequence encoding fragment of RPO30 gene of LSDV was amplified having 172 bp. 11 tested samples showed the characteristic PCR positive bands of 172bp size fragment of LSDV as indicated in the following picture below.

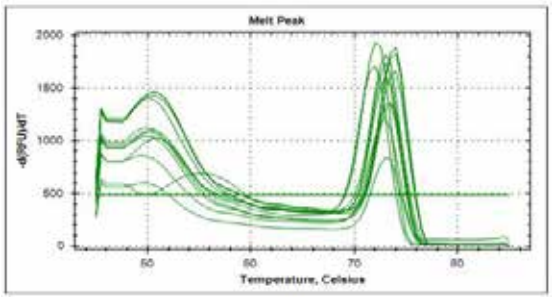

Figure.6: Differences in the melting curve for field isolates of LSDVs

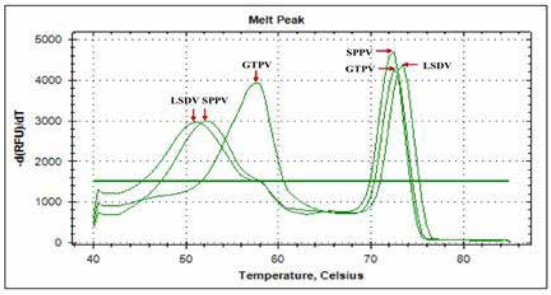

Figure 7: Differences in the melting curve for known positive isolates of CaPVs for comparison.

Real-time PCR

The extracted viral DNA was again subjected to real-time PCR. This was because the classical PCR could not differentiate LSDV from GTPV since both have 172bp PCR product; even though it could differentiate LSDV (172bp) from SPPV (151bp) as shown in the gel picture. The peak melting curve of the real time revealed that all 11 virus isolates were characterized as LSDV since their snapback of melting peaks were at 51oC while the second peaks were at 73.5oC (Fig.6). Known CaPV positive samples were tasted in all run as positive control for each three genotypes. Real-time PCR assay detected differences in the melting point temperatures for SPPV, GTPV and LSDV after fluorescence melting curve analysis from each other (Fig.7).

Discussion

The clinical findings, virus isolation and PCR diagnosis confirmed that the outbreak was caused by LSDV. Clinical signs observed on LSD infected cattle were fever, circumscribed skin nodules, enlargement of lymph nodes, in appetence, lacrimation, salivation, reduction of milk production and death. These signs have been documented as characteristic clinical features of LSD (Carn and Kitching, 1995; Radostits *et al.,* 2007).

The local name given to LSD by the farmers is "Gurbrb". According to the questionnaire survey result, LSD occurrences seasonally high at rainy season and decreases in the dry season. This result is supported by the works of other researchers who showed incidence of LSD to be high during wet seasons when biting-fly populations are abundant and it decreases during the dry season (Ali *et al.,* 2006; Radostits *et al.,* 2007; Gari *et al.,* 2010).

Laboratory confirmation was made by virus isolation on cell culture and LSDV genome identification based on convectional and real time PCR. Out of 11 skin biopsies, characteristic CPE of Capripox virus was observed in all samples following inoculation on Vero cell line. CPE characterized by rounded infected cells that formed singly, aggregation of cells and destruction of monolayer was observed. PCR reaction is a quick, sensitive and reliable method as antigenic resemblance of LSD virus with sheep and goat poxvirus makes the diagnosis through routine serological tests

difficult (Tuppurainen *et al.,* 2005; Anonymous, 2010).

All isolates were identified as LSDV after sequential diagnosis using convention PCR and real-time PCR. The present study is in agreement with the previously conducted study by Gelaye et al. (2015) that LSDV was isolated from nodular skin scrapping samples collected from cattle in different parts of Ethiopia.

In the present study, morbidity (7.22%) and mortality (2.1%) rates were observed regardless of vaccination history. there were problems associated with vaccine failure: It might be due to inappropriate storage of vaccine as a failure in one or more steps of the cold chain may occur, or the vaccine may be inactivated because of exposure to direct sunlight or high environmental temperatures during the vaccination process, improper use of protective dose and poor administration, vaccination of infected cattle (cattle which are at the incubation period of the disease that does not show clinical sign) and using of needles or diluents contaminated with virulent LSDV during the actual vaccination procedure may transmit the virus. These findings are in agreement with several literatures reported that the morbidity and mortality rates of LSD vary. The morbidity rate of the disease ranges from 5 to 100% (Woods, 1988; Fayez and Ahmed, 2011). While occasional mortality rates from 10 to 40% have been reported (EMPRES, 2013), but the rate of 1 to 5% is usually observed (Woods, 1988; Davis, 1991). These values vary with geography, climate, management conditions and immune status of animals, breed and strain of virus involved (Tuppurainen and Oura, 2012).

Conclusion and Recommendations

Lumpy Skin Disease was known to be the major cattle health problem causing severe economic loss due to reduced weight gain, permanent damage to hides, a prolonged debilitating clinical course, loss of milk production, temporary or permanent infertility or even sterility in bulls and abortion of pregnant cows. LSD is well known by the farmers and dairy farm owners in and around Kombolcha and Dessie and the virus circulating the area is LSDV. LSD caused moderately high morbidity and mortality in the study area which may be associated with direct and indirect economic losses (milk yield reduction, cost of dead animals, cost of treatment and additional feed cost for diseased animals until their recovery). Vaccination is the only effective method to control the disease in endemic countries like Ethiopia but the results of this study showed the presence of vaccine failure is in the study area. Based on the above conclusion the following recommendations are forwarded:

- Further isolation and molecular characterization of LSDV should be conducted so as to identify strain of the virus in the country in order to produce new vaccine.
- The government should establish strategic policies for effective control and eradication of the disease
- Animal health workers need to give emphasis in avoiding transmission of the disease while vaccinating animals

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SODIUM ARSENITE-INDUCED REPRODUCTIVE TOXICITIES IN MALE WISTAR RATS: ROLE OF TRIDAX PROCUMBENS LEAF EXTRACT

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Abstract

The cytotoxic potentials of arsenicals are well documented. Efforts at the mitigation of such effects are ongoing. In the present study, the effects of ethanol leaf extract of Tridax procumbens (ELETP); a notable medicinal plant, on the reproductive toxicities of sodium arsenite were investigated. Thirty-two male wistar rats (80-100g) allotted equally into four groups (A-D) were used. Rats in Group A (control) were treated with diluted olive oil {1:1(v/v)} at 1 ml/kg body weight (bwt.); Group B: sodium arsenite (SA); 2.5 mg/kg (positive control); Group C: 100mg/kg ELETP; and Group D: 100mg/kg ELETP and 2.5 mg/kg SA. ELETP was administered daily for 14 days while SA only was given on days 7 and 14 respectively. Spermatozoa characterization, epididymal morphometry, testicular morphometry, and histology of the testes were assessed. There was no significant difference ($p>0.05$) in the mean values of volume of spermatozoa and spermatozoa viability in Groups B to D as compared to Group A. On the other hand, the mean values of spermatozoa motility and concentration in Groups B to D as compared to Group A was significantly reduced ($p<0.05$). The percentage mean values of spermatozoa morphological characteristics observed in all the groups were found to be within the range of 10% to 20%. Furthermore, there was no significant difference ($p>0.05$) in the mean values of both testicular and epididymal morphometry in Groups B to D as compared to Group A. Sodium arsenite caused pathological effects on the testicular interstitial and germinal cells in Group B. However, these toxic effects were reversed in Group D. The ELETP seem toxic to the sperm cells rather than epididymal and testicular morphology, caution should therefore be exercised on the therapeutic application of Tridax procumbens in man and as feed for animals.

Key words: Tridax procumbens, arsenite, sperm characteristics, morphology, histology

TOXICITES REPRODUCTIVES INDUITES PAR L'ARSENITE DE SODIUM CHEZ LES RATS WISTAR MALES : RÔLE DE L'EXTRAIT DE FEUILLES DE TRIDAX PROCUMBENS

Resume

Les potentiels cytotoxiques des arsenics sont bien documentés. Les efforts visant à atténuer ces effets sont en cours. Dans la présente étude, les effets de l'extrait de feuilles d'éthanol de Tridax procumbens (ELETP), une plante médicinale remarquable, sur les toxicités reproductives de l'arsénite de sodium ont été étudiés. Trente-deux rats wistar mâles (80-100 g) répartis équitablement en quatre groupes (A-D) ont été utilisés dans l'étude. Les rats du groupe A (témoin) ont été traités avec de l'huile d'olive diluée 1: 1 (v / v) à 1 ml / kg de poids corporel (poids corporel) ; le Groupe B a reçu de l'arsénite de sodium (SA) ; 2,5 mg / kg (témoin positif) ; le Groupe C a reçu 100 mg / kg ELETP ; et le groupe D 100 mg / kg ELETP et 2,5 mg / kg SA. L'ELETP a été administré quotidiennement pendant 14 jours tandis que la SA n'a été administrée qu'aux jours 7 et 14. On a évalué la caractérisation des spermatozoïdes, la morphométrie épididymique, la morphométrie testiculaire et l'histologie des testicules. On n'a pas remarqué de différence significative ($p> 0,05$) dans les valeurs moyennes du volume des spermatozoïdes et de viabilité des spermatozoïdes dans les groupes B à D par rapport au groupe A. D'autre part, les valeurs moyennes de la motilité et de la concentration des spermatozoïdes dans les groupes B à D par rapport au groupe A ont été considérablement réduites ($p <0,05$). Les valeurs moyennes du pourcentage des

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caractéristiques morphologiques des spermatozoïdes observées dans tous les groupes se situaient dans la plage de 10 à 20%. En outre, on n'a pas noté de différence significative (p> 0,05) dans les valeurs moyennes de la morphométrie testiculaire et épididymique dans les groupes B à D par rapport au groupe A. L'arsénite de sodium a provoqué un effet pathologique sur les cellules interstitielles testiculaires et germinales du groupe B. Cependant, cet effet toxique a été inversé dans le groupe D. L'ELETP semble toxique pour les spermatozoïdes plutôt que la morphologie épididymique et testiculaire, il faut donc faire preuve de prudence dans l'application thérapeutique de Tridax procumbens chez l'homme et comme aliment pour animaux.

Mots-clés : Tridax procumbens, arsénite, caractéristiques du sperme, morphologie, histologie

Introduction

Tridax procumbens L. (Asteraceae) is a frequent tropical procumbent herb found especially in tropical Americas where it is native but, it has been introduced to tropical, subtropical and mid temperate regions worldwide (Salahdeen *et al.,* 2004). The plant is known to be valued for its pharmaceutical properties (Sahoo, 1998). It has been reported to possess medicinal properties against different conditions such as wound, malaria, dysentery, blood pressure, diarrhea, stomach ache, bronchial catarrh, and headache. It also prevents the falling off of hair and enhances the growth of hair (Saxena and Albert 2005; Rathi *et al.,* 2008) and stops hemorrhage from cuts and bruises (Ali, 2001). Its leaves and flowers have parasiticidal, insecticidal and antiseptic properties (Sahoo, 1998; Chandra *et al.,* 2016)

Investigation into its phytochemistry reveals the presence of chemical constituents like alkaloids, flavonoids, quercetin, carotenoids, beta-sitosterol, fumaric acid, luteolin, oxoester, lauric acid, myristic, palmitic, arachidic, linoleic acid, saponin, anthraquinone and tannin (Subramanian, 1968; Reddy, 2006; Singh, 2010). Ethnopharmacological activities like anti-diabetic, anti-inflammatory, analgesic, depressant action on respiration, immune-modulatory, anti-oxidant and hepatoprotection have been reported (Vyas, 2004; Reddipalli, 2008; Bhagwat, 2008; Samuel *et al.,* 2016a, b). Also, *in vitro* anticancer activity of the crude flower extract has been reported (Priya *et al.,* 2011; Vishnu, 2011).

There are reports that, arsenical compounds that are frequently used as herbicides, insecticides, rodenticides and food preservatives especially in Asia and Africa are challenging the aquatic environment through byproducts of used fossil fuel (Liu *et al.,* 2001, Waalkes *et al.,* 2003; Hubaux *et al.,* 2013). The main source of these compounds in most populations is the drinking water and food in which, inorganic form of arsenic predominates (Bates *et al.,* 1992, Pott *et al.,* 2001). However, arsenical compounds are environmental toxins with multiple effects in animal and human populations (Liu *et al.,* 2001, Waalkes *et al.,* 2003). Chronic dermal toxicity, nephrotoxicity, and skin cancer have all been reported to occur with arsenic exposure (NRC 1999). Arsenic is also a multi-site carcinogen in humans, causing tumors in a variety of tissues including lung, liver, skin, and bladder (NRC 1999; Waalkes *et al.,* 2003). Other studies indicate that the kidney, uterus and prostate may also be target sites of arsenic carcinogenesis in humans (NRC 1999; NRC 2014). Therefore, the urgent need for the protection or amelioration of arsenical compounds induced toxicities. In the present study, the role of Tridax procumbens L. on sodium arsenite-induced reproductive toxicities in male wistar rats was investigated.

Hypothesis

This study is designed to test the hypothesis that, Tridax procumbens L. will ameliorate the reproductive toxicities induced by sodium arsenite in male wistar rats.

Materials and Methods

Chemicals reagents

Sodium arsenite (0.05 M $NaAsO_2$, Sigma-Aldrich, USA) salt with dosage of 2.5 mg/kg body weight corresponding to 1/10th of the

oral LD_{50} was used. Freshly prepared solutions were used for each experiment and all reagents were of analytical grades.

Plant extract

Tridax procumbens L. (Asteraceae) leaves were collected from the University of Ibadan. Identification and authentication were done at the Department of Botany of our University and the specimen deposited has been given voucher number UIH-22542. The leaves were washed with potable water, air-dried and ground into fine powder. Extraction was done by soaking the ground leaves in 96% ethanol for 72hours and the mixture then filtered using Whatman No.1 filter paper. Thereafter, the filtrate collected was concentrated using rotary evaporator at a temperature of 40°C and later stored in an air tight container. Extract suspensions were freshly prepared in olive oil, which served as vehicle and negative control. The extract was administered orally to the rats at a dosage of 100 mg/kg body weight.

Experimental animals

Thirty-two (32) male wistar rats (80-100g) were obtained from the experimental animal house of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. All the experimental procedures were approved by institutional animal ethical committee with reference no: UI-ACUREC/App/12/2016/03. The animals were stabilized and observed for one week before they were used for this study. The healthy animals were housed in steel laboratory cages and under controlled conditions of temperature (25±2°C), relative humidity (50±15%) and normal photoperiod (12 h light and 12 h dark). The animals were fed on a standard rat diet and given water ad libitum in addition to good veterinary care as provided for in the broad International guiding principles for Biomedical Research Involving animals as developed by the Council for International Organisation of Medical Sciences (CIOMS) in 1985.

Experimental protocol

The Wistar strain rats were allotted into four groups (A to D) of eight rats each. The treatment groups were as follows: Group A (negative control) were treated with 1 ml/kg body weight (b.wt.) diluted olive oil 1:1(v/v), group B (positive control) sodium arsenite (SA) 2.5 mg/kg b.wt.. While animals in group C were administered 100mg/kg b.wt. extract daily for 14 days, and group D: 100mg/kg b.wt. extract for 14 days and SA on day 7 and day 14. Oral route of administration was used.

Reproductive Studies

The rats were anaesthetized with diethylether before they were sacrificed by cervical dislocation. In performing orchidectomy, a prescrotal incision was made and the testicles then exposed out of the incision site. Semen samples thereafter was collected from the caudal epididymis, the samples then analyzed immediately for morphology of the spermatozoa after the collection as described by Zemjanis (1977). The spermatozoa volume was determined by observing and reading it out in a calibrated measuring cylinder. The spermatozoa count and motility were determined as described by Pant and Srivastava, (2003). A total of 400 spermatozoa from each animal were examined for morphological alterations. These were determined from a total count of 400 spermatozoa in smears obtained with Wells and Awa stains. However, spermatozoa viability was determined as described by Wells and Awa (1970). The testes were excised from the animals and stored in bouin solution for tissue sections and subsequent histological examination using methods described by Chayen et al. (1973). The prepared slides were then viewed using an Ortholux microscope fitted with a Leitz camera unit and processed.

Statistics

The data generated were analyzed using one way analysis of variance (ANOVA). Graph pad prism 4 and Microsoft Excel Professional Plus (Microsoft Corporation, 2010) were used to carry out all procedures. The results were expressed as mean ±standard error of mean

(SEM) and level of significance set at $p<0.05$.

Results and Discussion

Findings from this studies showed that there was no significant difference ($p>0.05$) in the mean values of volume of spermatozoa and spermatozoa viability in Groups B to D as compared to Group A. However, there was significant reduction ($p<0.05$) in the mean values for spermatozoa motility {Groups B (16.67%), C (25.89%) and D (29.67%)} and concentration {Groups B (19.05%), C (32.30%) and D (39.44%)} as compared to Group A (Table 1).

Breeding soundness examination classification has been reported (Zemjanis, 1977) while various medicinal plants have different effects on spermatogenesis. Our observations in this study are dissimilar to what was reported for the studies carried out on Tribulus terrestris, Garcia kola and Curcuma longa. These plant parts were discovered to prevent peroxidative changes in the sperm cells and testicular membrane, thus enhancing sperm motility and decreasing spermatozoa abnormalities (Farombi *et al.,* 2007; Ishihara *et al.,* 2000). Hafez, (1993) and Ola-Davies et al. (2014) in their study stated that high percentage motility and viability will result in high fertility potential.

However, our findings corroborate the observations made by Parveen et al. (2003), Hauser et al. (2004) and Saba et al. (2009). The medicinal plants that were used in their studies had detrimental effects on sperm motility, sperm count and viability. Although, despite the fact that the sperm cells of the treated rats used in our study were deformed, it was observed that the mean values for the cells morphological parameters of all the groups were not significantly different ($p>0.05$) as

Table 1: Spermatozoa characterization

PARAMETERS	CONTROL	SA	TP	SA+TP
Motility (%)	90.0 ± 3.54^{abc}	75.0 ± 2.89^{a}	66.7 ± 3.33^{b}	63.3 ± 3.33^{c}
Viability (%)	97.3 ± 0.750^{a}	96.5 ± 0.866^{b}	97.0 ± 1.00^{c}	92.7 ± 3.93^{d}
Volume (cm^3)	5.18 ± 0.0250^{a}	5.15 ± 0.0289^{b}	5.20 ± 0.0000^{c}	5.17 ± 0.0333^{d}
Sperm concentration (x106 cells/ ml)	126 ± 4.32^{abc}	102 ± 6.69^{ad}	85.3 ± 3.18^{b}	76.3 ± 2.60^{cd}

Values are expressed as mean±SEM. Means with the same superscript within a row are significantly different ($p<0.05$). Control= Olive oil, SA= Sodium arsenite, TP= Tridax procumbens.

Table 2: Spermatozoa morphological parameters

PARAMETERS	CONTROL	SA	TP	SA+TP
Tailless head	1.11 ± 0.153^{a}	1.05 ± 0.188^{b}	1.24 ± 0.147^{c}	1.15 ± 0.209^{d}
Headless tail	1.05 ± 0.118^{a}	1.05 ± 0.178^{b}	1.23 ± 0.139^{c}	1.07 ± 0.226^{d}
Rudimentary tail	0.43 ± 0.121^{a}	0.56 ± 0.121^{b}	0.49 ± 0.144^{c}	0.49 ± 0.144^{d}
Bent tail	1.84 ± 0.143^{a}	2.05 ± 0.186^{b}	2.46 ± 0.232^{c}	2.39 ± 0.180^{d}
Curved tail	1.79 ± 0.166^{a}	2.05 ± 0.191^{b}	2.39 ± 0.210^{c}	2.06 ± 0.179^{d}
Curved mid-piece	2.04 ± 0.127^{a}	2.11 ± 0.151^{b}	2.39 ± 0.310^{c}	2.39 ± 0.292^{d}
Bent mid-piece	2.03 ± 0.162^{a}	2.17 ± 0.214^{b}	2.30 ± 0.211^{c}	2.03 ± 0.162^{d}
Looped tail	0.32 ± 0.078^{a}	0.43 ± 0.119^{b}	0.33 ± 0.085^{c}	0.33 ± 0.082^{d}
Total abnormal cells	10.61	11.47	12.83	11.91
Total normal cells	89.39	88.53	87.17	88.09

Values are expressed as mean±SEM. Means with the same superscript within a row are significantly different ($p<0.05$). Control= Olive oil, SA= Sodium arsenite, TP= Tridax procumbens.

Table 3: Testicular morphometry

PARAMETERS	CONTROL	SA	TP	SA+TP
Left testes weight (g)	0.73 ± 0.075[a]	0.80 ± 0.130b	0.69 ± 0.077c	0.79 ± 0.088d
Left testes length(cm)	2.40 ± 0.071a	2.30 ± 0.168b	2.10 ± 0.058c	2.08 ± 0.125d
Left testes diameter(cm)	3.03 ± 0.111a	3.15 ± 0.240b	2.93 ± 0.120c	2.90 ± 0.071d
Right testes weight (g)	0.80 ± 0.10a	0.78 ± 0.13b	0.68 ± 0.07c	0.72 ± 0.10d
Right testes length(cm)	2.13 ± 0.025a	2.23 ± 0.125b	2.10 ± 0.058c	2.48 ± 0.293d
Right testes diameter (cm)	2.93 ± 0.048a	2.85 ± 0.185b	3.03 ± 0.067c	3.08 ± 0.165d

Values are expressed as mean±SEM. Means with the same superscript within a row are significantly different (p<0.05). Control= Olive oil, SA= Sodium arsenite, TP= Tridax procumbens.

Table 4: Epididymal morphometry

PARAMETERS	CONTROL	SA	TP	SA+TP
Left epididymal weight (g)	0.18 ± 0.043[a]	0.19 ± 0.036[b]	0.13 ± 0.018[c]	0.19 ± 0.014[d]
Left epididymal length (cm)	3.43 ± 0.217[a]	3.40 ± 0.248[b]	3.43 ± 0.120[c]	3.65 ± 0.144[d]

Values are expressed as mean±SEM. Means with the same superscript within a row are significantly different (p<0.05). Control= Olive oil, SA= Sodium arsenite, TP= Tridax procumbens.

