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BLOOD PARASITES OF SEMI-DOMESTICATED AND WILD BIRDS IN KADUNA STATE, NIGERIA

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Abstract

Wild birds interact with poultry with likelihood of exchange of blood parasites between the wild bird and poultry highlighting the need to understand wild bird parasites so as to reduce cross infection at the wild bird-poultry interface. There is paucity of data on blood parasites of wild birds in Kaduna State, Nigeria. This study investigates the prevalence and diversity of blood parasites among wild birds in Kaduna State. Blood of wild birds were examined from Febuary to June, 2012 for parasites by microscopic examination. Data were analyzed using Quantitative Parasitology software. Of 297 birds examined, 23.9 % had blood parasites with 39.4 % families and 39.3 % species infected. Parasites identified were *Haemoproteus* (7.7%), *Plasmodium* (16.2%), *Leucocytozoon* (2.7%), Aegyptionella (1.3%), and microfilariae (1.3%). There was a significant difference (p=0.0) between the prevalence of Aegyptionella, *Leucocytozoon*, Hemoproteus and *Plasmodium*. Some birds (4.1 %) had multiple blood parasite infection. *Leucocytozoon* was dectected in Columba livia, Streptopelia senegalensis, Meleagris gallopavo, Francolinus bicalcaratus, Hirundo aethopia and Pychonotus barbatus. Live poultry markets prevalence were *Plasmodium* (47.8 %), *Haemoproteus* (15.8 %) and Aegyptionella (2.6 %). *Leucocytozoon* prevalence was 4.2 % in free flying birds. Anchau had *Plasmodium* prevalence of 31.7 %. This study concludes that cross infection of blood parasites can occur at the wild bird-poultry interface and LWBMs encourages blood parasites transmission between countries. There is need for more studies on blood parasites of wild birds to understand their impact on the survival of wild bird species in Nigeria.

Key words: Blood parasites, Kaduna State, Live wild bird markets, Nigeria, Wild birds

PARASITES SANGUINS DES OISEAUX SEMI-DOMESTIQUES ET DES OISEAUX SAUVAGES DANS L'ETAT DE KADUNA AU NIGERIA

Resume

Les oiseaux sauvages interagissent avec les volailles, et cette interaction présente un risque d'échange de parasites sanguins entre les deux, d'où la nécessité de comprendre les parasites des oiseaux sauvages afin de réduire l'infection croisée à l'interface oiseaux sauvages - volailles. Il existe peu de données sur les parasites sanguins des oiseaux sauvages dans l'État de Kaduna au Nigeria. La présente étude examine la prévalence et la diversité des parasites sanguins chez les oiseaux sauvages dans l'État de Kaduna. Du sang prélevé sur des oiseaux sauvages a été examiné de février à juin 2012 afin de détecter les parasites par examen microscopique. Les données ont été analysées en utilisant le logiciel de parasitologie quantitative. Des 297 oiseaux examinés, 23,9 % avaient des parasites sanguins, dont 39,4 % des familles et 39,3 % d'espèces infectées. Les parasites identifiés étaient Haemoproteus (7,7%), Plasmodium (16,2%), Leucocytozoon (2,7%), Aegyptionella (1,3%), et des microfilaires (1,3%). Une différence significative (p = 0,0) a été notée entre la prévalence de Aegyptionella, Leucocytozoon, Hemoproteus et Plasmodium. Certains oiseaux (4,1%) présentaient de multiples infections par des parasites sanguins. Leucocytozoon a été détecté chez Columba livia, Streptopelia senegalensis, Meleagris gallopavo, Francolinus bicalcaratus, Hirundo aethopia et Pychonotus barbatus. Sur les marchés de volailles vivantes l'étude a identifié Plasmodium (47,8 %), Haemoproteus (15,8%) et Aegyptionella (2,6%). La

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prévalence de Leucocytozoon était de 4,2% chez les oiseaux volant en toute liberté. Anchau avait une prévalence de Plasmodium de 31,7 %. L'étude conclut que l'infection croisée par des parasites sanguins peut se produire à l'interface oiseaux sauvages - volailles, et les marchés d'oiseaux sauvages vivants encouragent la transmission de parasites sanguins entre les pays. Il est nécessaire de mener d'autres études sur les parasites sanguins des oiseaux sauvages afin de comprendre leur impact sur la survie des espèces d'oiseaux sauvages au Nigeria.

Mots-cles : Parasites sanguins ; État de Kaduna ; Marchés d'oiseaux vivants ; Nigeria ; Oiseaux sauvages

Introduction

Wild birds are known to host a variety of parasites. Parasites usually affect population growth of species as well as interactions between species with the milder endemic parasites being able to play a major role in population regulation despite occasional devastating epidemics (Anderson, 1979; Anderson, 1980; Sumpton and Flowerdew, 1985). Parasites also exhibits other ecological implications in phenomena such as parasite mediated host competition, sexual choice, social behaviour, foraging tactic and predator-prey interactions (Price *et al.,* 1988; Hamilton *et al.,* 1990; Lozano, 1991; Hudson, 1992). The ability of these parasites to affect host life history and fitness coupled with their impact on host reproduction and survival highlights their role as formidable evolutionary forces (Rigby and Moret, 2000; Stjerman *et al.,* 2004).

The importance of parasites in the ecology of wild birds is being increasingly recognized and many health and parasite surveys have been conducted on wild birds throughout the world (Stjernman *et al.,* 2004). However, reports of studies on the ecology of WB parasites of free flying and live wild bird markets (LWBMs) wild birds in Sub Saharan Africa are scanty (Savage *et al.,* 2009). Consequently, because of the rich avi-fauna of Sub-Saharan Africa coupled with the fact that being home to wintering migratory birds from Europe and Asia (Gaidet *et al.,* 2008), it is vital to understand the parasite community in this region and its potential health effects on wild birds. This study determined the blood of free flying and LWBMs wild birds in Kaduna State, Nigeria. The study also appraised variation in parasite abundance, their prevalence in host populations, and intensity of infection in different families and species of wild birds. This study may be the first multispecies survey of blood parasites in free flying and LWBMs wild birds from West African Savannah.

Materials and Methods

Study Area

The study was carried out in Kaduna State, located in North Western Nigeria between latitude 8° 45"- 11°30" North and longitude 6°11" – 9oEast (RIM, 1993). It shares boundary with Kastina, Kano, Plateau, Niger, Zamfara, Bauchi, Nassarawa and FCT and has 23 local government areas that are inhabited by ethnic groups including Hausa, Fulani, Kaje and Kataf amongst others. Kaduna State has a population of 6 million people and 2,821,092 poultry of which 90% is local poultry raised extensively (RIM, 1993).

The annual temperature is 34°C with hottest months being March-April (40°C) and the coolest period (13.2°C) being December during severe harmattan. Rainfall varies between 1,000 mm and 1,500 mm and the rainy season last 100-150 days (Mid April – ending of October). The dry season occurs between October and April (RIM, 1993). Kaduna State has a land structure of undulating Plateau with major rivers including River Kaduna, River Wonderful in Kafanchan, River Kagom, River Gurara and Galma (RIM, 1993). The vegetation varies from the Guinea Savannah in the south to the Sudan Savannah in the North (RIM, 1993).

Sampling Technique

Wild bird in LWBMs, free flying and semi-domesticated birds from live poultry markets (LPMs) were sampled between February and June, 2012. Four sampling

locations were chosen based on poultry density, presence of LWBMs and LPMs; water bodies.

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

Sampling Units

Wild birds were sampled from three epidemiologic units namely live wild bird markets (LWBMs), free flying wild birds and live poultry markets (LPMs).

Live wild bird market

Live wild birds in Kaduna LWBMs were sampled after live wild bird sellers in Kaduna LWBMs were approached and consent obtained for participation in the study.

Free flying wild birds

Free flying birds are wild birds that were not in captivity. The birds were captured by mist nets, hunting and use of other traps. Hunters gave consent for hunted birds to be sampled. For free flying wild birds roasting on trees, faecal samples were collected by the use of a white paper. Free flying wild birds were sampled from Kaduna, Samaru, Anchau, Karoye and Sabon Gari.

Live poultry markets

Two semi domesticated species – guinea fowls and mallard ducks were identified due to their arboreal nature and likelihood of interacting with wild birds especially migratory birds and local poultry in human habitats. Live mallards and guinea fowls were sampled from Anchau LPM after obtaining consent from sellers to sample birds.

Identification of Wild Bird

Wild birds roasting on trees whose faecal samples were collected were also identified using a pair of binoculars with magnification 7x 50. All birds were visually identified with the aid of a field guide by Borrow and Demey (2004) and physically examined prior to sampling.

Blood Collection

About 0.5-2 ml of blood was collected by venipuncture into EDTA tubes, using a sterile needle and syringe from the wild birds. The amount of blood taken was dependent on size of the bird.

Blood Parasite Examination

A drop of blood was used to prepare thin blood smears on microscope slides. The thin blood smears were fixed in the field with absolute methanol, and later stained with Giemsa (pH 7.0).

Smears were initially examined at 100 X for 3–5 min (approximately 2 × 106 blood cells examined) for detection of blood parasites. Each slide was then examined under 1,000 X for an additional 5–8 min (approximately 3 × 104 blood cells). Smears from infected birds were then examined at 1,000 X for the entirety of the slide and parasite were identified by their morphologic characteristics and documented by photography. All slides are on deposit at the Department of Veterinary Medicine, Ahmadu Bello University - Zaria, collections.

Data Analysis

Positive bird was defined as any wild bird with at least one blood or external parasites. Prevalence, mean intensity and mean abundance values were analysed using Quantitative Parasitology 3.0 (Ro´zsa *et al.,* 2000).

The differences in prevalence between blood parasites, was determined using chi square test. The difference in mean intensity and abundance between parasites was determined using t-test. The median intensities were compared using Mood's median test (Ro´zsa *et al.,* 2000). Confidence intervals for prevalence and intensity were computed using Sterne's exact method, and bootstrapping (with 2,000 repetitions), respectively, using the computer program Quantitative Parasitology 3.0 (Reiczigel and Ro´ zsa, 2005).

Prevalence between and within families, species, epidemiologic units and sampling sites,

were compared by the chi-square test with p values ≤ 0.05 considered significant. Association of blood parasite and ectoparasite, were analyzed using cross-tabulations with Statistical Package for Social Sciences (SPSS) version 17.

Results

Of the 297 birds sampled in this survey, 23.9 % (71) were parasitemic with at least one species of blood parasites. In addition, 43.2 % (16/37) families and 38.7 % (24/62) species were infected, with prevalence within and between families ranging from 13.6 % (Phasianidae) to 69.6 % (Numididae) (Table 1) and from 1.6 % to 26.2 % respectively (Figure 1).

However, among the 24 species infected by blood parasites, Hirundo aethiopica had the highest species prevalence rate of 85. 3 % (5/6) with the lowest being Columba livia with 11.1% (1/9) (Table 1) though between species the highest prevalence was 22.5 % (16/71) for Numida meleagris (Figure 2).

Plasmodium species was the most prevalent blood parasites with 16.2 % (48/297) followed by Hemoproteus species with a prevalence of 7.7 % (23/297). *Leucocytozoon* species were observed in 2.7 % (8/297) of the birds, followed by Aegyptionella spp. with 1.3 % (3/297), and Microfilaria 1.3 % (4/297) (Figure 3). The mean intensity for *Plasmodium* was 3.29 (Table 2). There was a significant difference between *Plasmodium* and *Haemoproteus* prevalence ($X2 = 10.0$, $df = 1$, $p = 0.00$), however, there was no significant difference in their mean abundance, mean and median intensities.

There was a significant difference between the prevalence, mean abundance of *Plasmodium* and *Leucocytozoon* ($X2 = 31.55$, $df = 1$, $p = 0.00$); (bootstrap p-value = 0.04, t = 2.29) respectively. Conversely, there was no significant difference between the mean and median intensities of *Plasmodium* and *Leucocytozoon*. There was a significant difference between the prevalence of *Haemoproteus* and *Leucocytozoon* ($X2 = 7.66$, $df = 1$, $p = 0.01$). However, there was no significant difference between the mean abundance; mean and median intensities of *Haemoproteus* and *Leucocytozoon*.

The 95% confidence limits of Microfilaria prevalence was 0.36 – 3.1 % with mean and median intensity of 1.00. The mean abundance was 0.01 with confidence limits of 0.0-0.02 at 95%. However, Aegptionella mean and median intensities were 1.25 and 1 respectively.

Comparing prevalence of *Leucocytozoon*, Hemoproteus and *Plasmodium*, there was a significant difference between their prevalence ($X2 = 34.03$, $df = 2$, $p = 0.00$). There was no significant difference between their median intensities. Among the 14 bird families and 16 species infected with *Plasmodium*. Hirundae and great egret (Egretta alba) had the highest prevalence of 83.3 % (5/6) and 66.7% (2/3) respectively (Table 1). *Plasmodium* prevalence between the infected Families (Figure 4) and species ranged from 2.3 % to 30.2 % (Figure 5). Over 62.5 % (10/16) of infected families had *Haemoproteus* with prevalence within families ranging from 6.5 % to 50 % (Table 1). The proportionate prevalence among families was 9.1 % for Anatidae and Pyononotidae (Figure 6).

Fifteen species out of the 24 blood parasites infected species (62.5 %) were infected with *Haemoproteus* and their prevalence between species was 4.2 % (1/24) for black crake (Amaurornis flavirostra), allen gallinue (Porphyrio alleni) (Figure 7). The prevalence within species varied from 6.7 % (1/15) in Mallard duck to 66.7 % (2/3) in Great Egret (Table 1).

Five out of the 16 families (31.25 %) infected by blood parasites had *Leucocytozoon* infection. The families were Charadriidae, Columbidae, Phasianidae, Melagrididae and Pyononotidae with *Leucocytozoon* prevalence of 4.3 % (2/47), 9.7 % (3/31), 11.1 % (1/9) and 11.1 % (1/9) respectively within families. However, the proportionate prevalence among families was 25 % (2/8), 50 % (4/8) and 12.5 % (1/8) for Columbidae, Phasianidae, Melagrididae and Pyononotidae respectively. Similarly, 25 % (6/24) of infected species had *Leucocytozoon* with species proportionate prevalence of 12.5 % (1/8) for common bulbul, Ethopian swallow, rock dove, turkey and laughing dove with 37.5 % (3/8) for double-spurred francolin. The species prevalence was 11.1 % (1/9) for

Table 1: Prevalence of blood parasites among wild bird families and species in Kaduna State, Nigeria.

Infected Family/Species	P (No. infected/ sample)	*Plasmodium*	Parasitea *Haemo-proteus*	*Leucocytozoon*
ANATIDAE	**27.3% (6/22)**	**22.7% (5/22)**	**9.1 % (2/22)**	-†
Barn geese	14.3% (1/7)	-	14.3% (1/7)	-
Anas platyrhynchos	33.3% (5/15)	33.3% (5/15)	6.7% (1/15)	-
ARDEIDAE	**26.7% (4/15)**	**14.3% (2/14)**	**20% (3/15)**	**-**
Bubulcus ibis	16.7% (1/6)	-	16.7% (1/6)	-
Egretta alba	100.0% (3/3)	66.7% (2/3)	66.7% (2/3)	-
CHARADRIIDAE	**12.5% (2/16)**	**6.25% (1/16)**	**6.25% (1/16)**	**-**
Vanellus tectus	16.7% (1/6)	16.7% (1/6)	-	-
Vanellus spinosus	30.0% (3/10)	-	(1/10) 10.0%	-
CICONIIDAE	**25.0% (3/12)**	**25.0% (3/12)**	**-**	**-**
Ciconia ciconia	25.0% (3/12)	25.0% (3/12)	-	-
COLUMBIDAE	**18.5% (10/54)**	**11.1 % (6/54)**	**5.6% (3/54)**	**3.7 % (2/54)**
Streptopelia senegalensis	20.7 % (6/29)	13.8% (4/29)	6.9% (2/29)	3.4 % (1/29)
Streptopelia vinacea	20.0% (2/5)	20.0% (2/5)	-	-
Columba guinea	33.3% (1/3)	-	33.3% (1/3)	-
Columba livia	11.1% (1/9)	-	-	11.1 % (1/9)
JACANIDAE	**50.0% (1/2)**	**50.0% (1/2)**	**-**	**-**
Actophilornis africanus	50.0% (1/2)	50.0% (1/2)	-	-
MELAGRIDIDAE	**22.2% (2/9)**	**22.2% (2/9)**	**-**	**11.1 % (1/9)**
Meleagris gallopavo	22.2% (2/9)	22.2% (2/9)	-	11.1 % (1/9)
MUSOPHAGIDAE	**50.0% (2/4)**	**25.0% (1/4)**	**50.0% (2/4)**	**-**
Crinifer piscator	50.0% (2/4)	25.0% (1/4)	50.0% (2/4)	-
NUMIDIDAE	**69.6% (16/23)**	**56.5% (13/23)**	**21.7% (5/23)**	**-**
Numida meleagris	69.6% (16/23)	56.5% (13/23)	21.7%(5/23)	-
PHASIANIDAE	**13.2% (5/38)**	**7.9% (3/38)**	**-**	**7.9 % (3/38)**
Francolinus bicalcaratus	13.2% (5/38)	7.9% (3/31)	-	7.9 % (3/31)
PSITTACIDAE	50.0% (1/2)	50.0% (1/2)	-	-
Psittacus erithacus	50.0% (1/2)	50.0% (1/2)	-	-
PYNONOTIDAE	**44.4% (4/9)**	**11.1% (1/9)**	**22.2% (2/9)**	**11.1 % (1/9)**
Pychonotus barbatus	44.4% (4/9)	11.1% (1/9)	22.2% (2/9)	11.1 % (1/9)
RALLIDAE	**22.7% (5/22)**	**22.7% (5/22)**	**13.6% (3/22)**	**-**
Amaurornis flavirostra	23.1% (3/13)	30.8% (4/13)	7.7% (1/13)	-
Porphyrio alleni	50.0% (1/2)	50.0%(1/2)	50.0% (1/2)	-
Porphyrio porphyrio	16.7% (1/6)	-	16.7% (1/6)	-
RECURVIROSTRIDAE	**40.0% (2/5)**	**-**	**20.0% (1/5)**	**-**
Himantopus himantopus	40.0% (2/5)	-	20.0% (1/5)	-
HIRUNDINIDAE	**83.3 % (5/6)**	**66.7% (4/16)**	**-**	**16.7% (1/16)**
Hirundo aethiopica	83.3 % (5/6)	66.7% (4/16)	16.7 % (1/6)	16.7% (1/16)
MALACONOTIDAE	**16.7% (1/6)**	**-**	**16.7% (1/6)**	**-**
Laniarius barbarus	16.7% (1/6)	-	7.7% (23/297)	-
TOTAL	**23.9% (71/297)**	**16.2% (48/297)**		**2.7% (8/297)**

†= Negative.

common bulbul (Table1).

Three families, Charadriidae, Numididae (1/3) and Rallidae (2/3) were infected with Aegyptionella. However, the family prevalence was 6.25% (1/16), 4.3 % (1/23) and 9.1 % (2/22) for Numididae and Rallidae respectively. Species infected by Aegyptionella were African black crake and helmeted guinea fowl with prevalence of 15.4 % (2/13) and 4.3 % (1/22) respectively.

Ardeidae, Charadriidae, Phasianidae and Recurvirostridae were the only families infected with microfilaria in the study. The prevalence within family was 6.7 % (1/15), 6.25 % (1/16), 2.6 % (1/38) and 20 % (1/5) for Ardeidae, Charadriidae, Phasianidae and Recurvirostridae respectively. The microfilariae proportionate prevalence among families was 25 % (1/4) for each of the infected families.

The infected species from these families were spur-winged lapwing, double spurred francolin, black winged stilt and great egret with prevalence of 10 % (1/10), 12.6 % (1/38), 20 % (1/5) and 33.3 % (1/3) respectively within species.

Multiple infections, defined as infections with more than one type of blood parasite, were observed in 15.5 % (11/71) of the infected individuals, i.e. 3.7 % (11/297) of all birds sampled (Table 3). Among infected wild bird families, 68.8 % (11/16) had multiple infections with the Ardeidae, Columbidae, Numidiae, Pyononotidae and Rallidae having triple infections (Table 1).

The blood parasites prevalence in sampling units were 20.5 % (40/195) for free flying birds, 55.3 % (21/38) for LPM and 15.6 % (10/64) for LWBM. However, the proportionate prevalence among sampling units were 56.3 % (40/71) for free flying birds, 29.6 % (21/71) for LPM and 14.1 % (10/71) for LWBM ($p = 0.00$; $X2 = 23.02$; $df = 2$).

The *Plasmodium* prevalence within the different sampling units were 11.8 % (23/195) for free flying birds ($p = 0.00$; $X2 = 27.9$; $df = 2$) (Table 4). Among the birds infected with *Plasmodium*, 47.9 % (23/48) was free flying birds ($p = 0.00$; $X2 = 27.9$; $df = 2$) (Figure 8).

Prevalence of *Haemoproteus* infection in the sampling units was 7.2 % (14/195) for free flying birds 4.7 % (3/64) for LWBM (Table 4). Nevertheless, the Hemoproteus proportionate prevalence among the sampling units was 26.1 % (6/23) for LPM (Figure 8).

The *Leucocytozoon* prevalence was 0 % (0/38) in LPM (Table 4). However, comparing prevalence among the sampling units, 87.5 % (7/8) of *Leucocytozoon* infected birds were free flying birds (Figure 8).

The Aegyptionella prevalence was 2.6 % (1/38) in LPM (Table 4) with a proportionate prevalence of 33.3 (1/3) for LPM. The study revealed that only free flying birds were infected with microfilaria with a prevalence of 2.4 % (4/164).

The blood parasites prevalence within sampling location was 38.3 % (23/60) in Anchau, 15.4 % (10/65) in Kaduna; 21.3 % (35/164) and 40 % (2/5) in Samaru and Koraye respectively. The blood parasites prevalence in Sabon Gari was 33.3 % (1/3). However, the sampling location's contributions to the overall blood parasites prevalence rate was 16.4 % (10/61) in Kaduna ($p = 0.04$; $df = 5$; $X2 = 11.91$) (Figure 9).

Samaru had the highest *Plasmodium* prevalence of 50 % (24/48) between sampling location (Figure 10). However, the sampling locations' prevalence were 31.7 % (15/60) for Anchau ($p = 0.012$; $df = 5$; $X2 = 14.64$) (Table 4).

Haemoproteus was prevalent in all the

Table 2: Haemoparasite prevalence, mean/median intensity and mean abundance among wild birds in Kaduna State, Nigeria.

Parasite	Prevalence @ 95 % confidence limit	Mean intensity @ 95 % confidence limit	Median intensity @ 99.7 % confidence limit	Mean abundance @ 95 % confidence limit
Plasmodium	16.%2(12.7%-20.1 %)	3.29 (2.6- 4.2)	2.0 (2-3)	0.53 (0.4-0.7)
Hemoproteus	7.7% (5.2%-11.4 %)	8.17 (4.9-13.7)	3 (1-11)	0.63 (0.3-1.2)
Leucocytozoon	2.7 % (1.2–5.2 %)	6.88 (2.6-18.9)	3.5 (1-34)	0.22 (0.06-0.8)

Table 3: Distribution of multiple blood parasites infection among wild birds in Kaduna State, Nigeria.

Multiple infection combination	Infected species	Multiple infection prevalence	% multiple infection
Plasmodium/*Haemoproteus*	African black crake, Western plaintain eater, Allen gallinule, Mallard duck, Common bulbul	2.4 % (6/246)	60 % (6/10)
Plasmodium/Aegyptionella	Hamlet guinea fowl, African black crake	1.2 % (3/246)	30 % (3/10)
Plasmodium/Microfillaria	Great egret	0.4 % (1/246)	10 % (1/10)

Table 4: Blood parasite prevalence among wild birds withindifferent sampling units and locations in Kaduna State, Nigeria.

	***Plasmodium* prevalence**	***Haemoproteus* prevalence**	***Leucocytozoon* prevalence**	**Aegyptionella prevalence**
Sampling unit				
Free flying birds	11.8% (23/195)	7.2 % (13/144)	3.6 % (6/195)	1.5 % (3/144)
Live poultry markets	47.8 % (18/38)	15.8 % (6/38)	0 % (0/38)	2.6 % (1/38)
Live wild bird markets	10.9 % (7/64)	4.7 % (3/64)	1.6 % (1/64)	0 % (0/64)
Sampling location				
Anchau	31.7 % (15/60)	10 % (6/60)	5 % (3/60)	1.7 % (1/60)
Kaduna	7.7 % (5/65)	4.6 % (3/65)	4.6 % (3/65)	0 % (0/65)
Koraye	0 % (0/5)	0 % (0/5)	20 % (1/5)	0 % (0/5)
Sabon Gari	0 % (0/3)	33.3 % (1/3)	0 % (0/3)	0 % (0/3)
Samaru	14.6% (24/164)	7.9 % (13/164)	0.6 % (1/164)	1.8 % (3/164)

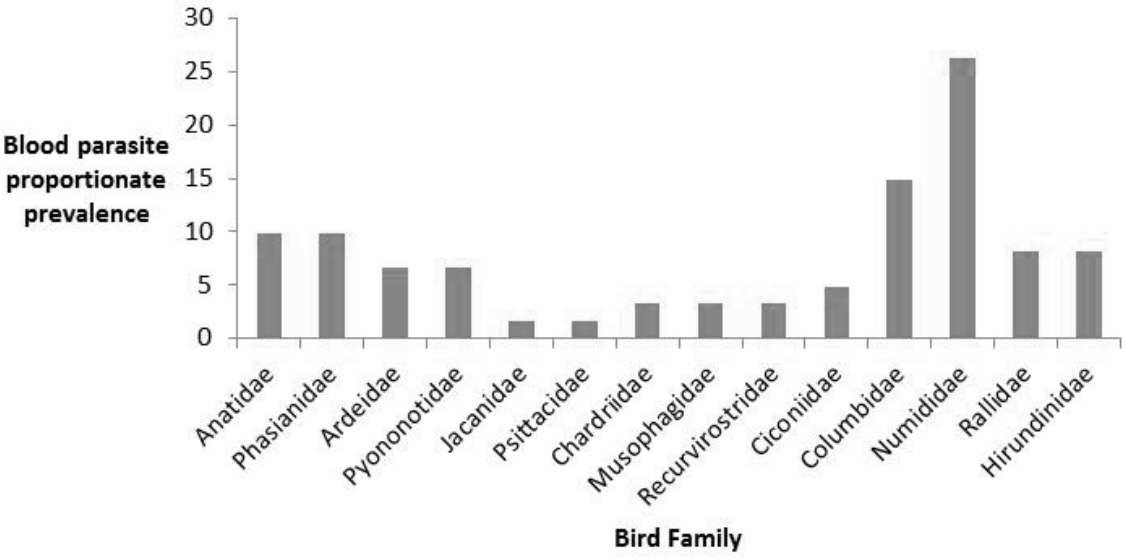

Figure 1: Proportionate prevalence of blood parasite among wild bird families in Kaduna State, Nigeria.

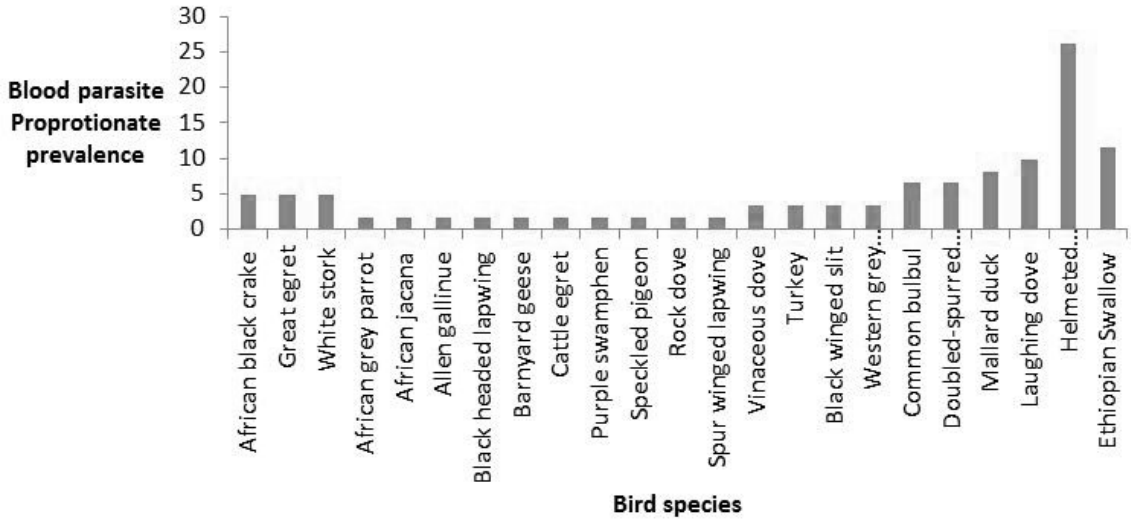

Figure 2: Proportionate prevalence of blood parasite among wild bird species in Kaduna State, Nigeria.

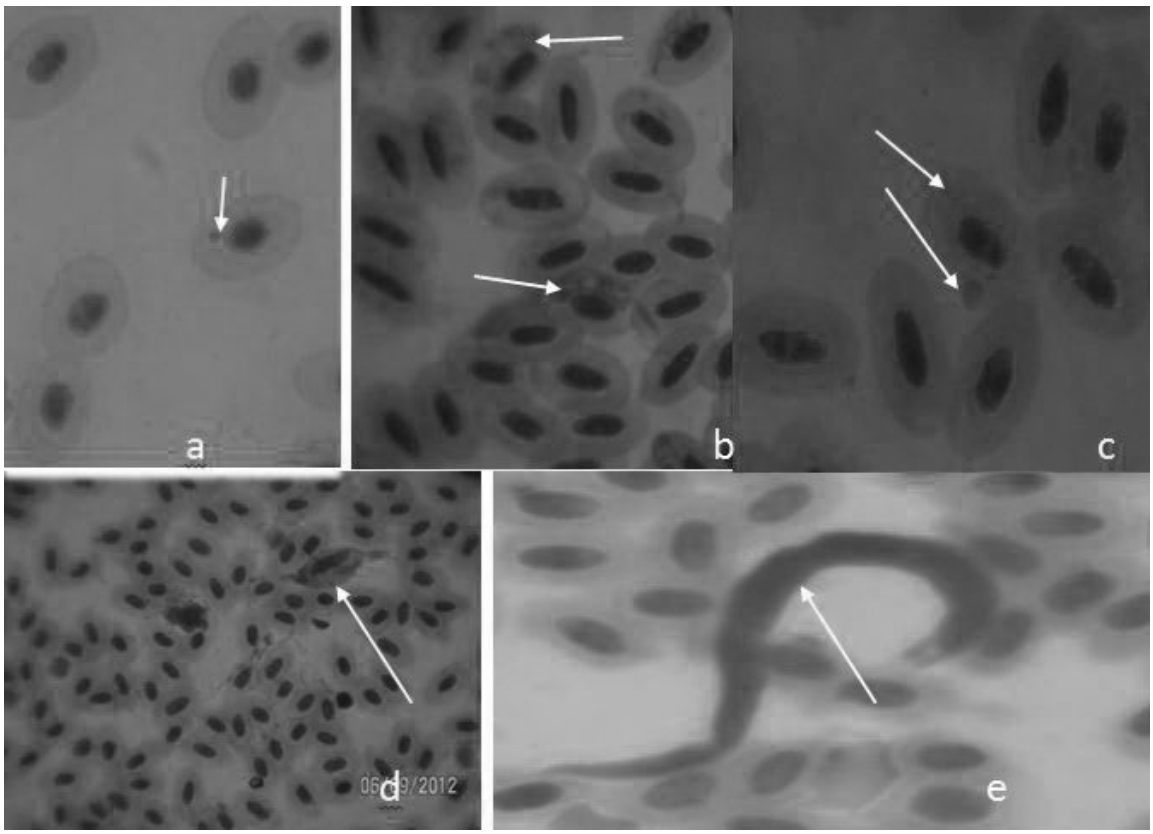

Figure 3: Blood parasites of wild birds in Kaduna State, Nigeria. (a) Aegyptionella spp from African black crake. (b) *Haemoproteus* spp from laughing dove. (c) *Plasmodium* spp from white stork. (d) *Leucocytozoon* spp from common bulbul. (e) Microfilaria from black winged stilt. (White arrows point to the parasites)

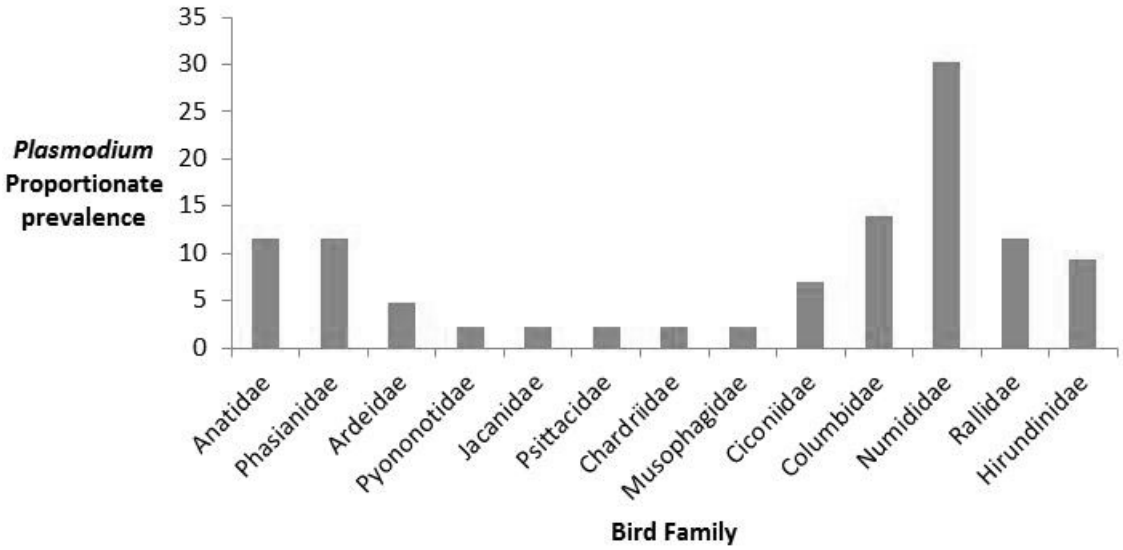

Figure 4: Proportionate prevalence of *Plasmodium* among wild bird families in Kaduna State, Nigeria.

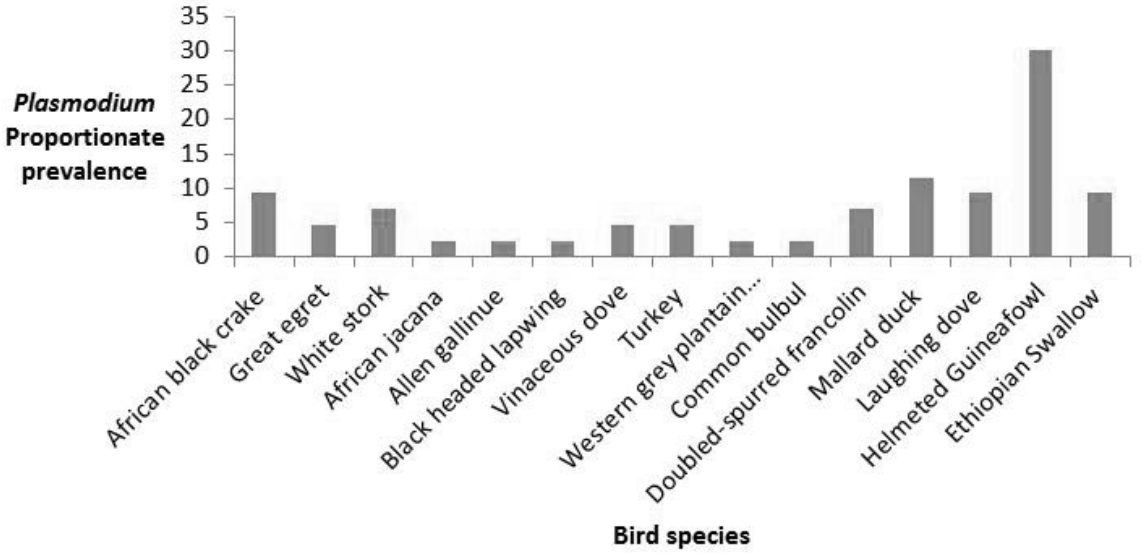

Figure 5: Proportionate prevalence of *Plasmodium* among wild bird species in Kaduna State, Nigeria.

sampled sites except Koraye (Table 4) although the proportionate prevalence among sampling locations was 13.0 % (3/23) for Kaduna (Figure 10). However, *Leucocytozoon* was prevalent in Anchau, Kaduna, Samaru and Koraye (Table 4) with prevalence between sampling location ranging from 14.3 % to 42.9 % (p=0.00; df=5; X2= 35.0).

Aegyptionella was prevalent in only birds from Anchau and Samaru (Table 4) though prevalence between sites was 25 % (1/4) for Anchau and 75 % (3/4) for Samaru. Similarly, microfilaria was prevalent in sampled birds from Koraye and Samaru with within site prevalence of 20 % (1/5) and 1.2 % (1/164) though the prevalence between sites was 33.3% (1/3) (p = 0.00; df = 5; X2 = 24.89).

Discussion

The study confirms previous reports of blood and external parasites infection among wild birds in Kaduna State (Adang *et al.,* 2009; Oladele *et al.,* 2012). These parasites, both singly and in combination, might be responsible for lost of resources from the birds, tissue injury, causing reduced immunity from disease and

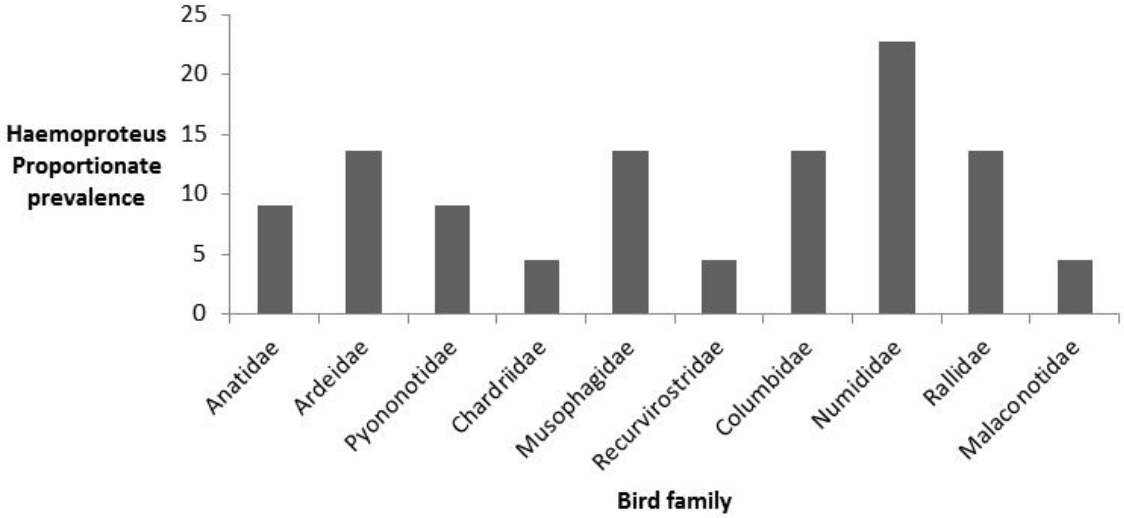

Figure 6: Proportionate prevalence of *Haemoproteus* among wild bird families in Kaduna State, Nigeria.

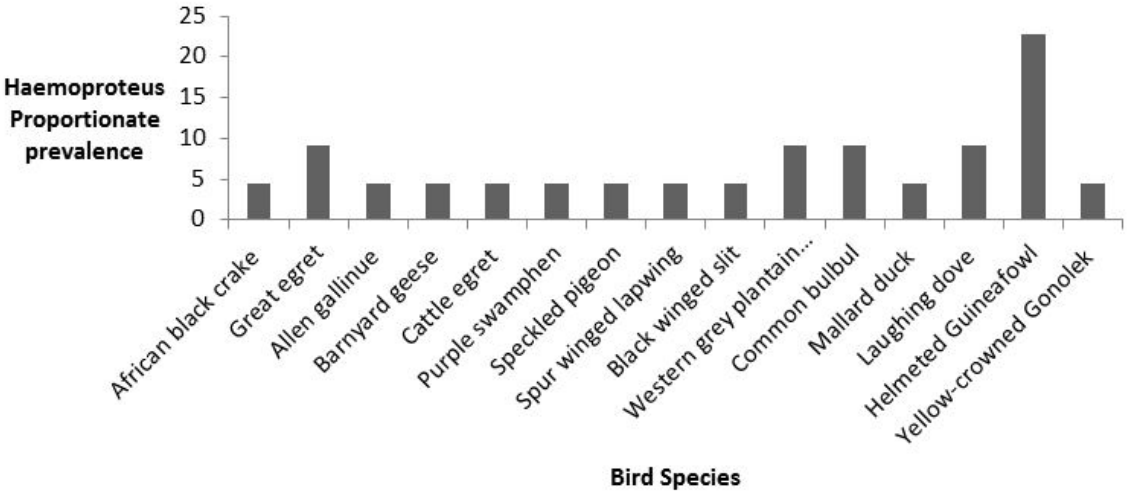

Figure 7: Proportionate prevalence of *Haemoproteus* among wild bird species in Kaduna State, Nigeria.

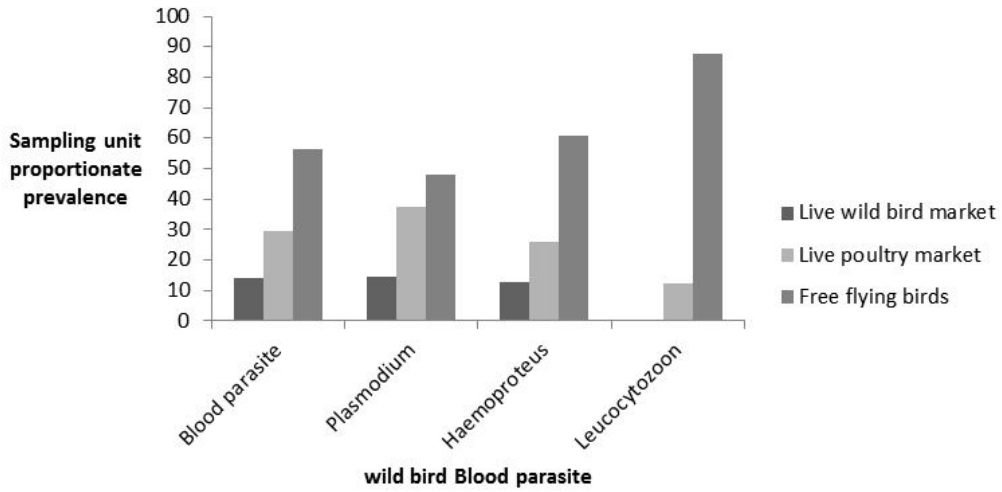

Figure 8: Proportionate prevalences of blood parasites, Plasmodium, *Haemoproteus* and *Leucocytozoon* among wild birds in different sampling units in Kaduna State.

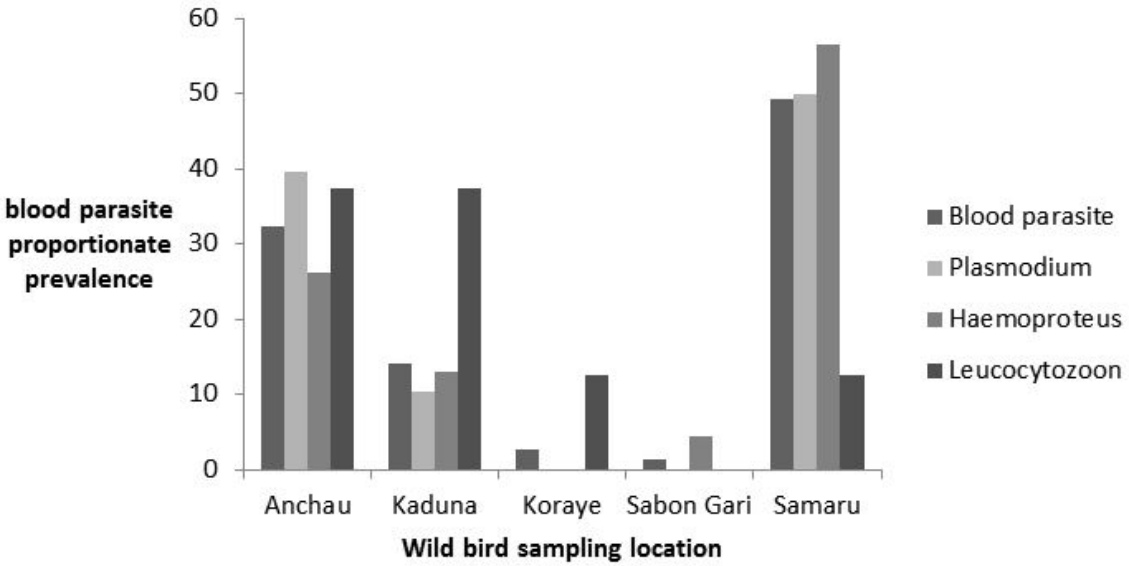

Figure 9: Proportionate prevalences of blood parasites, Plasmodium, *Haemoproteus* and Leucocytozoon among wild birds sampled at different locations in Kaduna State.

potentially decreased reproductive success of adults. The prevalence of parasitism observed in this study is within the ranges reported in several large-scale studies that examined birds for blood parasites in the Neotropics (White *et al.,* 1978), North America (Greiner *et al.,* 1975), Asia (McClure *et al.,* 1978), central Europe (Kucˇera, 1981) and Madagascar (Savage *et al.,* 2009). However, other studies in Africa revealed a significant variation of parasite prevalence, ranging from 72% and 61.9% in Zaire and Uganda, respectively, to 39.8% in Zambia, and 11.5% in Senegal (Bennett and Herman, 1976; Bennett *et al.,* 1978; Peirce, 1984; Valkiunas *et al.,* 2005). Variation in vector diversity and population size or avian composition of community might be responsible for the differences in prevalence reported (Savage *et al.,* 2009).

Although the number of families and species surveyed in this study was higher than that of previous studies, the family and species' blood parasite prevalence rate are lower with more families and species free from blood parasites in Madagascar. However, this might be due to difference in the ecological habitat as the previous study surveyed forest birds hence host-parasite dynamics (Savage *et al.,* 2009).

Numididiae had the highest prevalence between and within families increasing its role in the transfer of avian parasites between domestic poultry and wild birds since it is semi-domesticated and its ability to fly allows it to interact with wild birds distant from human habitation.

This study revealed that birds sharing anthropogenic habitats have higher prevalence emphasizing the success of ornithophilic vectors and susceptibility of birds around human habitats. This is likely due to abundance of vectors for these parasites since these environments would promote establishment of these vectors and maintance of the parasites by domestic local poultry. Secondly the encroachment of human development which reduces and change wild bird habitats is a source of stress to these birds which negatively impact on their immune system thereby affecting their ability to combat infection.

Plasmodium were the most frequently observed blood parasites in this study contrary to previous studies on avian blood parasites (Greiner *et al.,* 1975; White *et al.,* 1978; Bennett *et al.,* 1982; Peirce, 1984; Murata, 2002, Savage *et al.,* 2009). This is because birds remain infected for life at a chronic level that stimulates immunity to re-infection (Young *et al.,* 2004). Although, the high prevalence of the mosquitoes vector of *plasmodium* in the tropics ensures regular re-infection such that parasitemias do not drop to undetectable

chronic levels. However, whereas in the other studies, low prevalence of *plasmodium* relative to Hemoproteus and *Leucocytozoon* may largely be sampling artifact since low intensity chronic infection are extremely difficult to detect by microscopy (Atkinson, 2008).

Nevertheless, the prevalence of *Plasmodium* species is similar to prevalence in previous studies conducted in West Africa which reported a prevalence of 10 % (Savage *et al.,* 2009). It is contrary to studies in Madagascar, Uganda and Senegal where the prevalence were less than 2 % (Bennett *et al.,* 1993, 1978; Sehgal *et al.,* 2005; Savage *et al.,* 2009).

Previous studies revealed that *Plasmodium* infected birds sang fewer songs (Gilman *et al.,* 2007). This could have a significant impact on mate choice and reproductive success of infected males. Similarly, the behavioral effects of acute infections may lead to increased predation of infected hosts (Yorinks and Atkinson, 2000; Møller and Nielsen, 2007).