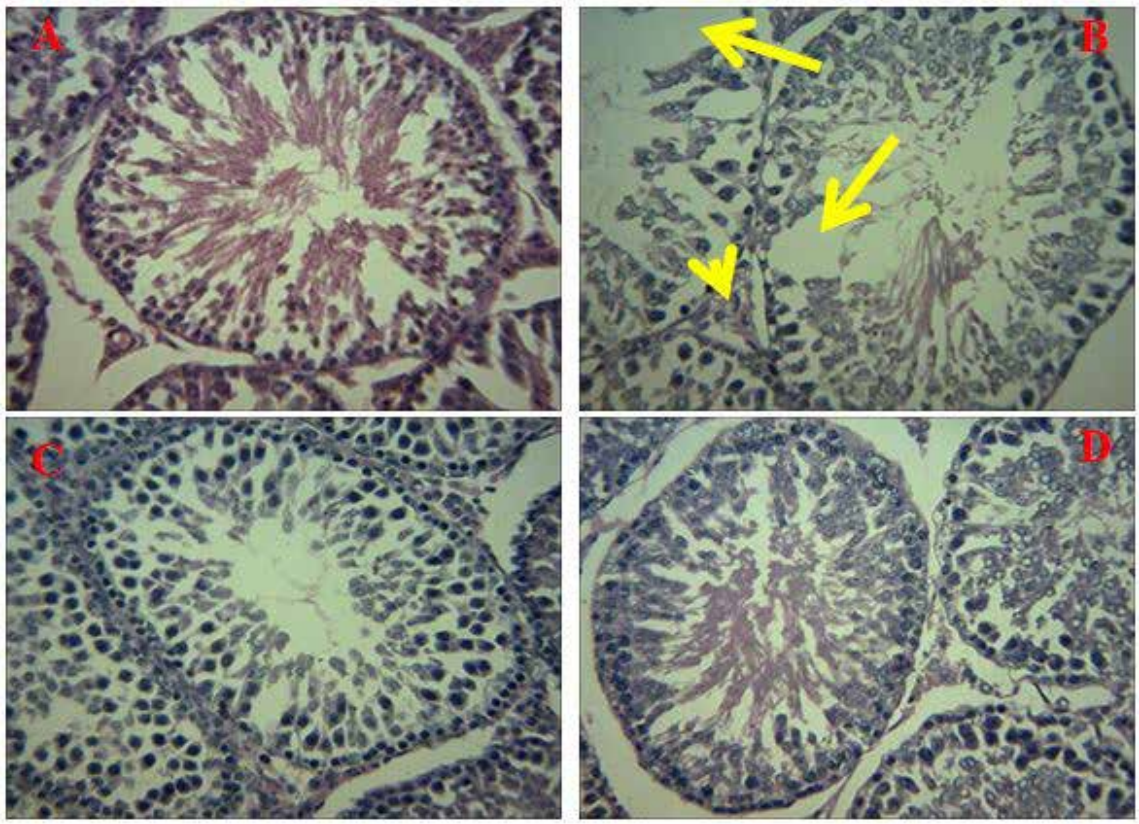

Figure 1: Testicular histology of rats in control and treated groups.
A (control): No visible lesions seen. B (sodium arsenite):There is a mild interstitial congestion (arrow head) and erosion of the germinal cells (arrow), C (extract): No visible lesions seen. D (extract plus sodium arsenite): No visible lesions seen. Mag x400.

compared to the negative control group (Table 2) and the total abnormal cells were found to be within the normal range of 10% to 20% (Table 2) (Bishop *et al.,* 1949; Zemjanis, 1977; Hafez, 1993).

Moreover, there was no significant difference ($p>0.05$) between the group mean values (Groups A to D) for both testicular and epididymal morphometry (Table 3 and Table 4). This is contrary to earlier report by Olayemi *et al.* (2011); that observed testicular degeneration and necrosis of the seminiferous epithelium in their experimental albino rats' model. Ola-Davies *et al.* (2014) also reported a significant reduction in testicular weight, testicular length, left epididymal weight, right and left epididymal length in their treated rats. Their findings also support the report of Simmon *et al.* (1995) in his extract treated male albino rats. This pathology as seen in the study of Olayemi *et al.* (2011), Ola-Davies *et al.* (2014) and Simmon *et al.* (1995) can interfere with sperm storage, transport and maturation function of the epididymis and this may lead to infertility. Therefore, Tridax procumbens might not have the potential to interfere with any of those functions.

Histological changes of the testes were observed (Figure 1). The sodium arsenite caused pathological effects on the testicular interstitial and germinal cells (Group B). However, the extract was able to ameliorate these toxic effects when co-administered with the sodium arsenite in Group D (Figure 1). This finding is in line with the earlier reported non-significant difference in mean values of the sperm morphological parameter of the extract treated group as compared to the control in this study. However, this observation does not corroborate the report of Saba *et al.* (2009). In their study, the plant extract of Lagenaria breviflora roberts used, exerts toxic effect on the seminiferous tubular epithelium of the testes. Furthermore, Adedapo *et al.* (2007) also reported that, the aqueous extract of Abrus precatorious, when administered to male rats, subsequently caused disruption of testicular architecture that was characterized with reduction in the number of epithelium cells.

Conclusion

It can be concluded that the leaf extract of Tridax procumbens seem toxic to the sperm cells rather than the epididymal and testicular morphology. Therefore, caution should be exercised on the therapeutic application of Tridax procumbens in man and as feed for animals.

Conflict of Interest

The authors declared no conflict of interest.

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IN VITRO ACARICIDAL ACTIVITY OF *CALLISTEMON VIMINALIS* AND *CUPRESSUS LUSITANICA* LEAF ESSENTIAL OIL AGAINST *AMBLYOMMA VARIEGATUM* TICK.

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Abstract

Tick infestations are among the main constraints to livestock productivity in sub-Saharan Africa but their control is tedious. Thus, a study on the acaricidal effect of two indigenous plants extracts on *Amblyomma variegatum* tick was carried out in the western highland of Cameroon. The leaf essential oil (EO) of *Callistemon viminalis* and *Cupressus lusitanica* were obtained by hydrodistillation and incorporated into soap. Three concentrations (0.16, 0.22 and 0.27 µL per gram of soap) and a control (soap without EO) with three replications for each treatment were used for *in vitro* trial. Each replication consisted of 10 ticks put into contact with a filter paper impregnated with soap foam and placed at the bottom of a Petrie dish. The mortality rate was significantly higher ($P<0.05$) from day 2 up to the end of the trial for both EO compared with control for the highest concentration (0.27µL/g). On day 8, the mortality rate for control was 16,6±5,7% whereas the highest concentration of *C. lusitanica* EO killed 96.2±6.4% of ticks. In contrast, the highest concentration of *C. viminalis* killed 100% of ticks from the fourth day of exposure, suggesting that the speed of action was faster for *C. viminalis* EO than for *C. lusitanica* EO. The LC_{50} for *C. viminalis* EO and *C. lusitanica* EO were 0.77 and 1.05 µL/g respectively, indicating that *C. viminalis* EO is more toxic than EO from *C. lusitanica*. The soap based on the EO from both plants is a promising alternative tool in tick control.

Key words: acaricidal effect, essential oil; *Callistemon viminalis*; *Cupressus lusitanica*; *Amblyomma variegatum*.

ACTIVITÉ ACARICIDE *IN VITRO* DE L'HUILLE ESSENTIELLE DE FEUILLES DE *CALLISTEMON VIMINALIS* ET *CUPRESSUS LUSITANICA* CONTRE LES TIQUES *AMBLYOMMA VARIEGATUM*

Résumé

Les infestations de tiques sont parmi les principales entraves à la productivité animale en Afrique Sub-saharienne, mais leur contrôle est fastidieux. Ainsi, une étude sur l'effet acaricide de deux extraits de plantes indigènes sur la tique *Amblyomma variegatum* a été réalisée dans les hautes terres occidentales du Cameroun. L'huile essentielle (HE) des feuilles de *Callistemon viminalis* et *Cupressus lusitanica* a été obtenue par hydrodistillation et incorporée au savon. Trois concentrations (0,16, 0,22 et 0,27 µL par gramme de savon) et un témoin (savon sans HE) avec trois répétitions pour chaque traitement ont été utilisés pour un essai in vitro. Chaque répétition était constituée d'un lot de dix tiques dans une boîte de Pétri tapissée de papier filtre uniformément imprégné de la mousse de savon. Le taux de mortalité était significativement plus élevé ($P <0,05$) à partir du jour 2 jusqu'à la fin de l'essai pour les deux HEs comparativement au lot témoin pour la concentration la plus élevée (0,27 µL / g). Au jour 8, le taux de mortalité pour le lot témoin était de 16,6 ± 5,7% tandis que la concentration la plus élevée de l'HE de *C. lusitanica* tuait 96,2 ± 6,4% des tiques. En revanche, la concentration la plus élevée de l'HE de *C. viminalis* a tué 100% des tiques à partir du quatrième jour d'exposition, ce qui fait penser que la vitesse d'action était plus rapide pour l'HE de *C. Viminalis* par rapport à l'HE de *C. lusitanica*. La LC50 pour l'HE de *C. Viminalis* et l'HE de *C. lusitanica* était respectivement de 0,77 et 1,05 µL / g, ce qui porte à croire que l'HE de *C. Viminalis* est plus toxique que l'HE de *C. lusitanica*. Le savon à base d'HE des deux plantes est un outil alternatif prometteur dans la lutte contre les tiques.

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Mots-clés : effet acaricide, huile essentielle ; *Callistemon viminalis*; *Cupressus lusitanica*; *Amblyomma variegatum*.

Introduction

Livestock industry is vital in the economy of developing countries. It is the main source of income for pastoralists, and a source of meat and milk for the entire population. Currently, livestock is one of the fastest growing agricultural subsectors in Cameroon with a growth rate estimated at 5.2 %; its share of agricultural GDP is estimated at 25 per cent (MINEPIA, 2013). However, the output of livestock is challenged by a number of constraints including poor feeding, social habits and ill-health among others (Yapi, 2007). Poor health in livestock is due to a variety of determining and supporting factors. Tick infestation is among these factors. Ticks affect the production of over 100 million cattle in the world and are among the most economically important ectoparasites of livestock in the tropics, including Sub-Saharan Africa (SSA) (Uilenberg, 1995). As blood sucking parasites, their direct pathogenic effect on livestock consist of anemia, stress, reduction in weight gain and milk yield, unrest, depreciation of hide value, hypersensitivity, mechanical irritation and toxicosis among others (Jongejan and

Uilenberg, 2004) while the indirect pathogenic effect is associated with their role as vector of pathogens to animals (Biryomumaisho *et al.,* 2012). These effects result in reduced production and poor productivity leading to economic losses (Beugnet *et al.,* 1994). Among Ticks, *Amblyomma variegatum* is one of the most devastating species of livestock causing both a direct and indirect pathogenic effect on livestock (Stachurshki, 2010). It is widely distributed in tropical area and found almost everywhere in Cameroon (Mamoudou *et al.,* 2015).

Tick control under large scale production system relies on the use of acaricides, burning of cattle pasture, cultivation of infested lands, improved drainage among other techniques (Jongejan and Uilenberg, 2004; Taylor *et al.,*2007). On the other hand, while poor local breeders control these ticks by manual removal, others still apply medicinal plants or use both methods (Pullan, 1980; Maina, 1986; Dharani *et al.,* 2015). Though the techniques used in intensive large scale production have yielded positive results, they are very expensive and environmentally unfriendly (Jongejan and Uilenberg, 2004; Taylor *et al.,* 2007). Thus, new alternative control strategies appear imperative.

In this line, it seems advantageous to use natural substances such as essential oil (EO) extracted from some plants, and shown to possess some therapeutic properties. Reports on the biological properties of these essential oils indicate that they are acaricidal, anti-pyretic, bactericidal, insecticidal, antiseptic and anti-inflammatory (Pamo *et al.,* 2003; Ndomo *et al.,* 2009; Mohamad and Ghaytha, 2016).

The leaves of *Callistemon viminalis*, widely found in tropical and subtropical regions (Ndomo *et al.,* 2009), have been documented to contain essential oils that are toxic to insects (Ndomo *et al.,* 2009). Similarly, the tree *Cupressus lusitanica*, originated from southern America and central America (Orwa *et al.,* 2009), is widely distributed in tropical Africa; the leaves of this tree have been shown to be toxic to insects (Bett *et al.,* 2016). Though these plants are reported to be toxic to insects, nothing is known about their acaricidal effect. Therefore the objective of this study was to assess the acaricidal effect of the EO from these plants on *Amblyomma variegatum* tick.

Material and Methods

Study Area

The study was carried out in the Western High Lands of Cameroon, between 5° and 6° North Latitude and between 10° and 11° East Longitude. The mean altitude of the region is 1420 m. The climate is equatorial. In this zone, rainfall varies between 1500 and 2000mm/year. Annual temperatures vary between 10°C in July and 25°C in February.

There are two main seasons in the region: a short dry season running from mid-November to mid-March and a long rainy season (corresponding to cultural season) from mid-March to mid-November (Pamo *et al.,* 2005). Subsistence agriculture, together with breeding and trade are the main economic activities of the region. The vegetation is the savannah with shrub, and sparse forests in some areas (Pamo *et al.,* 2004).

Extraction of essential oils of Callistemon viminalis and Cupressus lusitanica

Leaves of each plant were harvested, taken to the Laboratory and immediately pounded before extraction. Leaves of the plant were harvested in September (the rainy season) in the vicinity of the University of Dschang. *C. viminalis* was harvested at the flowering stage while *C. lusitanica* was harvested before its flowering stage. Oil was extracted by the hydrodistillation technique (Kuiate, 1993) using a modified Clevenger type apparatus. The technique consisted of placing the mixture of pounded plant leaves and water into a hot plate for eight hours. The EO was obtained through evaporation. The mixture of oil and water vapor condensed in the distillation apparatus, where cool water circulates permanently. A two-layered distillate with the upper part being the essential oil was collected into a graduated cylinder tube associated to the distillation apparatus. After allowing water to flow out through a tap, the oil was then collected in a container using the following formula:

$$\textbf{\textit{Yield}}(\%) = \frac{\textbf{\textit{weight of essential oil}}}{\textbf{\textit{weight of the plant material}}} \; \textbf{\textit{x}}\; \textbf{100}$$

The chemical composition of the oils from *C. lusitanica* and *C. viminalis*, as previously described by several authors is presented in table 1 and 2 respectively. The plant *C. viminalis* used in this study and the one used by Ndomo *et al.,* (2009) were both harvested in the same area. In contrast, the plant *C. lusitanica* was not harvested in the same area as those used by the authors referred to in table 1.

The Production of soap

In order to apply essential oils on tick, soap was chosen as vehicle as soap foam has been shown to be a cheap and efficient means of applying EO on ticks both *in vitro* and in vivo (Miégoué *et al.,* 2013; Kouam *et al.,* 2015). To produce the soap, solutions of soda and sikalite were mixed in a container and allowed to stand for 15 minutes. After this, palm oil was added and mixed by turning the preparation in the same direction for 15 minutes without resting during which essential oil was added. The final mixture was then put into appropriate moulds and left on the ground in darkness for seven days for solidification.

In vitro tests

Ticks used for *in vitro* tests were collected from the cattle held at the Teaching and Research Farm (TRF) of the University of Dschang located in the Western High Lands of Cameroon. The harvesting was done manually without any distinction of sexes and with great care, avoiding the destruction of their rostrum. Ticks were weighed on a scale of the type "kern" with a capacity of 120 g and a sensitivity of 0.001g. Their length was also measured using a millimeter paper. The average weight and length were 0.06 ± 0.01g and 7.5 ± 0.5 mm respectively. Selected adult ticks were without any preliminary treatment now identified as *Amblyomma variegatum* as previously described (Walker *et al.,* 2013). Once identified, ticks (adults) were ready for the tests.

The soap produced for bioassays had a weight of 450 g and contained 900 µl of essential oil, i.e. a concentration of 2 µL/g. The EO from each plant was used separately to produce the bioassay soap. To obtain applicable concentrations for *in vitro* tests, several dilutions of soap were made to find the most efficient concentration. The following concentrations were selected: 0.16, 0.22, and 0.27µL/g of soap. Soap without EO was used as control.

Tests consisted of the evaluation of *in vitro* toxicity by contact of soap foam on ticks. The concentrations obtained above were applied on ticks. With a 5 ml pipette, the solution was uniformly distributed in Petri

Table 1: Major chemical compounds of *Callistemon viminalis* leaf essential oil

Number of identified compounds	Major compounds	Percentage (%)	Reference
27	1,8-cineole	61,25	Mohamad *et al.,* 2016
	α-pinene	10,94	
	α-terpinol	9,73	
	p-cymene	5,88	
17	1,8-cineole	66,36	Gohar *et al.,* 2014
	α-pinene	20,43	
	α-terpinol	6,65	
13	1,8-cineole	58,49	Ndomo *et al.,* 2009
	3-carene	8,61	
	limonene	7,01	
	α-terpinol	5,83	

Table 2: Major chemical compounds of *Cupressus lusitanica* leaf essential oil

Number of identified compounds	Major compounds	Percentage (%)	Reference
49	Germacrene D	18,5	Ngo Teke *et al.,* 2013
	epi-zonarene,	8,2	
	cis-calamenene	8,2	
	terpinen-4-ol	6,3	
	linalool	6,0	
	umbellulone	6,0	
/	umbellulone	18,38	Bett *et al.,* 2016
	α-pinene	9,97	
49	α-pinene	40-82	Hassanzadeh *et al.,* 2010
	limonene	4-18	
	isobornyl acetate	10	
	cis-muurola-4(14),5-diene	7	

dishes with an area of 55.4cm^2, in which a round filter paper (Type Whatman No 1 with a diameter of 8.4cm) had already been placed. Each treatment had three replicates made of 10 ticks introduced in one of the above Petri dishes. Counting of dead ticks was done every 24 hours for eight days. The mortality rate in each dish was calculated following the Abott method (Abott, 1925) according to the following formula:

$$M_c = (M_0 - M_e) / (100 - M_e)$$ Where

M_0: mortality of ticks registered in treated replicates

M_e: mortality of ticks in the control

M_c: corrected mortality of ticks (%)

The LC50 was determined by the Finney Method (1971) based on the regression of the probit of the mortality depending on the logarithm of essential oil concentration.

Statistical Analysis

Data obtained were analyzed using the ANOVA test (Mc Clave and Dietrich, 1979) after correction of observed mortalities in relation to those of the control, and the differences between treatments when they existed, were separated at 5% significance level by Student "t" test. The results were expressed as means ± standard deviation

Results

Yield of extraction

The yield of the oil extraction was 0.035 and 0.067 % for *C. viminalis* and *C. lusitanica* respectively.

In vitro effects of the essential oil-based soap from C. viminalis leaves on A. variegatum

The effects of the essential oil depending on its concentration are presented in Fig. 1 and Table 3. From this figure, the mortality rate of *A. variegatum* increased both with the concentration of EO in the soap (µL/g) and with time. This mortality was maximal (100%) on the fourth day for the highest concentration, 0.27 µL/g. For the concentration 0.16 and 0.22 µL/g, the highest mortality on the 8th day was 40 % and 96.67 % respectively. Until the fourth day, no mortality was recorded in the control. The mortality remained weak in the control until the last day of the trial (16.67% on the 8th day). The difference in mortality between treated replicates and the control showed the toxicity of EO from *C. viminalis* leaves contained in soap on *A. variegatum*.

Data in table 3 showed some significant differences between treatments for different concentrations. At the end of the first day of exposure, no significant difference was observed between control and other treatments. On the second and third day, the mortality was significantly higher ($p<0.05$) for the highest concentration (0.27µL/g) compared with other concentrations, except for concentration 0.22 µL/g on the third day. The mortality in the treated groups was significantly different from each other on the fourth day but the lowest concentration was not significantly different from the control. This trend continued until the eighth day of the trial.

The adjustment of corrected mean cumulative mortalities (Abott, 1925) in relation to the concentration of the EO contained in the soap with time was used to draw a regression line (Fig. 2) with the equation $Y= 670.33X-70.571$ ($R2=0.8325$), where y is the mortality rate, X the concentration of treatment, and R2 the determination coefficient). The transformation of mortality rates into probits at the end of the second day in view of evaluating the LC_{50} permitted us to obtain the probit regression line in relation to the logarithm of concentration of *C. viminalis* EO with the equation $Y= 9.923X+6.0813$ ($R^2 = 1$) where y= probit of mortality rate and X= the concentration of treatments. From this regression line, it appeared that on the second day of exposure, LC_{50} is 0.77µL/g, thus confirming the toxicity of *C. viminalis* EO on *A. variegatum*.

In vitro effects of the essential oil-based soap from C. lusitanica leaves on A. variegatum

Data on the effects of the EO are presented in Fig. 3 and Table 4. As illustrated in Fig. 3, the cumulative mortality percentages increased with the concentration of treatment, and with time. The highest mortality (96.67%) was obtained with the highest concentration (0.27µL/g) on the fifth up to the eighth day of exposure. On the eighth day, the highest mortality for concentration 0.16 and 0.22 µL/g was 46.67 and 80% respectively. Until the end of the trial, the highest mortality for the control was 16.67%. This observation is an indication that EO from *C. lusitanica* leaves is toxic to *A. variegatum*.

No significant difference in the mortality was observed on the first day of exposure but on the second day, the mortality was significantly higher ($p<0.05$) for the highest concentration (0.27µL/g) compared with the lowest concentration (0.16µL/g) and control (0.0µL/g) (Table4). On the third day, the highest mortality was still obtained with the highest concentration (0.27µL/g), followed by concentration 0.22µL/g. On the

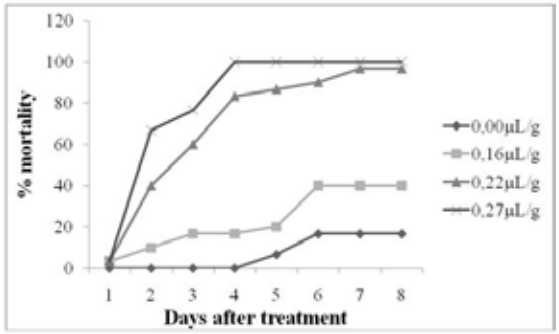

Figure 1: *In vitro* cumulative mortalities of *Amblyomma variegatum* exposed to foam soap containing various concentrations of *Callistemon viminalis* leaf essential oil (0.00µL/g is the control; 0.16, 0.22 and 0.27µL/g are the concentrations being tested in the study)

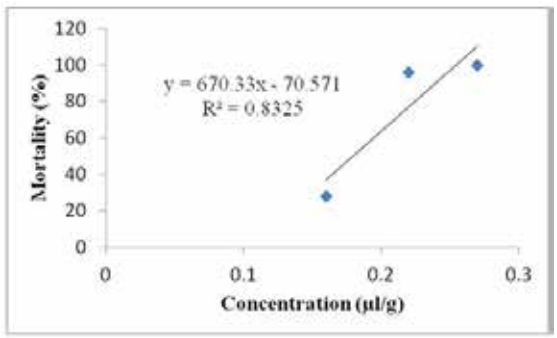

Figure 2: Evolution of mean corrected and cumulative percentage of *in vitro* mortality of *Amblyomma variegatum* in relation to the concentration of *Callistemon viminalis* leaf essential oil contained in the soap (y is the mortality rate, x the concentration of essential oil and R2 the determination coefficient)

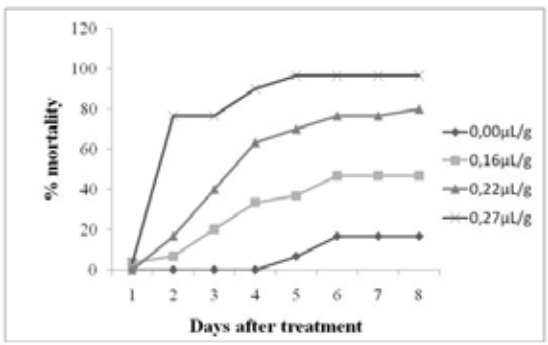

Figure 3: *In vitro* cumulative mortalities of *Amblyomma variegatum* exposed to foam soap containing various concentrations of *Cupressus lusitanica* leaf essential oil (0.00µL/g is the control; 0.16, 0.22 and 0.27µL/g are the concentrations being tested in the study)

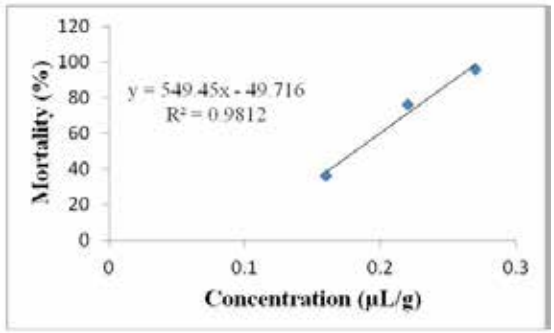

Figure 4: Evolution of mean corrected and cumulative percentage of *in vitro* mortality of *Amblyomma variegatum* in relation to the concentration of *Cupressus lusitanica* leaf essential oil contained in the soap (y is the mortality rate, x the concentration of essential oil and R2 the determination coefficient)

fourth day the mortality was significantly higher for concentration 0.22µL/g than for concentration 0.16µL/g. Up to this fourth day, no mortality was recorded for control. The sixth day, the mortality was still higher for the highest concentration in comparison to other concentrations. This trend continued until the end of the eighth day.