In this study *Haemoproteus* prevalence was lower and second to *Plasmodium* unlike previous studies (Savage *et al.,* 2009). This low prevalence might be due to low prevalence of ceratopogonid and hippoboscid vectors resulting in low re-infection rate such that parasitemias drop to undetectable levels. *Haemoproteus* have been reported to reduce survival, immunity, condition, and reproductive success (Allander and Bennett, 1995; Ots and Horak, 1998; Merino *et al.,* 2000; Sanz *et al.,* 2001; Sol *et al.,* 2003).

Although *Leucocytozoon* prevalence was low, this is the first report of *Leucocytozoon* in free flying birds in Nigeria and highlights the risk of introduction of new infection into resident bird population by migratory birds. However, *Leucocytozoon* was the only parasite reported in Columba livia while the other species with multiple infections such as Francolinus bicalcaratus, Pychonotus barbatus and Meleagris gallopavo had the same prevalence as *Plasmodium*. Streptopelia senegalensis's Leucytozoon prevalence was however lower than the *Plasmodium* prevalence.

The high intensity of these blood parasites might reduce bird energetic condition resulting in poor reproductive performance (DeGroote and Rodewald, 2010). Similarly, there are reports of a negative correlation between parasitemia and number of fledged offspring. Hence, these infections can have significant effects on host life history traits and therefore may act as important selective agents in wild bird populations (Asghar *et al.,* 2011).

The difference in *Plasmodium* and *Haemoproteus* prevalence confirms greater exposure of birds to *Plasmodium* vectors than Hemoproteus vectors. However, there was no difference in the birds' susceptibility to both parasites (Sol *et al.,* 2000). Therefore, the *Plasmodium* and Hemoproteus load do not significantly differ. However, intensity of hemoparasite infection has been reported to vary with the phase of infection likely to be influenced by the intricate interplay of host immunity, seasonal changes in photoperiod, and hormonal changes related with reproduction (Atkinson, 2008). Hence, the level of *Plasmodium* and Hemoproteus infection was not significantly different in the infected birds.

This study revealed that some wild birds which share anthropogenic habitats are likely to serve as reservoir hosts for domestic poultry infection (Huchzermeyer, 1993). However, multiple infection occurs in wild birds which is likely to be determined by resource competition between co-infecting parasites and immune-mediated competition (Ulrich and Schmid-Hempel, 2012).

Stress mediated changes in the immune system, food availability, concomitant infection with other parasites and exposure to predators have been identified to increase Hemoproteus intensity in mixed infection (Cox, 1987; Appleby *et al.,* 1999; Navarro *et al.,* 2004). Also, prevalence of mixed hemoparasites infection is influenced by habitat and season which are critically important in determining the distribution and abundance of vectors (Sol *et al.,* 2000; Mendes *et al.,* 2005).

There are reports that concurrent infection of *Plasmodium* infection with other haemosporidian parasites may also influence *Plasmodium* prevalence by maintaining infections at higher rate of recurrence than might be expected. This is because specific Mhc alleles associated with susceptibility to *Plasmodium* but confering resistance to a co-infecting strain of *Haemoproteus* may be maintained in a bird

population (Loiseau *et al.,* 2008).

Though *Plasmodium* has an overall high prevalence, four families (4/15) and seven species (7/22) had *Haemoproteus* prevalence higher than Plasmoduim. This was probably because these birds dwell in habitats that increase their contact with *Haemoproteus* vectors which are the ceratopogonid fly in the genus Culicoides and ectoparasitic hippoboscid flies (Atkinson, 2008).

The study revealed that though most of the infected birds were free flying birds, the LPM birds had the highest blood parasite prevalence probably due to their interaction with domestic poultry and their reside in anthropogenic habitats. These birds are likely to serve as reservoir host of blood parasites to domestic poultry. However, the low prevalence in LWBMs birds might be due to adequate provision of food and regular intervention with drugs by the wild bird sellers or the habitat from which they were trapped were free from the vectors transmitting blood parasites. However, birds from LWBMs are likely to be exposed to these blood parasites with infection resulting to clinical disease and mortality. The high *Plasmodium* prevalence in LPM birds confirm reports that birds sharing anthropogenic habitats have high prevalence to *Plasmodium* due to greater vector abundance, or increased host susceptibility (Bradley, 2012).

The high *Haemoproteus* prevalence of in free flying birds is as a result of these birds visiting water bodies inhabited by Hippoboscid flies, vector of *Haemoproteus*. However, the low *Leucocytozoon* prevalence reported in all the sampling units reflects the low prevalence of Simuliim, a vector responsible for transmitting *Leucocytozoon* within the sampling units. This also reveals that Ceratopogonidae, known to transmit both *Haemoproteus* and *Leucocytozoon* might not be contributing to the transmission of *Haemoproteus* in Kaduna State (Atkinson, 2008).

The study revealed that birds from Anchau are more likely to be infected with haemoparasites probably due to the wetlands in the area which is favourable for breeding and maintaince of the vectors for these haemoparasites. The high *Plasmodium* prevalence in Samaru and Anchau are related to these sites being anthropogenic habitats. However, with the high backyard poultry density within Samaru, with poor biosecurity practices there are risk of cross infection from wild bird to poultry and vise versa. These are likely to affect the life history of the wild birds there by affecting the wild bird diversity and species richness.

This study establishes baseline population data for future study of wild bird host-parasite interaction in Nigeria. There is need to investigate the pathogenic effects of these blood parasites to understand their interaction and true impact on Nigerian bird populations with changes in wild bird habitat.

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ACARICIDAL EFFECT OF FOAM SOAP CONTAINING ESSENTIAL OIL OF *OCIMUM GRATISSIMUM* LEAVES ON *RHIPICEPHALUS LUNULATUS* IN THE WESTERN HIGHLAND OF CAMEROON

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Abstract

Acaricidal effect of foam soap containing essential oil of *Ocimum gratissimum* leaves was tested on *Rhipicephalus lunulatus* in western highland of Cameroon. Five doses of essential oil (0.00; 0.04; 0.06; 0.08; 0.10 µl/g) with four replications for each dose were tested in vitro. Each replication consisted of 10 ticks in Petri dish with filter paper impregnated uniformly with the foam soap on the bottom. Four of those doses (0.00; 0.06; 0.08; 0.10 µl/g) in three replications were used in vivo. In this case, each replication was made up of 10 naturally ticks infested goats. Results of this study indicated that foam soap containing essential oil of O. gratissimum leaves is toxic to R. lunulatus. The in vitro mortality rate was observed to vary from 0 to 30.00% during the treatment with the controls as compare to 80.00% with the lowest dose (0.04 µl/g) on day 8 and 100.00% with the highest dose on day 6. Meanwhile, the in vivo mortality rate was observed to be 22.69% with control on day 8 after treatments whereas the highest dose killed 93.87% of the tick by this day 8. The LD50 of the foam soap containing essential oil was 0.061 µl/g for in vitro and 0.066 µl/g for in vivo on day 2. This indicates that this medicated soap is potentially highly efficient on this parasite.

Keys Words: foam soap, essential oil, *Ocimum gratissimum*, *Rhipicephalus lunulatus*, Cameroon

EFFET ACARICIDE DU SAVON MOUSSE CONTENANT DE L'HUILE ESSENTIELLE DE FEUILLES D'OCIMUM GRATISSIMUM SUR RHIPICEPHALUS LUNULATUS DANS LES HAUTES TERRES DE L'OUEST DU CAMEROUN

Résumé

L'effet acaricide du savon mousse contenant de l'huile essentielle de feuilles d'Ocimum gratissimum a été testé sur Rhipicephalus lunulatus dans les hautes terres de l'ouest du Cameroun. Cinq doses d'huile essentielle (0,00 ; 0,04 ; 0,06 ; 0,08 ; 0,10 µl/g) avec quatre répétitions chacune ont été testées in vitro. Chaque répétition était composée de 10 tiques dans une boîte de Petri avec du papier filtre imprégné de façon uniforme de mousse de savon à la face inférieure. Quatre de ces doses (0,00 ; 0,06 ; 0,08 ; 0,10 µl/g) en trois répétitions ont été utilisées in vivo. Dans ce cas, chaque répétition était composée de 10 chèvres naturellement infestées par des tiques. Les résultats de cette étude ont révélé que le savon mousse contenant de l'huile essentielle de feuilles d'O.gratissimum est toxique pour R. lunulatus. On a constaté que le taux de mortalité in vitro variait entre 0 et 30,00% pendant le traitement avec les doses témoins par rapport à 80,00% pour la plus faible dose (0,04 µl/g) au jour 8 et 100,00 % pour la plus haute dose au jour 6. Entretemps, on a constaté que le taux de mortalité in vivo était de 22,69 % pour la dose témoin au jour 8 après les traitements, tandis que la plus haute dose avait tué 93,87 % des tiques au jour 8. La dose létale LD50 du savon mousse contenant de l'huile essentielle était de 0,061 µl/g pour µl/g et 0,066 µl/g pour le traitement in vivo au jour 2. Ceci est une indication que ce savon médicamenté est potentiellement très efficace sur ce parasite.

Mots-clés : Savon mousse ; Huile essentielle ; Ocimum gratissimum ; Rhipicephalus lunulatus ; Cameroun

Introduction

Breeding of ruminants constitutes one of the main productions activities in many African regions in general and in particularly in Cameroon (Pamo *et al.,* 2002). In Cameroon, small ruminants are used as source of income and for many other purposes almost every where in the country (Pamo *et al.,* 2001; Pamo *et al.,* 2004). Specifically, goats for breeders are easily mobilizable investment, having a very short development cycle (Lhoste *et al.,* 1993). Following these considerations, it appears necessary to set interested in the breeding condition of goats in this zone where the demographic and hygienic conditions are not favorable for breeding activities.

Goat breeding is slowed down by various factors including nutrition; diseases and also ticks infestation which are the most important (Pamo *et al.,* 2005; Tendonkeng *et al.,* 2010). This *Rhipicephalus lunulatus* tick has been reported to be a common ectoparasite of goats in Cameroun and the surrounding countries (Pamo *et al.,* 2005).

In fact, ticks in general and *Rhipicephalus lunulatus* are one of the main causes of mortality in farm animals (IEMVT, 1989). They are also responsible for secondary infections which could be bacterial, viral or protozoa related (Soulsby, 1982). Furthermore, each of the conventional methods of tick control is quite costly and environmentally unfriendly (Pamo *et al.,* 2005). Attention is then shifted towards natural substances with therapeutic properties like essential oils extracted from some plants. Indeed, a substantial part of plants (leaves, fruits, flowers, stems and roots) contain antiseptic, anti-inflammatory, insecticidal, bactericidal healing substances (Kuiate, 1993). The acaricidal effect of essential oils of many plants has been documented in many studies (Pamo *et al.,* 2002; Pamo *et al.,* 2003; Pamo *et al.,* 2005). An important example is *Ocimum gratissimum* whose leaves were shown to contain essential oils which make them to be sometimes irritating and toxic (Daget & Godron, 1995; Tapondjou *et al.,* 2002; Pamo *et al.,* 2002). Those previous studies then bring up the problem of conditioning theses essentials oils in better way for their efficient utilization for on farm ticks control. This study is therefore aimed at finding an efficient, cheap and easily applicable method of using essential oils to fight against ectoparasites in general and ticks in particular.

Materials and Methods

Extraction of O. gratissimum essential oil

Fresh leaves of O. gratissimum were harvested and sun dried for 3days. Extraction of essential oil was done by hydrodistillation (Kuiate, 1993). Two kilograms of dried leaves were soaked in 6 l of water and boiled for 10h in the modified Clavenger vendor. The evaporate was collected in an open mouth bottle and filtered through an anhydrous sodium sulphate column to eliminate the trace of water present in the essential oil. The oil was stored in the dark at room temperature.

The yield of essential oil was calculated using the following formula:

$$\text{yield (\%)} = \frac{\text{(weight of essential oil)}}{\text{(weight of O.gratissimum leaves)}} \times 100$$

Collection and identification of ticks

Male and female R. lunulatus ticks, frequently found on ruminants in the highlands of west Cameroon, were collected by manual removal without breaking their rostrum. These ticks were fixed in ethyl acetate and identified as R. lunulatus according to Walker et al. (2002). To have an uninfested goat population, 10 West African dwarf goats were examined and all ticks removed. Another manual removal of ticks was done 30 days later. A total of 72 ticks were collected from the 10 goats. The average weight of engorging ticks was 0.5±0.1g and the average length was 6.5 ± 0.4 mm. These two parameters (weight and length) were used in the tests.

Preparation of medicated soap

Palm oil liquid soap was used as vehicle for the essential oil. A volume of 900 µl of essential oil was added to 450 g of liquid soap to obtain a concentration of 2 µl/g base on which all doses were prepared. The solution was poured into the molds and allowed to solidify.

In vitro study

R. lunilatus ticks were collected from various West African Dwarf goats and identified. A disc of N°1 Whatman filter paper measuring 62.63 cm2 surface area were soaked with soap of various concentrations of the essential oil (0.00; 0.04; 0.06; 0.08; 0.10 µl/g) and placed in clean dry Petri dishes with four replicates each at room temperature (24°C, humidity 70%). Ten ticks randomly placed in each Petri dish and covered. The plates were examine each morning during 8 days and dead ticks, if any, were counted and removed. One soap sample without essential oil served as control. The mortality rate of the tick was calculated as described by Abott (1925), and lethal dose 50 (LD50) was calculated according to Valette (1972).

$$Mc = \frac{(M0-Mt)}{(100-Mt)\ X100}$$

Where: Mc is the accrued and corrected death rate, M0 the death rate in Petri dishes treated and Mt the death rate in the control Petri dish (natural mortality).

In vivo study on the acaricide property of essential oil

The number of R. lunulatus ticks was counted at the preferential sites (ears, tail and head) (Tenekeu, 2002) on each of the 40 goats selected in this study. Soap with four concentration of essential oil selected after in vitro test (0.00; 0.06; 0.08; 0.10 µl/g) was applied on batches of 10 goats. The preferential sites were examined for the number of live/ dead ticks every 24 h for 8 days. Three replicates of 10 goats/group/dose (120 goats) were carried out during on-farm trial. Ticks removed by engorgement were not taken into consideration during analysis.

Statistical analyses

The cumulative and corrected mortality percentages were submitted to analysis of variance (Mc Clave and Dietrich, 1979) and the differences between the treatments were analyzed by student's t- test.

Results and Discussion

In vitro study

The yield of the oil extraction was 0.62 %. This yield was higher than that obtain by Pamo et al. (2003) which was 0.50 %. This difference can be explained by many factors including the distillation method and the period during which the plant was harvested.

All the three concentrations tested in the study showed some acaricidal effect on R. lunulatus. Efficiency increased as the concentration of essential oil increased and with the duration of exposure. The highest dose (0.10 µl/g) killed all ticks by day six of exposure while, the lowest dose (0.04 µl/g) caused 80 % of mortality within the eighth day of the trial. By the end of the study, control dose killed only 30% of ticks (Figure 1). This study established that the essential oil obtained from O. gratissimum, and incorporated in soap as carrier was toxic to ticks, and toxicity was directly proportional to the concentration of the essential oil in the soap. The toxicity of these soaps containing essential oil can be mainly attributed to the predominance of Phenolics and Terpenoics compounds present in the essential oil. The main compound of this essential oil is Thymol. This compound is a highly selective chemical substance attacking specific aspects of the endocrine system of insect, thus inducing a toxic effect (Ojimelukwe and Alder, 1999). The high toxicity of this essential oil suggest, that Thymol, known for his insecticidal and acaricidal effect (Tapondjou *et al.,* 2003; Tapondjou *et al.,* 2005; Ndomo *et al.,* 2009) react in synergy with other monoterpens like y-terpinen, p-cymen, terpenoids like β-caryophyllen, and eugenol which insecticidal activities have been documented (Tchoubougnang, 1996; Obeng-Ofory *et al.,*1997; Tapondjou *et al.,* 2005; Ndomo *et al.,* 2009). Action of phenolics compound such as linalool and y-terpinol is also important. These substances are recognized as important insecticides, fungicides and bactericides (Hassanali *et al.,* 1990; Obeng-ofory *et al.,* 1997). This reality was confirmed by Pamo et al. (2003) who demonstrated the acaricidal effect of crude essential oil from O. gratissimum leaves. Finding from these studies showed that these essential oils which were

mainly rich in monoterpen in general and in thymol in particular can induce not only toxic effect but also metabolic disorder in R. lunulatus that may impact their development and reproductive process. The high mortality record in the control group can be explained by other soap components such as soda.

The regression equation derived by comparing the average cumulative mortalities with the concentration of essential oil (Y= 656.25X +11.455; R2=0.9213; Figure 2) revealed that 97.96% of the correlation with mortality doses could be assigned to the concentration of the essential oil.

The adjustment of the average cumulative mortality percentages with doses in time led to the regression equation: Y= 656.25X + 11.455 (R2=0.9213).

Following the transformation of the mortality percentages to probits at the end of the second day of exposure, the regression line Y= 5.1015X+10.19 (R_2= 0.9621) showed that after 2 days of exposure, the LD50 was 0.061 µl/g. These confirm the degree of toxicity of soap containing essential oil of *O. gratissimum* on R. lunilatus.

In vivo study

The cumulative mortality of ticks was significantly higher in all treatment groups than the control by third day after treatment. This continued to increase with time in each group. By the end of study, the mortality was almost more than three time higher in group 2 and 3 and four time in group 4 as compared to control (Figure 3). Accordingly, soap containing essential oil at the rate of 0.10µl/g would be effective in completely eliminating the R. lunilatus ticks on goat within a week.

The regression equation (373.63X+5.473, R2= 0.999) on mortality versus concentration of essential oil suggests that increasing concentration of essential oil in the soap had increasing effect on ticks.

Similarly, the regression line (Y= 3.0771X+6.5929, R2= 0.9868) suggest that by the second day of the treatment, the LD50 was 0.066 µl/g. There was no appeared side-effect of the medicated soap application on behaviors and/or health of goats. The in vivo mortalities were relatively lower as compared to those achieved in vitro with LD50 of 0.061 µl/g. This could be due to the fact that on-farm, the application of medicated soap was carried out on ticks that were allowed to continue feeding. In the laboratory the ticks were not fed, and thus were under stress. This could have weakened them with regard to synergetic activity of the ingredients of the medicated soap.

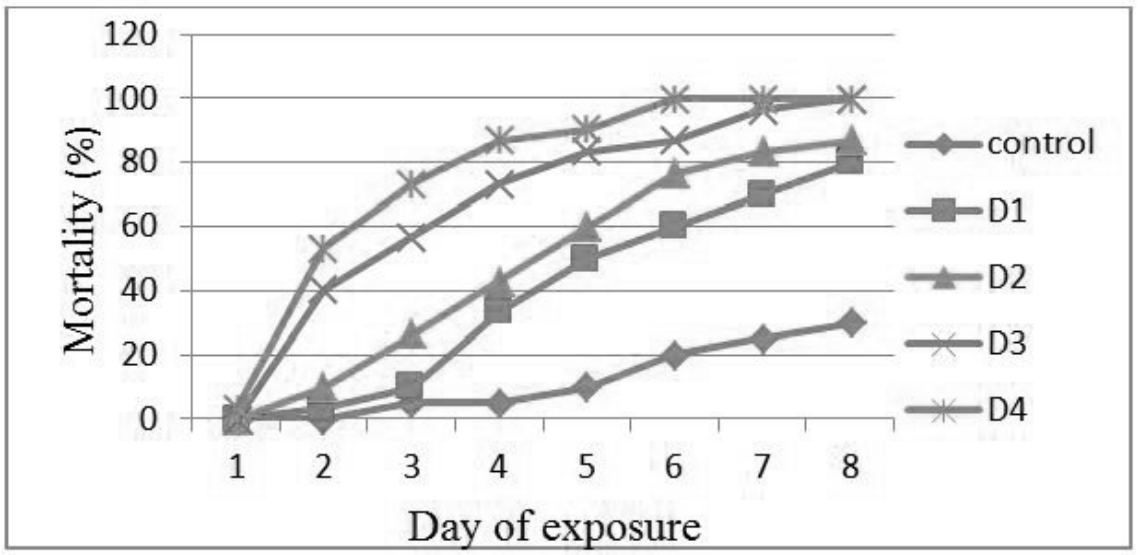

Figure 1: Cumulative ticks mortality (%) following in vitro treatment with soap containing different doses of O. gratissimum leaves essential oil.

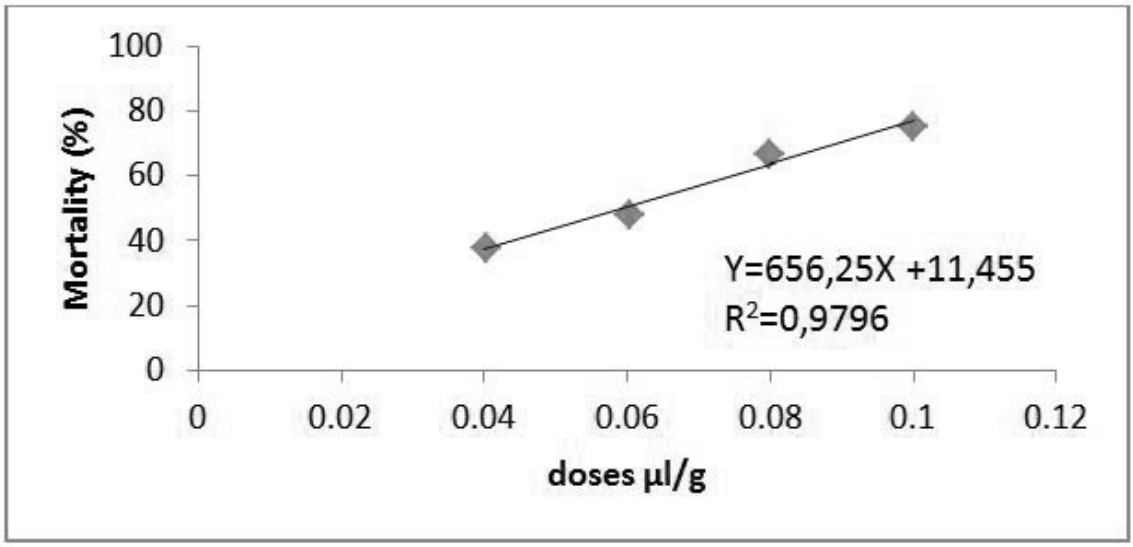

Figure 2: Regression line of the cumulative mortality of R. lunulatus and concentration of essential oil.

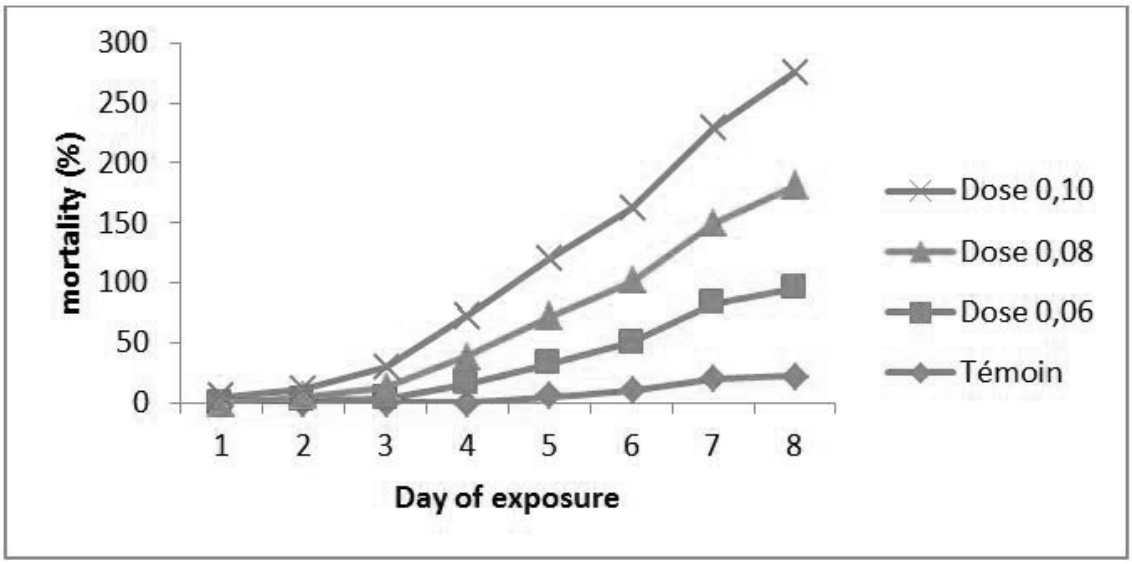

Figure 3: Cumulative ticks mortality (%) following in vivo treatment with soap containing different doses of O. gratissimum leaves essential oil.