The adjustment of corrected mean cumulative mortalities (Abott, 1925) in relation to the concentration of the EO contained in the soap with time was used to draw a regression line (Fig.4) with the following equation Y= 549.45X-49.716 (R2=0.9812). The transformation of mortality rates into probits at the end of the second day in view of evaluating the LC50 was used to draw the probit regression line in relation to the logarithm of concentration of EO from *C. lusitanica* with the equationY= 8.3461X+4.8096 (R^2 = 1). From this regression line, it appeared that on the second day of exposure, LC50 is 1.05µL/g, thus confirming the toxicity of *C. lusitanica* EO on *A. variegatum*.

Table 3: Abbott-corrected cumulative mortality (%) of *Amblyomma variegatum* submitted to different concentrations of the leaf essential oil of *Callistemon viminalis*

Days after treatment	Concentrations (µL/g)			
	0,0 cM[1]±s.d[2]	0,16 cM±s.d	0,22 cM±s.d	0,27 cM±s.d
1	0,0±0,0[a]	3,33±5,7[a]	3,33±5,7[a]	3,33±5,7[a]
2	0,0±0,0[a]	10,0±17,3[a]	40,0±26,4[a]	66,6±30,5[b]
3	0,0±0,0[a]	16,6±11,5[a]	60,0±26,4[ab]	76,6±25,1[b]
4	0,0±0,0[a]	16,6±28,8[a]	83,3±5,7[b]	100,0±0,0[c]
5	6,6±11,5[a]	16,6±28,8[a]	85,8±5,2[b]	100,0±0,0[c]
6	16,6±5,7[a]	26,8±27,9[a]	87,9±0,8[b]	100,0±0,0[c]
7	16,6±5,7[a]	26,8±27,9[a]	87,9±0,8[b]	100,0±0,0[c]
8	16,6±5,7[a]	26,8±27,9[a]	96,2±6,4[b]	100,0±0,0[c]

[1]= Cumumative mortality
[2]= Standard deviation
[a,b,c]= For each row, values with different superscripts are statistically different (P<0.05).

Table 4: Abbott-corrected cumulative mortality (%) of *Amblyomma variegatum* submitted to different concentrations of the leaf essential oil of *Cupressus lusitanica*

Days after treatment	Concentrations (µL/g)			
	0,06 cM[1]±s.d[2]	0,16 cM[1]±s.d[2]	0,22 cM[1]±s.d[2]	0,27 cM[1]±s.d[2]
1	0,0±0,0[a]	3,3±5,7[a]	3,3±5,7[a]	3,3±5,7[a]
2	0,0±0,0[a]	6,6±11,5[a]	16,6±28,8[ab]	76,7±20,8[b]
3	0,0±0,0[a]	20,0±20,0[ab]	40,0±20,0[bc]	76,7±20,8[c]
4	0,0±0,0[a]	33,3±5,7[b]	63,3±5,7[cd]	90,0±17,3[d]
5	6,6±11,5[a]	33,3±20,8[b]	67,5±10,8[c]	95,8±7,2[d]
6	16,6±5,7[a]	37,5±37,5[ab]	71,7±8,1[b]	95,8±7,2[c]
7	16,6±5,7[a]	37,5±37,5[ab]	71,7±8,1[b]	95,8±7,2[c]
8	16,6±5,7[a]	37,5±37,5[ab]	75,9±12,6[b]	95,8±7,2[c]

[1]= Cumumative mortality
[2]= Standard deviation
[a,b,c]= For each row, values with different superscripts are statistically different (P<0.05).

Discussion

The yield of the oil extraction was 0.035 and 0.067 % for *C. viminalis* and *C. lusitanica* respectively. The yield of the oil extraction for the fresh leaves of *C. viminalis* was lower than the yield reported by Mohamad and Ghaytha (2016) and Gohar et al.(2014) which were 0.28 % and 0.7 % respectively. Similarly, the extraction yield for the fresh leaves of *C. lusitanica* (0.067%) was lower than the yield obtained by Pamo et al.(2003). This difference can be explained by many factors including method of distillation, climate conditions between harvest sites, plant maturity, plant part, and the period during which the plant was harvested. Indeed, hydrodistillation can some time lead to loss of some quantity of EO during purification while, plant harvested in dry season can produce more oil than in rainy season (Kouam *et al.,* 2015). Besides yield, the other anti tick factors normally vary with season of collection and method of extraction hence efficacy of two different extracts collected during different seasons cannot be compared.

Soap based on EO from *C. viminalis* turned out to be toxic for *A. variegatum*. The toxicity may be attributed both to the

predominant presence of terpen compounds in the EO, and to tick sensitivity towards these compounds. The toxicity might be due to an attack by 1,8-cineole, of the central nervous system and some vital organs as a result of the inhibition of some enzyme activity such as acetylcholinesterase and nitric oxide synthetase. 1,8-cineole is one of the main compound of this EO (Hu *et al.,*2015); In contact with mange mite, 1,8-cineole has been shown to suppress the activity of enzymes including acetylcholinesterase and nitric oxide synthase (Hu *et al.,* 2015). Thus, tick death might have occur as a result of 1,8-cineole neurotoxicity. The toxicity might also be attributed to the action of α-terpinol, another major compound of the EO from *C. viminalis*. This compound, an antagonist to octopamine receptor, has been shown to kill insects including cockroaches (Fan, 2010) through modulation of octopamine receptors. α-terpinol may act the same way in ticks by modulating the octopamine receptors. Nevertheless, toxicity of this oil could not be attributed to 1,8-cineole and α-terpinol activity only but also to a synergistic action between these compounds, other EO compounds and soap ingredients.These results are in agreement with those of Ndomo et al.(2009) who showed that the insecticide effect of the EO from *C. viminalis* on Acanthoscelides obtectusne might be due to both the major and minor compounds of the EO.

Soap containing EO from *C. lusitanica* was found to be toxic for *A. variegatum*. The toxicity of this soap towards *A. variegatum* might be attributed to monoterpene compound (linalool, terpene 4-ol) of which the insecticide effects have been documented (Ryan and Byrne, 1998; Abdelaziz and Regnault-Roger, 2013). Indeed linalool and terpene 4-ol are both inhibitor of acetylcholinesterase (Ryan et Byrne, 1988; Regnault-Roger, 2008). However, the toxicity of the soap could be due to a possible synergistic action between these compounds, other EO components and soap ingredients, notably soda. This finding agree with the result of Bett et al. (2016) who showed that adult Acanthoselides obtectus are very sensitive to EO from *C. lusitanica* leaves.

The high value of the determination coefficient (0.83 and 0.98) for both EO showed that a large proportion (83% and 98%) of the variation of the corrected cumulative mortality rates is only due to the effect of treatment.

A. variegatum ticks were significantly affected by both EO from the second day of exposure for the highest concentration (0.27 µL/g). From the second day of exposure up to the trial end (eighth day), the mortality of ticks was significantly different from control but never reached 100% for *C. lusitanica* EO while in contrast, all ticks died from the fourth day for *C. viminalis* EO.. This observation suggests that the speed of action was faster for *C. viminalis* EO than for *C. lusitanica* EO. This could be explained by the difference in the chemical composition of both EO which share just some compounds including α-pinene (Bett *et al.,* 2016; Mohamad *et al.,* 2016). An additional explanation could be the difference in the susceptibility of ticks to EO similar to the phenomenon observed with insecticides for which factors affecting the speed of action of the insecticides have been shown to include the difference in susceptibility to insecticides (Koehler *et al.,* 1991, 1993).This finding about *C. viminalis* EO action speed is consistent with the finding of Ndomo et al. (2009) who observed 100 % mortality in Acanthoscelides obtectus insect exposed to *C. viminalis* EO on the fourth day.

The LC50 of soap based on *C. viminalis* EO (0.77µL/g) and of soap based on *C. lusitanica* EO (1.05µl/g) showed that soap based on EO from *C. viminalis* is more toxic than soap based on EO from *C. lusitanica*.This is probably related to the difference in the chemical composition of both EO, the EO from *C. viminalis* containing compound such 1.8-cyneole and α-terpinol (Ndomo *et al.,*2009; Gohar *et al.,* 2014; Mohamad *et al.,* 2016) not present in EO from *C. lusitanica* (Hassanzadeh *et al.,* 2010; Ngo Teke *et al.,*2013; Bett *et al.,* 2016).

Conclusion

Soaps containing EO of *C. viminalis* and *C. lusitanica* leaves were toxic to *A. variegatum*.

Mortality rates of ticks increased gradually with increasing EO concentration and exposure time. The obtained results suggest that soap based on EO from both plants is a promising alternative tool in tick control. However, in vivo trials to evaluate the field application of this tool will have to be done first. Low LC50 obtained (0.77 µL/g and 1.05 µL/g) for both soaps showed the high toxicity of the applied treatment. The analysis of the chemical composition and the study of the acaricidal effects of the different fractions of the EO of these plants will permit us to improve on our knowledge of the toxic effects of our treatment. It will be equally important to determine with accuracy the active ingredient of the applied treatments, and to study the safety of plant extract on the host animal.

Conflict of Interest:

The authors declare that they have no conflict of interest

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HAEMATOLOGY, SERUM BIOCHEMISTRY AND GROWTH PERFORMANCE OF PREGNANT KALAHARI RED GOATS FED CONCENTRATE DIETS AT THREE PROTEIN LEVELS IN NIGERIA

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Abstract

Haematological and biochemical indices of domesticated animals like goat can give some insight into their performance potentials. High performing does have the tendency of producing healthy kids with reasonable weight at birth compared to least performing does. A study was conducted to investigate the haematology, serum biochemistry and growth performance of grazing pregnant Kalahari Red does fed concentrate diets at three protein levels. A total of 33 dry primiparous Kalahari Red does between 2-21/2 years with an average body weight of 38.10±1.13kg were randomly allotted to three treatments consisting of 11 does per treatment for a feeding trial that lasted for 153 days. Concentrate diets with 3 crude protein (CP) levels i.e. Low Protein Diet- LPD (12.42% CP at 124.93gday-1), Medium Protein Diet- MPD (14.18% CP at 145.87gday-1) and High Protein Diet- HPD (16.35% CP at 168.19gday-1) were fed to the does at 3% of their body weight from mating till kidding. Data obtained were subjected to analysis of variance in a completely randomized design at 5% probability level using SAS® 9.1 Statistical package. Does fed MPD recorded highest value ($p<0.05$) for white blood cells (20.50x109/l) and serum albumin (2.86g/dl). It was observed at the end of the study that pregnant Kalahari Red does fed MPD recorded highest values ($p<0.05$) for weight gain (20.57kg); net weight gain (10.73kg); daily weight gain (135.87gday-1); and daily weight gain less foetal and afterbirth weights (71.06gday-1) compared to other does. From the results of this study, it can be concluded that dietary supplementation of grazing pregnant Kalahari Red does with concentrate diet containing 14.18% CP improved the serum albumin and had the best performance characteristics.

Keywords: Rhodes Grass, Grazing, Low Protein Diet, Medium Protein Diet, High Protein Diet

HEMATOLOGIE, BIOCHIMIE DE SERUM ET PERFORMANCE DE CROISSANCE DE CHEVRES KALAHARI ROUGES GESTANTES SOUMISES A DES REGIMES DE CONCENTRÉS À TROIS NIVEAUX DE PROTÉINES AU NIGERIA

Résumé

Les indices hématologiques et biochimiques d'animaux domestiques comme les chèvres peuvent donner une idée de leur potentiel de performance. Les chèvres hautement performantes ont tendance à produire des chevreaux en bonne santé ayant un poids raisonnable à la naissance, contrairement aux moins performantes. Une étude a été réalisée pour examiner l'hématologie, la biochimie sérique et la performance de croissance des chèvres Kalahari rouges gestantes au pâturage, soumises à des régimes de concentrés à trois niveaux de protéines. Au total, 33 Kalahari rouges primipares âgées de 2-21/2 ans avec un poids corporel moyen de 38,10 ± 1,13 kg ont été réparties de manière aléatoire à trois traitements

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composés de 11 chèvres chacun pour un essai alimentaire d'une durée de 153 jours. Des régimes de concentrés avec 3 niveaux de protéines brutes (CP), c.-à-d. un régime à faible teneur en protéines - LPD (12,42% CP à 124,93gday-1), un régime à teneur protéique moyenne - MPD (14,18% CP à 145,87gday-1) et un régime à teneur protéique élevée -HPD (16,35 % CP à 168,19gday-1) ont été donnés aux chèvres à 3% de leur poids corporel, depuis l'accouplement jusqu'à la mise bas. Les données obtenues ont été soumises à une analyse de variance selon un schéma entièrement aléatoire à un niveau de probabilité de 5% utilisant le progiciel statistique SAS® 9.1. Les chèvres nourries au MPD ont enregistré la valeur la plus élevée ($p < 0,05$) de globules blancs (20,50x109 / l) et d'albumine sérique (2,86 g / dl). L'on a noté à la fin de l'étude que les Kalahari rouges gestantes nourries avec MPD avaient enregistré les valeurs les plus élevées ($p < 0,05$) pour le gain pondéral (20,57 kg), le gain pondéral net (10,73kg), le gain pondéral quotidien (135.87gday-1), et le gain pondéral journalier moins les poids fœtal et post-natal (71.06gday-1) par rapport aux autres chèvres. Sur la base des résultats de cette étude, on peut conclure que la supplémentation des Kalahari rouges gestantes au pâturage par un régime concentré contenant 14,18% de CP a amélioré l'albumine sérique et a produit les meilleures caractéristiques de performance.

Mots-clés : herbe de Rhodes, pâturage, régime à faible teneur protéique, régime à teneur protéique moyenne, régime à forte teneur protéique

Introduction

Blood is known to be vital to the life of animals. This is because it is a medium through which nutrients are conveyed to various parts of the body system of a live animal. A readily available and fast means of assessing clinical and nutritional status of an animal on feeding trial can be the use of blood analysis (Olabanji *et al.,* 2007). Haematological parameter is also an important and reliable medium used to monitor and evaluate health and nutritional status of animals (Gupta *et al.,* 2007). It therefore becomes imperative to evaluate blood parameters of an animal particularly when unconventional feeds are fed to animals in order to determine the performance characteristics of such experimental animals as well as suitability of the feed fed on the specie of livestock.

A study was conducted to evaluate the response of 33 dry primiparous Kalahari Red does grazing on Rhodes grass (Chloris gayana) receiving supplementation with concentrate diets at three different protein levels to haematology, serum biochemistry and growth performance.

The Kalahari Red is an indigenous breed of goat that originates from South Africa with several characteristics such as adaptation to arid and semi-arid savannah, good foraging and excellent mothering abilities which are indicators that the goat will be of productive and reproductive uses to farmers if reared on the farm. It is regarded as a "minimum care/ maximum profit" breed (Ramsay *et al.,* 2001). The performance of goats is a major determinant of productivity and economic viability of commercial goat farms (Mellado *et al.,* 2006) especially during pregnancy. The reproductive process is regulated by genetic and environmental factors (Guerra *et al.,* 2011; Notter, 2012) such as feeding regime and other management practices.

Some of Kalahari Red goats were imported from South Africa to the Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR), Federal University of Agriculture, Abeokuta, Nigeria in September, 2011 for improvement of Nigerian indigenous goats in terms of meat and milk production. In order to manage the Kalahari Red goats efficiently and to also properly utilise them for the purpose of importation there is need to know their dietary requirements for optimal performance since little or no information is available on these, most especially reproductive and growth performances. Therefore, this study was conducted to investigate the haematology, serum biochemistry and growth performance of pregnant Kalahari Red does fed concentrate diets at three protein levels in south-western Nigeria.

Materials and Methods

Site of the Experiment

The study was conducted at the Kalahari Red Goat Unit of Livestock Production Research Programme under Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR), Federal University of Agriculture Abeokuta (FUNAAB), Ogun State, Nigeria. The region is 76m above the sea level and falls within latitudes 7o18'2"N and 7o18'30"N; and longitude 3o22'10"E and 3o22'41"E. The climate is humid and located in the forest zone of South-Western Nigeria. The mean annual precipitation and the temperature are 1,330mm and 29.30C respectively, with an average relative humidity of 80% throughout the year (ORBDA, 2013).

Animal Management and Experimental Procedure

The study was conducted with the use of 33 matured Kalahari Red does. The does were dry and primiparous with age range of 2 to 21/2 years. The average live body weight of the does at the start of the study was 38.10±1.13kg. Flushing of the does was done two weeks before the commencement of the study with the experimental diets. Flock treatment was also carried out by the veterinary doctors on the farm two weeks before mating was done in order to ensure that only does that were in good health conditions were used. Oxytetracycline 20% LA (Oxytetracycline 200mg/ml as dihydrate) was administered intramuscularly at 1ml per 20kg bodyweight, while Vita-Strong® Injection was administered intramuscularly at 1ml per 10kg bodyweight as vitamin supplement and anti-stress. Ivanor® Ivermectin Injection (Ivermectin Injection 10mg/ml) was also administered subcutaneously at 1ml per 50kg bodyweight to control gastro-intestinal worms, fly larvae, lice, ticks and mites.

Experimental animals used were selected from Kalahari Red flock of 68 dry does. Three healthy, experienced and vigorous Kalahari Red bucks were used to detect goats on heat. The average weight of the bucks was 44.70kg within 2-21/2 years. To ensure that only does on heat were selected, three (3) experienced and proven vigorous Kalahari Red bucks were introduced into the flock to detect does on heat. Does that exhibited signs of heat were removed from the flock, and then placed in a holding pen. The selected does were ear-tagged and randomly assigned to three pen houses used for the study. Each of the pen houses measured 18.79m x 5.15m out of which 9.09m lengthwise was covered with slates as shed against adverse weather conditions. The floor of the pens is cemented, with a slight slope to ease draining of urine and other liquid substances. There was also perimeter fencing round the pen houses with strong iron net to confine and restrict the movement of the does. The surrounding of the pen houses was kept clean always, with bush cut to ground level at all time. Weight of individual doe was determined using Avery weigh-Tronix® Digital scale before they were pen-mated. The mating was done by introduction of 3 Kalahari Red bucks into the pen houses (i.e. a buck per pen). Proper observation was done to ensure that mating was successful before data collection commenced.

The does, after mating were fed experimental concentrate diets at three levels of protein (CP) which were- Low Protein Diet- LPD (12.42% CP; 14.95 MJ/kg DM), Medium Protein Diet- MPD (14.18% CP; 15.64 MJ/kg DM) and High Protein Diet- HPD (16.35% CP; 15.60 MJ/kg DM) as supplements to forage (Chloris gayana) on the paddock which resulted to daily CP intakes of 124.93gday-1; 145.87gday-1; and 168.19gday-1 from the respective diet. LPD, MPD and HPD were fed to the pregnant does at 3% of their body weight on dry matter basis. Locally available ingredients such as Maize; Unpeeled Cassava Root Meal; Wheat Offal; Palm Kernel Cake, Groundnut Cake, Bone Meal; Salt and Premix were used to compound the experimental diets (Table 1). Each experimental diet was fed to eleven (11) pregnant does.

Feeding Trial

The pregnant does were fed for a period of 153 days (22 weeks). Live weight

of each doe was taken at the beginning of the experiment (when mating was confirmed successful) for three consecutive days and the mean weights were recorded as initial weight. Thereafter, individual animal was weighed on weekly basis before feeding throughout the experimental period. Final live weight of each doe was taken on week 22 i.e. within 24 hours prior to kidding and within 24 hours after kidding.

Table 1: Gross compositions (%) of experimental diets fed to Kalahari Red does in Nigeria

	Experimental concentrate diets		
Ingredients	**Low Protein**	**Medium Protein**	**High Protein**
Maize	10.0	10.0	10.0
Unpeeled Cassava Root Meal	50.0	19.5	1.0
Wheat Offal	10.0	30.0	50.0
Palm Kernel Cake	24.0	34.5	28.0
Groundnut Cake	1.5	1.5	6.5
Bone Meal	3.0	3.0	3.0
Salt	1.0	1.0	1.0
*Premix	0.5	0.5	0.5
Total (kg)	100	100	100

**contains Vitamin A (I.U.) 10,000.000; Vitamin D2 (I.U.) 2,000,000; Vitamin E (I.U) 20,000; Vitamin K (mg) 2,250; Riboflavin (mg) 5000; Pyridoxine (mg) 275; Biotin (mg) 50; Pantothenic acid (mg) 7500; Vitamin B1 (mg) 175; Vitamin B12 (mg) 15.0; Niacin (mg) 27,500; Folic acid (mg) 7500. Choline Chloride (mg) 400; Antioxidant (mg) 125; Fe (g) 20.0; Zn (g) 50.0; Mn (g) 80.0; Cu (g) 5.0g; I (g) 12.0; Co (mg) 200; Se (mg) 200.*

Table 2: Nutrient composition of experimental concentrate diets and Rhodes grass (Chloris gayana) fed to pregnant Kalahari Red goats

	Experimental concentrate diet			
Parameters (%)	**LPD**	**MPD**	**HPD**	**Rhodes grass**
Dry Matter	88.00	90.00	90.00	39.00
Crude Protein	12.42	14.18	16.35	3.24
Ether Extract	7.49	4.50	8.44	0.15
Ash	7.38	6.03	8.19	3.33
Nitrogen Free Extract	57.23	63.29	53.38	29.39
Organic Matter	92.62	93.97	91.81	96.67
Neutral Detergent Fibre	54.67	55.72	57.22	58.92
Acid Detergent Fibre	42.46	40.32	41.73	46.72
Acid Detergent Lignin	23.36	25.96	25.63	24.82
Hemicellulose	12.21	15.40	15.49	12.20
Cellulose	19.10	14.36	16.10	21.90
*ME (MJ/kg DM)	14.95	15.64	15.60	14.56

** Calculated using MAFF (1984) equation*

LPD- Low Protein Diet; MPD- Medium Protein Diet; HPD- High Protein Diet; ME- Metabolisable Energy

The does were fed the experimental diets (i.e. concentrates) throughout the feeding trial twice a day. Half of the portions was fed in the morning by 8:00h and the remaining half in the evening by 16:30h. The does after feeding on concentrate diets were allowed to graze on an established 16Ha sole-pasture planted with Rhodes grass (Chloris gayana) by 10:00h. This allowed some level of sunshine before grazing commenced. By 16:00h, the does were returned, then the remaining portion of the concentrate diets were offered. Total concentrate refused each day was recorded before fresh concentrates were offered the following day. Fresh and clean drinkable water was made available to the does daily ad libitum.

Aliquots were taken from experimental concentrate diets and Rhodes grass to determine nutrient composition of the samples. The samples were oven-dried at 600C for 3days until constant weight was obtained, then milled with industrial blender into fine particles. The blended samples were kept until when needed for laboratory analysis. The nutrient composition was determined by following the procedure of A.O.A.C. (2005) at Food Processing Laboratory in the Department of Food Science and Technology, Federal University of Agriculture Abeokuta, Nigeria. The nutrient composition of the concentrate diets fed and Rhodes grass grazed upon by the pregnant Kalahari Red goats is presented in Table 2.