Conclusion

The essential oil of O. gratissimum is toxic to R. lunilatus ticks, both in vitro and in vivo. The toxic effect of the medicated soap containing the essential oil on ticks increased as the concentration of essential oil increased and persisted during the entire period of the study. A low LD50 (0.061 µl/g for in vitro and 0.066 µl/g for in vivo application) of the essential oil suggests that it bar potential or acaricidal agent for R. lunilatus ticks. Further studies on the chemical nature of the components of essential oil as well as the separation of the different fractions would improve our Knowledge and understanding. Identification of the active ingredient in the essential oil would improve quality and efficiency of the product. The effects of this essential oil on other tick species need to be studied. Likewise, the effect of repeated application of the medicated soap on adults ticks and their others life stage needs some evaluation.

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CARACTÉRISATION PHÉNOLOGIQUES DE LA POULE BARRÉE DE L'OUEST CAMEROUN

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Resume

Entre mai et Juin 2011, les performances de croissance et les caractéristiques phénologiques de la poule barrée des hautes terres de l'Ouest Cameroun ont été évaluées à la Ferme d'Application et de Recherche de l'Université de Dschang. Les données sur le poids vif, les mensurations corporelles et les caractéristiques de la carcasse ont été enregistrées sur 120 poules. Les matrices de corrélation et les courbes de régression du poids vif sur les mensurations corporelles ont été établies. Les résultats ont révélé une grande variabilité dans les caractères morphologiques et biométriques de la poule barrée. A 20 semaines, le poids vif moyen (1204,09g et 1566,87g respectivement chez les femelles et les mâles) et les valeurs des différentes mensurations chez les mâles étaient significativement supérieure ($P<0,05$) à celles des femelles. Par ailleurs, les coefficients de corrélation du poids vif sur les mensurations corporelles ont été moyens et positifs chez les femelles (0,53 à 0,67). Chez les mâles, ils ont été positifs et faibles (0,28 à 0,50) par rapport à ceux des femelles. Dans l'ordre d'importance croissant, les caractères pouvant servir à la prédiction du poids chez les mâles ont été le pourtour thoracique, la longueur du tarse et du corps mais avec une précision plutôt faible. A l'exception du rendement carcasse, du poids du gésier, du gras abdominal et du bréchet qui étaient comparables entre mâles et femelles, tous les autres paramètres ont été significativement ($P<0,05$) plus élevés chez les mâles comparés à ceux des femelles.

Mots clés : Performances de croissance, mensurations corporelles, poule locale

PHENOLOGICAL CHARACTERISTICS OF LOCAL BARRED CHICKEN IN WESTERN CAMEROON

Abstract

Between May and June 2011, the growth performance and phenological characteristics of local barred chicken of the Western Highland Cameroon was carried out in the Teaching and Research Farm of the University of Dschang. The data on body weight, body measurements and carcass characteristics were collected on 120 chickens. Matrices correlation and regression curves of body weight on body measurements were established.

The results revealed high variability in morphological and biometric characters of barred chicken. At 20 weeks, the average body weight was 1204.09 and 1566.87 g respectively for female and male. The values of the different measurements were significantly ($P < 0.05$) higher in males as compared to the females. Otherwise, the correlation coefficients of body weight on body measurements were positive in females (0.52 to 0.67). In males, they were positive and weak (0.28 to 0.50) as compared to the females. The characters that can be used to predict weight in males were thoraxic perimeter, tarsus and body length but with a rather low accuracy. Appart from the carcass yield, weight of gizzard, abdominal fat and breast muscle which were comparables between males and females, all other parameters studied including carcass characteristics and various visceral organs were significantly ($P < 0.05$) higher in males as compared to the females.

Key words: Growth performance, Phenological characteristics, Local barred chicken

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Introduction

En Afrique sub-saharienne, l'aviculture villageoise est pratiquée dans un système extensif en milieux rural et peri-urbain et répond mieux aux méthodes culinaires et aux goûts des populations africaines (Kperegbeyi *et al.,* 2009). Elle joue un rôle capital dans la couverture des besoins des populations en protéines animales jusqu'alors déficitaires. Elle a une très faible productivité et sa caractérisation préalable en vue de son amélioration génétique reste un problème majeur (Kéambou *et al.,* 2007a). Cette activité revêt une importance très significative comme source de revenus particulièrement chez les femmes (Zaman *et al.,* 2004) elle constitue une caisse d'épargne facilement mobilisable en cas de besoins urgents (maladie, rentrée scolaire…). Outre sa valeur économique, la poule locale joue un rôle important dans la vie socioculturelle des africains. Elle est utilisée dans les cérémonies de mariage, les sacrifices de tout genre, la pharmacopée traditionnelle et le maintien de la cohésion sociale au sein des communautés traditionnelles à travers des dons et la réception de visiteurs de marque (Guèye, 1998; Fotsa *et al.,* 2007).

La recherche en aviculture a été pendant très longtemps orientée vers le développement de la production des produits de croisement des poules de races standards, négligeant la production de la poule villageoise dont l'intérêt s'accroit de façon remarquable au fil du temps. Malgré toute l'attention portée sur les poules sélectionnées, la demande en viande et en œufs de table en Afrique et surtout au Cameroun reste encore très élevée par rapport à l'offre. Les produits issus de l'aviculture villageoise représente environ 50% des produits avicoles du pays (Poné, 1998; Teleu et Ngatchou, 2006). Il est par conséquent nécessaire d'envisager une intensification de sa production qui passe par une meilleure connaissance du matériel animal. La poule locale camerounaise n'est pas suffisamment connus et le défaut de connaissance des différents types génétiques et des contraintes liées à leur production rend extrêmement difficile leur exploitation rationnelle et la mise sur pied d'un programme de développement qui puisse bénéficier aux populations locales (Kéambou *et al.,* 2007a).

L'objectif de cette étude est de contribuer à l'amélioration des connaissances sur la croissance de la poule villageoise en vue d'initier un programme de sélection visant à l'intensification de la production de viande et d'œufs de table.

Matériel et méthodes

Présentation de la zone de l'étude :

Cette étude a été menée à la Ferme d'Application et de Recherche de la Faculté d'Agronomie et de Sciences Agricoles (FASA) de l'Université de Dschang (Cameroun) entre mai 2010 et juin 2011. Dschang est situé à environ 1420 m d'altitude (LN 5-7°, LE 8-12°). Le climat est de type Soudano-guinéen avec environ 2000 mm de pluie par an, répartie sur une seule saison allant de Mars à Novembre. La température moyenne est de 20°C et l'humidité relative est généralement supérieure à 60%.

Matériel animal

Au total 120 poussins d'un jour pesant en moyenne 33g obtenus par incubation artificiel des œufs de parentaux présents à la ferme ont été utilisés dans cet essai. Les poussins ont reçu une couverture sanitaire en vigueur dans la zone; il s'agit des traitements préventifs contre la coccidiose et des vaccinations contre les maladies virales telles que la pseudo-peste aviaire, la bronchite infectieuse et la maladie de Gumboro. Les poussins ont été élevés en groupes de 30 sujets en phase démarrage sur litière faite de copeaux de bois à une densité de 8 animaux /m2 pendant 12 semaines. Au terme de cette période, les males au nombre de 50 ont été élevés séparément des femelles, sur caillebotis à une densité de 4/m² pendant huit semaines correspondant à la phase croissance. Les femelles (70) sont restées dans le bâtiment de départ a une densité de 5/m2 jusqu'à l'âge de 20 semaines (phase croissance). Chaque loge était équipée d'une mangeoire conique en plastique et d'un abreuvoir de dix litres. Les animaux ont été identifiés à l'aide des bagues métalliques placées au niveau du tarse.

Rations expérimentales

A chaque phase de l'étude, une ration faite à base de maïs, de son de blé, de tourteau de soja, de coton, de coquillage a été distribuée ad libitum aux animaux. Ces rations ont été formulé sur la base des besoins nutritionnelles des pondeuses d'œufs de table avec des caractéristiques qui variaient en fonction de l'âge des animaux et de la phase d'élevage (Tableau 1) soit ; démarrage (1-12 semaines) et croissance (13-20 semaines).

Collecte des données

La consommation alimentaire hebdomadaire a été calculée en faisant la différence entre la quantité d'aliment distribuée au courant de la semaine et les restes à la fin de la même semaine. Les animaux ont été pesés à l'éclosion et tous les sept jours par la suite, en même temps que les aliments. Pendant les deux premières semaines, les poussins ont été pesés en groupe. Par la suite, des bagues d'identification ont permis d'effectuer les pesées individuelles. Le gain de poids hebdomadaire a été obtenu en faisant la différence entre deux poids hebdomadaires consécutifs.

A l'âge de vingt semaines, 16 mâles et 16 femelles ont été choisi au hasard, mis en diète alimentaire pendant 24 heures puis pesés, saignés, plumés et éviscérés tel que préconisé par Jourdain (1980). Le poids vif, Le cœur, le foie, le pancréas, le gésier et la graisse abdominale, la carcasse, la tête, les pattes, les cuisses, le bréchet, les ailes et l'intestin ont été pesés à l'aide d'une balance électronique de précision 1g.

Le poids relatif de chaque organe a été calculé en faisant le rapport du poids de l'organe sur le poids vif.

La longueur de l'intestin a été mesurée de la loupe duodénale jusqu'au cæcum à l'aide d'un mètre ruban. La densité de l'intestin a été calculée à partir de la formule suivante :

$$\text{Densité intestin (g/cm)} = \frac{\text{(Poids de l^' intestin (g))}}{\text{(Longueurde l^' intestin (cm))}}$$

Au cours de l'essai les mensurations corporelles ont été prises sur les sujets toutes les deux semaines à l'aide d'un pied à coulisse et d'un mètre ruban :

- Le pourtour thoracique (périmètre thoracique) qui est la circonférence de la poitrine prise en dessous des ailes et au niveau de la région saillante du bréchet ;
- La longueur du bec, égale à la distance entre le bout de la mandibule supérieure et la commissure des deux mandibules ;
- La longueur du corps mesurée entre la fin de la nuque (trou occipital) et le croupillon,
- Longueur de la patte mesurée entre l'articulation du bassin et la cheville ;
- Longueur du tarse: distance entre le calcanéum articulation du genou et la cheville ;
- Diamètre du tarse : mesuré entre le calcanéum et la cheville un peu au-dessus de l'ergot ;
- Longueur de l'aile étendue depuis la jonction de l'humérus à la colonne vertébrale jusqu'au bout de l'aile (sans plume).

Analyse statistique

Les résultats ont été exprimés en moyennes ± écart types et soumis à la statistique descriptive. Les coefficients de corrélation et de régression ont été utilisés pour évaluer les relations entre poids vif et mensurations corporelles des poules (Steel et Torrie, 1980). Le logiciel SPSS 14.0 a été utilisé pour ces analyses.

Resultats

Performances zootechniques

La consommation d'aliment a varié avec l'âge et le sexe. La quantité d'aliment cumulée consommée par une poule barrée entre 1 et 12 semaines et entre 13 et 20 semaines a été respectivement de 3169,75g et de 5221,90g. Par ailleurs, entre 13 et 20 semaines, la consommation moyenne cumulée des mâles 5632,23g a été plus élevée par rapport à celle des femelles 4735,95g. En effet, les femelles ont consommée 11,86% moins d'aliment que les mâles.

Tableau 1: Valeurs nutritives et coût de production des rations expérimentales

Composition (kg)	Démarrage (0 à 12 semaines)	Croissance (13 à 20 semaines)
Composition chimique calculée		
Protéine brute (%)	23,2	20,7
Energie métabolisable (kcal/kg)	2913	3013
Calcium (%)	1,48	1,51
Phosphore (%)	0,69	0,73
Lysine (%)	1,29	1,10
Méthionine (%)	0,43	0,40
EM/PB	125	145

Tableau2: Performances zootechniques de la poule barrée des hautes terres de l'ouest Cameroun.

Période (semaine)		Paramètres			
		Consommation alimentaire cumulé (g)	Poids vif (g)	Gain de poids cumulé (g)	Indice de consommation
1-12	mâle et femelle	3169±306	781±14,8	747±34,1	4,14±1,97
	mâle	5632±513	1566±15,9	652±52,6	6,94±2,88
13-20	femelle	4735±864	1204±59,9	446±75,1	9,70±2,48
	mâle et femelle	5221±875	1401±194	549±125	8,89±5,29
1-20	mâle et femelle	8391±1182		1361±12,9	5,89±4,15

PV= poids vif ; LA= longueur de l'aile; LC= longueur du corps ; LP= longueur de la patte ; PT= pourtour thoracique ;LT= longueur du tarse ; DT= diamètre du tarse ; LTe= longueur de la tête ; LB=longueur du bec ; N=effectif ; ET = Ecart Type.

Tableau 3 : Caractéristiques morphologiques de la poule barrée à l'âge de 20 semaines (mensurations corporelles en centimètre et poids vif en gramme)

	Sexe	N	Moyenne ± ET	Minimum	Maximum	P
PV	mâle	16	1565±160[b]	1186	1846	
	femelle	16	1104±114[a]	950	1321	0,000
	mâle et femelle	32	1334±117	1103	1518	
LA	mâle	16	23,5±1,73 [b]	20	26	
	femelle	16	20,6±1,14 [a]	18	22	0,000
	mâle et femelle	32	22,0±1,02	20	24	
LC	mâle	16	38,1±2,93 [b]	32	41	
	femelle	16	33,5±3,17 [a]	27	39	0,002
	mâle et femelle	32	35,8±2,27	32	40	
LP	mâle	16	33,4±3,16 [b]	28,5	39,7	
	femelle	16	27,7±3,42 [a]	21,5	34,0	0,000
	mâle et femelle	32	30,6±2,88	25,0	36,9	
LT	mâle	16	9,32± 0,58 [b]	8,3	10,3	
	femelle	16	7,31± 0,59 [a]	6,7	8,5	0,000
	mâle et femelle	32	8,32± 0,45	7,8	9,3	

	Sexe	N	Moyenne ± ET	Minimum	Maximum	P
L Te	mâle	16	4,70± 0,31[b]	4,2	5,0	
	femelle	16	4,21± 0,19 [a]	4,0	4,5	0,019
	mâle et femelle	32	4,46± 0,19	4,3	4,8	
L B	mâle	16	3,72± 0,24 [b]	3	4	
	femelle	16	3,23± 0,22 [a]	3	4	0,000
	mâle et femelle	32	3,48± 0,16	3	4	
PT	mâle	16	31,9±2,22 [b]	30	36	
	femelle	16	26,5±1,47 [a]	25	28	0,000
	mâle et femelle	32	28,9±1,60	25	31	
DT	mâle	16	1,33 ± 0,12 [b]	1,2	1,5	
	femelle	16	1,08 ± 0,08 [a]	1,0	1,2	0,000
	mâle et femelle	32	1,20 ± 0,09	1,1	1,4	

[a ,b] : Dans la même colonne, les valeurs affectées de la même lettre ne sont pas significativement différents (p>0,05) pour le même caractère.

Tableau 4 : Régression entre le poids vif et quelques paramètres morpho métriques

Sexe	Paramètres	Equation de régression	r^2	R	N
mâle	PV- PT	PV= 71,3PT-789	0,46	0,50	52
femelle		PV = 45,8PT - 270	0.56	0,60	68
mâle et femelle		PV = 67,8PT - 813	0,87	0,93**	120
mâle	PV – LA	PV=23,2LA+713	0,13	0,36	52
femelle		PV=23,1LA+450	0,29	0,53	68
mâle et femelle		PV= 69,8LA - 525	0,78	0,88**	120
mâle	PV- LT	PV= 228LT - 734	0,55	0,49	52
femelle		PV= 207LT - 610	0,42	0,65	68
mâle et femelle		PV= 194LT - 524	0,88	0,93**	120
mâle	PV-DT	PV = 1187DT - 171	0,54	0,37	52
femelle		PV = 886DT +39,6	0,36	0,65	68
mâle et femelle		PV = 148DT - 605	0,59	0,91**	120
mâle	PV- LC	PV = 37,3LC - 46,0	0,35	0,48	52
femelle		PV = 46,1LC - 521	0,52	0,67	68
mâle et femelle		PV = 49,7LC - 606	0,87	0,93**	120
mâle	PV- LP	PV = 37,3LP - 46,0	0,35	0,28	52
femelle		PV = 46,1LP - 521	0,52	0,53	68
mâle et femelle		PV = 52,0LP - 363	0,88	0,90**	120

** corrélation significative (p < 0,01), N = nombre de poule, r 2 = coefficient de détermination, r = coefficient de corrélation, PV= poids vif, PT = pourtour thoracique, LA= longueur de l'aile, LT= longueur du tarse, DT= diamètre du tarse, LC=longueur du corps et LP= longueur de la patte

Tableau 5: Les caractéristiques de la carcasse, des organes viscéraux et des différentes parties de la poule barrée de 20 semaines d'âge

Variables	Femelles	Mâles	Mâles/Femelles	P
Nombre	16	16	32	
Poids à l'abattage (g)	1104±86,9[a]	1583±68,9[b]	1343±58,9	0,00
Rendement carcasse A(%)	71,4±5,15[a]	66,6±4,60[a]	70,2±1,66	0,28
Densité intestin (g/cm)	0,32±0,03[a]	0,42±0,06[b]	0,37±0,30	0,00
Surface Intestin (cm2)	217±15,2[a]	268±36,0[a]	243±15,2	0,04
Poids de la tête (g)	33,8±4,98[a]	58,5±3,70[b]	46,1±4,28	0,00
Poids des pattes (g)	32,8±5,30[a]	56,9±3,27[b]	44,8±3,76	0,00
Poids gésier (g)	23,9±3,35[a]	29,9±5,50[a]	26,9±4,30	0,22
Poids du cœur (g)	5,21±1,07[a]	10,2±1,10[b]	7,73±0,98	0,00
Poids du gras abdominal (g)	16,3±10[a]	10,9±4,29[a]	13,6±4,68	0,56
Poids du foie (g)	18,5±2,11[a]	28,5±4,49[b]	23,5±2,17	0,00
Poids du bréchet (g)	208±13,12[a]	242±23,9[a]	197±58,1	0,26
Poids cuisse et du pilon(g)	234±21,1[a]	356±10,7[b]	295±13,7	0,00
Poids des ailes (g)	109±10,6[a]	142±18,9[b]	125±11,6	0,00

[a,b,c] : Les moyennes portant la même lettre sur la même ligne ne sont pas significativement différentes (p>0,05).
ET = Ecart Type, carcasse A= carcasse prête à cuir conventionnelle,

A l'éclosion le poussin tout sexe confondu a pesé en moyenne 35,20g. Entre 13 et 20 semaines, le poids vif des mâles a été significativement (P<0,05) plus élevé comparé à celui des femelles (Tableau 2).

L'indice de consommation entre 1 et 12 semaines et entre 13 et 20 semaines a été respectivement de 4,14 et de 8,89. Par ailleurs, entre 13 et 20 semaines l'indice de consommation des femelles (6,94) a été inférieur à celui des males (9,70) même comme la différence n'a pas été n'a pas été significative (P>0,05).

Caractéristiques phénologiques

Aussi bien chez les mâles (1186g à 1846g) que chez les femelles (950g à 1321g) à l'âge de 20 semaines, le poids vif a été très variable en fonction du sexe. Une variation de 660g et de 371g ont été enregistrée respectivement chez les mâles et les femelles. Le taux de variation des mâles (42% du poids vif) a été plus fort que celui des femelles (33% du poids vif), suggérant que ce paramètre peut être exploité en vue d'une sélection des males pour la production de la chair. Quelles que soient les mensurations prises, les valeurs des différents paramètres chez les mâles ont été significativement (P<0,05) plus élevées comparé à celles des femelles (Tableau 3).

Entre 1 et 20 semaines, les corrélations entre poids vif et les mensurations corporelles ont été très fortes et significatives (Tableau 4). Le coefficient de corrélation a été de 0,88 avec la longueur de l'aile et de 0,93 avec la longueur du tarse et du corps. Par ailleurs, les coefficients de détermination entre le poids vifs et la longueur de l'aile ont été très faibles chez les mâles (0,13) et les femelles (0,29) par rapport à tous les autres paramètres morphométriques étudiés.

Caractéristiques de la carcasse

A l'exception du rendement carcasse, du poids relatif du gésier, du gras abdominal et du bréchet pour lesquels les mâles et les femelles étaient comparables (P>0,05), tous les autres paramètres étudiés ont été significativement (P<0,05) supérieures chez les mâles comparés aux femelles (Tableau 5).

Discussion

La consommation cumulée d'aliment de la poule barrée pendant la période de démarrage (3169,75g) est légèrement inférieure au 3515,92g rapportée par Kreman et al. (2012) chez cette poule dans la même zone. Par contre, pendant la période de croissance la consommation enregistrée dans cette étude (5221,90 g) a été plus élevé que celle rapportée par cet auteur (3615,55g). En effet, les sujets utilisés dans la présente étude sont les descendants (F2) des sujets de Kreman et al. (2012). Ceci suggère que la consommation s'améliore d'une génération à l'autre pendant la phase croissance.

Le poids vif de la poule barrée à 12 semaines (781,84g) enregistré dans cette étude est inférieur à celui de rapporté par Kreman et al. (2012) chez le même phénotype (886,62g). Par contre, Fosta (2008), a enregistré un poids à 12 semaines de 511,40g chez la poule locale de la Région du centre Cameroun qui est très inférieur à celui de la présente étude. A vingt semaines, le poids moyen des mâles (1566,87g) est comparable à celui rapporté par Kreman et al. (2012). Par contre, le poids vif des femelles (1204,09g) enregistré dans cette étude est supérieur au 1088g rapporté par ces auteurs.Au Congo, Akouango et al. (2010), ont enregistré des poids vifs à 5 mois plus faibles (1239g et 897g respectivement pour les mâles et les femelles) comparés aux résultats enregistrés dans la présente étude. Par ailleurs, Tike et Ronny, (2006) ont enregistré chez la poule locale en Indonésie des poids à vingt semaines de l'ordre de 1507 et 2290g respectivement pour les femelles et les males qui sont largement supérieurs à ceux de la présente étude. Cette grande variabilité de poids pourrait être due à la diversité génétique qui caractérise les poules locales et aux conditions d'élevage.

Dans cette étude, l'expression du dimorphisme sexuel pour le poids en faveur des mâles s'est établie dès la 6ème semaine. Ce résultat est en accord avec ceux rapportés par Keambou et al. (2007b), Fosta et Manjeli, (2010) et Kreman et al. (2012) chez les poules locales du Cameroun, et par Momoh et al. (2010), au Nigeria. Ceci suggère par ailleurs qu'un programme de sélection des coqs pour la production de viande serait plus avantageux qu'avec les femelles. Le dimorphisme en faveur des mâles s'est exprimé plus tardivement dans notre étude comparée aux résultats de Hako Touko et al. (2009) et Yapi-gnaoré et al. (2009) qui ont rapporté que le dimorphisme s'établie dès la quatrième semaine chez la poule locale africaine. Cet écart pourrait être dû au matériel génétique, à l'environnement et à l'alimentation et surtout au type génétique.

Les différences observées entre les mâles et les femelles pour les mensurations corporelles sont en accord avec les travaux de Pérez et al. (2004). Cette différence lié au dimorphisme sexuel qui apparait dès l'âge de 6 semaines et s'accentue avec l'âge, a également été observée chez la poule villageoise Sénégalaise par Guéye et al. (1998) et par Keambou et al. (2007b) chez les poules locales du Cameroun.

Durant la période allant de 1 à 20 semaines, les coefficients de corrélation du poids vif sur les mensurations corporelles ont été moyens et positifs chez les femelles (0,53 à 0,67). Les coefficients de corrélation les plus élevés ont été obtenu avec la longueur du corps, de la tête, du tarse et le diamètre du tarse. Ceci suggère que ces caractères peuvent être utilisés pour la prédiction du poids vif chez la femelle entre 13 et 20 semaines. Chez les mâles, ils ont été positifs et faibles par rapport à ceux des femelles (inférieur à 0,5). De même, pendant cette période, on a observé des coefficients de détermination plus faibles chez les mâles par rapport aux femelles. Ceci pourrait signifier que dans cette souche il est plus facile de prédire le poids vif avec les mensurations corporelles chez les femelles que chez les mâles. Comme déjà observé Guéye et al. (1998) et par Keambou (2007b) chez la poule villageoise, dans l'ordre d'importance croissant, les caractères pouvant servir à la prédiction du poids chez les mâles ont été le pourtour thoraxique, la longueur du tarse et du corps mais avec une précision plutôt faible.