Data Collection

Haematology and Serum Biochemistry

At week 21 of the feeding trial, blood samples were collected from each of the experimental animals via jugular vein punctured with new hypodermic needle fitted on a new 10ml calibrated syringe in the morning before feeding. 10ml of blood sample was collected from each of the pregnant does, out of which 5ml was put in a bottle containing Ethylene Diamine Tetra-acetic Acid (EDTA) as anti-coagulant for haematological analysis for parameters such as white blood cells, red blood cells, haemoglobin, haemocrit (packed cell volume), mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and red blood cells distribution width. The remaining 5ml of blood collected was put in a plain bottle without anti-coagulant for serum biochemistry for parameters like albumin, globulin, total protein and glucose.

Haematological analyses on the blood collected were conducted in the Department of Animal Physiology Laboratory, FUNAAB using Auto-Haemoanalyzer. While the serum biochemistry concentrations were determined in the Department of Veterinary Physiology and Pharmacology Laboratory, FUNAAB using spectrophotometer. Total protein was determined spectrophotometrically according to the method of Tietz (1995) as described in Randox Kit total protein manual; Serum Albumin Determination was measured spectrophotometrically according to the method of Doumas et al. (1971); and Serum glucose was assayed spectrophotometrically without deproteinization according to the method of Barham and Trinder (1972) as described in Randox Glucose kit manual.

Serum globulin was determined as follows:

Serum Globulin= Total Protein- Serum Albumin

Performance

The following data were collected on the performance characteristics of the does. Different weight parameters were taken using an Avery Weigh-Tronix® Digital scale.

i. Initial weight of the does: this was the weight of the experimental does taken at mating;
ii. Final weight of the does: this was the weight of the does taken within 24 hours before kidding;
iii. Does' Live-weight after Kidding: this was the weights of the does taken within 24 hours after kidding. Before the weight was taken, it was ensured that the placenta, umbilical cord together with other foetal membranes like allantois, chorion and amnion (together with amniotic fluid) were expelled;
iv. Live-weight Changes of Does during

Gestation: this was determined by taking the initial weight of the does at mating followed by subsequent weighing of the pregnant does weekly until the last doe kidded on the 22nd week (153rd day) of the study;

v. Feed consumption and refusal: this was determined by taking the weight of concentrates remaining each day throughout the experimental period. The difference between total concentrate remaining and total concentrate fed daily gave feed consumed per day;
vi. Gestation Length of the Does: this was estimated by determining length of time (in days) between successful mating and kidding for each of the does fed the experimental diets;
vii. Litter Weight: this was obtained by taking weight(s) of all the new-born kid(s) per doe together after their bodies were dried off either by natural air or with the use of dry towel to absorb fluid on their bodies within 24 hours after kidding;
viii. Afterbirth Weight: This was the weights of placenta, umbilical cord and foetal membranes such as allantois, chorion and amnion (together with amniotic fluid) that were expelled from uterus after kidding. This was determined by taking the weights of does that were about to kid (within 24 hours before kidding) and the weights of does that kidded within 24 hours post-kidding (after the expulsion of placenta). The resultant difference between the weight before kidding and doe's weight within 24hrs post-kidding plus litter weight per doe gave the afterbirth weight.

Performance Characteristics Formulae

Weight gain (kgW-0.75)= [(Initial Weight (kg) + Final weight (kg))/2]0.75

Initial Weight (kgW-0.75)= (Initial Weight (kg))0.75

Final Weight (kgW-0.75)= (Final Weight (kg))0.75

Weight Gain (kg)= Final weight (kg)- Initial weight (kg)

Weight Gain (gday-1)= (Weight gained (g)) / Gestation Length (days)

Gross weight gain (kg)= Weight within 24hrs before kidding (kg)-Weight at Mating (kg)

Net weight gain (kg)= Weight within 24hours after kidding (kg)- (Weight at Mating (kg))

Weight gain less foetal and afterbirth weights (gday-1)= (Net weight gain (g)) / (Gestation Length (days))

Afterbirth Weight (kg)= Weight within 24hours before kidding (kg) - (weight within 24hours after kidding (kg) + Litter weight within 24hours but after drying (kg))

Data Analysis

Data collected at the end of the study were subjected to One-way Analysis of Variance (ANOVA) by following the procedure of SAS® 9.1 Statistical package (SAS, 2002). Levels of significance were taken at 5% probability, while the significant means were separated using Duncan's Multiple Range Test (Duncan, 1955).

Results and Discussion

Blood parameters on Haematology and serum biochemistry of pregnant Kalahari Red does fed concentrate diets at three protein levels are presented in Tables 3 and 4. The concentrate diets exerted significant effects ($p<0.05$) on white blood cells and albumin across the dietary treatments. There was not significant effect of the diets on other blood parameters analysed for. White blood cell range for this study was 16.90-20.50 x109/l; with a mean value of 18.51 x109/l, while albumin range of between 2.10-2.86g/dl with a mean value of 2.57g/dl was obtained. Medium and high protein diets had the highest and lowest values for these blood parameters. Radostits et al. (2000) reported that clinically normal (but not pregnant) goats should have white blood

Table 3: Haematology of pregnant Kalahari Red does fed concentrate diets at three protein levels

	Concentrate diets			
Parameters	**LPD**	**MPD**	**HPD**	**SEM**
Haematology				
White Blood Cells (x109/l)	18.14[ab]	20.50[a]	16.90[b]	0.68
Red Blood Cells (x1012/l)	14.54	13.29	15.28	0.42
Haemoglobin (g/l)	76.60	76.60	81.75	2.44
Haemocrit (%)	23.00	23.48	24.73	0.93
Mean Corpuscular Volume (fl)	15.80	17.80	16.23	0.57
Mean Corpuscular Haemoglobin (pg)	5.20	5.72	5.30	0.12
Mean Corpuscular Haemoglobin	335.00	328.40	332.25	6.30
Concentration (g/l)				
Red Blood Cells Distribution Width (%)	24.38	24.44	25.18	0.41

[ab] Means on the same row having different superscripts were significantly different (p<0.05)
LPD, Low Protein Diet; MPD, Medium Protein Diet; HPD, High Protein Diet; SEM, Standard Error of Means

Table 4: Serum biochemistry of pregnant Kalahari Red does fed concentrate diets at three protein levels

	Concentrate diets			
Parameters	**LPD**	**MPD**	**HPD**	**SEM**
Albumin (g/dl)	2.76ab	2.86a	2.10b	0.15
Globulin (g/dl)	1.90	1.88	1.94	0.09
Total Protein (g/dl)	4.66	4.74	4.04	0.16
Glucose (mg/dl)	69.40	65.00	76.20	4.17

[ab] Means on the same row having different superscripts were significantly different (p<0.05)
LPD, Low Protein Diet; MPD, Medium Protein Diet; HPD, High Protein Diet; SEM, Standard Error of Means

cell range of 4.0–13.0 x109/l which implied that the increase in white blood cells might be as a result of pregnancy, this was corroborated by Waziri et al. (2010) who reported that white blood cells increases as pregnancy progresses. Contrary to the result obtained for white blood cells, Waziri et al. (2010) reported a lower value (13.08 x106/µl) for pregnant Sahel goats at week 20 of pregnancy. Hefnawy et al. (2011) reported a higher albumin value of 37.6g/l for some pregnant goats. The reason for these variations may be attributed to the management systems (Olayemi *et al.,* 2009), ages and breeds (Addass *et al.,* 2010) of goats used. In addition, increase in the white blood cells could be due to increase in the bone marrow activity as well as pregnancy stress (Waziri *et al.,* 2010).

The Growth Performance characteristics of pregnant Kalahari Red does fed concentrate diets at three protein levels is presented in Table 5. Kalahari Red does supplemented with MPD was most superior in terms of performance characteristics. This was because it recorded highest values for all the performance parameters taken such as weight gain, net weight gain, and daily weight gain. When crude protein (CP) in excess of what is required by ruminants is supplied through the diet, there will be a resultant loss of nitrogen through the faeces and urine (Oderinwale, 2014). In addition to this, pregnant Kalahari Red does fed MPD recorded best crude protein digestibility, nitrogen retention and digestibility according to Oderinwale (2014). This suggested that does fed MPD were able to utilise the concentrate diet for necessary body metabolic activities,

thus improved performance characteristics of the does. The initial live weight of the does before pregnancy and weight of the does within 24hours before kidding were not statistically different, but does assigned to LPD were marginally heaviest at mating compared to other dietary supplementations. It was observed that dietary concentrate treatments influenced weight taken within 24hours post-kidding. Some authors (Akingbade *et al.,* 2001; Rastogi *et al.,* 2006) reported that weights taken within 24hour post-kidding were not affected. This may be due to initial weights and nutrient utilisation by the does used in these studies. Gross weight gain range of 15.18-20.57kg was obtained. Medium and high protein diets recorded the highest and lowest values respectively. Akingbade et al. (2001) and Rastogi et al. (2006) reported gross weight gains of 9.71-12.57kg and 4.0-7.2kg respectively, where does placed on higher protein diets recorded the highest values in their respective studies. Similarly, some authors (Bawala *et al.,* 2006; Olomola *et al.,* 2008) reported lower values of gross weight gains for pregnant West African Dwarf goats. The values reported by these authors were lower than what was obtained for this study; the reason may be attributed to variation in the period the final weights were taken.

Net weight gain of the does (i.e. gross weight gain less products of pregnancy) ranged from 7.08 to 10.73kg, the value which was highest and lowest for medium and high protein diets fed does respectively. This improvement in net gain was possible because does placed on MPD recorded the lowest values for all pregnancy variables especially afterbirth weight (Oderinwale *et al.,* 2016). The reduction in these pregnancy variables indicated that there was a nutrient balance between the does and the developing foetuses for maternal body tissues development. The does were gaining more body weight simultaneous as the pregnancy advanced. Akingbade et al. (2001) and Rastogi et al. (2006) reported lower values which ranged from -0.50 to 2.94kg and 1.3 to 2.9kg in their respective studies. This may be due to variation in breeds, and higher weights of products of pregnancy obtained (in comparison to weights of the does) in their studies. This is because weight of products of pregnancy is dependent on the breed and weight of the dam. Heavy breeds of goat with much weight tend to have much weight for products of pregnancy and vice versa. Daily weight gain

Table 5: Growth Performance characteristics of pregnant Kalahari Red does fed concentrate diets at three protein levels

	Experimental concentrate diets			
Parameters	**LPD**	**MPD**	**HPD**	**SEM**
Initial live weight (kg)	40.68	37.34	36.20	1.13
Final live weight (kg)	57.53	57.91	51.38	1.48
Live weight within 24hours post-kidding	49.22^{a}	48.07^{ab}	43.28^{b}	1.15
Weight gain (kgW-0.75)	15.33	14.91	14.11	0.28
Initial live weight (kgW-0.75)	16.10	15.08	14.75	0.34
Final live weight (kgW-0.75)	20.87	20.97	19.19	0.41
Gross weight gain (kg)	16.85^{ab}	20.57^{a}	15.18^{b}	0.97
Net weight gain (kg)	8.54	10.73	7.08	0.74
Daily weight gain (gday-1)	111.76^{ab}	135.87^{a}	100.57^{b}	6.43
Daily weight gain less foetal and afterbirth weights (gday-1)	56.56^{b}	71.06^{a}	46.89^{c}	3.00

^{abc} *Means on the same row having different superscripts were significantly different ($p<0.05$)*
LPD- Low Protein Diet; MPD- Medium Protein Diet; HPD- High Protein Diet; SEM- Standard Error of Means
***Initial weight** is live weight at buck's introduction; **Final weight** is live weight taken within 24hrs before kidding*

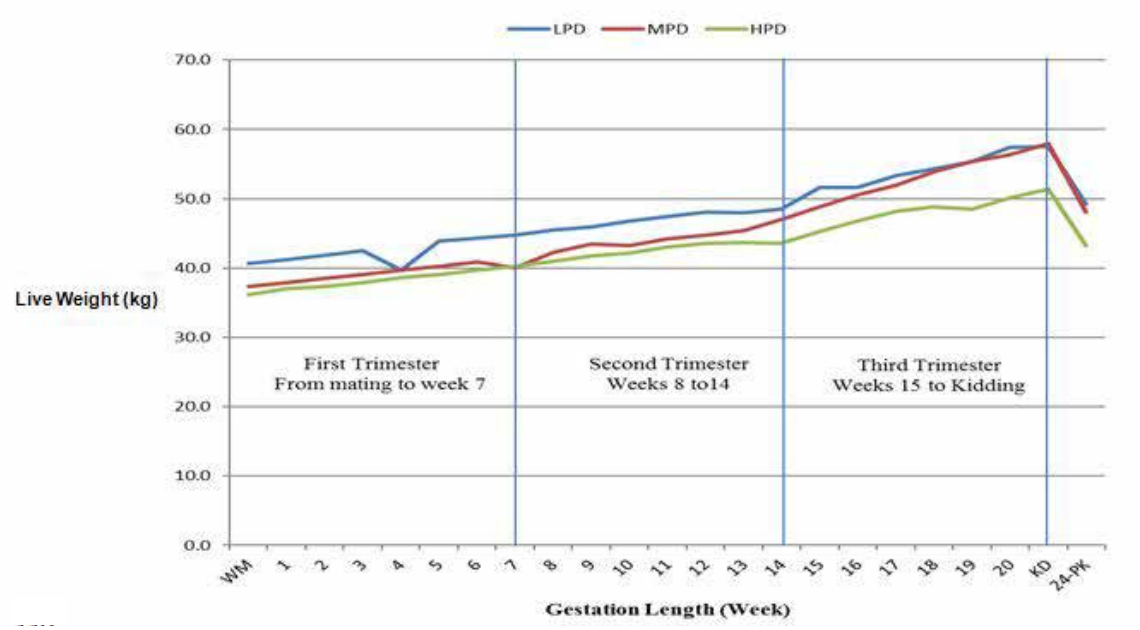

Figure 1: Weight changes from mating to kidding and within 24hour post-kidding of pregnant Kalahari Red does fed concentrate diets at three protein levels

range of 100.57-135.87gday-1 was obtained for the does. Does fed medium and high protein diets recorded highest and lowest values respectively. Similarly, average daily weight gain (less foetal and afterbirth weights) of between 46.89-71.06gday-1 was obtained, where does supplemented with medium and high protein diets also recorded highest and lowest values respectively.

Figure 1 shows weight changes from mating to kidding and within 24hour post-kidding of pregnant Kalahari Red does grazing on Rhodes grass (Chloris gayana) supplemented with three concentrate diets. The usual changes in the live weight during gestation are often assumed to be indicative of prenatal development of foetus(es) (Amoah *et al.,* 1996). Therefore changes in the weight of pregnant does can be used to monitor the development of foetus(es) and health of the does. However, reductions at some points in time during pregnancy may be due to some factors other than nutrition and health of the doe which are unexplainable. Weight changes of the does from mating to kidding and within 24hours after kidding for this study showed that weight gain across pregnancy was progressive in nature, which produced a non-linear graph. This indicated that at a point in time, weight of the does either dropped or same value recorded which picked up later in subsequent week(s). For low protein (12.42%) diet supplemented does, there were reductions in weight at weeks 4 (from 42.5kg to 39.7kg), 13 (from 48.1kg to 48.0kg), 15 and 16 (51.6kg, no weight gained). Furthermore, for does fed medium protein (14.18%) diet, weeks 7 (from 40.9kg to 40.0kg), and 10 (from 43.5kg to 43.2kg) experienced reductions in their gross weight gain. Weeks 12-14 recorded same weight (43.6kg) and there was a reduction in weight at week 19 (from 48.9kg to 48.6kg) for does supplemented with high protein (16.35%) diet. This weight changes from mating until kidding were similar with the results of other authors (Dayeh *et al.,* 1996; Akingbade *et al.,* 2001) who reported graphically, non-linear nature of pregnancy graphs and reductions in weight gain during pregnancy.

LPD- Low Protein Diet; MPD- Medium Protein Diet; HPD- High Protein Diet; SEM- Standard Error of Means

WM- Weight taken at Mating; KD- Weight taken at Kidding; 24-PK- Weight taken

within 24hours Post-Kidding

Conclusion

It can be concluded based on the results obtained from this study that pregnant Kalahari Red does grazed on Rhodes grass (Chloris gayana) with medium protein diet (14.18% CP; 145.87gday-1) supplementation recorded the highest serum albumin with best performance in terms of weight gains which are indicators of improved milk and meat yields of the animals.

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Conflict of Interest Statement:

The authors declare that they have no conflict of interest.

Statement of Animal Rights:

All applicable International, National, and Institutional guidelines for the care and use of animals were followed in the conduct of this research.

Informed Consent:

Informed consent was obtained from all individual participants included in this study.

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PREVALENCE OF RUMINAL AND RETICULAR FOREIGN BODIES IN CATTLE SLAUGHTERED AT JIMMA MUNICIPAL ABATTOIR, SOUTH WESTERN ETHIOPIA

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Abstract

A cross-sectional study was conducted from November, 2015 to April, 2016 at Jimma municipal Abattoir, Oromia Regional State, with the objectives of to assess the prevalence of rumen and reticulum foreign bodies, identify types of foreign bodies and associated risk factors for the occurrences of foreign bodies. Both antemortem and postmortem examinations were employed to examine the live animal and for the recovery of foreign bodies from rumen and reticulum after slaughter, respectively. The study animals were selected by using simple random sampling. From the total of 384 local male animals examined, 47(12.23%) were found positive for the occurrence of foreign bodies in rumen and reticulum. From these plastics (5.5%), cloth with rope (2.9%), wire with cloth (1.8%), wire (1.0%), wire with rope (0.5%) and nail (0.5%) were the positive cases, respectively. Prevalence of foreign bodies occurrence recorded in age groups were 7.93%, 11.56% and 20.75% in young, adult and old, respectively with highly statistically significant difference (P>0.05). The prevalence rate recorded within body condition scores were 15%, 10.20% and 14.06% for poor, medium and good body condition, respectively with non-significant difference in prevalence within body condition scores (P>0.05). The highest frequencies of foreign bodies observed in cattle originated from Gomma district (63.8%) while the lowest from Seka district (4.2%). From the total prevalence of 47(12.23%), 72.3%, 12.7% and 14.8% were observed from rumen, reticulum and both rumen and reticulum, respectively. And rumen harbored mostly plastic materials while reticulum was the major site for the retention of metallic objects. It is concluded that, detection of the foreign bodies in forestomach suggested as health risk to ruminants and contributes a lot for reduced production. Therefore, appropriate solid waste disposal system need to implement in the study area to prevent health risk of ruminants and also to protect the environment.

Key words: Abattoir, Cloth, Cross-sectional study, Metals, Plastic, Risk factors, Rope, Wire

Introduction

Ethiopian's livestock population is often said to be the largest in African. Excluding the Afar and Somali regions there were approximately 45.57 million cattle, 26.1 million sheep, 21.7 million goats, 2.1 million horses and mules, 5.6 million donkeys, 1 million camel and 39.6 million poultry. For the latter two regions, estimated numbers vary greatly between conventional and aerial censuses, but total less than 15% of the non-nomadic regions (CSA, 2009).

Ethiopia has great potential for increased livestock production, both for local use and for export. However, expansion was constrained by inadequate nutrition, disease, lack of support services and inadequate information on how to improve animal breeding, marketing and processing. Thus, the country is not utilizing this huge potential livestock resource and an improvement in this sector. Therefore, has the potential to contribute significantly to national income and to the welfare of the majority of rural families. The high concentration of animals in the high lands, together with the fact that cattle are often kept for status, reducing the economic potential of Ethiopia livestock (CSA, 2009).

Cattle play significant contribution in Ethiopian economy as source of meat, milk, drought power, income and foreign exchange. However, as other livestock in the country their contribution is below their expected potential due to prevalent livestock diseases, poor management system and poor genetic performance (Abebe, 1995).

Gastrointestinal foreign bodies are among the most common surgical emergency in veterinary medicine. Cattle are more susceptible to foreign body syndrome than small ruminants because they do not use their lips for prehension and are more likely to eat chopped feed; lack of oral discrimination in cattle may lead to ingestion of foreign bodies would be rejected by other species (Desiye and Mersha, 2012).

Traumatic reticuloperitonitis (TRP) is a relatively common disease in adult cattle caused by the ingestion and migration of a foreign body in the reticulum. The typical foreign body is a metallic object, such as a piece of wire or a nail, often greater than 2.5 cm in length. The majority of affected cattle (87%) are dairy cattle and 93% are older than 2 years of age. It has been hypothesized that dairy cattle are more commonly affected than beef cattle since they are more likely to be fed a chopped feed, such as silage or hay (Hailat *et al.,* 1996). A large number of adult dairy cattle have metallic foreign bodies in their reticulum without signs of clinical disease. It is likely that a predisposing factor in otherwise normal cows, such as tenesmus or a gravid uterus, causes migration of the foreign body into the reticular wall (Rebhun *et al.,* 1995).

Ingestion of foreign body in cattle is result a condition of great economic importance and causes severe loss of production and high mortality rate. The ingestion of foreign body is mainly related with nutritional deficiencies and feeding management and cause various problem in different organ of the animal, mainly in rumen and reticulum. The problem that are caused vary with the duration that the foreign body has been present, the location of the foreign body, the degree of obstruction that is caused as well as problems associated with the material of the foreign body. Ruminant are notorious for ingestion of foreign bodies. The disease of rumen and reticulum are great economic importance because of severe losses on productivity of the animals sometimes leading to the death of the animals (Radostits *et al.,* 2007).

Entrance and migration of foreign bodies through the body tissues lead to many complications that differ according to the nature of the foreign body and the way of its entrance in to the tissues. Traumatic reticuloperitonitis (TRP) relatively common disease in cattle caused by the ingestion of foreign bodies in the reticulum swallowed metallic objects such as nail or pieces of wire fall directly on the reticulum or pass into the rumen and subsequently carried over the rumeno-reticular folds in to the cranioventral part of the reticulum (Jones *et al.,* 1997).

Nonmetallic foreign bodies in the reticulo-rumen cause recurrent rumen tympani in adult dairy cattle, over a period of time, these materials, form large tight balls inside the rumen leading to anorexia decreased production and progressive loss of body condition (Jafarazadeh *et al.,* 2004). The presences of foreign bodies in the rumen and reticulum also hamper the absorption of volatile fatty acids (VFA) and consequently reduction in the rate of animal fattening. The perforation of the wall of the reticulum allows leakage of ingesta and bacteria which contaminates the peritoneal cavity, resulting in local or diffuse peritonitis is the swallowed objects can also penetrate pleural cavity causing pleuritis and pneumonitis and into the pericardial sac causing pericarditis (Cavedo *et al.,* 2004). The condition is serious in our country usually in urban and peri-urban areas where extensive building are carried out and proper plastic material disposal is no conditioned and so thrown on roads and near the fence or anywhere and that is way our dairy cattle are dying mainly associated with foreign bodies (Ramaswamy and Sharama, 2011).

In Ethiopian formation regarding the magnitude and occurrence of fore stomach foreign bodies is very limited. The fact that rumen impaction by these foreign bodies is mainly a symptomatic in nature and only diagnosed in live animals if the material is accumulated in large amount and thus, it can be adequately studied in abattoirs (Desiye and Mersha, 2012).