Comme observé par Gawande et al. (2007), les caractéristiques de la carcasse et les organes viscéraux de la poule villageoise varient avec le sexe, celles du mâle étant en général supérieures à celle de la femelle. Abstraction faite du sexe, le rendement carcasse prêt à

cuire (69,04%) enregistré dans cette étude est inférieur aux données obtenues par Kreman et al. (2012) au Cameroun sur même la poule âgée de 20 semaines (71,45%) et par Akouango et al. (2010) au Congo pour le coq local à 6 mois d'âge (78%). Par contre, ce résultat est supérieur à celui rapporté par Gawandé et al (2007) sur la poule villageoise indienne âgée de 5 mois (65,78%) et à celui rapporté par Koko et al (2006) à Madagascar sur les rendements carcasses des poules villageoises de Madasgacar âgées de 5 mois (64 à 66%). En effet, ce rendement s'élève avec l'âge car le volume sanguin, les plumes et les viscères augmentent moins vite que le poids vif (Leclercq 1990).

Conclusion

La poule barrée présente une grande variabilité dans ses caractères morphologiques et biométriques. Le dimorphisme sexuel apparait dès l'âge de 6 semaines pour le poids vif et les mensurations corporelles et s'accentuent avec l'âge. Les caractères pouvant servir à la prédiction du poids chez les mâles sont le pourtour thoraxique, la longueur du tarse et du corps, mais avec une précision plus faible.

La grande diversité pour le poids vif, les caractéristiques de la carcasse et des différents organes viscéraux enregistrées dans la présente étude fait penser qu'une sélection bien organisée pourrait permettre à terme de produire des animaux plus lourds à 20 semaines d'âge.

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GROWTH PERFORMANCE OF MALE RABBITS EXPOSED TO DIETARY FUMONISIN

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Abstract

An experiment was conducted in a completely randomised design to evaluate growth response of male rabbits fed fumonisin contaminated diets for 28 weeks. 48 weaned rabbits with average weight of 757±50.50g were randomly allotted to four dietary treatments containing 0.1, 5.0, 7.5 and 10.0 ppm fumonisin B1 (FB1), constituting diets 1 (control), 2, 3 and 4 respectively. The results showed that final cumulative live weight and daily weight gain of the animals were significantly depressed (P<0.05) in rabbits fed 7.5 and 10.0 ppm FB1 to about 88.11% and 85.27% respectively, relative to the mean daily weight gain of 7.74g of those that fed the control diet. Feed consumption of rabbits fed diets 2, 3 and 4 apparently declined to about 95.92, 91.51 and 84.77% respectively relative to the mean dry matter intake of the control rabbits. Feed conversion ratio was significantly (P<0.05) lowered in animals fed control diet compared to those that fed the test diets. Percents mortality of 8.33 and 16.67 were recorded in rabbits that fed 7.5 and 10.0 ppm FB1 respectively as compared to no mortality among the animals that fed diets 2 and the control. Dry matter and crude protein digestibility were depressed (P<0.05) in rabbits fed 7.5 and 10.0 ppm FB1. The results suggest that exposure of rabbits to diet formulated with ingredient contaminated with fumonisin B1 up to 5.0ppm and above will depress growth performance and impair nutrient digestibility in rabbits.

Key words: Dietary Fumonisin, Growth response, Rabbits

PERFORMANCE DE CROISSANCE DES LAPINS MÂLES EXPOSÉS À LA FUMONISINE D'ORIGINE ALIMENTAIRE

Résumé

Une expérience a été menée dans un dispositif complètement aléatoire dans le but d'évaluer la réponse de croissance de lapins mâles nourris pendant 28 semaines avec des régimes contaminés par la fumonisine. 48 lapereaux sevrés d'un poids moyen de 757 ± 50,50g ont été répartis de manière aléatoire à quatre traitements diététiques contenant 0,1 ; 5,0 ; 7,5 et 10,0 ppm de fumonisine B1 (FB1), constituant respectivement les régimes 1 (témoin), 2, 3 et 4. Les résultats ont montré que le poids vif final cumulatif et le gain pondéral quotidien des animaux ont diminué considérablement (P <0,05) chez les lapins recevant 7,5 et 10,0 ppm FB1 respectivement à environ 88,11 % et 85,27 %, par rapport au gain pondéral quotidien moyen de 7,74g de ceux nourris au régime témoin. La consommation alimentaire des lapins soumis aux régimes 2, 3 et 4 a apparemment baissé respectivement à environ 95,92 ; 91,51 et 84,77 % par rapport à la consommation moyenne de matière sèche des lapins témoins. L'indice de consommation alimentaire a significativement baissé (P<0,05) chez les animaux soumis au régime témoin par rapport à ceux nourris aux régimes d'essai. Les pourcentages de mortalité de 8,33 et 16,67 ont été enregistrés chez les lapins recevant respectivement 7,5 et 10,0 ppm FB1 par rapport à la mortalité zéro chez les animaux soumis aux régimes 2 et témoin. La digestibilité des matières sèches et des protéines brutes a diminué (P<0,05) chez les lapins recevant 7,5 et 10,0 ppm FB1. Les résultats indiquent que l'exposition de lapins aux aliments contenant un ingrédient contaminé par la fumonisine B1 jusqu'à 5,0ppm et plus diminue la performance de croissance et altère la digestibilité des nutriments chez les lapins.

Mots-clés : Fumonisine d'origine alimentaire ; Réponse de croissance ; Lapins

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Introduction

Mycotoxin contaminants that do result in poor quality feed always lead to decline in the rate and level of performance in the livestock industry. These toxins are secondary metabolites of fungal origin that are harmful to both humans and animals. The disease caused following the ingestion of feed or foods made toxic by these fungal metabolites are known as mycotoxicosis. Irrespective of how nutritive the diet is to meet maintenance and production nutritional requirement of the animals, contamination of such feed with the mycotoxin renders it toxic and detrimental to the health of the animal.

Fumonisin is one of the mycotoxins produced by Fusarium spp. That has been implicated in causing different physiological and pharmacological responses in both plants and animals. Fumonisin producing fungus – Fusarium verticillioides is one of the most prevalent food–borne fungi associated with maize (Zea mays L) intended for humans and animals consumption throughout the world [1]. Maize is a high caloric feed ingredient commonly used in ration formulation for livestock and also consumed as staple in most parts of Nigeria. F. verticillioides contamination of home grown maize has been associated with the high incidence of human oesophageal cancer in areas of the Transkei, Southern Africa [2]. Other effects such as leucoencephalomalacia in horse, porcine pulmonary oedema in pigs, hepatotoxic and carcinogenic activities in rats have been reported following the consumption of F. verticillioides cultured materials [1, 3, 4, 5, 6, 7]. The realisation of potential threat posed to both animals and humans has drastically increased the research interest in fumonisin.

With the above in mind coupled with the fact that tropical humid environment favour fungal growth and toxin production, initiated the design of this experiment to assess the growth response of rabbits exposed to fumonisin-contaminated diets for a long period.

Materials and Methods

Experimental site

The feeding trial was carried out at the Rabbitry Unit of the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria, and further laboratory analyses carried out at the Animal Physiology Laboratory of the Department of Animal Science, University of Ibadan, Ibadan, Nigeria (7020'N, 3050'E; 200m above sea level).

Experimental diets

Fusarium verticillioides cultured maize grains to produce FB1 was generated at the Plant Pathology Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, according to the method described by Nelson et al. [8]. Ground cultured maize was substituted for autoclaved, noncultured maize in various proportions to formulate four diets containing 0.13, 5.0, 7.5 and 10.0 ppm FB1, as determined using fumonisin quantitative test kit (Neorgen Corp., USA), constituting diets 1 (control), 2, 3 and 4 respectively.

Experimental animals and feeding trial

After a 2-week physiological adjustment period, 48 crossbred (New Zealand x Chinchilla) male rabbits of about 5-7 weeks of age averaging 757±50.50g were housed individually in hutches and randomly allotted to the four dietary treatments, such that each diet has 12 replicates. The animals were fed their respective diets ad libitum daily at 0800 h and 1600 h. Potable water was made available to the animals throughout the experimental period. The animals were placed under hygienic condition throughout the feeding period. The compositions of the diets fed for weanling and matured phases are shown in Tables 1 and 2 respectively, and satisfied the nutrient requirements of the animals as recommended by National Research Council [9]. The experiment lasted for 196 days.

Digestibility study

During the last seven days of the experiment, faecal droppings from each animal were collected, weighed, mixed and aliquots taken daily. The daily aliquots and the respective feed samples for each animal were oven-dried in an air-circulating oven at 1050C for 24 hours (to determine their moisture contents) for further analyses. The chemical compositions

of the experimental diets and faecal samples collected, which were used to calculate the apparent digestibility of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), Crude fibre (CF), ash and nitrogen-free extract (NFE), were determined by the method of AOAC [10].

Statistical analyses

The design used for this experiment was Completely Randomized Design (CRD). The data collected on performance indices and nutrient digestibility were subjected to statistical analysis using analysis of variance procedure of SAS [11]. The treatment means were compared using the Duncan's Multiple Range Test procedure of the same software. Mortality observed was subjected to descriptive statistics (percentage).

Results and Discussion

The performance data in terms of cumulative final weights, average daily dry matter intake, mean daily weight gains, relative weekly weight gain, feed conversion ratio and percentage mortality of rabbits fed varied dietary fumonisin B1 are shown in Table 3. The final live weight was significantly ($P<0.05$) depressed with increase in dietary fumonisin level to about 92.09% and 90.15% for animals fed 7.5 ppm and 10.0 ppm respectively relative to the final live weight of control animals. The daily weight gain, cumulative weekly weight gain and relative weekly weight gain were significantly ($P<0.05$) lowered in rabbits fed 7.5 and 10.0 ppm FB1 compared to those that fed control diet. The daily weight gain of rabbits that fed diets 3 and 4 significantly reduced to about 88.11% and 85.27% respectively, relative to daily mean weight gain (7.74g) of rabbits fed control diet. The significantly depressed final live weight in treated rabbits may probably be attributed to the cumulative effect of the toxin in the animals which inhibited nutrient utilization and indirectly impeded cell growth and depressed weight gain of the rabbits fed 7.5 and 10.0 ppm FB1. The percentage reduction in weight gains of animals fed 7.5 and 10.0 ppm compared to control adjudged the potential of the toxin to reduce body weight when rabbits are expose to dietary fumonisin for a longer period. This result agreed with the findings of Gelderblom et al. [12] who reported that the mean body weight of rats fed diets containing 1g fumonisin B1 /kg were 50% lower than those of non-treated rats. In another study with male Fischer rats fed same FB1 concentration in 26-day initiation study observed that the body weight gain were 80% lower than the control [13]. Voss et al. [14] also reported that exposure of Sprague-Dawley rats to culture containing fumonisin resulted in decreased body weight. Similar effect of depressed body weight as a result of the toxin in livestock diets has been reported [14, 15, 16, 17].

The dry matter intake was not significantly influenced among dietary treatments but apparently declined to about 95.92, 91.51 and 84. 77% for rabbits fed diets 2, 3, and 4 respectively, relative to mean daily dry matter intake (95.53g) of the control rabbits. This result corroborates the report of US National Toxicology Program [18] that there was no significance different in feed consumption of male rats fed fumonisin B1 when compared to rats fed control diet [18]. Gbore et al. [19] observed the same effect of the toxin in weanling pigs exposed to dietary FB1 for 6 weeks. Observed feed intake record in this study was at variance with the observation of Bondy et al. [20] who reported significant depression in feed intake of female Sprawgue-Dawley rats administered purified fumonisin B1 at gavage doses of 35 and 75 mg FB1/kg body weight per day.

Feed conversion ratio was significantly ($P<0.05$) lowered in control rabbits compared to animals on test diets with highest value in rabbits fed diet 4 containing 10.0 ppm FB1. Feed conversion ratio in this study revealed that the rate at which the animal placed on control diet were utilizing and converting the feed to flesh was higher than those fed the test diets. This could be attributed to the toxin effect which has been reported to interfere and inhibit the absorption of nutrients from the gastro-intestinal tract of animals [16]. Dry matter and crude protein digestibility were depressed ($P<0.05$) in rabbits fed 7.5 and 10.0 ppm FB1 in this study (Table 4). Inhibition of sphingolipid by fumonisin has also been

Table 1: Percentage Composition [g/100gDM] of Experimental Diets for weanling rabbits

	Dietary Treatments			
Ingredients	**1(Control)**	**2 (5.0 ppm)**	**3 (7.5 ppm)**	**4 (10.0 ppm)**
Non-infected maize	30.00	28.26	27.39	26.52
Infected maize	-	1.74	2.6	3.48
Rice Bran	23.00	23.00	23.00	23.00
Wheat Offal	27.00	27.00	27.00	27.00
Soybean Meal	15.00	15.00	15.00	15.00
Fish Meal	2.00	2.00	2.00	2.00
Bone Meal	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50
Premix (grower)*	0.45	0.45	0.45	0.45
Methionine	0.03	0.03	0.03	0.03
Lysine	0.02	0.02	0.02	0.02
Total	**100.00**	**100.00**	**100.00**	**100.00**

*To provide per Kg diet: Vit. A (10,000 i.u), Vit.D (20,000i.u), Vit E (5 i.u), Vit. K (2.5mg), Choline (350mg), Folic acid (1mg), Manganese (56mg), Iodine (1mg), Iron (20mg), Copper (10mg), Zinc (50mg), and Cobalt (1.25mg).

Table 2. Percentage Composition of Experimental Diets for Matured Rabbits

	Dietary Treatments			
Ingredients	**1(Control)**	**2 (5.0 ppm)**	**3 (7.5 ppm)**	**4 (10.0 ppm)**
Non-infected maize	30.00	28.00	27.00	26.00
Infected maize	-	2.00	3.00	4.00
Rice Husk	30.00	30.00	30.00	30.00
Wheat Offal	25.00	25.00	25.00	25.00
Soybean Meal	10.00	10.00	10.00	10.00
Fish Meal	2.00	2.00	2.00	2.00
Bone Meal	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50
Premix (grower)*	0.45	0.45	0.45	0.45
Methionine	0.03	0.03	0.03	0.03
Lysine	0.02	0.02	0.02	0.02
Total	**100.00**	**100.00**	**100.00**	**100.00**

*To provide per Kg diet: Vit. A (10,000 i.u), Vit.D (20,000i.u), Vit E (5 i.u), Vit. K (2.5mg), Choline (350mg), Folic acid (1mg), Manganese (56mg), Iodine (1mg), Iron (20mg), Copper (10mg), Zinc (50mg), and Cobalt (1.25mg).

reported to adversely affect normal epithelial morphology [21] coupled with tunica mucosa erosion of stomach and small intestine induced by the same toxin [16] may probably impeded absorption of nutrients of the experimental diets. This effect may have responsible for the significant decrease in the dry matter and crude protein digestibility of same animals as the toxin level increases in the diets (Table 4).

The accumulated effect of the toxin in this regards probably responsible for 8.33 and 16.67% mortality recorded for rabbits exposed to 7.5 and 10.0 ppm FB1 respectively. Organ histological examination revealed severe hepatic/necrotic lesion of the tissues examined in samples of dead animals diagnosed and others that were sacrificed at the end of the feeding trial [22]. Observed also in this study

Table 3. Performance of Rabbits Fed varied Levels of Dietary Fumonisin

	Dietary Treatments				
Parameters	**1(control)**	**2 (5.0ppm)**	**3(7.5ppm)**	**4 (10.0ppm)**	**SEM**
Initial live weight (g)	758.00	756.00	758.00	758.00	3.20
Final live weight (g)	2275.21^{a}	2128.78^{ab}	2095.54^{bc}	2051.33^{c}	46.80
Dry matter intake (g/day)	95.53	91.63	87.42	80.98	4.20
Weekly weight gain(g)	54.18^{a}	49.00^{ab}	47.74^{bc}	46.20^{c}	3.46
*Rel. weekly wt gain(%)	7.15^{a}	6.48^{b}	6.30^{b}	6.09^{c}	0.05
Daily weight gain (g)	7.74^{a}	7.00^{ab}	6.82^{bc}	6.60^{c}	1.16
Feed conversion ratio	20.37^{b}	28.91^{a}	30.25^{a}	33.33^{a}	2.44
Mortality (%)	0.00	0.00	8.33	16.66	

^{abc}: Means in the same row with different superscripts are significantly ($P < 0.05$) different.
SEM: Standard Error of Mean
*Relative to initial weight

Table 4: Apparent nutrient digestibility (0-1) of rabbits fed varied levels of dietary Fumonisin

	Dietary Treatments				
Parameters	**1(control)**	**2 (5.0ppm)**	**3(7.5ppm)**	**4 (10.0ppm)**	**SEM**
Dry matter	0.65^{a}	0.63^{b}	0.62^{b}	0.61^{b}	0.034
Crude protein	0.67^{a}	0.66^{ab}	0.64^{b}	0.54^{c}	0.051
Ether extract	0.59	0.62	0.58	0.59	0.033
Crude fibre	0.69	0.69	0.69	0.68	0.031
Ash	0.55	0.50	0.52	0.52	0.023
Nitrogen free extract	0.73	0.73	0.75	0.76	0.032

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

was one of the animals fed 10.0 ppm FB1 went into comma, incoordination and paralysis of hind quarters for about 36 to 48 hours at 26th week into feeding trial and recovered thereafter without any medication.

Conclusion

Feeding farm animals most especially rabbits with diet formulated with F.verticillioides infected grains that would liberate up to 7.5 ppm fumonisin B1 will impair growth performance of the animal by depressing live weight and weight gain as a result of poor feed utilization. It can also induce mortality, thereby resulting into economic loss to livestock farmers and livestock industry at large. Therefore effort should be geared towards preventing grains from fusarium infection and fumonisin-contaminated grains should be avoided in feed mills when formulating ration for livestock most especially rabbits.

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FACTORS ASSOCIATED WITH RABIES AWARENESS AND ATTITUDE TO DOG BITE IN A UNIVERSITY COMMUNITY

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Abstract

Preponderance of stray dogs at the study site necessitated assessment of awareness on rabies and associated factors, attitude to dog bite and knowledge on rabies among students and staff members in a University community.

We reviewed hospital records for dog bite cases from 2005 to 2010 and administered structured questionnaire to 326 randomly selected respondents. Incomplete entries were removed and the remaining 315 entries analyzed using Epi-Info 3.5.1. Chi square test and multivariate analysis were done at 95% confidence interval.

Ten dog bites cases were extracted from hospital records while 65 were reported from survey. Of the 65 cases, 67% were between the ages of 13 – 18 years; 41.4% had bite on the legs; 52.3% reported at the hospital within a few hours after the dog bite incidence. Of 34 respondents who reported at the hospital, only 37.6% received both wound treatment and post exposure vaccination. Of 315 respondents, 79% were aware of rabies. Majority (43%) reported to have heard from friends. Having at least secondary school education (Adjusted Odds Ratio (AOR) = 3.7, Confidence Interval (CI) = 1.8 – 7.8), owning a dog (AOR = 8.2, CI = 2.4 – 27.8) and knowing bat as source of rabies (AOR = 4.7, C = 1.6 – 14.0) were significantly associated (P<0.05) with rabies awareness.

Majority of the respondents have fair knowledge on rabies despite high awareness level. Educational level and dog ownership are associated with rabies awareness. Dog bite cases are under reported and friends can serve as good source of information dissemination.

Keywords: Knowledge; awareness; dog-bite; rabies, friends, University

FACTEURS ASSOCIÉS À L'INFORMATION SUR LA RAGE ET À L'ATTITUDE VIS-À-VIS DES MORSURES DE CHIEN DANS UNE COMMUNAUTÉ UNIVERSITAIRE

Resume

Le grand nombre de chiens errants sur le site de l'étude a nécessité une évaluation de l'information sur la rage et des facteurs associés, de l'attitude vis-à-vis des morsures de chien et des connaissances sur la rage parmi les étudiants et les membres du personnel d'une communauté universitaire.

Nous avons examiné les fiches d'hôpitaux pour les cas de morsure de chien enregistrés de 2005 à 2010, et administré un questionnaire structuré à 326 personnes choisies de manière aléatoire. Les réponses incomplètes ont été ignorées, et les données des 315 restantes ont été analysées en utilisant le logiciel Epi -Info 3.5.1. Le test du Chi carré et une analyse multivariée ont été effectués à un intervalle de confiance de 95%.

Dix cas de morsures de chien ont été extraits des dossiers d'hôpitaux tandis que 65 ont été signalés dans l'enquête. Des 65 cas, 67 % étaient âgés de 13 à 18 ans ; 41,4 % avaient des morsures sur les jambes ; 52,3 % se sont présentés à l'hôpital quelques heures après la morsure par un chien. Des 34 répondants qui se sont présentés à l'hôpital, seuls 37,6 % avaient reçu à la fois un traitement des plaies et une vaccination post-exposition. Des 315 répondants, 79 % étaient renseignés sur la rage. La majorité (43 %) a déclaré en avoir entendu parler par des amis. Le fait d'avoir au moins une formation secondaire (quotients de probabilité ajustés (AOR) = 3,7 ; l'intervalle de confiance (IC) : 1,8 à 7,8) ; le fait d'avoir un chien (AOR = 8,2, IC = 2,4 à 27,8) et de savoir que la chauve-souris est une source potentielle de la rage (AOR = 4,7, C = 1,6 à 14,0) étaient des facteurs significativement associés (P <0,05) avec la sensibilisation à la rage.

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La majorité des répondants ont une connaissance moyenne de la rage en dépit du niveau de sensibilisation élevé. Le niveau de formation et le fait d'avoir un chien sont associés à la connaissance de la rage. Les cas de morsure de chien sont sous-déclarés ; et les amis peuvent être une bonne source de diffusion de l'information.

Mots-cles : Connaissance ; Sensibilisation ; Morsure de chien ; Rage ; Amis ; Université

Introduction

Rabies is a viral zoonosis that causes acute encephalitis (inflammation of the brain) in warm blooded animals. It occurs in a wide range of hosts including bats, cats, skunks, foxes, raccoons, dogs and humans. In humans, rabies is most commonly associated with bite from an infected animal (Jawetez and Anderlberg, 1980). Rabies is almost invariably fatal if post exposure prophylaxis is not administered prior to the onset of severe symptoms (Mani and Murray, 2006). The case fatality is almost 100% (Greene and Dreesen, 1990).

The disease occurs throughout the world although a few countries are free of the disease due to successful eradication programmes and enforcement of rigorous quarantine measures (Jenkins *et al.,* 1996). There are an estimated 55,000 human death annually from rabies worldwide, with about 31000 in Asia and 24,000 in Africa and many of these deaths occur in children (WHO 2002).

There is inadequate surveillance of rabies in most parts of the world, (Warrell and Warrel, 2004) and there are no reliable data on the disease in developing countries, like Nigeria, where the disease has an endemic status (Osunnubi *et al.,* 1999). Dog is the principal animal reservoir in developing countries like Nigeria. There are many stray dogs in the University of Ibadan and most of these stray dogs as well as some home dogs are not vaccinated (Nottidge and Omobowale, 2008). The expansion and improvement of knowledge and awareness of any disease around the world is critical to the prevention and control of such diseases (GARC 2012). Effective rabies education can help prevent new infection by providing people with information about rabies and how it is passed on , and in so doing equip individuals with knowledge to protect themselves from becoming infected, (GARC 2012, Hanlon *et al.,* 1999). Ignorance about the risk of uncomplicated treatment, financial constraints and scarcity of vaccine may be implicated in the outcome of rabies (Oginni *et al.,* 2002). Thus, we set out to assess the awareness level on rabies and contributing factors; attitude to dog bite and knowledge on rabies amongst students and staff members at the University of Ibadan in order to determine effective strategy to prevention and control of dog bite and rabies amongst students and staff members at the University of Ibadan.

Materials and Methods

Study Design

The study was a cross sectional survey among students and staff members at the University of Ibadan, Ibadan from July to October 2011.

Study Location

The study site was the University of Ibadan, Ibadan, which is on latitude 7o26'490"N and longitude 3o54'359"E (Geographical positioning system, etrex, Garmin, Taiwan). The University of Ibadan is the oldest University in Nigeria with a student population of about 20,000 (Anon. 2008). The University has a staff school, two secondary schools (Abadina College and The International School), Staff quarters, nine Faculties and a University College Hospital.

Study Population

The respondents were primary school pupils from the University of Ibadan Staff School; junior and senior secondary pupils from the International School, Ibadan; tertiary students from various faculties at the University of Ibadan and residents of some staff quarters at the University of Ibadan.

Sample size and Sampling

A total of 326 respondents were involved in the study. Based on previous studies we powered the study to detect prevalence

(p) of 90% awareness (Matibag *et al.,* 2007). We considered precision of 3.5%; non-response rate of 10. The students were simple randomly selected from a frame of students present during class hours; while the staff members living on streets selected simple randomly from the list of streets at the University of Ibadan who also showed willingness to participate were recruited in the study. Informed consent was obtained from all respondents; respondents who declined to participate were excluded from the study.

Data collection

We reviewed the University of Ibadan Health Centre records for dog bite cases from 2005 to 2010. A pre-tested structured questionnaire was used to obtain information on demography, dog bite history, dog bite management and vaccination, awareness and general knowledge on rabies. Two other interviewers were trained on how to administer the questionnaire. The questionnaires were both self and interviewer administered: It was interviewer administered to the primary school pupils, junior secondary school students, and some of the staff members. All of the questionnaires to the senior secondary school and university students were self-administered.

All three hundred and twenty six filled questionnaires were imputed on a spreadsheet (Microsoft excels, 2003). The data were cleaned and eleven incomplete entries deleted. The remaining 315 entries, which were 34 primary school pupils, 80 junior secondary school students, 81 senior secondary school students, 41 university students and 79 staff members were analyzed.

Data analysis

Descriptive statistics was done while statistical significance was determine using Chi squares test at 95% confidence intervals with Epi-Info version 3.5.1. Multiple regressions was conducted to control for confounding and to identify factors that were independently associated with rabies awareness. Factors that were significant in bivariate analyses were included in the logistic model. The logistic model coefficients were determined to be significant if their p value was less than 0.05 on the Wald Chi-squared test. The knowledge on mode of transmission and clinical signs were scored using a 2 point knowledge scale; a score less than 2 was rated fair. Knowledge on prevention was scored using a 6 point knowledge scale; a score less than 3 was rated fair. Probability level of less than 5% ($p < 0.05$) was accepted as significant.

Results

Record review and demography

A total of 10 dog bite cases were reported at the University Health Centre from 2005 to 2010 (Figure 1). The median age of respondents was 15 years (Range 7 – 59years). Of the 315 respondents, most (40.3%) were within the age group 13 – 18years; 51.1% were males; 33% attained highest educational level of having primary education while 74.6% were students (table 1).

Dog bite history, Management and Vaccination

Sixty five (20.6%) of 315 respondents reported either them or a family member to have been bitten by a dog within 2005 and 2010. Out of the 65 dog bite cases reported, 61.5% occurred within 2005 and 2009; while 38.5% occurred in 2010. Thirty three (10.5%) respondents reported to have been bitten by a dog within 2005 and 2010. Of the 65 reported cases of dog bite, 67% of the dog bite victims were between the ages of 13 – 18 years while 53.9% was due to provocation. About 47% of the dog bite cases occurred on the street. Majority (41.4%) of the respondents were bitten on the leg. Thirty four (52.3%) reported at the hospital within a few hours after the dog bite incidence. Of the 34 respondents who reported at the hospital, only 37.6% received both wound treatment and post exposure vaccination.

Of the 315 respondents, 24.1% owned at least a dog; of which about 49% were exotic breed. About 52% of the dogs were males and 53% were above 6 months old. Most (58%) of the dogs were kept in strict confinement and about 75% of the respondents that had dogs have vaccinated their dogs against rabies as at study time.

Awareness

Of 315 respondents, 249 (79.0%) reported to have heard of rabies; most (43.2%) reported to have heard from friends. About 46.0% of the respondents knew the cause of rabies to be virus. Majority (83.5%) knew the mode of infection to be through a bite from an infected dog. Only 23% of the respondents reported that rabies can also be contacted through bats. Majority of the respondents (70%) knew that irregular vaccination of a dog is a risk to having rabies; 68% also believed that playing with strange/ownerless dog is a risk to having rabies. Most of the respondents (64%) knew that a change in behaviour of dog is one of the signs of rabies; while only 44% knew that a rabid dog can also be calm and not always aggressive. Only 40% of the respondents answered in the affirmative their ability to recognize a rabid dog. About 76% of the respondents believed rabies is preventable. Of the 315 respondents, 36.5% claimed their first line of action when bitten by a dog will be to report to a clinic, 30.2% will give only first aid, 29.2% will treat at home while the rest will do nothing about it. On bivariate analysis, factors significantly associated ($p<0.05$) with rabies awareness were age, educational level, owning a dog, taking one's dog for vaccination and knowing that bat is a possible source of rabies (table 2). Educational level, owning a dog and knowing that bat is a possible source of rabies remained significant independent factors that were associated with rabies awareness in the logistic model (table 3).

General knowledge on rabies

Overall, 145 (46.0%) and 67 (21.3%) had correct knowledge of the cause of rabies and how one can contact rabies respectively. Ninety seven (30.8%) respondents had correct knowledge of the clinical signs of rabies in dogs; majority (73.0%) knew that rabies is a deadly disease; about half of the respondents (50.2%) had correct knowledge of what to do in the event of a dog bite while 53.0% correctly knew what to do to prevent contracting rabies. There is a significant difference ($P<0.05$) in the knowledge on aetiology, mode of transmission, prevention of rabies and knowledge on what to do when there is a dog bite among the various

Table 1: Demographic features of respondents

Variables	N = 315 n (%)
Age group (yrs)	
7-12	77 (24.4)
13-18	127 (40.3)
19-49	108 (34.3)
>= 50	3 (1.0)
Sex	
Female	154 (48.9)
Male	161 (51.1)
Marital status	
Single	257 (81.6)
Co-habiting	2 (0.6)
Married	47 (14.9)
Divorced	7 (2.2)
Widow	2 (0.6)
Religion	
Christian	217 (68.9)
Islam	95 (30.2)
Others	3 (0.9)
Highest educational qualification	
None	35 (11.1)
Primary	104 (33.0)
Secondary	91 (28.9)
Tertiary	85 (27.0)
Student status	
Primary	34 (10.8%)
Junior secondary	80 (25.4%)
Senior secondary	81 (25.7%)
Tertiary	41 (13.0%)
Staff members	79 (25.1%)

Data are frequencies and percentages (%).

Table 2: Factors associated with rabies awareness among respondents

Variable	Aware n (%)	Not aware n (%)	OR (95% CI)	P value
Age group (yrs)				
≤ 18	153 (61.4)	51 (77.3)	0.47 (0.24 – 0.91)	0.02*
> 18	96 (38.6)	15 (22.7)		
Sex				
Male	127 (78.9)	34 (21.1)	0.98 (0.55 – 1.75)	0.95
Marital status				
Married/Co-habiting	41 (16.5)	8 (12.1)	1.43 (0.60 – 3.51)	0.50
Highest education				
Secondary/tertiary	155 (62.2)	21 (31.8)	3.53 (1.91 – 6.57)	0.00*
None/primary	94 (37.8)	45 (68.2)	0.60 (0.29 – 1.25)	
Status				
Students	182 (73.1)	54 (81.2)	1.84 (0.82 – 4.26)	0.20
Staff members	67 (26.9)	12 (18.2)		0.16
Dog bite history				
Had history of dog bite	56 (29.0)	9 (13.6)	8.71 (2.65 – 28.63)	0.00*
Dog ownership				
Owned a dog	73 (29.3)	3 (4.5)	1.84 (0.12 – 53.84)	1.00
Sex of dog				
Female	35 (47.9)	1 (33.3)	0.46 (0.02 – 6.93)	0.61
Male	38 (52.1)	2 (66.7)	0.0	
Breeds of dog				
Exotic	35 (47.9)	2 (66.7)		0.02*
Non-exotic	38 (52.1)	1 (33.3)		
Vaccination status of dogs				
Vaccinated	56 (76.7)	0 (0.0)	5.82 (2.04 – 16.62)	0.00*
Not vaccinated	17 (23.3)	3 (100.0)		
Knowledge of bat as source of rabies				
Had knowledge	68 (27.3)	4 (6.1)		

Data are frequencies of those who have heard of rabies and those who have not by age, gender, educational level and other factors (%) with their odds ratios (OR), 95% confidence interval (CI) and Probability value (P). *Significant

Table 3: Unconditional logistic regression of factors associated with rabies awareness

Variables	Adjusted Odds Ratio	95% Confidence Interval	P-Value
Age group (> 18years/ ≤ 18years)	1.28	0.53 - 3.00	0.59
Gender (Male/ Female)	1.07	0.58 - 1.96	0.82
Educational level (Sec. & Ter./ None & Pry.)	3.71	1.77 - 7.77	0.001*
Owning a dog (Yes/ No)	8.22	2.44 - 27.75	0.001*
Knowing bat as source of rabies (Yes/ No)	4.74	1.60 – 14.03	0.01*

*significant difference at $p<0.05$

Table 4: Knowledge level on rabies among students and staff members

Status	Good knowledge n (%)	Fair knowledge n (%)	Odds Ratio (95% CI)	P value
Aetiology				
Primary (n = 34)	11 (7.6)	23 (13.5)	Ref	
Junior sec. (n = 80)	34 (23.4)	46 (27.1)	1.55 (0.62 – 3.92)	0.42
Senior sec. (n = 81)	31 (21.4)	50 (29.4)	1.30 (0.51 – 3.30)	0.70
Tertiary (n = 41)	21 (14.5)	20 (11.8)	2.20 (0.77 – 6.31)	0.15
Staff member (n = 79)	48 (33.1)	31 (18.2)	3.24 (1.28 – 8.28)	0.01*
Mode of transmission				
Primary (n = 34)	4 (6.0)	30 (12.1)	Ref	
Junior sec. (n = 80)	8 (11.9)	72 (29.0)	0.83 (0.21 – 3.60)	0.75
Senior sec. (n = 81)	19 (28.4)	62 (25.0)	2.30 (0.66 – 8.80)	0.24
Tertiary (n = 41)	14 (20.9)	27 (10.9)	3.89 (1.02 – 16.09)	0.047*
Staff member (n = 79)	22 (32.8)	57 (23.0)	2.89 (0.84 – 10.97)	0.11
Clinical signs				
Primary (n = 34)	10 (10.3)	24 (11.0)	Ref	
Junior sec. (n = 80)	29 (30.0)	51 (23.4)	1.36 (0.53 – 3.56)	0.63
Senior sec. (n = 81)	27 (27.8)	54 (24.8)	1.20 (0.46 – 3.14)	0.85
Tertiary (n = 41)	14 (14.4)	27 (12.4)	1.24 (0.42 – 3.72)	0.85
Staff member (n = 79)	17 (17.5)	62 (28.4)	0.66 (0.24 – 1.81)	0.51
Rabies case fatality rate				
Primary (n = 34)	21 (9.1)	13 (15.3)	Ref	
Junior sec. (n = 80)	59 (25.7)	21 (24.7)	1.74 (0.68 – 4.44)	0.29
Senior sec. (n = 81)	60 (26.1)	21 (24.7)	1.77 (0.69 – 4.51)	0.27
Tertiary (n = 41)	29 (12.6)	12 (14.1)	1.50 (0.51 – 4.39)	0.57
Staff member (n = 79)	61 (26.5)	18 (21.2)	2.10 (0.81 – 5.46)	0.14
Rabies prevention				
Primary (n = 34)	24 (15.2)	10 (6.4)	Ref	
Junior sec. (n = 80)	5 (3.2)	75 (47.8)	0.03 (0.01 – 0.10)	0.00*
Senior sec. (n = 81)	37 (23.4)	44 (28.0)	0.35 (0.14 – 0.89)	0.03*
Tertiary (n = 41)	33 (20.9)	8 (5.1)	1.72 (0.52 – 5.71)	0.47
Staff member (n = 79)	59 (37.3)	20 (12.7)	1.23 (0.46 – 3.28)	0.83
Rabies prevention				
Primary (n = 34)	14 (8.4)	20 (13.5)	Ref	
Junior sec. (n = 80)	30 (17.9)	50 (33.8)	0.86 (0.35 – 2.11)	0.87
Senior sec. (n = 81)	38 (22.8)	43 (29.0)	1.26 (0.52 – 3.07)	0.72
Tertiary (n = 41)	28 (16.8)	13 (8.8)	3.08 (1.08 – 8.92)	0.03*
Staff member (n = 79)	57 (34.1)	22 (14.9)	3.70 (1.47 – 9.40)	0.003*

*significant difference at $p<0.05$

student status (primary, junior secondary, senior secondary schools, tertiary institution) and staff members – table 4. Junior secondary pupils and senior secondary pupils are 0.03 and 0.35 respectively less likely to know what to do when there is a dog bite than primary school pupils. Tertiary students and staff members were 3 and 4 times respectively more likely to know about rabies preventive measures that primary and secondary school pupils (table 4).

Discussion

This study reported more cases of dog bite over the 5-year period from the University community survey than from the University health center records within the same time period. The difference indicates the possibility of under-reporting of dog bite cases and possible sentiment of dog bite victims to seeking hospital health care. The under reporting of cases of dog bite is similar to the observation of Abubakar and Bakari (2012) who reported low hospital incidence of dog bite injuries in a tertiary hospital in Northern Nigeria; and Aghahowa and Ogbevoen (2012) who also reported rarity of dog bite cases in another tertiary hospital in Eastern Nigeria. Another reason for the lower number of dog bite cases from hospital records than from the survey could be the trivialization of some cases of dog bite by victims especially when the degree of injury is not type III and thus may not go to the hospital. Type III degrees of injury are reported most in hospitals (Abubakar and Bakari, 2012, Oginni *et al.,* 2002). This ought not to be if dog bite victims are aware of the risk of rabies a "mere bite from a street dog" could pose. Majority of dog bite victims and other community people are not aware of the fatality and importance of vaccination and post-exposure prophylaxis (Herbert *et al.,* 2012, Wasay *et al.,* 2012). The high cost of obtaining post-exposure prophylaxis (PEP) in developing countries has been adduced as one of the reasons dog bite victims seek alternatives rather than proper medical care (Warrell, 2012, Sudarshan *et al.,* 2006, Ichhpujani *et al.,* 2006, Wilde *et al.,* 2005). The pain from injection could also discourage some from seeking hospital health care (Ichhpujani *et al.,* 2006) as well as inadequate knowledge of health care workers on rabies and other zoonoses could also be adduced as reasons for under-reporting (Gonen *et al.,* 2012; Kakkar *et al.,* 2012).

We reported 20.6% prevalence of dog bite among respondents and their family members within the 5-year period and 38.5% within the previous year. The prevalence of dog bite the previous year in our study is higher than that (13%) reported by Hergert and Nel (2012) in Kwazulu-Natal, South Africa and 18% by Wasay and others (2012) in Karachi, Pakistan. More so, the 10.5% dog bite case among respondents only is higher than that (2.5%) reported by Wasay and others (2012) in Karachi, Pakistan. The higher dog bite cases reported in this study could be as result of many stray dogs (Nottidge and Omobowale, 2008) and because the other two studies were Province population-based survey as opposed to University community-based survey carried out and thus the possibility of higher concentration of dog bite cases.

We reported more dog bite cases (67%) in ages less than 18 years, this is similar to the findings in most previous studies (Abubakar and Bakari, 2012, Tenzin *et al.,* 2012, Salahuddin *et al.,* 2012, Sudarshan *et al.,* 2006). The reason for this could be because of the inquisitive and playful tendencies of pupils in this age group and thus may want to play with or care for stray dogs. In addition more than half of the dog bite victim reported the event was due to provocation. This study reported that about half of the dog bite cases occurred on the street. This is similar to the findings of Sudarshan and others (2006) who reported 63% dog bite from stray dogs in a national multi-centric rabies survey; and Tenzin and others (2012) who reported 71% stray dog bite in Eastern Bhutan. Poverty has been adduced as a reason why some dogs stray and a bane to canine rabies elimination in developing world (Taylor, 2012, Schneider *et al.,* 2012).

We also reported that majority of the respondents were bitten on the leg; this is consistent with previous findings (Abubakar and Bakari, 2012, Aghahowa and Ogbevoen, 2012, Sudarshan *et al.,* 2007, Shetty *et al.,* 2005, Agarwal and Reddajah, 2004).

This study also reported that about half of dog-bite victims reported at the hospital within a few hours after the dog bite incidence. This response is low compared to 82% reported by Hergert and Nel (2012) in Kwazulu-Natal, South Africa. This may be due to some of the reasons earlier adduced for the difference in numbers of dog bite cases between the hospital records and the survey. It may also be an indication of gap in the information dissemination on rabies prevention and control. However, among dog-bite victims who reported at the hospital, those who received both wound treatment and at least one post exposure vaccination (37.6%) from our study is similar to that reported by Hergert and Nel (2012) (35%) in Kwazulu-Natal, South Africa and Dey and others (2012) (15.7%) among pediatrics in Bangladesh. In contrast, Abubakar and Bakari (2012) reported high (87.7%) PEP in victims of dog bite from a hospital based study in Northern Nigeria. Some reasons adduced for decrease administration of PEP include: non-indication by the health care givers (Salahuddin, 2012, Beaujean, 2008) - this position may pose some risks as most of these bites are from stray dogs (Tenzin *et al.,* 2012, Dey *et al.,* 2012, Nottidge and Omobowale, 2008) and samples from those dogs may not be available for any diagnostic test (Wacharapluesadee *et al.,* 2012); unavailability and unaffordable cost of vaccine (Warrell, 2012, Sudarshan *et al.,* 2006) or inadequate knowledge of health care givers on rabies control and prevention (Gonen *et al.,* 2012).

About 24.1% of respondents in our study had at least a dog; this is similar to what obtains in sub-Saharan African and Asia but slightly higher than that reported in Tanzania (14%) by Knobel and others (Knobel *et al.,* 2008) and in India (17%) by Sudarshan and others (2006). In contrast, high (77%) dog ownership was reported in Bohol, Philippines by Davlin (2012) and 56% by Bingham (2012) in Bravos county, Texas, United States of America. Factors such as having livestock, poultry, cats, being wealthy, being a Christian and having male as head of the household have been associated with dog ownership (Knobel *et al.,* 2008); though other factors were not considered in this study, however, majority (69%) of our respondents were Christians and having 49% exotic breed is an indication of some level of wealth. Most (75%) of the respondents that had dogs in this study had recently vaccinated their dogs against rabies; 53% were above 6 months old and about 58% of the dogs were kept in strict confinement. This is similar to the observation of Davlin and others (2012) who reported 64% recent vaccination against rabies in owned dogs; however Fielding and others (2012) and Sudarshan and others (2006) reported lower percent (41.6% and 32.9%) of vaccinated dogs in Port-au-Prince, Haiti and India respectively. Increasing age of dogs and confinement of dog both day and night have been associated with increased dog vaccination (Davlin *et al.,* 2012).

We also reported that 79% of the respondents have heard of rabies; this is consistent with most studies (Herbert *et al.,* 2012, Hergert and Nel, 2012, Bingham *et al.,* 2012). In contrast lower rabies awareness level had been reported by Wasay and others (2012) (58%) and Ichhpujani and others (2006) (68.7%). The high awareness level in this study could be because it is a University based community survey and the presence of a Veterinary Teaching Hospital within the University community. The most reported source of rabies awareness was through friends, this is contrary to the findings of Hergert and Nel (2012) who reported Government Veterinary Services as the most reported source. It is possible that the source of information might have emanated from Veterinary services, but our findings underscore the role of friends in information dissemination in community among various age groups. About 83.5% of the respondents knew that rabies could be contacted through a bite from an infected dog; similar to the findings of Ichhpujani and others (2006). This is expected because many people living in urban areas are aware of canine urban rabies and had almost erroneously and exclusively attributed rabies incidence to dog bite Ichhpujani *et al.,* 2006. However, 23% of the respondents were also aware that bat could be a potential source of rabies; similar to Robertson and others (2012) who reported 10% awareness level in Thailand. The low awareness level is expected since less attention is being given to bat as a potential source of rabies in Nigeria; emphasis has been

on control and prevention of rabies due to dog bite. Factor significantly associated ($p<0.05$) with rabies awareness in this study were age, educational level, owning a dog, taking one's dog for rabies vaccination and knowing that bat is a possible source of rabies. Similarly Herbert and others (2012) found significant association between gender, age, educational status and rabies awareness. Moreso, Bingham and others (2012) reported significant association between respondents who believed bats could be a potential source of rabies and reporting a dog bite. However, Hergert and Nel (2012) found that non-dog owners were more likely to have heard of rabies than dog owners; also Bingham and others (2012) reported no association between dog ownership and reporting a dog bite. Educational level, owning a dog and knowing that bat is a possible source of rabies remained significant independent factors on multivariate analysis. The indication of this is that age and taking one's dog for rabies vaccination might have been a confounder in this association. Though Hergert and Nel (2012) did not find any association between dog ownership and rabies awareness; this however remained a significant factor on logistic regression. The percentage of respondents that are dog owners was not indicated by Hergert and Nel (2012); if this is very low compared to non dog owners, it may account for the differences observed in association in the two studies.

Evaluation of the depth of knowledge on rabies of individual respondent revealed that 46.0% and 21.3% of total respondents had correct knowledge of the cause of rabies and how one can contact rabies respectively. Ninety seven (30.8%) respondents had correct knowledge of the clinical signs of rabies in dogs; majority (73.0%) knew that rabies is a deadly disease; about half of the respondents (50.2%) had correct knowledge of what to do in the event of a dog bite while 53.0% correctly knew what to do to prevent contracting rabies. These results generally indicate a poor to fair knowledge on rabies in spite of the fact that majority reported to have heard about rabies. However, majority knew that rabies is very fatal; this is similar to the findings of Agarwal and Reddajah (2004) in India; but in contrast to what some studies reported (Herbert *et al.,* 2012, Wasay *et al.,* 2012, Bingham *et al.,* 2012). It is not expected that respondents should have in-depth knowledge on rabies but those information that could assist in prevention and control like clinical signs of a suspected rabid dog; rabies fatality; what to do in the event of unsuspected dog bite; and prevention measures. Our results revealed the gaps in the message content on rabies in the community.

We attempted to look at the areas where there was poor to fair knowledge on

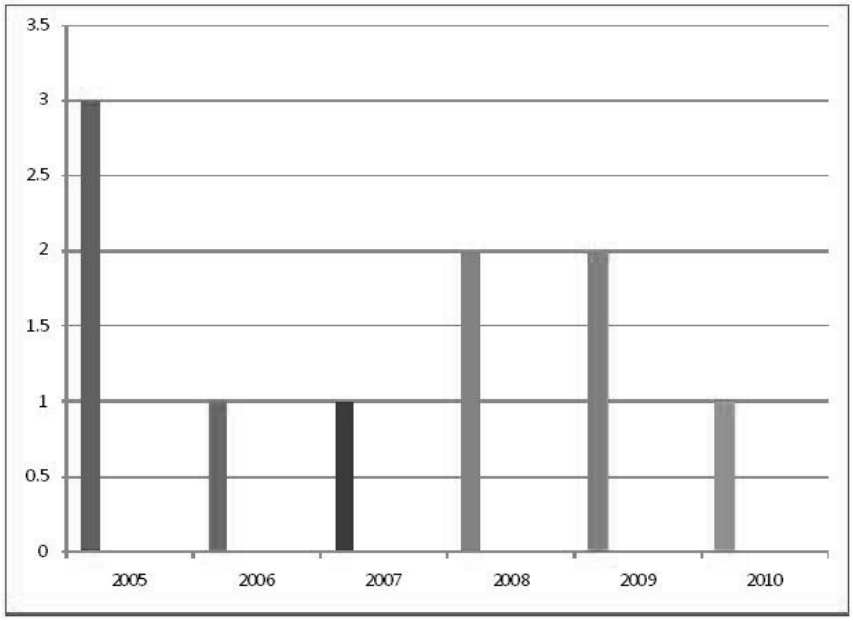

Figure 1: Number of dog bite cases reported at the University Health Centre, Jaja, from 2005 to 2010

rabies and identify groups attention should be focused on. Tertiary students were more likely to know bat as a potential source of rabies than other category of respondents; surprisingly Junior secondary pupils and senior secondary pupils are 0.03 and 0.35 respectively less likely to know what to do when there is a dog bite than primary school pupils; tertiary students and staff members were 3 and 4 times respectively more likely to know about rabies preventive measures that primary and secondary school pupils. Though there is no significant difference in knowledge on clinical signs of a suspected rabid dog among the category of respondents, the knowledge level is poor. The indication of this is that all categories of respondents should be taught clinical signs of a suspected rabies dog especially the fact that not all rabid dogs are furious some cold be calm. More so, attention should be focused on teaching secondary school pupils on what to do in the case of a dog bite. The possibility of bat as a possible source of rabies infection should be taught to all especially primary and secondary pupils.