Therefore the objectives of this study were to assess the prevalence of rumen and reticulum foreign bodies in cattle slaughtered at Jimma municipal Abattoir and to identify the prevalence and type of rumen and reticulum foreign bodies and to study the risk factors associated with the ingestion of those foreign bodies in cattle.

Materials and Methods

Study Area

The study was conducted at Jimma municipal Abattoir, the zone located at southwestern of Ethiopia in Oromia Regional State. The town is located at about 352 km away from Addis Ababa in South western direction. Geographically the town is located at latitude of about 7°13' -8°56' N and longitude of about 35°52' - 37°37' E and at elevation ranging 880-3360 meter above sea level. Ecologically the area lies in wet land ecosystem and area receives a mean annual rainfall of about 1530ml, which comes from long and short rainy seasons. The annual minimum and maximum temperature is about 14.4 and 26.7°c, respectively. Jimma district have livestock population of 18,354 bovine, 846 caprine, 3,310 ovine and 1,490 equine.

Study Animals

The study was conducted on 384 male apparently healthy slaughtered cattle at Jimma municipal Abattoir from November, 2015 to April, 2016. The animals were local breed, which are originated from various localities. The geographical origin of all animals slaughtered at Jimma municipal Abattoir brought from Gomma, Dedo, Seka, Kersa districts. Age, body condition and breed were considered as risk factors for occurrence of foreign bodies. During the study time the animals were categorize into three based on age ≤5 years (young), 5-10 years (adult) and ≥10 years (old) and also grouped based on body condition as poor, medium and good. Estimation of age was carried out by examination of the teeth eruption using the approach forwarded by De Lahunta and Habel (1986). The body condition scoring was classified into three categories as according to Nicholson and Butterworth (1986).

Study Design

A cross sectional study was conducted to assess the prevalence of the rumen and reticulum foreign body's and to identify the types of foreign bodies and their associated risk factors. Out of total cattle slaughtered at Jimma municipal Abattoir during the study period, 384 animals were selected and examined by using simple random sampling method.

SamplingTechnique and Sample Size Determination

Simple random sampling technique was employed to select the study animals and rumen and reticulum of individual animals were examined. To determine the sample size, the expected prevalence in the study area was assumed to be 50% at 95% confidence interval because of absence of previous study. Therefore, the sample size was calculated based on the formula given by Thrusfield (2005) and 384 were sampled.

Study Methodology

Antemortem examination

Antemortem examination on individual animals was done for assessment of age, and body condition. Age was categorized into young, adult and old based on dentition pattern and body condition also poor, medium and good. Each animal selected for the study was further identified by providing a unique identification number that could be used for both ante-mortem and post-mortem examinations of the animal and each animals mark for the identification by writing a code on its gluteal muscle by using ink.

Postmortem examination

In the postmortem examination rumen and reticulum was examined immediately after slaughter in the evisceration stage, the stomach was carefully removed from the abdominal cavity and rumen and reticulum were thoroughly examined by visual inspection and palpation with open and explore for the prevalence of any foreign non dietary material by visualization and palpation. All the contents were examined thoroughly for the presence of foreign bodies. Any foreign bodies were obtained during inspection washed with water to remove adhering feed material and identify type of foreign bodies. When the finding was positive, the location and type of the foreign bodies was recorded otherwise recorded as negative in postmortem record sheet.

Data Analysis

The data obtained was coded in Microsoft excel and subjected to descriptive statistics and chi square (X2) in order to assess the magnitude of the difference of comparable variables using SPSS version 20.0 software. Pearson chi square (X2) test was employed to assess the existence of association between prevalence of the foreign bodies and different potential risk factors considered. For (X2) test, P-value < 0.05 was considered significant. The total prevalence of rumen and reticulum foreign bodies was calculated as percentage by dividing total number of positive cattle for foreign bodies to the total number of cattle examined.

Results

Occurrence

From the total of 384 male cattle examined for the presences of any foreign bodies in their rumen and reticulum 47 (12.24%) of them were found positive for foreign bodies. The types of foreign bodies were plastic, wire with rope, wire with cloth, wire, clothe with rope and nail.

Prevalence of foreign body in relation to age

Age wise prevalence of the foreign bodies was observed and its rate was 5 (7.93%), 31 (11.6%) and 11(20.75%) in young, adult and old equines, respectively. And the prevalence was found to be highly statistically significant (P>0.05) (Table1).

Prevalence of foreign body with regard to body condition scores

From total of 384 cattle 60,196 and 128 were categorized under with poor, medium and good body condition scores, 9 (15%), 20 (10.02%) and 18 (14.06%) were positive for foreign bodies, respectively with nonsignificant difference (P >0.05) among the body condition scores and the occurrences of foreign bodies (Table2).

Prevalence of Foreign Body with Regard to animals' origin (districts)

Table 1: Age wise distribution of foreign bodies in rumen and reticulum of cattle at Jimma Municipal Abattoir

Age	Type of foreign bodies							
	Negative for foreign bodies	Plastic	Wire with rope	Wire with clothe	Wire	Cloth with rope	Nail	Total
Adult	237(88.4%)	16(6%)	0(0%)	7(2.6%)	2(0.7%)	5(1.9%)	1(4%)	268
Young	58(92.1%)	1(1.6%)	2(3.2%)	0(0%)	0(0%)	2(3.2%)	0(0%)	63
Old	42(79.2%)	4(7.5%)	0(0%)	0(0%)	2(3.8%)	4(7.5%)	1(1.9%)	53
Total	**337(88.8%)**	**21(5.5%)**	**2(0.5%)**	**7(1.8%)**	**4(1.0%)**	**11(2.9)**	**2(0.5%)**	**384**

$\chi 2 = 28.111, P = 0.005$).

Table 2: Occurrence of foreign bodies based on body condition score in cattle at Jimma Municipal Abattoir

Body condition score	Plastic	Wire with rope	Wire with clothe	Wire	Clothe with rope	Nail	Total
Poor	3(5%)	0(0%)	2(3.3%)	1(1.7%)	3(5%)	0(0%)	60
Medium	11(5.6%)	1(5%)	4(2%)	1(0.5%)	3(1.5%)	0(0%)	196
Good	7(5.5%)	1(8%)	1(8%)	2(1.6%)	5(3.9%)	2(1.6)	128
Total	**21(5.5%)**	**2(0.5%)**	**7(1.8%)**	**4(1.0%)**	**11(2.9%)**	**2(0.5)**	**384**

$\chi 2 = 10.015, P=0.615$

Animals for which the postmortems were conducted and slaughtered were come from Gomma, Seka, Dedo, kersa districts. The highest frequencies of rumen and reticulum foreign bodies were observed in cattle originated from Gomma (63.8%) while the lowest from Seka (4.2%) statistically with significant difference (P< 0.05) (Table 3).

Prevalence of Foreign Bodies with Regard to Location Site

From total 47 positive cases of foreign body, 34(72.3%) were occurred in rumen while 6(12.7%) in reticulum and 7(14.8%) in both rumen and reticulum and rumen harbored mostly plastic materials while reticulum was the major site for the retention of metallic objects. Statistical analysis revealed that a highly significant difference (P< 0.05) was observed among different stomach compartments and in the occurrences of foreign bodies (Table-4).

Discussion

Ingestion of indigestible foreign

Table 3: Origin of animals was found determining the frequency of occurrence of rumen and reticulum foreign body in cattle slaughtered at Jimma Municipal abattoir

Origin (district)	Type of foreign bodies							
	Negative for foreign bodies	Plastic	Wire with rope	Wire with clothe	Wire	Cloth with rope	Nail	Total
Gomma	54(16%)	14(66.7%)	1(50%)	5(71.4%)	3(75%)	5(45.5%)	2(100%)	84
Seka	89(26.4%)	2(9.5%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	91
Dedo	102(30.3%)	2(9.5%)	0(0%)	0(0%)	1(25%)	6(54.5%)	0(0%)	111
Kersa	92(27.3%)	3(14.3%)	1(50%)	2(28.6%)	0(0%)	0(0%)	0(0%)	98
Total	**337**	**21**	**2**	**7**	**4**	**11**	**2**	**384**

$\chi 2 = 28.111, P = 0.005$).

Table 4: Frequency of occurrences of rumen and reticulum foreign body in cattle

Location	Plastic	Wire with rope	Wire with cloth	Wire	Clothe with rope	Nail	Total
Rumen	21(63.6%)	1(3.03%)	0(0%)	1(3%)	11(33.3%)	0(0%)	34
Reticulem	0(0%)	0(0%)	1(16.7%)	3(50%)	0(0%)	2(33.3)	6
Reticulem and Rumen	0(0%)	1(14.3)	6(85.7%)	0(0%)	0(0%)	0(0%)	7
Total	**21(5.5%)**	**2(0.5%)**	**7(1.8%)**	**4(1.0%)**	**11(2.9%)**	**2(0.5)**	**47**

$\chi 2 = 1.1583, P=0.000$

materials by ruminants is a common worldwide problem previously reported from Nigeria (Remi-Adewumi *et al.,* 2004, Igbokwe *et al.,* 2003), Jordan (Hailat *et al.,* 1998) and Sudan (Ghurashi *et al.,* 2009; Bakhiet, 2008; Mohammed *et al.,* 2006). This study revealed an overall prevalence of 12.23% in male cattle slaughtered at Jimma abattoir. The present prevalence rate of foreign bodies is almost similar with Desiye and Mersha (2012), who reported 13.22 % of rumen and reticulum foreign body in cattle slaughtered at Jimma Municipal Abattoir and slightly lower than the report of Rahel (2011) who reported 17.07% of prevalence of forestomach foreign bodies in Hawasa Municipal Abattoir, Ethiopia and Dawit et.al (2012), who reported 23.9% different types of foreign bodies in their rumen and/or reticulum of cattle at Hirna municipal abattoir, Ethiopia. The variation in the prevalence of foreign bodies in the studies areas could due to differences in the waste management systems between the study areas. Moreover, the time of the study also could play a role for the differences where in recent times the rate of intensification of animal management is increasing and as a result the probability of animals to be exposed to foreign materials might be declined as the animals are staying in a limited confinement for longer time. The highest frequency of occurrence of rumen and reticulum foreign bodies were detected in animal's ≥10 year (20.75%) followed by 5-10 years (11.6%) and ≤5 years (7.93%) age group of animals. Highest prevalence (25.6%) of foreign bodies was detected in cattle greater than 10 year than other age group. This finding is in agreement with Desiye and Mersha (2012), who recovered 81.25% of foreign bodies in cattle greater than 10 years age and also significant prevalence rate of 59.14% was reported in old Achai cattle by Hailat et al. (1998). Rahel (2011) also reported 17.85% of the animals had higher frequency of foreign bodies in rumen and reticulum in the old age. Radostitis et al. (2007) reported old dairy cattle are the most commonly affected group. Ismael et al. (2007) from Jordan also reported the metallic foreign bodies were found in 10(32.25%) of the cows from medical records of 31 old dairy cows suffering from the recurrent rumen tympany. This might be associated with increase of exposure through life and many were found accumulate and lead the undead animals to be positive. The highest frequency of occurrence of rumen and reticulum foreign bodies was detected in poor (15%), medium (10.02%) and good body condition (14.06%) animals. The highest frequency of occurrence of rumen and reticulum foreign bodies was detected in poor body condition animals. Our finding is in agreement with Desiye and Mersha (2012), who found 72.72 % in poor body condition score animals followed by medium (35.95%) and good body condition score animals (7.33%) is the least. Rahel (2011) and Tesfaye et al (2012) also reported higher frequency of foreign body occurrence in animals having poor body condition than in good body conditioned animals. Poor body condition by itself might be due to the contribution of the foreign body that is the animal loss weight after it has been exposed or it might be due to the interference of foreign body with the absorption of volatile fatty acid (VFA) and thus causes reduced weight gain reported by (Rahel,

2011; Ismael *et al.,* 2007; Remi-Adewunmi *et al.,* 2004). Hairball sometimes occur in ruminant in forestomachs and abomasums (Maxie, 2007) and long period of time, these materials form large tight balls over inside the rumen leading to anorexia, decreased production and loss of body condition (Tyagi, and Singh, 1993) as such foreign bodies hinders the process of fermentation and mixing of contents leadings to poor body condition. The highest prevalence of foreign body was observed in animals originated from Gomma district (63.82%) and the lowest in those originated from Seka district (4.26%). Abebe and Nuru (2011) had stated that urban and semi-urban areas are polluted with plastics, ropes, hairs, wool and are growing problem for grazing animals because of the poor management system and inadequate availability of feed especially during long dry seasons. Metallic foreign bodies were most frequently recovered from reticulum. Radostits et al. (2007) reported that in industrialized countries, metallic foreign bodies present in the reticulum up to 90% of normal animals. The reason might be due to retention of these foreign bodies by honey comb structure of the reticular mucosa and their heavy weight give chance to be attracted to the lumen of the reticulum due to gravitational attraction force of heavy foreign bodies to the ventral part of forestomach. This study indicated that most foreign bodies occurred in the rumen 34(72.3%) than reticulum 6(12.7%) and both rumen and reticulum 7(14.8%) from the total 47(12.23%) positive cases. Our result is in line with Desiye and Mersha (2012), who reported from 64 positive cases of foreign bodies 49 (79.68%), was detected in rumen. Jagos (1969) also reported higher overall prevalence of foreign body in adult cows (51%) where 63% and 15% of the foreign bodies was observed in rumen and reticulum, respectively. The current results also agree with Remi-Adewunmi et al. (2004), who found 58.45% in rumen and 19.32 % in reticulum of Achai Cattle. The highest frequency of occurrence of rumen and reticulum foreign bodies was detected at rumen. This may be due to the fact that many ingested feed goes to the rumen. This study also indicated that Metallic foreign bodies were most frequently recovered from reticulum, while nonmetallic foreign bodies were detected from rumen. The types of foreign bodies detected in this study were plastic, wire with rope, wire with clothe, wire, nail and clothe with rope. The result of this study indicated that plastics 21(44.6%) were the most common observed foreign body followed by cloth with rope. This result agree with Desiye and Mersha (2012) reported that plastics were the most common observed 22 (34.37 %) of the total 64 positive cases. This may be due to improper disposal of plastics and other ingestible foreign materials with in plastics. Roman and Hiwot (2010), Hailat *et al.,* (1998), Igbokwe et al. (2003) and Remi-Adewunmi et al. (2004) reported plastics were the most common cause of rumen impaction found in 13% of the cases in the rumen. Kahn *et al.,* (1999) also reported that due to relatively large size, plastic materials are preferentially retained in rumen and at certain time may cause impaction of the rumen leading to death of animals. Ismail *et al.,* (2007) also observed that the presence of large amounts of these materials in the reticulo-rumen, this may be due to the impaction of bodies such as plastic bags interfered with flow of ingesta leading to the distention of rumen and consequently impairs the digestion process.

Conclusion and Recomendations

Ingestion of metallic and non- metallic foreign bodies is the most common problem encountered in cattle not only because of its mortality and morbidity but also it causes decrease in productivity. It is common in developing countries where the standard of animal management is unsatisfactory. In present study poor body condition cattle were the most affected groups compared to that of good body condition and also old animals were more affected. The highest prevalence of foreign body was observed in animals originated from Gomma district and the lowest in those originated from Seka district. The types of foreign bodies detected in this study were plastic, wire with rope, wire with clothe,

wire, cloth with rope, nail. Plastics were the most common observed foreign body followed by cloth with rope. Most of the nonmetallic foreign bodies lodged in rumen while metallic foreign bodies lodged in reticulum. In order to avoid risk of foreign body ingestion, ruminants should be kept away from new construction site and unclear grazing site.

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EPIDEMIOLOGICAL STUDY ON HAEMONCUS CONTORTUS, IN SMALL RUMINANTS SLAUGHTERED AT JIMMA TOWN MUNICIPAL ABATTOIR, OROMIA REGION, ETHIOPIA.

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Abstract

Haemonchosis caused by Haemonchus contortus is a predominant, highly pathogenic, and economically important disease of sheep and goats. Cross sectional study using random sampling from November 2015 to march 2016 in a total of 384 sheep was conducted with objectives of determining the prevalence and associated risk factors of Haemonchosis in sheep slaughtered at Jimma town municipal abattoir in southwest part of Ethiopia in Oromia Regional State. The overall prevalence of Haemonchus contortus was 264 (68.75%). There was insignificant variation (P>0.05) in the prevalence of poor body condition 94 (85.5%), medium body condition 81 (66.9%) and in good body condition 89 (58.2%). The prevalence in adult sheep was 195 (69.6%) varies significantly (P<0.05) from that of young sheep 69 (67%), respectively. The prevalence of 85 (73.3%) in sheep that originated from Seka district compared to Dedo district 62 (68.9%), Mana district 40 (67.8%), Gomma district 25 (67.6%) and Kersa district 52 (63.4%) showed no significant variation (P>0.05). The mean Packed Cell Volume (PCV) of parasitaemic sheep was 23.73±3.7 and the mean Packed Cell Volume (PCV) of aparasitamic sheep was 37.66±4.736 statistically with significantly variation (P<0.05). The highest monthly mean worm burden was found in November (443.9) and lowest monthly mean worm burden was revealed on March (200). Statistical analysis showed there was significance (P<0.05) difference in monthly mean burden. It is concluded that the present finding revealed that significant numbers of sheep were in a high infection rate with Haemonchus contortus was observed. Hence strategic deworming and appropriate control measure with good husbandry practice should be implemented.

Keyword: Aparasitamic; Haemonchus contortus; Parasitaemic; Packed Cell Volume, Risk factors

ÉTUDE ÉPIDÉMIOLOGIQUE DE HAEMONCUS CONTORTUS CHEZ LES PETITS RUMINANTS ABATTUS À L'ABATTOIR MUNICIPAL DE JIMMA EN OROMIE (ÉTHIOPIE)

Resume

L'hémonchose causée par Haemonchus contortus est une maladie répandue, hautement pathogène et économiquement importante des moutons et des chèvres. Une étude transversale utilisant un échantillonnage aléatoire a été menée de novembre 2015 à mars 2016 sur un total de 384 moutons, dans le but de déterminer la prévalence et les facteurs de risque associés à l'hémonchose chez les ovins abattus à l'abattoir municipal de Jimma dans l'État régional d'Oromia (sud-ouest de l'Éthiopie). La prévalence globale de Haemonchus contortus était de 264 (68,75%). On a noté une variation négligeable (P> 0,05) de la prévalence d'un état physique médiocre 94 (85,5%), d'un état physique moyen 81 (66,9%) et d'un bon état physique 89 (58,2%). La prévalence chez les moutons adultes était de 195 (69,6%) et variait de manière significative (P <0,05) par rapport à celle des jeunes moutons 69 (67%). La prévalence de 85 (73,3%) chez les moutons originaires du district de Seka par rapport au district de Dedo 62 (68,9%), au district de Mana 40 (67,8%), au district de Gomma 25 (67,6%) et au district de Kersa 52 (63,4%) n'a pas montré de variation significative (P> 0,05). L'hématocrite moyen (PCV) des moutons parasitémiques était statistiquement de 23,73 ± 3,7 et l'hématocrite moyen (PCV) des moutons aparasitémiques était de 37,66 ± 4,736 avec une variation significative (P <0,05). La charge mensuelle moyenne la plus élevée de verres a été notée en novembre (443,9) et la charge parasitaire mensuelle moyenne la plus élevée a été enregistré en mars (200). L'analyse statistique a montré une différence significative (P <0,05) au niveau de la charge mensuelle

moyenne. Sur base de ces résultats, il a été conclu que des nombres significatifs de moutons avaient un taux d'infection élevé par Haemonchus contortus. Par conséquent, le déparasitage stratégique et une mesure de contrôle appropriée avec de bonnes pratiques d'élevage devraient être mis en œuvre.

Mots-clés : aparasitamique; Haemonchus contortus ; parasitémique ; hématocrite, facteurs de risque

Introduction

In many countries particularly, small ruminants play a great role in the economy of the country, as sources of meat, milk, fiber, cash income, and skin and they can live in extreme climatic conditions, they can use herbage, which is unsuitable for large ruminants, and they require few labor-intensive inputs (Fraser, 1991). Sheep and goats assume a great share in the socioeconomic activities of about 85% of the population (Ayele *et al.,* 2003). The other important attributes of sheep and goats over the other livestock are they are highly adaptable to broad ranges of environment, have short generation cycles and have high reproductive rates which leads to high production efficiency and is also important to those poor people can afford few ewes since cost of them is less than other large animals. With little inputs, small ruminant play an important role in the rural economy through provision of meat, milk, generation of income by selling them and their products, saving of capital, manure and to the national economy thorough the export of live animals and their products to different regions of the world (Tibbo *et al.,* 2003).

Ethiopia lies within the tropical latitudes of Africa and has an extremely diverse topography, wide range of climatic features, and multitude of agro-ecological zones, which make the country suitable for different agricultural production systems. This in turn has contributed to the existence of a large diversity of farm animal genetic resources in the country. Ethiopian livestock production systems are broadly characterized as low input, mixed crop-livestock, agro-pastoral, and pastoral systems, as well as medium input, per-urban, and urban enterprises. These livestock are almost entirely managed by the poor smallholder farmers and pastoralists (Sisay *et al.,* 2007). Ethiopia has the largest livestock and draft animal population in the African continent which is approximately 56,706,389 cattle, 29,332,382 sheep, 29,112,963 goats, 2,033,115 horses, 400,329 mules, 7,428,037 donkeys, 1,164,106 camels and 56,866,719 chickens are found in the country (CSA, 2014).

The prevalence of gastrointestinal nematodes (GIN) in tropical and subtropical areas has adversely affected the production potential of sheep and goats, leading to countless deaths and insidious economic losses in livestock sector (Al-Quaisy *et al.,* 1987). Due to suitable geographic and climatic conditions of the country, parasitic GI nematodes are perhaps the leading cause of productivity losses in small ruminant production in Ethiopia (Biffa *et al.,* 2007). In the tropics, the most important nematode species affecting small ruminants are Haemonchus contortus, Trichostrongylus species, Nematodirus species, Cooperia species, Bunostomum species and Oesophagostomum species. H. contortus is blood sucking nematode parasite, primarily occurring in the abomasum of small ruminants, notably sheep and goats. This nematode is also called the barber pole worm because of its red and white striped appearance in the female. The female is capable of producing over 5,000 eggs a day, which are passed through the feces onto pasture. It has been ranked as the most important parasite of small ruminants in all regions across the tropics and subtropics and causes an insidious drain on production, retarded growth, loss of appetite, anemia, edema, decrease in protein and even mortality in young animals (Miller, 2004, Paddock, 2010 and Bhat *et al.,* 2011).

Among GIN, Haemonchus contortus is considered main culprit causing anemia and hypoproteinaemia in ruminants (Reinecke, 1983). The primary effect of Haemonchosis is economic losses due to mortality and loss in production (Barger and Cox, 1984). The cardinal sign of Haemonchosis is pallor of the skin and mucous membranes, loss of

plasma protein results in anasarca frequently manifested externally as a sub maxillary edema (bottle jaw). Feces are well formed; diarrhea occurring only in infections complicated by the presence of such species as Trichostrongylus and Cooperia species. Lambs and kids are the most affected members of the flock and older sheep and goats under stress also may have total anemia (Bowman, 2003). It is characterized by causing retarded growth, loss of productivity, loss of appetite, anemia, edema, weight loss (Iqbal and Jabber, 2005).