Conclusions and Recommendations

Our study revealed that there is under reporting of dog bite cases and that about half of dog bite victim will not visit hospital; also that less people among those who visited receive PEP. There is high rabies awareness, however majority of the respondents have poor knowledge depth on rabies. Friends/ peer group played a major role in information gathering and dissemination. Educational level, dog ownership and being aware of bat as a potential source of rabies are associated with rabies awareness. Knowledge gaps are identified in the areas of causes of rabies, sources of infection, clinical signs of rabies in dogs, what to do in case of dog bites and preventive measures. Information dissemination should be focused on primary and secondary pupils.

In view of the study findings we recommended that the content on rabies information should be made to emphasize issues like causes of rabies, sources of infection and what to do in the event of a dog bite and preventive measures. The role of friend/ peer group should also be explored in the information dissemination. A multi-center community survey on the above subject should be carried out.

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GUMBORO DISEASE OUTBREAKS CAUSE HIGH MORTALITY RATES IN INDIGENOUS CHICKENS IN KENYA

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Abstract

Infectious bursal disease is a disease of economic importance which affects all types of chickens and causes variable mortality. To establish the importance of this disease in the indigenous chickens in Kenya a comparative study of natural outbreaks in flocks of layers, broilers and indigenous chickens was done. Thirty nine outbreak farms (5 keeping broilers, 19 keeping layers and 15 keeping indigenous flock) were visited; vaccination history collected, clinical signs observed, flock size and number of dead birds recorded. Diagnosis was done through Gross pathology and Agar gel precipitation tests (AGPT). Haemorrhages in skeletal muscles and destruction of the bursa of Fabricius were seen in all flock types. Bursa of Fabricius presented with haemorrhage, oedema and necrosis. Indigenous chickens had the highest average mortality rate (39.2%), followed by layers (31.1%) and broilers (13.4%) Difference in average mortality rates between layers, broilers and indigenous flocks was, however, not statistically significant (P>0.05). There was also no association between flock type and level of mortality rate (P>0.05). Effective control strategies should be developed to target the three chicken flock types in Kenya.

Key words: Infectious bursal disease, vaccination failure, disease control, chicken production

DES EPIDEMIES DE LA MALADIE DE GUMBORO ENGENDRENT DES TAUX DE MORTALITE ELEVES CHEZ LES POULETS INDIGENES AU KENYA

Résumé

La bursite infectieuse est une maladie d'importance économique qui affecte tous les types de poulet et engendre une mortalité variable. Dans la perspective de déterminer l'importance de cette maladie chez les poulets indigènes au Kenya, une étude comparative des épidémies naturelles chez des troupeaux de poulets de chair, de pondeuses et de poulets indigènes a été réalisée. Trente-neuf fermes affectées par l'épidémie (5 fermes de poulets de chair, 19 fermes de pondeuses et 15 de poulets indigènes) ont été visitées ; l'historique des vaccinations recueillie, les signes cliniques observés, la taille des troupeaux et le nombre d'oiseaux morts enregistrés. Le diagnostic a été établi par l'examen pathologique macroscopique et l'épreuve de précipitation en gélose (AGPT). Des hémorragies des muscles squelettiques et une destruction de la bourse de Fabricius ont été observées chez tous les types de troupeau. Les bourses de Fabricius présentaient des hémorragies, des œdèmes et des nécroses. Les poulets indigènes avaient le taux de mortalité moyen le plus élevé (39,2 %), suivis des poules pondeuses (31,1 %) et des poulets de chair (13,4%). Cependant, la différence au niveau des taux de mortalité moyens entre les pondeuses, les poulets de chair et les troupeaux indigènes n'était pas statistiquement significative (P>0,05). On n'a relevé aucune association entre le type de troupeau et le niveau de mortalité (P>0,05). Des stratégies de contrôle efficaces devraient être élaborées pour cibler les trois types de troupeau de poulets au Kenya.

Mots-clés : Bursite infectieuse ; Echec vaccinal ; Contrôle des maladies ; Production de poulets

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Introduction

Poultry sub-sector positively impacts on economic growth and contributes to poverty reduction in Kenya (King'ori *et al.,* 2010). Annually, the country produces about 20 tones of poultry meat worth 3.5 billion Kenya shillings and 1.3 billion eggs worth 9.7 billion Kenya shillings (Agriculture Sector Development Strategy, 2010). Chickens constitute more than 80% of the poultry (Danda *et al.,* 2010). Over 70% of the 32 million chickens in Kenya are of indigenous type, kept in villages by poor and vulnerable individuals who are mainly women (Bebora *et al.,* 2002). Indigenous chickens contribute 71% of the total egg and poultry meat produced in Kenya (Nyaga, 2008). An increased demand in quality protein in developing countries necessitates great increase in production of poultry (Mengesha and Tsega, 2011). Among a number of reasons that favour increased production of chickens is the relatively small space or land allocation that the enterprise demands as compared to the larger livestock types and crop enterprises (Danda *et al.,* 2010). Commercialization of indigenous chickens is an emerging phenomenon in Kenya which is fast gaining popularity. Not only do the products from these chickens fetch good prices in the market but the meat is also said to be tastier (Mengesha, 2012). One of the main challenges facing farmers in poultry production is diseases. Controlling diseases of chickens especially in indigenous stock will improve the economy of this country. Among diseases that cause huge economic losses to poultry industry in Kenya are viral diseases (Nyaga, 2008; Njagi *et al.,* 2010).

Infectious bursal disease virus (IBDV) the aetiological agent of infectious bursal disease (IBD) commonly known as Gumboro disease was observed more than 40 years ago and is an important threat to commercial poultry industry worldwide (Müller *et al.,* 2003). The first case in Kenya was reported in 1991 in commercial birds at the Kenyan coast (Mbuthia and Karaba, 2000). The subclinical form of the disease is common in chicks less than three weeks of age and it is characterized by severe immune-suppression and lack of clinical signs (Lukert and Saif, 2003). The clinical form of the disease mainly affects 3-6 week old chicks and usually has a sudden onset and a short incubation period. Affected birds manifest prostration, vent pecking, profuse watery diarrhea followed by death or rapid recovery; in severe cases, there is extensive destruction of lymphocytes especially in the bursa of Fabricius. This form is characterized by variable morbidity and mortality rates, both of which can reach up to 100% depending on the pathogenicity of the virus and the susceptibility of the flock (Lukert and Saif, 2011). Heavy mortality, reduction in growth rate and immuno-suppression in affected flocks contribute to economic importance of the disease (Shome *et al.,* 1997). Control strategies of the disease in Kenya target mainly the exotic layers and broilers simply because they are kept for commercial purposes. While natural outbreaks of the disease in indigenous chickens in Kenya and the associated mortality rates have not been documented, the disease does occur in these birds. This paper documents the importance of this disease in indigenous chickens; it compares the mortality rates in natural outbreaks in indigenous chickens, layers and broilers in Kenya.

Materials and Methods

Study design

Infectious bursal disease outbreaks in Kenya in the period between August 2011 and April 2012 were investigated. Permission from the Director of Veterinary Services to utilize departmental staff and institutions to identify these outbreaks was sought and granted. The National Central Veterinary Laboratories, Kabete; Regional Veterinary Investigation laboratories (RVIL) at Mariakani, Ukunda, Nakuru, University of Nairobi Poultry clinic and Nakuru Veterinary Resource Center were engaged to identify outbreaks and collect samples. These institutions were selected based on previous reports of the disease. All the institutions were supplied with sample collection bottles, cool boxes and other sampling materials. Carcasses of chicken submitted to these institutions for diagnosis were opened and full postmortem examination

done (Charlton *et al.,* 2006). History of the outbreak was collected and outbreak farms visited for collection of more fresh carcasses. On postmortem diagnosis of infectious bursal disease, bursa of Fabricius samples were aseptically collected, placed in sterile universal bottles and transported in a cool box to the University of Nairobi virology laboratory. The disease confirmation was done using agar gel precipitation test (AGPT).

Flocks in the study

A total of 39 flocks of chickens with IBD outbreaks were studied. Nineteen of these were flocks of commercial layers, 5 were broiler flocks and 15 were flocks of indigenous chickens. The layers, broilers and 8 of the indigenous flocks were being produced commercially under intensive production system while 7 of the indigenous flocks were under free range extensive system where they were scavenging for food. The flock sizes ranged from 100 to 1100 birds among commercial flocks while in the indigenous free-range birds the flock sizes ranged from 8 to 40 birds. The birds were of mixed ages ranging from 3 weeks to 14 weeks old. Twenty two of the flocks were vaccinated against IBDV by the farmers while seventeen were not.

Farm visits and collection of dead birds

Farm visits to the outbreak farms were made as outbreaks were reported. Clinical signs presented by the sick birds were observed and recorded. For each outbreak, information on when the outbreak started and the number of birds dead was collected. Fresh carcasses were collected for postmortem examination. A follow up phone call was made at the end of the outbreak to establish the total number dead. Laboratory sample submission form was filled to document all the history pertaining to the outbreak. History collected included type of birds affected, number affected, number dead, duration of outbreak, vaccination history and the clinical signs observed.

Postmortem examination and sample collection

Carcasses were opened in the laboratories and postmortem examinations done (Charlton *et al.,* 2006). From each fresh carcass opened the bursa of Fabricius was aseptically collected and placed in sterile universal bottles and chilled. The samples were transported in a cool box to the virology laboratory at the University of Nairobi for AGPT.

Agar gel precipitation test (AGPT)

Bursal tissues were sliced and homogenized into 50% (w/v) suspension in phosphate buffered saline. The homogenate was centrifuged at 2000 rpm for 30 minutes and the supernatant was harvested and tested for viral antigen (OIE, 2008; Chuahan and Roy, 1998). In a layer of agar on a petri-dish, a hexagonal pattern of one central and six peripheral rounded wells 6 mm in diameter 3 mm apart were cut using a template and tubular cutter (OIE, 2008). The well at the center was filled with 50µl of standard antiserum; while the five peripheral wells were filled with test sample(s) [bursal homogenate(s)] alternated with known standard antigen. Results were read by checking for an opaque white line of precipitation between the central well and peripheral wells; this happened in places where homologous antigens and antibodies met in optimal concentration (OIE, 2008). Standardized antigens J.nr. 75.60353 (freeze-dried bursa) and standardized antisera, CUX 52/70 75.60352 used had been supplied for a previous study to RVIL Mariakani by A.M. Bojesen of The Royal Veterinary Agricultural University, Denmark.

Data analysis

Descriptive statistics generated included the mortality rates for each farm and the average mortality rates for the vaccinated and unvaccinated flocks. A t-test was used to determine difference in mortality rates between the flock types. Chi square analysis was used to check for any association between level of mortality rate (20% and above was considered high) and two independent variables; type of flock and vaccination history. A p-value of $P<0.05$ was considered statistically significant (Preacher, 2001).

Figure 1: Ruffled feathers (short arrows) and drowsiness (long arrow) in infectious bursal disease outbreak affected chicks submitted to laboratory

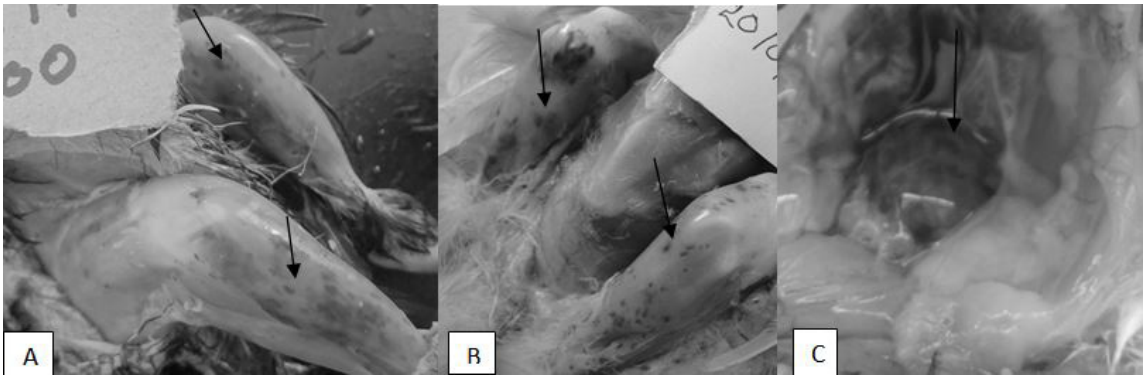

Figure 2: Haemorrhages on thigh muscles of indigenous chicken (black arrows in A), layer (black arrows in B) and haemorrhagic bursa in broiler (black arrow in C) from outbreaks of infectious bursal disease

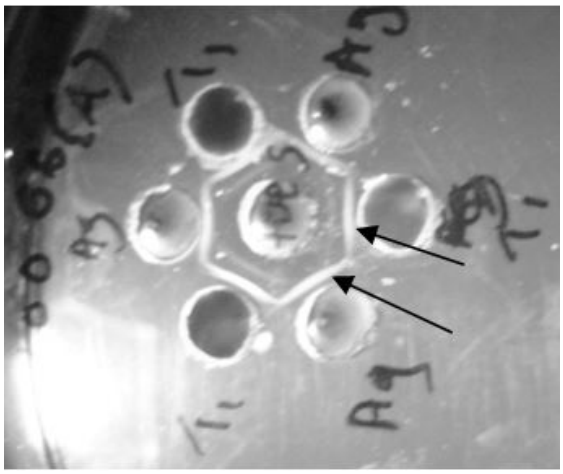

Figure 3: Agar gel precipitation test plate showing precipitation lines (arrow) in positive cases

Results

Clinical signs

In all outbreak flocks, birds showed drowsiness (Figure 1), depression (Figure 2), drooling of saliva, ruffled feathers (Figure 1 and 2), white watery diarrhea, severe prostration and death. The affected birds were from both vaccinated and unvaccinated flocks of layers, broilers and indigenous flocks.

Gross findings at postmortem examination

Lesions observed in the carcasses that were opened, from all three flock types, were typical of IBD infection; haemorrhages were observed on the bursa of Fabricius, thigh and breast muscles (Figure 3). Bursae of Fabricius were either enlarged, due to oedema, and

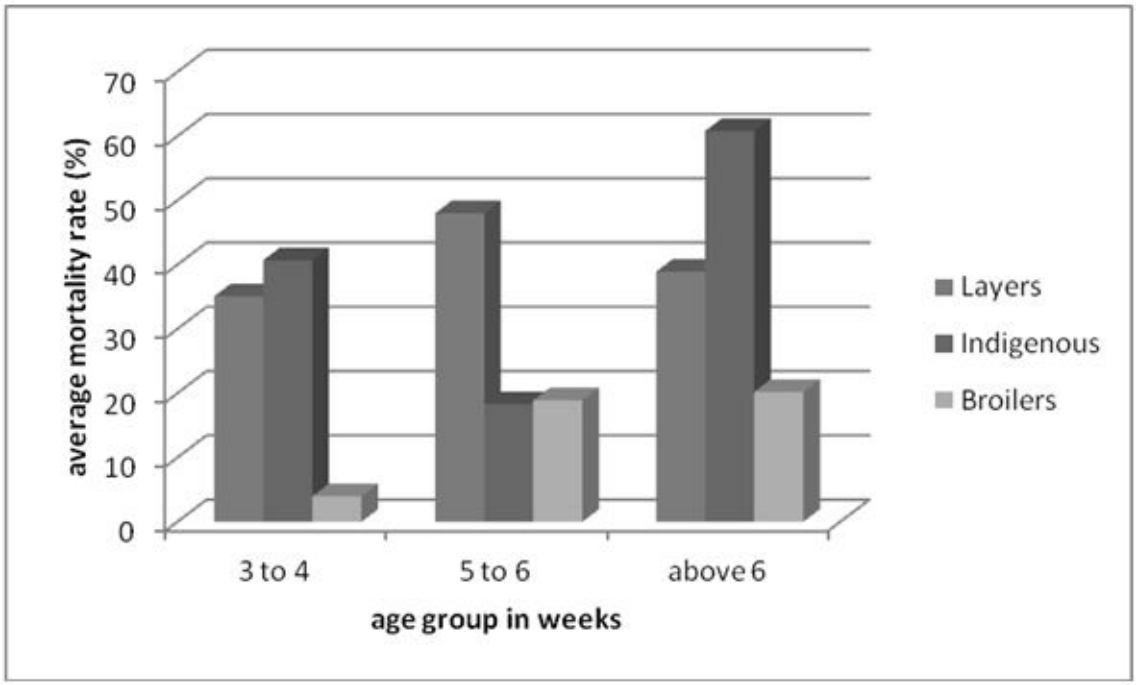

Figure 4: Average mortality rate per age group

hyperaemic; or shrunken, with necrotic debri. Liver and spleen were enlarged. Kidneys were pale and swollen with dilated tubules. Haemorrhages were also seen in proventriculus.

Confirmation of outbreaks

Bursal suspensions prepared from the outbreak cases yielded clear white lines of precipitation in AGPT (Figure 4). This was a confirmation of the presence of IBD viral antigen in the bursae of the birds. Outbreaks in layers broilers and indigenous birds were positive on AGPT.

Mortality rates and history of vaccination

Flocks of indigenous chickens had the highest mortality rates which ranged from 1.3% to 100%, with an average of 39.2%; followed by layers with an average of 31.1%. Mortality rates in layers ranged from 0.67% to 86%. Flocks of broilers had the least mortality rates ranging from 0.4% to 33.3%, with an average of 13.4%. The difference in mortality rates between indigenous flocks and layers was not statistically significant (P=0.5076); between indigenous flocks and broilers was also not statistically significant (P=0.1240). The same was the case for difference between indigenous flocks and exotic flocks (broilers and layers combined) (P=0.309). In this study 73.7% of the outbreaks in layers were in vaccinated flocks. Figure 5 shows carcasses collected from an outbreak in vaccinated layers. In comparison 40% of the outbreaks in broilers and indigenous flocks were in vaccinated flocks. This study established that most of the farmers who vaccinated their indigenous chickens were raising them commercially, through intensive production. Those keeping the birds through the traditional extensive free range system, where chickens were kept for subsistence purposes, did not vaccinate their birds. Outbreaks in vaccinated indigenous flocks recorded the highest average mortality rate (46.98%), followed by unvaccinated indigenous flocks (34.3%), vaccinated layers (32.8%) and unvaccinated layers (26.6%), as shown in Table 1 and Chart 1. Mortality rates recorded in outbreaks in vaccinated broilers were however lower (7.3%) than in unvaccinated ones (17.5%). Vaccination was not associated with low level of mortality rate (less than 20%) in outbreak flocks (P=0.709).

When compared between age groups, indigenous chickens had highest mortality rate in the 3-4 weeks and above 6 weeks age groups.

Highest mortality rate in layers was recorded in the 5-6 weeks old age group (Chart 2). Broiler flocks had the least mortality rate in all the age groups compared to flocks of layers and indigenous chickens. In broilers, the mortality rate increased with age. In this study, level of mortality rate (high or low) was not associated with any flock type (P>0.05). Vaccination was also not associated with low level of mortality (P=0.709)

Discussion

Generally, indigenous chickens are said to be hardy and resistant to many diseases as compared to exotic ones (Kityali, 1998). This contrasts observations made in this study. Infectious bursal disease (IBD) outbreaks caused high mortality in indigenous flocks in Kenya. Though mortality rates ranged from low to high in outbreaks in indigenous as well as in the exotic chickens, the highest average mortality rate was in outbreaks in indigenous flocks. Genetic differences in susceptibility of chicken lines to infection with IBDV have been documented to occur (Bumstead *et al.,* 1993). Earlier researchers found that the disease caused higher mortality rates in lighter breeds than in heavy breeds (Bumstead *et al.,* 1993; Lukert and Saif 1991; Abdul, 2004), while certain lines of Brown and White Leghorns have been shown to be highly susceptible to IBDV infection (Bumstead *et al.,* 1993). This is similar to a study done in Nigeria, where local Nigerian chickens were found to be more susceptible to IBDV than an exotic breed (Okoye and Aba-Adulugba, 1998). Variation in mortality rates was also demonstrated in 5 breeds of Egyptian chicken (Hassan *et al.,* 2002). In his study, mortality in native breeds varied from 11% to 85.3% compared with 20% in the White Leghorns.

Severity of IBD outbreak depends on the virulence of the virus and the susceptibility of the chicken among other factors. In another study higher mortality was observed in Fayaomi than in white leghorn chicks infected with IBD (Chakraborty *et al.,* 2010). After finding variable susceptibility to very virulent IBDV (vvIBDV) among various Egyptian chickens it was suggested that innate non immunogenic factors may play a critical role in resistance (Hassan *et al.,* 2002). Highly pathogenic strains of IBDV were reported to cause high mortalities exceeding 90% in highly susceptible chicken flocks in Japan (Nunoya *et al.,* 1992). This compares with findings of this study, where some outbreaks recorded a mortality rate of 100% in indigenous flocks. This finding suggests that some Kenyan indigenous flocks are highly susceptible to the disease. In contrast indigenous chickens and cross breeds in Nigeria were found susceptible to IBD but the mortality rates between the two, though not different, was significantly lower than in the exotic chickens (Okoye *et al.,* 1999; Oluwayelu *et al.,* 2002). In Baglandesh IBD was reported in local sonali and fayoumi chicks reared under semi scavenging system and in indigenous chicks under confinement (Biswas *et al.,* 2005; Chakraborty *et al.,* 2010).

Other studies found a high sero-prevalence of IBDV (over 40%) in indigenous chickens in Kenya and suggested it to be one of the major causes of chick mortality in Newcastle disease vaccinated chicken flocks (Ndanyi, 2005). Indigenous village chickens are normally not immunized against IBDV. The chicks are highly susceptible since they do not have maternal antibodies. This explains why there was high mortality rates recorded in the 3-4 weeks old age group in indigenous flocks. Survivors gained immunity; hence the low mortality rates in 5-6 weeks old age group. The observation made in this study, that as the chicks grew older they seemed to lose respective immunity, could be because there was no vaccination done in the indigenous flocks. This increased susceptibility is shown by the fact that there was high mortality rate in the above 6weeks old age group. Outbreaks in young indigenous chicks are not usually reported to the Veterinary department. Failure to suspect IBD coupled with the assumption that indigenous chicken are hardy and resistant to many diseases as compared to exotic ones (Kityali, 1998) could be the reason why few farmers vaccinate their indigenous chickens against IBD. However outbreaks occur even in vaccinated flocks (Yahia *et al.,* 2008; Islam *et al.,* 2005; Mutinda, 2011) including vaccinated indigenous flocks as found in this study. It is,

thus, important that effective control strategies should be developed for all types of chickens; the exotic commercial flocks, indigenous commercial and free range flocks in Kenya.

Conclusion

Infectious bursal disease is a disease of economic importance in indigenous and exotic chickens in Kenya. When mortality rate is used as a measure of severity, indigenous chickens in Kenya have been shown to be as severely affected as the exotic chickens. Infectious bursal disease vaccination failures seen in exotic chickens are also experienced in indigenous chickens. Control strategies should, therefore, also target the indigenous, as they target the exotic chickens.

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IMMUNOGENIC RESPONSE OF RABBITS TO MONOVALENT AND POLYVALENT ANTISERA OF MANNHAEMIA HAEMOLYTICA BIOTYPE A

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Abstract

This work was carried out in University of Surrey UK Department of Microbiology. In this study, the efficacy of monovalent and polyvalent vaccines made from Mannhaemia haemolytica antigens, were evaluated by measuring specific serum antibody titers produced against the bacteria in immunized rabbits. Eleven biotype A strains of Mannhaemia haemolytica were used for raising polyvalent antisera in rabbits. Monovalent antisera were produced against the strains A1, A2, A6, and A9. Different routes of inoculations were applied for injecting rabbits with multivaccine; separate strains in different sites subcutaneously, mixed strains in different sides subcutaneously, mixed strains at one site subcutaneously and mixed strains intravenously. Slide agglutination and indirect haemagglutination tests were used for detecting and measuring specific antibodies against the strains used. Antisera against polyvalent immunogens protected 83-100% of rabbits against A1, A7 and 50% of rabbits against A2 challenge while the lowest protection (16-33%) was seen in serotype A6.

The results showed that the indirect haemagglutination test was more sensitive than the somatic agglutination tests and intravenous injection produced higher antibodiesresponses when compared to others routes of injection.

key words: Mannhaemia, rabbits, immunization, indirect haemagglutination, immunization.