Haemonchus is one of the important endoparasites of sheep and goats. The first and second stages of larvae are free-living organisms and the host ingests the third stage larvae starting the infection. Adults of the parasite are found on the surface of the mucosa (the lining of the stomach). Both the larvae (L4) and the adults of Haemonchus species suck blood (Shapiro, 2005).The major impacts of H. contortus in small ruminants is associated with the blood sucking activity of the parasites which responsible for extensive loss of blood, each worm suck 0.05 milliliter of blood per a day (Urquhart *et al.,* 1996).

Epidemiologically, haemonchosis, is prevalent wherever sheep and goats are raised. However; it exerts the greatest economic losses in temperate and tropical regions (Chaudary *et al.,* 2007). The disease has also found in the colder climates and recently been found as far north as the Arctic Circle (Durrani *et al.,* 2007). The prevalence of the haemonchosis in sheep and goat in Ethiopia is very high. The research conducted in Hawassa town, in southern nations nationalities and people's regional state by Molalign et al. (2011) showed prevalence of 75.9% and 55.9% in sheep and goats, respectively and In Gondar town, in Amhara regional state by Tewodros and Girja (2012) reported that the overall prevalence was found 80.21%.

Previously there was not any recently documented data with regard to the prevalence of the haemonchosis in sheep regardless of the high populations of sheep in the study area and most previous studies in Ethiopia were based on coprological examinations, which are less sensitive in identifying the nematode species. Therefore, this study was conducted with the objectives to determine the prevalence of sheep haemonchosis based on postmortem examination and to assess the influence of host related risk factors such as age, body condition and origin on the occurrence of sheep haemonchosis in Jimma town municipality abattoir.

Materials and Methods

Description of Study Area and Study period

The present study was conducted from November 2015 to March 2016 in Oromia regional state in Jimma town municipal abattoir of Jimma zone administration Southwest part of Ethiopia. Jimma is located 356 km far away from Addis Ababa , capital city of Ethiopia and the area geographically lies within 70 013' -800 56' North latitude and 350 052'-370 037' East longitude and of an altitude of 880meter to 3360 meter above sea level (m.a.s.l.). The study area receives a mean annual rainfall of about 1530 millimeters which comes from the long and short rainy seasons. The annual mean minimum and maximum temperature during the study period were ranges 14.40c and 26.7 0c respectively.

Study Animals

The study animals were 384 sheep slaughtered in Jimma town municipal abattoir of Jimma zone. The animals were presented to the abattoir from different local markets (seka, Dedo, Mana, Gomma and Kersa districts) available around Jimma. Animals were indigenous breeds kept under traditional management system and with different body condition and age were the age of the sheep was characterized using teeth eruption by Gatenby, 1991 and body condition scoring method as per Russel, 1991.

Study design and type of Study

A cross-sectional epidemiology using simple random sampling technique was conducted from November 2015 to March 2016 to determine the prevalence and

associated risk factors of Haemonchosis in sheep slaughtered at the study area.

Study Design

The cross-sectional types of study was designed and simple random sampling technique was used for the research with the assumption that it would help to get an understanding of the current status of problem by describing it in relation to determine the prevalence and associated risk factors of haemonchosis in sheep slaughtered at Jimma municipal abattoir (Dohoo et al, 2003).

Sample size determination

Sample size was determined according to Thrusfield (2007) formula, considering 95% confidence interval, 5% desired absolute precision and 50% prevalence of haemonchosis among sheep since there was no previous study specifically in the study area. Formula for sample size determination is given as follow.

$$n = \frac{1.962 \times P\,(1-P)}{d2}$$

When: n = required sample size; P = expected prevalence; d = desired absolute precision;

1.96 = the value of Z of 95% confidence level. Hence, by using this formula, a total of 384 sheep were randomly selected.

Study Methodology

Three times weekly regular examination was made to the abattoir. Ante mortem examination was performed a few hours before slaughtering from randomly selected sheep. Body condition, origin month and age were recorded prior to slaughter and were given an identification number. All the slaughtered animals were male. Based on their age, animals were categorized into young (<2 years old) and adult (>2 years old). Based on their body condition, animals were classified into poor, medium and good. The age and body condition of sheep were determined before the animals were slaughtered by standard methods given by Gatenby, 1991 and Russel, 1991, respectively.

Hematocrit (PCV) determination was conducted from blood samples collected from the jugular vein of randomly selected each animal using heparinized ethylene diamine tetra acidic acid (EDTA) and each blood sample were properly level on EDETA and separated case books. Then blood was taken in microhaematocrit capillary tubes that filled 3/4 of the height and sealed with Crystal Sea. Sample from each animal was labeled using codes describing the specific animal. The sealed microhaematocrit capillary tubes containing blood were centrifuged by microhaematocrit centrifuge for 5 minutes at 12,000 rpm. After centrifugation, the pack cell volume (PCV) value was recorded for estimation of anemia using Hematocrit according to Uilenberg (1998).

During postmortem examination a total of 384 of sheep abomasums were collected and examined for the presence of adult haemonchosis. The abomasums were collected within 30 minute of evisceration. Each abomasums was legated at both ends with string and separated from omasum and duodenum. The abomasum was opened along its greater curvature and its contents were washed in to a bucket up to a total volume of 2 litters from which an aliquot of 200ml was transferred to a labelled plastic container and preserved in 10% formalin. 20ml of the sub-sample was taken onto a Petri dish a drop of iodine (approximately 2 – 3 ml) were added, for examination of abomasal worms under stereomicroscope and the worms which were preserved in 10% formalin were poured in to petri dishes and examined under a stereomicroscope and identification was made according to keys developed by Hansens and Perry (1994). For those positive abomasal samples, the number of worms found from 20ml of sub sample was multiplied by 100 to get total worm number in each abomasums according to standard procedure described by Hansens and Perry (1994).

Statistical Analysis

All data were collected and recorded in Microsoft excel 2010 spreadsheet and statistical analyses was conducted using SPSS

statistical software version 20.0. Descriptive statistics was used to compute percentages, proportions and frequency distributions of the data. Logistic regression statistical tool was used to measure the association between prevalence of the Haemonchosis with the age, body condition and origin. Mean worm burdens between different months of the study period and the mean PCV of the infected animals with that of non-infected animals were compared by ANOVA. Confidence level was held at 95% and statistical analysis for the difference in prevalence of H. contortus among risk factors were considered significant when $P < 0.05$.

Results

In present study, a total of 384 sheep were examined using postmortem to determine prevalence and associated risk factors of Haemonchus contortus, 264 (68.75%) were positive for haemonchosis. The overall prevalence of haemonchosis in sheep was found to be 68.75% in the study area. From the 153 examined good body condition sheep, about 89 (58.2%) sheep were positive for haemonchosis, among the 121examined medium body condition sheep, about 81(66.6%) sheep were positive of haemonchosis and from 110 examined poor body condition sheep, about 94(85.5%) sheep were positive for heamonchosis. Statistical analysis showed there was significance ($P<0.05$) difference in prevalence between the body condition (Table 1).

During the study period, sheep were classified based on their age less than two years were categorized young and greater than two years were categorized as adult. Based on their classification of animal in age, prevalence and associated risk factor of haemonchosis was studied. From 103 examined young sheep, about 69 (67%) sheep were positive of haemonchosis and among the 281 examined adult sheep, 195(69.6%) of them were positive for haemonchosis. Statistical analysis revealed that there is no significance ($P>0.05$) difference in prevalence between the age groups (Table 1).

A total 384 examined sheep for Prevalence of Haemonchus, 264(68.75%) sheep were positive and 120 were negative in which 37 were from Gomma district, 90 were from Dedo district, 116 were from Seka district,59 were from Mana district and 82 were from Kersa district. The prevalence of haemonchosis was different with different origin of sheep; and it was found that (67.6%) in Gomma district, (68.9%) in Dedo district, (73.3%) in Seka district, (67.8%) in Mana and (63.4%) in Kersa district sheep. Statistical analysis showed there was no significance ($P>0.05$) difference in prevalence between origin (Table 1).

The mean PCV of parasitaemic and aparasitaemic sheep in the study area.

Out of the 384 examined sheep for haemonchosis, 264 were parasitaemic with Mean PCV of 22.73 ± 3.7% and 120 of them were aparasiteamic with Mean PCV of 37.66 ± 4.736 %. Statistical analysis showed there was significance ($P<0.05$) difference in Mean PCV between parasiteamic and aparasiteamic (Table 2).

Monthly mean worm burden of Haemonchosis in sheep of study area

In present study, 384 sheep were examined to determine monthly mean worm burden of heamonchosis in November, December, January, February and March, and it was found that 443.9, 279.45, 382.28, 306.49 and 200 monthly mean worm burden of Haemonchosis in sheep, respectively. The highest monthly mean worm burden was found in November (443.9) and lowest monthly mean worm burden was revealed on March (200). Statistical analysis showed that there was significance ($P<0.05$) difference in monthly mean burden (Table 3).

Discussion

The overall prevalence in the study area was 68.75% parallel to the study conducted in and around Finoteselam with prevalence of 71.03% (Mengist *et al.,* 2014). However, the current finding of overall prevalence was found to be lower than 96.5% reported by Abebe and

Table 1: Influence of risk factors on prevalence of haemonchosis

Risk factors	Groups	No examined	Prevalence (%)	OR	95% CI	P-value
Body condition score	Good	153	89(58.2%)	4.225	2.273-7.852	0.000
	Medium	81	81(66.9%)	2.901	1.512-5.566	
	Poor	110	94(85.5%)			
	Total	**384**	**68.75%**			
Age	Young	103	69(67%)	0.895	0.552-1.45	0.653
	Adult	281	195(69.4%)			
	Total	**384**	**68.75%**			
Origin (District)	Gomma	37	25(67.6%)		0.691	
	Dedo	90	62(68.9%)	0.941	0.414-2.137	
	Seka	116	85(73.3%)	0.76	0.341-1.694	
	Mana	59	40(67.8%)	0.990	0.411-2.383	
	Kersa	82	52(63.4%)	1.202	0.528-2.734	
	Total	**384**	**68.75%**			

Table 2: Relative Mean PCV of sheep in the study area.

Status	No examined	PCV(Mean± SD)	F	P –Value
Parasitaemic	264	22.73 ± 3.7	11.6	0.001
Aparasitaemic	120	37.66 ± 4.736		
Total	**384**			

Table 3: Monthly mean worm burden of Haemonchosis in sheep relation to months

Months	Monthly mean of haemonchus	95%CI		F	P-value
		Lower boundary	Upper boundary		
November	443.9	359.93	527.88	3.548	0.007
December	279.45	209.14	349.77		
January	382.28	284.4	480.16		
February	306.49	226.3	386.68		
March	200	107.89	292.11		

Esayas (2001) in sheep in arid and semi-arid zone of eastern Ethiopia, 91.2% reported by Kumsa and Wossene (2006) in Ogaden region slaughtered at Debrezeit ELFORA abattoir and 80.2% reported by Tewodros and Girja, (2012) in Gonder town, but higher than 40.9 % reported by Lidya and Berihun (2014) slaughtered at different restaurants in Wukro, Ethiopia. The variation of this finding in prevalence reported from different parts of Ethiopia might be due to variation in temporal distribution of the parasite, differences in agro-ecological systems, sheep management and production systems, population density, the types of diagnostic methods used to determine the prevalence, environmental factors, host, age, breeding status, level of education and economical capacity of the community and anthelmintic usage which influences the development, distribution and survival of parasites. The high prevalence of haemonchosis in the study area might be due to the fact that sheep's were managed under extensive managements system with the high stocking density, where large numbers of

animals graze together throughout the year in communal grazing land, inadequate nutritional status and lack of community awareness.

Among the potential predisposing factors assessed in body condition scores, infection rate were 85.5%, 66.9% and 58.2% in poor, medium and good body condition, respectively statistical analysis showed there was significant (P<0.05) association between prevalence of haemonchosis in sheep. The present study was concur with Lidya and Berihun (2014) in Wukro, who reported statistically significance (P<0.05) difference between prevalence of haemonchosis in sheep in body condition scores. However disagrees with the research reported by Ragassa et al. (2006). This result is agreement with the previous reports of Gonfa et al. (2013), who reported prevalence of 77.21% and 84.44% in good and medium body condition animal, respectively. Similarly, Tewodros and Girija,(2012) indicated that the rate of the parasite was higher in medium body condition sheep compared to that of good body condition with the prevalence of 81.2% and 73.6%, respectively. The highest infection rate recorded in poor body condition may be due to the effect of heavy infection rate of abomasal nematode parasites and other factors, which lead to significant weight loss, differences in seasonal change of feed, poor management system and the presence of other concurrent diseases that decreases the ability of the host to cope with the adverse consequences of parasitism and resistances of the host to overcome parasitism by limiting the establishment, development and fecundity of the parasites.

The current study revealed that there was no significant difference based on age (P>0.05) with the prevalence of 67% and 69.6% in young and adults, respectively. This finding was in line with Gonfa et al. (2013) in Debre Ziet and Tewodros and Girja (2012) in Gonder, who reported there was no significant (P >0.05) association based on the age. All age groups of sheep are susceptible to haemonchus species infection and most of the researchers have observed higher rate of haemonchosis in young (Gauly, 2006 and Kuchai, 2011). But Mengist et al. (2014) reported prevalence of Haemonchosis in young 67.50% and 71.43% in adult that is agreed with our study. It is probably due to the facts that adults might be stressed on seasonal changes and developed resistance against the frequently used anthelminthic and the adult animals can withstand higher infection without much adverse effect leading to chronicity of infection. Also there was the likelihood that the young animals had been given anthelmintic which made them eliminate helminthes infection.

Our study revealed that there was no statistically significant (P<0.05) difference based on origin (districts) with the prevalence of 73.3%, 68.9%, 67.8%, 67.6% and 63.4% in Seka, Dedo, Mana, Gomma and Kersa districts, respectively. During the study period, the highest prevalence of haemonchosis was recorded in those sheep brought from Seka district with prevalence of 73.3%. This might be due to the difference of agroecology, production system, management system, exposure of sheep's to the haemonchosis in the study area and the environmental factors (warm and humidity) those facilitates the distribution of the parasite to the grazing pasture.

During study period, out of 384 examined sheep's for hemonchosis, 264 were parasitic with mean PCV (Packed Cell Volume) of 22.73 ± 3.7 % and 120 of them were aparasitic with mean PCV (Packed Cell Volume) of 37.66 ± 4.736 %. Statistical analysis showed that there was significance difference between mean PCV and haemonchosis in sheep. This might be due to blood sucking parasite haemonchosis so; heavy infestation of adult worm in abomasums could result to anemic. In addition to this, it might be due to the change in appetite in infected sheep's attributed to hormonal changes like that of gastrin level which affects feed intake (Hoste, 2001). Similarly, (Soulsby, 1986) reported that haemonchosis is an acute syndrome characterized by severe anemia associated with the rapid acquisition of large worm burdens.

The monthly mean adult worm burden of haemonchus contortus was 443.9, 279.45, 382.28, 306.49 and 200 in November,

December, January, February and March, respectively. The monthly mean worm burden of Haemonchus contortus was the highest in November, (443.9) and the least in March (200). Statistical analysis revealed that there was significant ($P<0.05$) difference for total worm burden within months in sheep. This finding was in line with Kumsa and Wossene (2006) and Abebe and Esayas (2001), who reported there was statistically significant difference ($P<0.05$) with monthly mean adult worm burden of haemonchus contortus. This is due to the effect of climatic factors on worm burden revealed a significant positive correlation with rainfall and relative humidity which favorably support the larvae survival and development. Amenu (2005), who reported that the higher worm burden occur during Months of rainy season. The present study was shown that the parasitic burden in the wet season is significantly higher than the dry season owing to the general understanding that moisture is one of the bionomic factor that support the development of the infective stage of most parasites (Hansen and Perry, 1994; Urquhart *et al.,* 1996) which is also true phenomenon in sub-Saharan Africa (Teklay, 1991).

Conclusion

The present study was conducted on prevalence of haemonchosis in sheep for the period of five month in Jimma town municipal abattoir showed high infection rate with H. contortus. Among the predisposing risk factors included in the study, body condition scores and months were significantly associated with prevalence of haemonchosis. The distribution of the parasite was higher in poor body condition sheep than medium and good body condition. Higher prevalence was observed in adult than young sheep. High prevalence was shown in sheep that originated from Seka compared to Dedo, Mana, Gomma and Kersa districts. The mean PCV of parasitaemic sheep varies significantly from that of aparasitamic. In general, epidemiological evidence of the present investigation showed that haemonchosis is considerably prevalent diseases in the study area and the disease need due attention from farmers and veterinarians.

Authors' Contribution

Amarech Habte being with her advisor Dr. Nuraddis Ibrahim developed the proposal and she collected all the relevant data for the paper. Dr. Nuraddis Ibrahim designed the paper, engaged in follow-up during data collection, and analysed the data. All authors contributed to the writing of the paper and approved submitted version of the paper.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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PHYTOCHEMICAL AND ANTI-MICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF *MORINGA OLEIFERA*, *ASPILIA AFRICANA* AND *AZADIRACHTA* INDICA LEAVES USING RABBIT CAECUM

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Abstract

The antibacterial activity of leaf extracts of Moringa oleifera Lam, Azadirachta indica, Aspilia Africana (Pers) C. D. Adams against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus saphrophyticus* were determined using agar diffusion method. The minimum inhibitory concentration (MIC) was determined. The minimum inhibitory concentration (MIC) was determined. The result showed that the MIC ranges from 21.3-42.5 mg, 42.5-85.0 mg and 85.0 mg for *M. oleifera*, *A. indica* and *A. africana* leaf extracts respectively against *E. coli* and *S. aureus*. The phytochemical screening of the leaves revealed the presence of secondary metabolites such as tannin, saponin, alkaloid, flavonoid and phenol. The secondary metabolites detected in the leaves are responsible for the observed antibacterial activity of the plant and hence, its potential use as medicinal herb in the treatment of infections caused by the test organisms.

Keywords: Methanolic extract, Antibacterial activity, Minimum Inhibitory Concentration, Phytochemical, Moringa, Neem, Aspilia.

ACTIVITÉ PHYTOCHIMIQUE ET ANTI-MICROBIENNE DE L'EXTRAIT MÉTHANOLIQUE DE FEUILLES DE Moringa oleifera, Aspilia africana et Azadirachta indica UTILISANT LE CAECUM DE LAPIN

Résumé

L'activité antibactérienne des extraits de feuilles de Moringa oleifera Lam, Azadirachta indica, Aspilia Africana (Pers) C. D. Adams contre les bactéries *Escherichia coli*, Pseudomonas aeruginosa, Staphylococcus aureus, *Staphylococcus saphrophyticus* a été déterminée à l'aide d'une méthode de diffusion sur l'agar. La concentration minimale inhibitrice (MIC) a été déterminée. Le résultat a montré que le MIC variait entre 21,3-42,5 mg, 42,5-85,0 mg et 85,0 mg respectivement pour les extraits de feuilles de *M. oleifera*, *A. indica* et *A. africana* contre *E. coli* et *S. aureus*. L'examen phyto-chimique des feuilles a révélé la présence de métabolites secondaires tels que le tanin, la saponine, l'alcaloïde, le flavonoïde et le phénol. Les métabolites secondaires détectés dans les feuilles sont responsables de l'activité antibactérienne observée de la plante et, partant, son utilisation potentielle comme herbe médicinale dans le traitement des infections causées par les organismes testés.

Mots-clés : extrait méthanolique, activité antibactérienne, concentration inhibitrice minimale, phyto-chimique, Moringa, Neem, Aspilia.

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Introduction

Plants contain many biologically active compounds which have potential for development as medicinal agents. Herbal medicines already form the basis of therapeutic use in the developing countries, but of recent, there has been an increase in the use of herbal medicines in the developed world too (De and Ifeoma, 2002; El-Mahmood *et al.,* 2010). Medicinal plants have been found useful in the cure of a number of diseases including bacterial diseases as the plants are a rich source of antimicrobial agents (Mahesh and Satish *et al.,* 2008) that are naturally toxic to bacteria organisms (Basile *et al.,* 1999). These active compounds are generally called phytochemical. Presence of certain phytochemical components such as alkaloids, saponins, tannins, polyphenols, are the bioactive bases for the medicinal properties.Azadirachta indica is one of such plants belonging to the Meliaceae family. It is popularly known as Neem (English) or "dongoyaro" (Yoruba-Western Nigeria) in Nigeria. The plant is perhaps one of the most studied and widely used medicinal plants of all ages. Aspilia africana (Pers) C. D. Adams in the family of Asteraceae. It is used in African ethno-medicine for the treatment of haemorrhage (Katsayal, 2002). Moringa oleifera popularly called the "miracle tree" is a monogeneric plant in the family Moringaceae. In ethno medicine, Moringa oleifera leaves have been used by local traditional healers in treatment of various ailments. The leaves have also been found to possess anti-tumour, anti-pyretic, anti-epileptic, anti-inflammatory, anti-ulcer, anti-spasmodic, diuretic, antihypertensive and antioxidant properties (Bukar *et al.,* 2010).Due to wide uses of these leaves, the present study was planned to investigate the antibacterial potential of the leaf extracts on E. coli, P. aeruginosa, S. aureus and S. saphrophyticusin an attempt to provide a profile that gives a scientific backing to various tradomedical claims and the uses.

Materials and Methods

Plant Material and Identification

Fresh leaves of M. oleifera Lam, A. africana(Pers.) C. D. Adamsand A. indica were harvested during dry season within the arboretum of Ogun State, Nigeria state. The leaves were identified taxonomically by Dr. J. A. Olanite at the Department of Pasture and Range Management, Federal University of Agriculture Abeokuta, Ogun State, Nigeria.

Extraction of leaf material

The leaves were air-dried and milled to obtain a leaf meal product. Methanolic extraction of M. oleifera, A. africanaand A. indica was prepared in cold condition. In cold extraction, the coarse dried powdered leaves were macerated.

The extract was done at 25oC for 72 hrs.These were carried out by suspending 250 g of the powdered leaves were soaked into 1.5 L of methanol in a conical flask cover with rubber cork with periodic shaking. The extracts were then decanted and filtered through a Whatman filter paper. The filtrate was evaporated to dryness in a water bath at 25oC which gives a semi-solid residue.

Source of test organisms

Caecal content of rabbits were aseptically collected after slaughtering and cultured overnight on nutrient agar. It was incubated at 37oC for 18-24 hrs at ambient temperature. Each bacterium isolate was biochemically characterized and identified appropriately according to Cowan and Steel (1996). These include at least four isolates: E. coli, P. aeruginosa, S. aureus and S. saphrophyticus as the test organisms.

Screening of the Extracts for Antibacterial Activity

Agar disc-diffusion assay was performed to evaluate the antibacterial activity of M. oleifera, A. africana and A. indicaextract. Twenty ml of molten sterile nutrient agar was poured into petri dishes. After solidification, an overnight broth culture of E. coli, P. aeruginosa, S. aureus and S. saphrophyticus was introduced

unto the surface of the sterile plate each and sterile glass spreader were used for even distribution. Wells were made aseptically with 7 mm sterile cork borer and1 g of each extract was reconstituted in normal saline to obtain extract concentration of 100 mg/ml i.e. 1000 mg in 10 mls of normal saline. The plates were incubated aerobically for 24 hours at 37oC and were examined for zone of inhibition, which indicate the degree of susceptibility of the test organism. The same procedure was carried out using a standard disc antibiotic (procaine penicillin) as the positive control and the prepared extract was used as negative control.

Determination of Minimum Inhibitory concentration (MIC) of the Extracts

The MIC of the extract on isolates was determined by micro broth dilution assay following the recommendation of (Kumar *et al.,* 2007). Equal volume of 1000 mg/ml concentrations of each extracts and broth medium were mixed in Mueller-Hinton broth by serial dilutions to make up 100 mg of solution were assayed against the test organisms and incubated for 24 hrs at 37oC.

The experiment was conducted in duplicate for all the test isolates. Tubes of Mueller-Hinton broth containing only the 100 mg/mlsuspension of the test organisms without the extract, and the tubes of Mueller-Hinton broth containing different concentrations of the extract without test organisms, were used as controls.The lowest concentration of the extract to inhibit the growth of microorganisms after incubation period was taken as the MIC.

Phytochemical analysis

The extract were subjected to quantitative phytochemical analyses for secondary metabolites to assess the presence of tannins, saponins, alkaloids, flavonoids, phenol, following the standard method (Harborne, 1998; Houghton and Raman, 1998 and Parekh, 2006).

Statistical Analysis

Data collected were subjected to one way analysis of variance (ANOVA) in a Completely Randomized Design using SPSS (Release 20.0) statistical package (SPSS, 2011). Treatment means were separated using Duncan's Multiple Range Test.

Results and Discussion

The results of the antibacterial effectiveness of the methanol leaf extracts as compared with the activity of standard antibiotics (proclaim penicillin) was shown in Table 1. The extracts inhibited the growth of two Gram negative (E. coli and P.aeruginosa) and positive (S. saphrophyticus and S. aureus). The antibacterial activity of theantibioticinhibited higher zones of inhibition than the plant extracts. The antibiotics and extracts of M. oleifera, A. indica and A. africana produced inhibitory zone of 20, 13 and 11 mm against E coli respectively. The extract of M. oleifera showed high activity with the diameter of Zone of inhibition of 17cm against S. aureus and least activity of11 cm against P. aeruginosa. The sensitivity of the S. aureus, S. saphrophyticus and Pseudomonas aeruginosato the extracts of A. africana in this study corresponds with the work of Adeniyi and Odufowora (2000) that showed that extracts of A. africana possessed a broad spectrum antibacterial activity against both gram positive and negative bacteria. Mild effect of the extract of A. africana on E. coli was inline with the findings of Salit et al. (2014) who reported a mild effect of A. africana leaf extract against E. coli. The leaf extract of A. indica had a mild effect against E. coli, P. aeruginosa13, 14 mmand a susceptible effect on S. aureus and S. Sapophyticu 18 mm for each respectively. Failure of some of the extract to exert antibacterial effect on the test organism is not enough to conclude that the leaf does not contain substances that can exert antibacterial activity against the test organisms because the potency of extract depends on the method used to obtain the extract (Unaeze and Abarikwa, 1986). The sensitivity of the leaf extract shown in this study, was only able to inhibit the growth of organisms but did not exert a killing effect on the bacteria isolates. This shows that the methanolic extract was

bacteriostatic and not bactericidal.

Table 2 shows the minimum inhibitory concentration (MIC) of the methanol extraction of M. oleifera, A. africana and A. indica leaves against E. coli and S. aureus. The MIC of A. Indica had the highest value compared to M. oleifera and A. africana. This indicated that the leaf of A. indica has similar potency on S. aureus and E. coli. The findings is in line with the study carried out by National Library of Medicine at the National Institutes of Health who reported that in test tubes, A. indica has been shown to have significant effects on both gram positive and gram negative organisms and other bacteria that cause a wide array of human and animal diseases. The result revealed that the MIC of the methanol leaf extract of M. oleifera on E. coli and S. aureus were 42.5 mg/ml and 21.3 mg/ml respectively. The MIC of A. africana leaf extract on E. coli and S. aureuswere 42.5 mg/ml and 85 mg/ml respectively. However, the extract of A. indica at 85 mg/ml concentration exhibited antibacterial activities against the test organisms.

The secondary metabolites of the leaves of these study plants revealed the presence of alkaloid, saponin, tannin, phenols and flavonoid as the major phytochemical components as shown in Table 3. The findings is similar with the work of Geyid *et al.,* (2005) ;Tedong *et al.,*(2006) and Veeramuthu *et al.,*(2006)who reported that plant have variety of secondary metabolites as metioned above. The phytochemical composition level of the selected browse plants studied are below 2.5% recommended by (NRC, 1985) with the exception of M. oleifera and A. indica which recorded little above the recommended value in flavonoid and alkaloid.

A. indica contained significantly ($P<0.05$) higher concentrations of tannin (1.25%) and alkaloid (2.74%) than M. oleifera and A. africana leaves. Tannin has inhibitory effect on many enzyme due to protein precipitation and the presence could be why the observed plants are used locally as treatment of wound and skin disease (Trease and Evans, 2002; Bruneton, 1999). Higher concentration (0.86%) was recorded for saponins in A. africanathan M. oleifera and A. indicaleading to a significant ($P<0.05$) differencewith A. indica having the least value (0.38%). The presence of saponin in plant leaves confirmed the plant as anti-inflammatory, antifungal, anti parasitic (Spray *et al.,*2004) and antimicrobial activity (Barile *et al.,* 2007; Ayoola *et al.,* 2008). Cheeke (1971) and Eleazu et al. (2010) reported that saponin have effect on erythrocyte, haemolysis, reduction of blood and liver cholesterol, depression of growth rate, bloat inhibition of smooth muscle activity, enzyme inhibition and reduction in nutrient absorption. High levels of saponin in feed affect feed intake and growth rate in animals (Sim *et al.,* 1984; Dei etal., 2007) and bitterness Sodipo et al. (2000). The phenol content in this study ranged between 0.30-0.59%.

Conclusion

Table1: Antimicrobial activity of the leaf extracts of M. oleifera, A. africana and

Gram	Diameter zone of inhibition (mm)				
	Bacteria isolates	**Procaine penicillin**	***M. oleifera***	***A. indica***	***A. africana***
Negative	*Escherichia coli*	20	13	13	11
Negative	*Pseudomonas aeruginosa*	19	11	14	18
Positive	*Staphylococcus saphrophyticus*	22	16	18	16
Positive	*Staphylococcus aureus*	20	17	18	16

Table2: Minimum Inhibition Concentration of the leaf extracts of M. oleifera, A. africana and A. indica (mg/mL)

Methanol based Extract				
Test organism	***M. oleifera***	***A. africana***	***A. indica***	***Gram***
Escherichia coli	42.5	42.5	85.0	Negative
Staphylococcus aureus	21.3	85.0	85.0	Positive

Table 3: Phytochemical content of dried leaves of the selected browse plants

Dried leaves of the selected browse plants				
	M. oleifera	***A. africana***	***A. indica***	SEM
Components		**Concentration (%)**		
Tannin	0.45^{c}	0.93^{b}	1.25^{a}	0.02
Flavonoid	2.74^{a}	1.92^{b}	1.63^{c}	0.01
Alkaloid	0.85^{c}	1.47^{b}	2.74^{a}	0.01
Phenol	0.59^{a}	0.30^{c}	0.37^{b}	0.00
Saponin	0.74^{b}	0.86^{a}	0.38^{c}	0.01

*^{abc} Means along the same row with different superscripts are significantly different (P *< 0.05). SEM- Standard Error of Mean.*

The methanol leaf extracts of M. oleifera, A. indica and A. africana possessed good antibacterial activity confirming an appreciable inhibitory activity against the test organisms. The activity of the leaf extract against gram positive (S. sapophyticus and S. aureus) were more sensitivethan gram negative (E. coli and P. aeruginosa)bacteria. The inhibitory effect of the extract is an indication of the presence of broad-spectrum bioactive compounds in the leaf. Therefore, the leaves could be a promising natural antimicrobial agent with potential applications in livestock industry for controlling bacteria used in this work.

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PATHOGENICITY AND ANTIBIOTIC SENSITIVITY PATTERN OF *SALMONELLA* SPECIES ISOLATED FROM POND-REARED *CLARIAS GARIEPINUS* (BURCHELL, 1822)

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Abstract

Salmonella species has been isolated from fish ponds, and two different clinical cases of *Salmonella* infections were reported in tropical fishes. It therefore becomes important to investigate the occurrence of *Salmonella* in farmed *Clarias gariepinus*, and evaluate fish and public health significance.

Bacteria isolated on *Salmonella*-Shigella agar from blood and upper intestinal tract of 50 *C gariepinus* juveniles were biochemically characterised and those identified as *Salmonella* sp were evaluated for antibiotic sensitivity using the disc diffusion method. Two groups, A and B of *C. gariepinus* juveniles were each divided into 4 subgroups of 10 fish. Three subgroups in A and B were experimentally infected via oral and intraperitoneal route respectively with 1.2 x 106, 1.2 x 107 and 1.2 x 108 cfu of *Salmonella*. The forth subgroup is the control. Daily observation was made for clinical signs, lesions and mortality for 14 days.

Biochemical tests confirmed 72% and 32% occurrence of *Salmonella* sp in the intestinal tract and the blood of sampled fish respectively. Antibiotic resistance frequency of 100% (augmentin, ceftriazone and nitrofurantoin), 89% (gentamicin and amoxycilin), 78% (cotrimoxazole and tetracycline), 33% (ofloxacin) and 11% (ciprofloxacin and pefloxacin) was observed. The multiple antibiotic resistance indices of the observed isolates ranged from 0.5 to 0.9. No clinical signs, lesions or mortality was observed in experimentally infected fish.

Farmed *Clarias gariepinus* may be a reservoir for *Salmonella* sp probably acquired from faecal contamination of pond water, or consumption of contaminated feed. Risk of *Salmonella* infection to consumers and producers may be high, with serious public and animal health significance. Multi-drug antibiotic resistance is an indication of misuse of antibiotic in our society. There is the danger of possible spread of multi-drug resistant *Salmonella* sp, and choice of drug for treatment of *Salmonella* infection in man and livestock may be limited.

Keywords: Isolation, *Salmonella* sp, Experimental infection, Antibiotic sensitivity.

PATHOGÉNICITÉ ET PROFIL DE SENSIBILITÉ AUX ANTIBIOTIQUES DES ESPÈCES DE *SALMONELLA* ISOLEES CHEZ DES *CLARIAS GARIEPINUS* ELEVES EN ETANG (BURCHELL, 1822)

Résumé

Des espèces de *Salmonella* ont été isolées dans des étangs de poissons et deux cas cliniques différents d'infections à *Salmonella* ont été signalés parmi les poissons tropicaux. Il devient donc important d'étudier la présence de *Salmonella* chez les *Clarias gariepinus* élevés et d'évaluer sa signification pour la santé publique et des poissons.

Les bactéries isolées sur la gélose *Salmonella*-Shigella provenant du sang et du tractus intestinal supérieur de 50 *C. gariepinus* juvéniles ont été caractérisées biochimiquement et celles identifiées comme *Salmonella* sp ont été évaluées pour leur sensibilité aux antibiotiques à l'aide de la méthode de diffusion par disque. Deux groupes, A et B de juvéniles de *C. gariepinus* ont chacun été divisés en 4 sous-groupes

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de 10 poissons. Trois sous-groupes dans A et B ont été infectés expérimentalement par voie orale et intrapéritonéale respectivement avec 1.2 x 106, 1.2 x 107 and 1.2 x 108 cfu de *Salmonella*. Le quatrième sous-groupe est le groupe témoin. Une observation quotidienne a été faite pour détecter les signes cliniques, les lésions et la mortalité pendant 14 jours.

Les tests biochimiques ont confirmé une présence de 72% et 32% de *Salmonella* sp respectivement dans le tractus intestinal et le sang de poissons échantillonnés. On a observé une fréquence de résistance aux antibiotiques de 100% (augmentin, ceftriazone et nitrofurantoïne), 89% (gentamicine et amoxyciline), 78% (cotrimoxazole et tetracycline), 33% (ofloxacine) et 11% (ciprofloxacine et pefloxacine). Les multiples indices de résistance aux antibiotiques des isolats observés variaient de 0,5 à 0,9. Aucun signe clinique, aucune lésion ou mortalité n'a été observé chez les poissons expérimentalement infectés.

Les *Clarias gariepinus* élevés peuvent être un réservoir de *Salmonella* sp probablement acquis à partir de la contamination fécale de l'eau des étangs ou la consommation d'aliments contaminés. Le risque d'infection à *Salmonella* chez les consommateurs et les producteurs peut être élevé, avec des répercussions graves pour la santé publique et animale. La résistance à de multiples antibiotiques est une indication de l'utilisation abusive d'antibiotiques dans notre société. Il existe un risque de propagation possible de *Salmonella* sp résistant à plusieurs médicaments et le choix du médicament pour le traitement de l'infection par *Salmonella* chez l'homme et les animaux peut être limité.

Mots-clés : Isolement, *Salmonella* sp, infection expérimentale, sensitivité antibiotique.

Introduction

Salmonella species are endemic bacteria of great public health concern (Le Minor *et al.,* 1974; Minette, 1986). Fish serve as reservoir of some bacteria and source of infection to both humans and animals (Gaulin *et al.,* 2002; Musto *et al.,* 2006). Pathogenic bacteria of fish manifest in three forms, namely as normal micro-flora, as enteric bacteria and as contaminant bacteria (Lyhs, 2009). Two clinical cases of salmonellosis in fish have been reported (Kodama *et al.,* 1987), while experimental exposure of fish to *Salmonella* (Bocek *et al.,* 1992; Horse *et al.,* 1978; Baker and Smitherman, 1983) has not been observed to produce any clinical infection to the best of our knowledge. Wedekind *et al.* (2010), observed that experimental infection of fish with pathogens rarely produce disease. This was supported by Song *et al.,* (2008) with the observation that there must be interaction between the host, the environment and the infectious agents for an infection to take place. *Salmonella* has been isolated from gills, muscles and skin of fish with high pathogen concentration in the gut and peritoneal fluid (Joseph and George, 2010). Gartner et al. (2008), is of the opinion that *Salmonella*, is not a normal micro-flora of fish intestines, but have reached the aquatic biosphere through faecal contamination by mammals, birds and reptiles (Nadia *et al.,* 2011; Tauxe, 1997). Other sources of contamination of *Salmonella* in the aquatic environment includes contaminated feeds, non-point run-off water, aquatic insect, improper disposal of human and animal waste in the aquatic bodies, etc. (Lunestad *et al.,* 2007; Budiati *et al.,* 2012, Ilkan, 2012). Therefore, Salmonellosis is a major public health problem and also of great research interest.

Materials and methods

Sample collection, bacterial Isolation and characterisation

Fifty juveniles of *C. gariepinus* were purchased from ten different commercial catfish farms in Ibadan, Oyo State, Nigeria, and were transported to the laboratory for further analysis.

The weight and length of sampled fish were measured using the weighing balance and meter rule respectively, and observed to be between 50-75g and 15-21cm respectively. The fish were rinsed and sterilized with the use of deionized water and 70% ethanol.

Blood samples and gut samples were aseptically collected (Noga, 2010) and inoculated into prepared Selenite broth base (Oxoid) (for maximum recovery of *Salmonella* species) for 18 hours at 37^0C in orbital shaker incubator. The inoculum were then transferred

onto *Salmonella* Shigella agar (Oxoid) for 24 hours at 37^{0}C, and colonies of *Salmonella* were selected based on colony morphology, and sub-cultured for pure *Salmonella* isolates. Motility test was carried out on the isolates as described by Stewart and Beswick (1977), by making a watery emulsion of the bacteria from the pure culture on a glass slide and observing under the microscope for bacterial motility at 400X magnification. Biochemical tests that were conducted include Gram reaction, catalase test, Simmons citrate test, indole test, H_2S production test and sugar fermentation tests (specifically glucose, sucrose, lactose and sorbitol).

Antibiotic Sensitivity Test

The disk diffusion method was used in this study to evaluate the antibiotic sensitivity of 3 randomly selected isolates morphologically and biochemically characterised as *Salmonella*, and designated as B1, B2 and B3 (from blood samples) and G1, G2 and G3 (from the gut). Diameters of growth inhibition zones were measured and analysed for Multiple Antibiotic Resistance Index (MARI)

Experimental Infection Test

Plate Count Method was used to quantify the isolate that was used for experimental infection of fish as described by Arora and Arora (2007).

New set of 80 apparently healthy *C. gariepinus* juveniles were purchased from a commercial fish farm in Ibadan, Nigeria, grouped into two (A and B), and acclimatized for two weeks. They were further subdivided into 4 subgroups of 10 fish each, comprising of the control and three other subgroups experimentally infected with 1.2 x 106, 1.2 x 107 and 1.2 x 108 cfu of *Salmonella* via the oral and the intra-peritoneal route respectively. Daily observation was made for clinical signs, pathological lesions and mortality thereafter, for 14 days.

Results

Bacterial growth on *Salmonella*-Shigella agar was in small, black and circular colonies, while on nutrient agar they were small, cream and circular. The colonies consist of Gram negative, rod shaped bacteria cells that were motile. The organism fermented sugars that include glucose, sucrose, lactose and sorbitol, and produced catalase, citrate and hydrogen sulphide but not indole.

Table 1: Sensitivity pattern of *Salmonella* isolates to antimicrobial agents

Antimicrobial agents	Percentage (%) of resistant isolates	Percentage (%) of sensitive isolates	Percentage (%) of intermediate isolates
Augmentin	100	0	0
Ceftriazone	100	0	0
Nitrofurantoin	100	0	0
Gentamicin	89	11	0
Cotrimoxazole	78	11	11
Ofloxacin	33	67	0
Amoxycilin	89	11	0
Ciprofloxacin	11	67	22
Tetracycline	78	22	0
Pefloxacin	11	89	0

Table 2: Multiple Antibiotic Resistance Index (MARI) observed for *Salmonella* isolates

Isolates	AUG	CRO	NIT	GEN	COT	OFT	AMX	CPX	TET	PFX	MARI
B1	R	R	R	R	R	S	R	I	S	S	0.6
B2	R	R	R	R	I	S	S	S	R	S	0.5
B3	R	R	R	R	R	R	R	R	R	S	0.9
G1	R	R	R	R	R	S	R	S	R	S	0.7
G2	R	R	R	S	S	R	R	S	R	S	0.6
G3	R	R	R	R	R	S	R	I	S	S	0.6

KEYS:
AUG – Augmentin *CRO – Ceftriazone* *NIT – Nitrofurantoin* *GEN – Gentamicin* *COT – Cotrimoxazole*
OFL – Ofloxacin *AMX – Amoxycilin* *CPX – Ciprofloxacin*
TET – Tetracycline *PFX – Pefloxacin* *R – Resistant* *I – Intermediate*
S - Sensitive *MARI - Multiple Antibiotic Resistance Index* *B1-B3 – Isolates from the blood*
G1-G3 – Isolates from the gut

$$\text{Multiple Antibiotic Resistance Index (MARI)} = \frac{\text{No. of antibiotics to which the isolate was resistant}}{\text{Total No. of antibiotics tested}}$$

Out of 50 fish that were subjected to bacteriological examination, result showed that 32% had *Salmonella* in the blood and 72% had *Salmonella* in their gut contents. However, experimental infection via both routes applied produced neither clinical signs nor pathological lesions, and no mortality was observed at the end of two weeks.

Furthermore, as shown in table 1, all isolates (100%) tested for antibiotic sensitivity were resistant to augmentin, ceftriazone and nitrofurantoin. Resistance was also high for various antibiotics including gentamycin (89%), cotrimoxazole (78%), amoxycilin (89%) and tetracycline (78%). The quinolones were observed to be a much more effective group of antibiotics with 89% of the isolates being sensitive to pefloxacin and 67% sensitive to both ciprofloxacin and ofloxacin. The percentage of isolates resistant to ofloxacin was however higher (33%) compared to ciprofloxacin (11%) and pefloxacin (11%).

Multiple Antibiotic Resistant Index (MARI) for all isolates from both the blood and the gut was observed to be high compared to the standard (0.2) for Enterobacteriaceae. The MARI observed in this study ranged from 0.5 to 0.9 for isolates from the blood and 0.6 to 0.7 for those from the gut.

Discussion

Salmonella organisms were present in the guts and blood of *Clarias gariepinus* that were purchased from ponds in Ibadan, Oyo State, Nigeria. Presence of *Salmonella* species in *Clarias gariepinus* is a strong indication that catfish is an important reservoir of *Salmonella* organisms in aquaculture (Budiati *et al.,* 2012) and cultured fish are not exempted like the naturally existing ones such as those reared in lakes, rivers, seas and oceans (Awuor et al. 2011; Gaertner *et al.,* 2008; Nadia *et al.,* 2011). *Salmonella* that was isolated from the gut was 40% higher than the one that was isolated from the blood. This may be due to possible fish feed contamination with *Salmonella*, as earlier reported by Lunestad (2007) who observed that fish feeds serve as a medium by which *Salmonella* organisms reach the aquatic environment and infect fish. Apart from feeds, other sources by which the organism contaminates fish in the earthen pond in commercial aquaculture should not be underestimated. These include non-point water run-off, run-off of organic matter into ponds including the waste of wild and domesticated animal, pond fertilization with manure, and contaminated source of water which are due to low standard of hygiene practices (Ilkan, 2012; Joseph and George, 2010). Nadia et al. (2011) also reported that aquatic insects are bio-

indicator of transmission or of contamination of *Salmonella* in the aquatic environment.

Another contributing factor to the presence of *Salmonella* in fish is also the use of water bodies as a means of disposing industrial and human waste (such as agricultural activities, forestry, agro-based industries and rural and urban settlement) which is a common practise world-wide (Awuor *et al.,* 2011).

The presence of *Salmonella* in the blood is also a confirmation of the septicaemic infection encountered both in Australia in 2006 and Sapparo in 1987 (Musto, *et al.,* 2006; Kodama *et al.,* 1987). Although, there was no sign of septicaemia in the present study, it is possible that *Salmonella* could have cross the cellular barrier of intestinal mucosa of fish or could have been transmitted into the circulatory system through wounds created on the skin or gills by ectoparasites.

According to Difco manual, *Salmonella* arizonae are indole negative, Simmon Citrate positive, Hydrogen Sulphide positive, catalase positive, glucose positive, sucrose negative, sorbitol positive and 10 to 89% lactose negative. The *Salmonella* organism isolated from *C. gariepinus* were able to ferment both lactose and sucrose when subjected to biochemical test. Grimont (2007) published that *S. enterica diarizonae* are 75% lactose positive while *S. enterica arizonae* are 25% lactose positive too. Both, arizonae and diarizonae are associated with cold-blooded animals and environment and are more likely to be the isolates encountered in this study. Some of the serovars of *Salmonella* that are lactose positive are *S. tennessee*, *S. virchow*, *S. indiana*, *S. agona*, *S. typhimurium*, *S. oranienburg*, *S. tuebingen*, *S. newport*, *S. typhi*, *S. java*, *S. toulen* (McDonough, 2000).

Salmonella contamination in fish has not directly imposed much threat on the health of piscine creature by inducing clinical infection and occurrence of *Salmonella* infection in fish is uncommon. Earlier observation revealed that experimental infection of any piscine species with the organism has not produce any infection (Karin *et al.,* 2011). This may be due to the ability of the fish to shed the organism after some days in water; this was observed in silver carp that were experimentally inoculated for 14 days. It may also be due to the ability of fish to produce immunity as seen in Tilapia aurea which produced fivefold antibody titre to *S. typhimurium* after 30 days of post infection. An elevated antibody titre is not necessarily indicative of active bacterial infection (Baker and Smitherman, 1983). *Crassius auratus* (goldfish) experimentally infected with *Salmonella* did not come down with any known infection nor showed any clinical sign. It was observed that *Salmonella* organisms colonized the visceral of the fish and they were also shed in the faeces of the fish in question (Horse et al, 1978). Probably factors that may be responsible for this is that, fish will most-likely come down with bacterial infection only when they are subjected to environmental stress (Song *et al.,* 2008). However, even where disease is not induced in fish by the presence of the bacterial organism, fish may serve as a reservoir host and veritable source of infection to both human and animals whose source of protein is fish (Ilkan, 2012), and also as occupational hazard to fish handers such as fish mongers and fish culturists. Moreover, since one of the characteristics of *Salmonella* organism is converting nitrate to nitrite, then its presence in the aquatic biosphere still portends more danger, because nitrite at level as low as 0.10 mg/L is very toxic to fish.

Another interesting thing about the isolates is the strong antibiotic resistance displayed by the organism. In Nigeria, antibiotics are used for prophylaxis, treatment and as growth promoter in animal husbandry and aquaculture, creating the basis for possible development of resistance of pathogens to many antibiotics in animal husbandry and aquaculture. Australian fish importers are also known to make use of antibiotics in their fish tanks as preventive measure against illness from aquatic fish pathogen (Musto et al, 2006). In this study, it was observed that the isolate were 100% resistance to Augmentin, Ceftriazone and Nitrofurantoin, 78-89% resistance to Tetracycline, Amoxicillin, Gentamicin and Cotrimoxazole. Susceptibility exhibited for the class of Fluroquinolones (i.e. Ofloxacin,

Ciprofloxacin, and Pefloxacin) was high at 67 – 89%. All the drugs displayed very high MARI, used in the determination of drug misuse. Whenever the MARI of a bacterium is greater than 0.2, the organism is deemed to have been isolated from an area where antibiotics had been misused. Therefore, all isolates observed in this study have been exposed to antibiotics mis-use at one time or the other. Resistance to Tetracycline and Amoxicillin could be related to their excessive use in catfish farming in Nigeria. Musto *et al.* (2006) reported multi-drug resistance *Salmonella* isolate from tropical fish in Australia, and the drugs were ampicillin, streptomycin, tetracycline, chloramphenicol, sulphonamides and spectinomycin. In Malaysia, Budiati *et al.* (2012) also observed that some *Salmonella* isolates were also resistance to clindamycin, tetracycline and chloramphenicol. The class of Fluroquinolones are the only drug that exhibit high level of efficacy which is an indication that these drugs are not widely used by aquaculturist. Susceptibility to ciprofloxacin and its family was not only demonstrated in this study but was also noticed in Malaysia by Budiati *et al* (2012). In case of ceftriazone, resistance was observed in this study but susceptibility was reported in Malaysia by Budiati *et al.* (2012). Furthermore, it has been observed that multi-drug resistance *Salmonella* isolates are more virulent than non-multiple drug resistant *Salmonella* isolates (Fluit, 2005; Foley and Lynne, 2008; Budiati *et al.,* 2012), and the spread of multi-drug resistance gene to other fish bacteria is also possible through mutation. Apart from this, we should be aware that these antibiotic resistance strains can spread internationally and may become endemic in countries importing tropical fish (Musto et al, 2006).

Conclusion

There is high prevalence of *Salmonella* in the blood and the gut of *Clarias gariepinus* that are cultured in earthen pond. The risk of *Salmonella* being transmitted to consumers (both human and animal) and producers (farm workers) is considered to be highly significant. Furthermore, the presence of *Salmonella* may be used to monitor faecal pollution in aquatic biosphere. This is because when these bacteria are present in native fishes, it may be strong retrospective indices of faecal pollution (Lawton and Morse, 1980). On another note, qualities of water bodies are seriously endangered when *Salmonella* is present, because of production of nitrite by the organism,

The occurrence of antimicrobial resistance among *Salmonella* isolates from catfish as observed in this study is considered to be very high. Hence, there is the danger of possible spread of multi-drug resistance among *Salmonella* serovars and also spread of resistance to other bacterial pathogens of fish. This spread may not only be limited to Nigeria alone but could spread internationally due to export. *Salmonella* organism is known to retain its resistant gene even when the drug is no longer in use and when all other microorganism has lost their own resistant gene. If thorough control of drug resistance *Salmonella* is not ensured, there can be a limit to the choice of drug for the treatment of salmonellosis in man and livestock. Strict personal hygiene is important for fish farmers and fish handlers, while effective biosecurity measures become very essential on fish farms.

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TEMPORAL AND SPATIAL PATTERNS OF THEILERIOSIS IN ZIMBABWE: 2000-2014

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Abstract

A retrospective study was conducted to determine the temporal and spatial patterns of theileriosis in Zimbabwe and the factors that may influence these patterns. Data were obtained from the Department of Field Veterinary Services (DFVS) theileriosis database covering the period from January 2000 to December 2014. Temporal patterns were analysed through examining the monthly, yearly, and seasonal patterns. The spatial spread of theileriosis and its main vector over the study period was done by projecting the geo-referenced records of cases and the tick vector into a Geographical Information System. The chi-square (X2) test for association and odds ratios (OR) at 95% confidence level was used to evaluate the association between theileriosis cases and seasons. The month of January recorded most cases, and the rainy season also accounted for the majority of cases (64%), with the hot-dry season recording the lowest number of cases (1%). The rainy season was also significantly associated with the occurrence of theileriosis (X2 =70.44, P<0.005). The communal areas recorded the highest number of cases (31%), whilst the resettlement areas had the lowest reported cases (10%), though the difference was not significant (P>0.05). Evidence from this study suggest theileriosis is spreading to most parts of the country thus improved availability of acaricides to enable more regular dipping of cattle to control the tick is recommended.

Key words: theileriosis, Rhipicephalus appendiculatus, temporal, spatial

REPARTITION TEMPORELLE ET SPATIALE DE LA THEILERIOSE AU ZIMBABWE : 2000-2014

Résumé

Une étude rétrospective a été menée pour déterminer la répartition temporelle et spatiale de la theilériose au Zimbabwe, ainsi que les facteurs qui peuvent influencer cette répartition. Les données ont été obtenues à partir de la base de données de la theilériose du Département des services vétérinaires de terrain (DFVS), et couvrent la période de janvier 2000 à décembre 2014. Les tendances temporelles ont été analysées par examen de modèles mensuels, annuels et saisonniers. La propagation spatiale de la theilériose et son vecteur principal pendant la période d'étude a été déterminée par projection des enregistrements géoréférencés des cas et la tique-vecteur dans un système d'information géographique. Le test chi-carré (X2) pour les rapports d'association et de probabilités (OR) à 95% de niveau de confiance a été utilisé pour évaluer l'association entre les cas de la theilériose et les saisons. Le mois de janvier a enregistré de nombreux cas, et la saison des pluies a également représenté la majorité des cas (64%), la saison sèche chaude représentant le plus petit nombre de cas (1%). La saison des pluies a également été significativement associée à l'apparition de la theilériose (X2 = 70,44, P <0,005). Les zones collectives ont enregistré le plus grand nombre de cas (31%), tandis que les zones de retour comptent le plus faible nombre de cas déclarés (10%), bien que la différence n'ait pas été significative (P> 0,05). Les données factuelles de cette étude font apparaître que la theilériose se propage dans la plupart des régions du pays ; il est donc recommandé d'améliorer la disponibilité des acaricides pour permettre une immersion plus régulière des bovins afin de contrôler les tiques.

Mots-clés : theilériose, Rhipicephalus appendiculatus, temporel, spatial

Introduction

Bovine theileriosis, caused by the protozoan parasite Theileria parva, is a lymphoproliferative disease characterised by high mortality in exotic and crossbred cattle, with indigenous breeds being less susceptible. Other species of Theileria occur in domestic animals of Africa, but these cause benign infections, thus T. parva is the most important in Zimbabwe and southern Africa in general (Norval *et al.,* 1992). The parasite is transmitted mainly by the brown ear ticks Rhipicephalus appendiculatus and R. zambeziensis.

Rhipicephalus appendiculatus is confined to the wetter areas of the country in the Highveld and Middleveld (Lawrence *et al.,* 1994), while R. zambeziensis replaces R. appendiculatus in the hotter and drier areas of the country including the large valleys of the Zambezi, Save and Limpopo rivers (Norval, *et al.,* 1992) as the tick is able to withstand extreme weather conditions (Madder *et al.,* 2005). However, their distributions overlap where there are gradual transitions between wet and dry areas with R. appendiculatus being always much abundant than R. zambeziensis. Norval *et al.,* (1988) attributed this situation to host resistance mechanisms which act more effectively against R. zambeziensis than R. appendiculatus.

Zimbabwean theileriosis occurs sporadically in the Highveld and Middleveld, where it usually follows the distribution of R. appendiculatus. Most clinical cases are reported in agro-ecological regions (AR) II and III (Manicaland and Mashonaland East, Central and West provinces) where there is high rainfall (Madzima and Mutugi, 1996). However, isolated cases occur in the Lowveld where these are attributed to R. zambeziensis transmission since the tick is usually found in low lying areas of Zimbabwe.

In Zimbabwe, theileriosis poses a considerable threat to livestock production; as a result of cattle morbidity and mortality, production losses associated with sick and recovering cattle, treatment of sick animals as well as from the costs of measures taken to control the tick vectors and the disease (Gachohi *et al.,* 2012). The disease further prevents the introduction of more productive exotic breeds of cattle, thus adversely affecting the development of the livestock sector. Mortality due to Zimbabwean theileriosis may reach 90% in severe outbreaks (Lawrence *et al.,* 1994). Subclinical infection also leads to decreased weight gain, reduced fertility and reduced milk production (Norval *et al.,* 1992). The reduction of beef and dairy production will also lead to diminished meat and milk supply to consumers thus negatively affecting household nutrition. However, like in other areas of pest management, there are few reliable estimates of economic losses resulting from theileriosis. The problem is further complicated by the occurrence of more than one tick-borne disease in most areas and the need to control ticks. Since the turn of the millennium, the Zimbabwean cattle farming has undergone tremendous management changes associated with the introduction of the agrarian reform programme in the year 2000, economic depression faced by the country, increased frequency of drought periods due to climate change and the occurrence of illegal animal movements among other factors. These factors could have resulted in the changes in the epidemiological trends of many infectious diseases of cattle that include theileriosis. In spite of these challenges, a great deal of epidemiological data on many of these diseases has been collected by the Department of Livestock and Veterinary Services and stored in a central database. However, analysis of the data to determine the spatial and temporal distribution of theileriosis over the years has not been conducted. This study was conducted to determine the temporal and spatial epidemiology of theileriosis in cattle in Zimbabwe, using data collected on routine monitoring of the disease.

Materials and Methods

Data collection

A retrospective study was conducted using data obtained from the monthly and

annual reports of theileriosis in the Department of Field Veterinary Services (DFVS) covering the period from January 2000 to December 2014. The data extracted covered monthly and annual disease reports of the DFVS. The DFVS is responsible for the compilation and summarization of all disease reports from the different provinces around the country. The diagnosis of theileriosis was based on both clinical and laboratory confirmation. The clinical diagnosis of field theileriosis was confirmed by experienced State veterinarians. Diagnosis was confirmed by laboratory tests, where schizonts were demonstrated by microscopic examination of smears made from lymph node aspirates. All the tests were carried out at the Central Veterinary Laboratory in Harare. Only the cases with laboratory investigated cases were stored in the national data base.

Data analysis

The collected data was scrutinized for repetitions of reporting prior to electronic entry. Microsoft Excel 2013 was then used to verify and edit the data, and generate descriptive statistics of cases and deaths. Analysis of temporal patterns was through examining the monthly, yearly, as well as the seasonal patterns. The four seasons of the year were defined as; rainy (December to February), post-rainy (March to May), cold-dry (June to August), and hot-dry (September to November) (Chikerema *et al.,* 2012), and were used for analysing the seasonal patterns of theileriosis cases and outbreaks. The chi-square (X2) test for association and odds ratios (OR) at a confidence level of 95% was used to evaluate the association between theileriosis cases and seasons using Epi-Cal 2000. The period under study was also subdivided into three groups, (2000-2004, 2005-2009 and 2010-2014), each five years long. Farming sectors were divided into communal, commercial, small scale and resettlement areas. Natural regions were divided into five agro-ecological regions on the basis of the amount of rainfall, soil quality and vegetation, among other factors (Moyo, 2000), with the quality of the land resource declining from AR I through to AR V. Prevalence was estimated based on the number of theileriosis cases and the census at the time of diagnosis. The chi-square test for association and odds ratio (OR) at a confidence level of 95% was used to evaluate the association between theileriosis cases and different sectors using Epi-Cal 2000. To project the spatial spread of theileriosis cases and its main vector, geo-referenced records of cases and the tick vector were imported from Microsoft Excel into a Geographical Information System (ILWIS Academic v. 3.1) for initial editing for errors and repetitions. Final mapping of the records were done in DIVA-GIS (www.diva-gis.org/download).

Results

Temporal patterns of theileriosis cases

A total of 2 223 cases were recorded during the period 2000–2014. The monthly occurrence of cases were pooled for this period, with the highest number of cases occurring in the month of January, followed by March and February, with the rest of the months having no or very few cases (Figure 1).

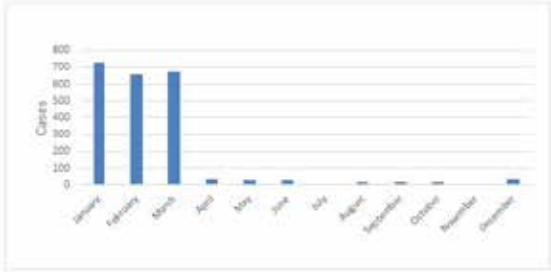

Figure 1: Monthly distribution of theileriosis cases in cattle for the period January 2000 to December 2014.

The rainy season accounted for the majority of cases (64%), followed by the post-rainy season (33%), the cold-dry season (2%), with the hot-dry season recording the lowest number of cases (1%). The rainy season was also significantly associated with the occurrence of theileriosis (X2 =70.44, P<0.005), and the disease was found to be approximately twice (OR= 1.78, 95% CI, 1.45, 2.34) as likely to occur in the rainy season compared to other seasons.

The post-rainy season, compared to the cold-dry and the hot-dry season, was also found to be significantly associated with the occurrence of the disease (X2=48.4, P<0.001) and (X2=136.63, P<0.001) and the disease more likely to occur by approximately three (OR= 3.12, 95% CI, 2.23, 4.37) and six (OR=6.40, 95% CI, 4.47, 9.16) times respectively.

The highest numbers of cases were recorded in the year 2001, that year accounting for 634 cases and the lowest in 2009, accounting for the 26 cases as shown in Figure 2. The first third (2000-2004) of the study period had a markedly high number of cases accounting for 66% of the cases, followed by the last third (2010-2014) with 21% of the cases and the second third (2005-2010) had the lowest cases (13%).

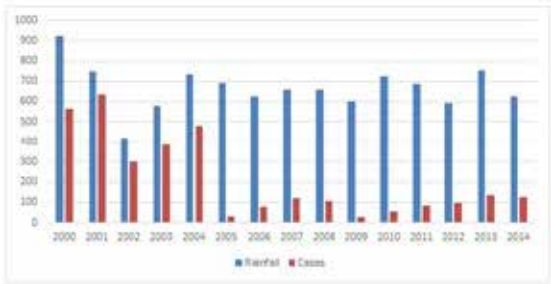

Figure 2: Annual distribution of theileriosis and total annual rainfall for the period 2000 to 2014.

Spatial distribution of theileriosis cases

The majority of cases were reported in the communal areas (31%) and small scale areas (31%) followed by the commercial areas (28%), whilst the resettlement areas had the lowest number of cases (10%) (Figure 3), but the difference was not significant (P>0.05).

Of all the cases which occurred in the post-rainy season, the majority of them were in the commercial area (38%), followed by the resettlement area (22%) and small scale area (21%), whilst only 19% occurred in the communal area. During the cold-dry and hot-dry season the majority of cases occurred in the commercial area (41%), followed by resettlement area (30%), small scale area (20%) and lastly the communal area (9%).

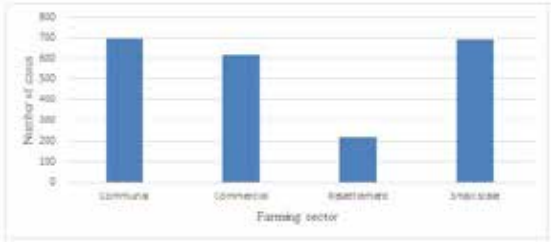

Figure 3: Distribution of theileriosis cases in different farming sectors of Zimbabwe (2000-2014).

Matabeleland North province accounted for the highest number of reported cases of about 26% in the whole study period. On the other hand, Matabeleland South had the lowest number of reported cases accounting for only 0.01% of the cases in the study period.

In 1996, both R. appendiculatus ticks and theileriosis outbreaks were mainly concentrated in the north and eastern regions of Zimbabwe. However, 18 years later (2014), both the tick vector and theileriosis had spread to areas of the country (Figure 4).

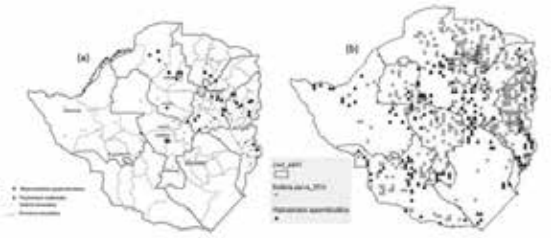

Figure 4: Distribution of theileriosis and R. appendiculatus in (a) 1996 and (b) 2014.

AR II accounted for most of the cases (34%), followed by AR V (30%), AR III (16%), AR IV (14%) and AR I had the lowest number of cases (6%).

Discussion

This retrospective study was conducted to determine the spatial and temporal patterns of theileriosis in Zimbabwe and to investigate the factors that may influence these patterns. Notwithstanding the drawbacks of using retrospective data collected from routine disease monitoring, the analysis of such data could be a convenient and inexpensive source of information to study important epidemiological

information on theileriosis. Considering that this study focused on only the reported cases of theileriosis, this could be an underestimate of the extent of the problem posed by the disease at national level. Poor disease surveillance due to budgetary constraints and fewer veterinary personnel during the years under study, lack of knowledge and awareness amongst rural farmers (Chikerema *et al.,* 2012), as well inaccessibility especially in resettlement areas are some of the factors that could have attributed to underreporting of theileriosis cases and outbreaks. Overestimation in some instances could also have occurred due to misdiagnosis of theileriosis. However, the fact that all the cases recorded in the national data base were based on laboratory confirmed cases reduced the bias of misdiagnosis.

The majority of cases occurred in the month of January and during the rainy season. The results concur with field observations on the seasonal occurrence of theileriosis outbreaks as only a single generation of R. appendiculatus ticks occurs in the country, whereby the adults are most abundant in the rainy season (Koch, 1990), and are responsible for the main transmission of theileriosis. The occurrence of the majority of cases in the post-rainy season could be attributed to adult ticks as they are still active up until the month of April (Latif *et al.,* 2001). The cold dry season experienced a lower number of cases as this period is characterized by the predominance of immature stages of the tick, the nymph and the larvae. Nymphal transmission in Zimbabwe usually occurs between June and December but is usually limited by presence or absence of wild animals which act as alternative hosts (Latif *et al.,* 2001). Nymphs have been reported to transmit theileriosis but at a very low rate (Latif *et al.,* 2001)

The period from the year 2000 to 2004 witnessed the highest number of cases. This could be attributed to the large movement of cattle as farmers migrated from the mostly overgrazed communal land areas to the former commercial farms where there was good grass cover which is conducive for survival of the R. appendiculatus tick. This could have led to more tick burden on the animals and increased number of theileriosis outbreaks and cases. The country also went through an economic recession which started in 2000 and peaked in 2008. The decreased budgetary allocation for veterinary services and hence erratic provision of dipping services affected mainly the communal land farmers since they rely mostly on government to supply acaricide (Ndhlovu *et al.,* 2009), leading to more cases of theileriosis.

The number of cases was highest in communal areas. The high number of cases in the communal land areas could be attributed to the fact that communal farmers own the majority of cattle in Zimbabwe (Tavirimirwa *et al.,* 2013). The majority of cases also occurred during the rainy season when conditions are more conducive for adult tick survival whilst other seasons are characterized by very fewer numbers of ticks. This may also partly explain the pronounced seasonal pattern of the disease in the communal land areas.

The fewer cases reported from the small scale sector compared to other sectors could be due to the fact that most small scale farms are found in AR II and III, regions that are less suitable for the tick survival. In addition, as this sector occupies only 4% all land in Zimbabwe, the fewer number of cattle contributes to the low number of cases. This also partly explains the low number of cases in the resettlement areas.

Spatial analysis of the distribution of the theileriosis and its vector revealed an increased spread of R. appendiculatus and theileriosis from the northern and eastern regions to the rest of the country. The increased spread of the disease and its vector could be attributed mainly to the uncontrolled movement of cattle during the land reform programme which may have resulted in introduction of the vector by infested cattle in these areas. Increased number of cases might also have been due to reduced acaricide treatment of cattle by the newly resettled farmers.

Occurrence of theileriosis in different areas exhibited a wide range of variation among the different agro-ecological zones from as low

as 6% in AR I to as high as 34% in AR II. These differences could be a reflection of differences in the intensity of R. appendiculatus activity as well as control practices on individual properties in the different regions (Muhanguzi *et al.,* 2014). Within the different regions, various ecological and cattle management systems affect the occurrence of theileriosis. The high number of cases in Regions II and III could be due to the higher rainfall and good grass cover in these areas creating more ideal conditions for R. appendiculatus survival. Whilst AR I has ideal conditions for R. appendiculatus survival, the low number of cases could be due the fact that it occupies only 4% of the whole country and most of the land is dedicated to cash crop production, thus the low numbers of cattle may have contributed to the low number of cases.

Conclusions

This work detected an increase in the number of cases of theileriosis and the spatial spread of the disease and its tick vector across the country. We recommend availing of more regular supplies of acaricides so that cattle owners can dip their cattle more regularly in order to effectively control the tick vector.

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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 2017

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The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Interafrican Bureau for Animal Resources (AU-IBAR) is a scientific journal which publishes articles on research relevant to animal health and production including wildlife and fisheries contributing to the human wellbeing, food security, poverty alleviation and sustainable development in Africa. The bulletin disseminates technical recommendations on animal health and production to stakeholders, including policy makers, researchers and scientists in member states. The Bulletin is the African voice on animal resources issues specific to Africa.

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- *Books:* Durbin R, Eddy SR, Krogh A, Mitchison G, 1999. Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids. London, Cambridge University Press.

- *Chapter in a Book:* Leach J, 1993. Impacts of the Zebra Mussel (Dreissena polymorpha) on water quality and fish spawning reefs of Western Lake Erie. In Zebra Mussels: Biology, Impacts and Control, Eds., Nalepa T, Schloesser D, Ann Arbor, MI: Lewis Publishers, pp: 381-397.
- *Reports:* Makarewicz JC, Lewis T, Bertram P, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. US EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.
- *Conference Proceedings:* Stock A, 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.
- *Thesis:* Strunk JL, 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, Unpublished PhD thesis, Michigan State University, East Lansing, MI.
- *Web links:* Cerón-Muñoz M F, Tonhati H, Costa C N, Rojas-Sarmiento D and Solarte Portilla C 2004 Variance heterogeneity for milk yield in Brazilian and Colombian Holstein herds. Livestock Research for Rural Development. Volume 16, Article #20 Visited June 1, 2005, from http://www.lrrd.org/lrrd16/4/cero16020.htm

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