RÉPONSE IMMUNOGÈNE DES LAPINS AUX ANTISÉRUMS MONOVALENTS ET POLYVALENTS DE MANNHAEMIA HAEMOLYTICA BIOTYPE A

Résumé

Ce travail a été réalisé par le Département de microbiologie de l'Université de Surrey au Royaume-Uni. Dans cette étude, l'efficacité des vaccins polyvalents et monovalents à base d'antigènes de Mannhaemia haemolytica a été évaluée en mesurant les titres d'anticorps sériques spécifiques produits contre les bactéries chez des lapins immunisés. Onze souches de Mannhaemia haemolytica biotype A ont été utilisées pour produire des antisérums polyvalents chez les lapins. Des antisérums monovalents ont été produits contre les souches A1, A2, A6, et A9.

Différentes voies d'inoculation ont été utilisées pour injecter le multi-vaccin chez les lapins: souches distinctes dans différents sites par voie sous-cutanée ; souches mixtes dans différents sites par voie sous-cutanée ; souches mixtes dans un site par voie sous-cutanée ; et souches mixtes par voie intraveineuse. Les tests d'agglutination sur lame et d'hémagglutination indirecte ont été utilisés pour la détection et la mesure d'anticorps spécifiques contre les souches utilisées. Les antisérums produits contre les immunogènes polyvalents ont protégé 83 à 100 % des lapins contre A1 et A7 et 50% des lapins contre A2, tandis que la plus faible protection (16-33 %) a été observée pour le sérotype A6.

Les résultats ont montré que le test d'hémagglutination indirecte était plus sensible que les tests d'agglutination somatique, et que l'injection intraveineuse a produit des réponses défensives plus élevées par rapport aux autres voies d'injection.

Mots-clés : Mannhaemia ; Lapins ; Immunisation ; Hémagglutination indirecte ; Immunisation

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Introduction

Pasteurella group consist of pleomorphic Gram – negative bacteria, occur in cocco-bacilli form and sometimes in short rods. Capsules of varying degrees are not uncommon specially in virulent strains of Mannhaemia (M.) haemolytica and Pasteurella multocida are associated with pneumonia in sheep and cattle with pathological damage of the ovine respiratory tract. They are the most important respiratory pathogens affecting domestic ruminants, especially in sheep and cattle, together causing fibrinous and necrotic Pneumonia. This disease, commonly called "shipping fever" and is a leading cause of] economical losses in sheep industries. Factors such as transportation, viral infection, and overcrowded housing, may predispose the opportunistic pathogens to induce the infection. M. haemolytica is divided into two biotypes based on carbohydrate fermentation patterns and 21 serotypes based on surface antigens (Diker *et al.,* 2006).All serotypes can be involved in diseases however; there is a wide variation in the prevalence of individual serotypes isolated from pneumonic pasteurellosis (Lesley *et al.,* 1985). The organism possesses several components that may function as virulence factors. For most among these is leukotoxin which is secreted during the logarithmic growth phase and is lethal to ovine leukocytes and lymphocytes (Akan *et al.,* 2006; Mohammed and Abdelsalam, 2008). The capsular polysaccharide is another virulence factor, impairs the ability of neutrophils to ingest and kill the microganisms. In addition, the bacterium has numerous potential immunogens, such as LPS, OMPs, and fimbriae (Ulrike *et al.,* 2005)Vaccine development for the prevention of pneumonic pasteurellosis remains a critical issue for the feedlot industry. The assessment of Pasteurella vaccines by field trails is not successful due to the sporadic nature of the disease, and in many studies that have been conducted, a poor immune response to the serotype A2, which is the main serotyped involved in pneumonic pasteurellosis, was achieved (Gilmour and Angen, 1983.;Adlam, 1989; Zamri – Saed *et al.,* 1994).Akan et al. (2006) reported that the One Shot Ultra 8 vaccine in sheep induced specific antibody production against M. haemolytica using ELISA tests, with a higher specific antibody titers in vaccinated groups than in the control ones. Cassirer et al. (2001) tested whether a combination of an experimental P. trehalosi and M. haemolytica and a commercially available bovine P. multocida and M. haemolytica vaccine would increase lamb survival following a pneumonia epidemic. They found that lamb survival differed among flocks ranging from 22% to 100% and antibody titers were higher in ewes prior to vaccination, and the vaccines failed to enhance antibody titers in treated ewes. McVicker et al. (2002) reported that the development of an effective vaccine could be possible and based on the fact that animals naturally infected did develop resistance to subsequent infection. Most currently available Pasteurella vaccines are formulated to stimulate immunity by either providing an adequate antigenic mass in the administered dose or by relying on subsequent production of antigens by in vivo growth of live or killed vaccine. Researchers have found that vaccines provide a good protection against homologous strains while they were insufficiently protective against heterologus ones (Chasdrassekran *et al.,* 1991; Diker *et al.,* 2000). At present, several commercial vaccines have beendeveloped to control pasteurellosis in sheep, including bacterins, live attenuated, leukotoxin, capsule, lipopolysaccaride and subunit vaccines, sodium salicilate and Potassium thiocyanate extract. The following vaccines for Pasteurella are now commercially available and they are used worldwide : Ovipast, Hepatovax, Polyserum vaccine, Ovine Pasteurella (USA), Onderstepoort Pasteurella (SA) , Ovivac –P- plus (Intervet UK Ltd 2005a ;Intervet Ltd. 2005b; Intervet South Africa, 2005).The objectives of the present a study were to evaluate the immunogenic response of rabbits to mixed vaccines of M. haemolytica with different combinations of serotypes and to assess the relationship between the routes of immunization and the immune responses.

Materials and methods

Strains used:

M. haemolytica biotypes A1 (NCTC=10609)

A2 (NCTC=101365), A5, A6

(NCTC=10632), A7, A8, A9 (NCTC=10638) and A11, A12, A13 and A14 strains used in this study are field strains obtained from pneumonic sheep lungs. Isolates were identified and biotypes according to their phenotypic characteristics by an indirect haemagglutination test according to Biberstein et al, (1960) and Carter (1984).

Vaccines production:

Mucoid colonies of 11 biotypes A strains of Mannhaemia haemolytica were inoculated individually in 5 ml brain heart infusion broth. The cultures were incubated at 37°C for 18 hours. Formalin was added to each culture to give a final concentration of 0.3% to obtain formalin – killed vaccines.

Immunization of rabbits and collection of the antisera:

Experiment (1):

Four New Zealand white rabbits were used for production of monovalent antisera and vaccinated as: rabbit No.1: with strain A1, rabbit No.2: with strain A2, rabbit No.3: with strain A6, and rabbit No.4: was with strain A9. Before vaccination sera were collected from each rabbit for use as serum control in serological tests. 0.5 ml of formalin-Killed vaccine of each strain was injected subcutaneously, then 1.0, 2.0, 3.0 and 3.0 ml intravenously at 3 days intervals.

Experiment (2):

Different strains and routes of injection were applied for production of polyvalent antisera in the same group of rabbits after 4 weeks: Rabbit No.1: was injected subcutaneously (s/c) with 0.5 ml of formalin-killed vaccine. A1 in left shoulder, A2 in right shoulder, A5 in left flank, A6 in right flank, A7 in left flank and A8 right thigh s/c. Rabbit No. 2: Equal quantities of 0.5 ml formalin-killed cultures of the strains A1, A2, A5, A9, A11 and A12 were mixed together thoroughly and injected into each site as described for rabbit No.1. Rabbit No. 3: formalin-killed cultures of strains A1, A2, A6, A7, A9, A11, A13 and A14 were mixed together. 3 ml of the mixture was injected subcutaneously. Rabbit No. 4: the method and the strains used was the same as rabbit No. 3 but the route of injection was intravenously. 3 ml of antisera was collected from individual animals at week 4, 5, 6, and 7 post vaccination and 25 ml after the last injection. The collected antisera were stored at -20°C.

Serological tests:

Rapid slide agglutination and the indirect haemagglutination tests were used for measuring the antibody titres in the harvested monovalent and polyvalent antisera.

Rapid slide agglutination test:

The rapid slide agglutination test was carried out according to Mackie and McCartney, (1996). A drop of undiluted antiserum of M. haemolytica (50 µl) was placed on a clean glass surface, and then a small amount of M. haemolytica colony from blood agar plates was picked up by an inoculating needle and mixed with the serum. A strong positive reaction in the form of clumping and clearing occurred as the mixture was stirred with the needle. In experiment (1) antisera collected at day 6 after the last injection, while in experiment (2) antisera collected at 8 week post vaccinations. Antiserum from each rabbit was tested against the 11 biotype A strains at dilutions1, 1/5th and 1/25th of the sera were made in 0.85% Nacl. Titers were expressed as the reciprocal of the highest serum dilution at which positive agglutination occurred. Clumping of the mixture within 60 seconds indicated positive agglutination. Control negative test was done by adding saline solution to the bacterial suspension.

Indirect haemagglutination test:

The antibody titres for the monovalent and polyvalent antisera were determined by the modified indirect haemagglutination test as described by Takouswada et al. (1982). Antisera were collected at week 4, 5, 6, 7 and 8 post vaccinations. The heat extracted antigens were prepared by culturing representative strains onto dextrose starch agar and incubated overnight at 37°C in moist chamber. Then each growth was harvested in 1ml of 0.02M phosphate buffer saline (pH 7.0) and placed

into 1.5µL plastic microcentrifuge tubes. The harvested cultures were heated at 100oC for one hour in an oven then cells were pelleted by centrifugation at 10.000X g for 20 minutes. The supernatant fluid was assigned as heat-extracted antigens. Glutraldehyde-fixed sheep blood cells (GA-SRBCS) were sensitized with heat extracted antigens by mixing of 1 ml of 10% suspension of GA-SRBCS of each strain. The mixture was incubated for one hour and shaken at each 15 minutes interval. The sensitized SRBCS were washed three times by centrifugation and then suspended in PBS containing 0.1% sodium azide and 0.25% bovine serum albumin to yield a final concentration of 0.5% of SRBCS (vol./vol.). The optimal dilutions of the antigens used were determined by box titration against homologous rabbit antiserum and the titres were expressed as the reciprocal of the highest dilution of antiserum showing positive reading (flat uniform sediment of the RBCs compared to negative controls (dense dot in the centre of the well).

Results

In rapid slide agglutination test, positive reactions were found in all individual rabbits antisera and the degree of positivity varied from moderate to more pronounced (Fig.1). No reaction was seen in serum collected before vaccination. All the tested antisera showed positive reactions in the indirect haemagglutination test, the antibody titres found from 20 to 320. Due to this low antibody titres, 3 additional doses of M. haemolytica live vaccine were given to each rabbit.

Antisera were collected 6 days after the injection of the final dose. For polyvalent vaccines, rapid slide agglutination test was used at dilution 1, 1/5th and 1/25th. Strong reactions were observed in the antiserum of the rabbit injected intravenously. Cross reactions were seen in strain A7 and A 12 when tested against antiserum collected from rabbit No.1 and rabbit No.2 respectively. The antibody titres obtained from rabbits injected with polyvalent vaccines in different routes, were failed to produce sufficient antibodies against corresponding strains in the vaccine when tested with indirect haemagglutination test. Intravenous immunization produced slightly higher antibody titres when compared to the other rabbits injected subcutaneously in different sites. A9 strain produced higher antibodies (2048), while strain A2 as expected produced the lowest titre (32). A1 and A6 strains produced titres of 512 and 1024 respectively. No agglutinations were seen on either saline or serum controls (Table 1 and 2).

Discussion

M. haemolytica is the organism most commonly associated with ovine pneumonia worldwide and various serotypes may involve in the disease (Confer, 1993). The organism continues to be an important pathogen in sheep. Vaccine development for the prevention of pneumonic pasteurellosis remains a critical issue for sheep industry. Despite a number of live and killed vaccines have been developed and used for control of pneumonic pasteurellosis in sheep, their efficacy in field trials have been variable (Jones *et al.,* 1986).

Direct bacterial agglutination for measuring the antibody titres was generally found practicable for monitoring the overall antibody response of the rabbits and not as laborious as indirect haemagglutination tests. In the recent study differences were observed in the immunogenicity of whole cell preparations of M. haemolytica serotypes used and relatively low antibody titers against biotype A2 was observed which was in agreements with previous report of Jones *et al.,* (1986)who found that A2 serotype was poorly immunogenic in lambs, mice and rabbits. The antibody titres recorded in the monovalent antisera ranged from 4 to 64 compared to the indirect haemagglutination titres which ranged from 32 to 2048. Slide agglutination results of the polyvalent antisera suggested that the test was also less sensitive than the indirect haemagglutination test and this is in agreement with the findings of Frank and Wessman (1978). The indirect haemagglutination tests are generally found to be more sensitive than the somatic agglutination. It was observed that all rabbits responded well to the strains in the individual vaccines, although variation in the antibody titres was found. the study showed there is no

Table 1: Slide agglutination test

Rabbit no.	Strains in the vaccine	Concentration of the antisera	Strains tested										
			1	2	5	6	7	8	9	11	12	13	14
1	1, 2, 5,	1	++++	+	++	+++	++	+	-	-	(+)	-	-
	6, 7,	1/5th	+++	-	+	++	-	-	-	-	(+)	-	-
		1/25th	++	-	+	-	-	-	-	-	-	-	-
2	1, 2, 5, 9, 11, 12	1	+++	+	+++	-	(+)	-	++	+++	+++	-	-
2		1/5th	+++	+	+++	-	-	-	+	++	++	-	-
2		1/25th	+	-	+	-	-	-	-	-	+	-	-
3	1, 2, 6, 7, 9, 11, 13, 14	1	+++	-	++	+++	++	-	-	-	-	+++	-
3		1/5th	++	-	++	++	+	-	-	-	-	++	-
3		1/25th	-	-	-	-	-	-	-	-	-	-	-
4	1, 5, 6, 7, 9, 12, 13	1	+++	-	++++	++	-	+++	++	-	+++	+++	-
4		1/5th	++	-	+++	++	-	++	+	-	+++	++	-
4		1/25th	+	-	++	+	-	+	-	-	++		

Table 2: shows the antibody titres of the four rabbits measured by the indirect haemagglutination test

Rabbit No.		Tested strains										
		1	2	5	6	7	8	9	11	12	13	14
1		+	+	+	+	+	+	-	-	-	-	-
	Reaction:	16	32	8	8	64	32	0	0	0	0	0
2	Titre:	+	+	+	-	(+)	-	+	+	+	-	-
		2	16	8	0	(4)	0	16	64	64	0	0
3		+	+	-	+	+	-	+	+	-	+	+
		2	16	0	16	64	0	8	32	0	64	32
4		+	-	+-	+	-	+	+	-	+	+	-
		32	0	32	16	0	64	32	0	64	128	0

significant differences in the immune response of the rabbits inoculated subcutaneously, but intravenous injection resulted in higher antibody titres than the subcutaneous routes, but other investigators (Wiki and Norris ,1976) reported that the appearance of Mannhaemia haemolytica antibodies in the serum of rabbit is independent of the routes of immunization and this finding was in disagreements with our findings in that intravenous immunization gave rise to higher antibody titres in immunized rabbit. Subramanian et al, (2011) evaluated the effect of repeated immunization with a multivalent Mannheimia and Bibersteinia cultures supernatant vaccine on protection of big horn sheep (BHS) against experimental M. haemolytica challenge and reported that if BHS can be induced to develop high titers of Lkt-neutralizing antibodies and antibodies to surface antigens, they are likely to survive M.

haemolytica challenge which is likely to reduce BHS population decline due to pneumonia. Feasibility of producing polyvalent antisera and their use for vaccine serotyping of Mannhaemia haemolytica was found applicable, and further studies are needed for production of antisera with sufficient levels of antibodies for serotyping of the bacterium.

Impact

In this study, Slide agglutination and indirect haemagglutination tests were used for detecting and measuring of the specific antibodies against different combination of strains of Mannhaemia haemolytica. The results showed that the indirect haemagglutination test was more sensitive than the somatic agglutination tests. Moreover, the monovalent vaccines produced higher immune responses that the multivalent vaccines. Similarly intravenous injection produced higher antibodies responses when compared to others routes of injection. Particular attention would be directed towards correct representation of the trains in the multivaccine as well as vaccination schedules and number of strains in each vaccine for production of a vaccine with high potency and wide range of coverage for control of animal pasteurellosis.

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EFFECTS OF MOLASSES AND STORAGE PERIOD ON THE CHEMICAL, MICROBIAL AND FERMENTATION CHARACTERISTICS OF GUINEA GRASS - CASSAVA LEAVES SILAGE

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Abstract

The study was conducted to determine the effects of molasses and storage periods on the chemical composition, microbial and fermentation characteristics of silage produced from guinea grass and cassava leaves mixture. Guinea grass was harvested at 2 months regrowth from an established pasture and cassava tops collected immediately after root harvest consisting of only the tops with the green stem and its leaf canopy. The forages were wilted and thoroughly mixed in the ratio of 70:30 (guinea grass: cassava leaves); mixed with molasses at the rate of 0, 2, 4 and 6 % and ensiled for 30 and 60 days respectively for fermentation. The DM, CP, NDF, HCN and tannin contents of the ensiled guinea grass and cassava leaves significantly reduced as the level of molasses addition and storage period increased. The acetic acid fermentation was high in the 0, 2, 4 and 6% molasses addition with values ranging from 40.2 to 42.4 g/kg DM while the lactic acid content was only 18.4 to 30.3 g/kg DM at the 0% molasses addition. However, lactic acid fermentation increased significantly as both the storage periods and molasses addition increased from 0 to 60 days. The highest bacterial count (8.4 log cfu/g) was recorded at the 0% molasses addition and this significantly reduced as molasses addition increased from 0 to 6% and as the ensiling periods elongate from 0 to 60 days. However, a sharp decline in fungi count was observed with increase in the percentage of molasses added. The bacteria isolated from the silage were Staphylococcus aureus, Streptococcus spp., Enterobacter spp., Proteus spp., Escherichia coli and Lactobacillus buchneri. It is concluded that ensiling mixture of guinea grass and cassava leaves with molasses increased lactic acid and suppressed the production of acetic and butyric acids with drastic reduction in microbial load.

Keywords: Guinea grass, cassava, storage period, molasses, fermentation, chemical composition

EFFETS DE LA MELASSE ET DES PERIODES DE STOCKAGE SUR LES CARACTERISTIQUES CHIMIQUES, MICROBIENNES ET DE FERMENTATION DE L'ENSILAGE DE L'HERBE DE GUINEE / FEUILLES DE MANIOC

Résumé

La présente étude a été menée dans le but de déterminer les effets de la mélasse et des périodes de stockage sur la composition chimique, les caractéristiques microbiennes et de fermentation de l'ensilage produit à partir d'un mélange d'herbe de Guinée et de feuilles de manioc. L'herbe de Guinée a été récoltée à 2 mois de repousse dans un pâturage établi ; et les cimes de manioc ont été recueillies immédiatement après la récolte des tubercules, et étaient composées seulement de pseudo-troncs verts et de leurs nappes foliaires. Les fourrages ont été flétris et soigneusement mélangés dans la proportion de 70:30 (herbe

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de Guinée : feuilles de manioc) ; mélangés ensuite avec de la mélasse au taux de 0, 2, 4 et 6 % et ensilés respectivement pendant 30 et 60 jours pour la fermentation. La teneur en MS, en PB, en FDN, en HCN et en tanin de l'ensilage herbe de Guinée/ feuilles de manioc a baissé considérablement parallèlement à l'augmentation du niveau de mélasse et de la période de stockage. La fermentation de l'acide acétique était élevée dans l'ajout de de mélasse à 0,2, 4 et 6 %, les valeurs allant de 40,2 à 42,4 g / kg de matière sèche tandis que la teneur en acide lactique variait seulement entre 18,4 et 30,3 g / kg de MS à une addition de mélasse à 0%. Cependant, la fermentation de l'acide lactique a augmenté de manière significative parallèlement à l'extension des périodes de stockage de 0 à 60 jours et à l'adjonction accrue de mélasse. La numération bactérienne la plus élevée (8,4 log cfu/g) a été enregistrée à l'adjonction de mélasse à 0%, et elle a baissé de manière significative avec l'augmentation de la mélasse de 0 à 6 % - et avec l'extension des périodes d'ensilage de 0 à 60 jours. Cependant, une forte baisse de la numération fongique a été observée parallèlement à l'augmentation du pourcentage de mélasse ajoutée. Les bactéries isolées dans l'ensilage étaient Staphylococcus aureus, Streptococcus spp, Enterobacter spp, Proteus spp, Escherichia coli et Lactobacillus buchneri. Il est conclu que le mélange ensilé d'herbe de Guinée et de feuilles de manioc avec adjonction de mélasse a augmenté l'acide lactique et supprimé la production des acides acétique et butyrique, avec une réduction drastique de la charge microbienne.

Mots-clés : Herbe de Guinée ; Manioc ; Période de stockage ; Mélasse ; Fermentation ; Composition chimique

Introduction

Preservation of herbage in the form of silage is a useful strategy for livestock farming, particularly in the tropics and in an environment where forage availability is seasonal. Ensiling enables the storage of herbage until feeding with minimal nutrient loss or significant changes in its chemical composition (McDonald et al. 2002). Although, ensiling is not commonly practiced in the tropics, increasing attempts are being made to develop a suitable process for tropical grass ensiling (Ohmomo et al. 2002). Tropical grasses are commonly used for silage with reports of low content of dry matter and low concentrations of water soluble carbohydrates (WSC) and crude protein (CP) (Mtengeti *et al.,* 2006; Zanine *et al.,* 2006). The composition of the herbage makes it difficult to produce high quality silage without the use of additives. The addition of molasses as a source of water solube carbohydrates (WSC) has been widely used to promote a low pH and higher proportions of lactate in such silages (Melotti, 2004: Mtengetti, *et al.,* 2006). Monitoring of microbial community in silage, for which traditional culturing methods are routinely used, can provide substantial information about the dynamics of the ensiling process (Ennahar et al. 2003). Rodriguez et al. (1989) found an initial prevalence of lactic acid in guinea grass silage, but also found that, after several weeks, the level of lactic acid decreased while the level of acetic acid increased. This shift from lactic acid to acetic acid fermentation was retarded when crops were wilted to 300-400 g dry matter (DM) kg-1, suggesting that the assessment of ensiling tropical grass could be influenced by DM and storage period (Nishino et al. 2011).

The need for higher quality silages requires new strategy by the introduction of unconventional protein rich leaves and forage species. Tree foliage can be used to produce silage with higher CP concentrations and thus be an avenue to replace the feeding of conventional concentrates (Cardenas et al. 2003). Although, there are many tree foliage and crop by-products that are rich in protein, one of these is cassava which is considered as one of the world's most important economic crops of the millennium. It is increasingly growing in importance as the main source of sustenance both in the domestic and industrial circles. On the world scale, Nigeria is the largest producer of this commodity (FAOSTAT, 2007) with an estimated annual production of over 34 million tonnes. Invariably, this scale of production suggests that large amount of by-products will be available following the industrial processing of cassava into various products, with an attendant problem of disposal of the by-products and a disturbing repercussion on the environment. Cassava leaves, a by-product of cassava root harvest has high protein content (16.7 - 39.90%) (Yousuf et al. 2007)

with almost 85% of the crude protein as true protein. This is due to the presence of tannin which forms tannin-protein complex that by pass the rumen (Wanapat et al. 1997). Cassava leaves is also rich in minerals, vitamins B1, B2, C and carotenes (Adewusi and Bradbury, 1993). Cassava leaves have been used as a protein source when collected at the time of root harvesting (Oni et al. 2010). The addition of cassava leaves increases the nitrogen content of the silage and can promote the growth of fermentative microbes in N-deficient feeds. The objective of this experiment is to determine the effect on chemical composition and fermentation characteristics when cassava leaves is introduced for ensiling in mixtures with components of guinea grass and molasses. The hypothesis tested was that the introduction of cassava leaves, as a component of silage to improve the nutritional value of silages as supplementary feed in the tropics, will produce silages with chemical composition and fermentation characteristics comparable to traditional silages based on guinea grass and molasses as an additive.

Materials and Methods

Ensiling

The guinea grass was harvested at 2 months regrowth from the established pasture of the Pasture and Range Management Department (University of Agriculture, Abeokuta, Nigeria) Experimental Farm. Cassava tops were collected right after root harvesting consisting of only the tops with the green stem and its leaf canopy with an average of 40 to 60cm length. The harvested cassava leaves and guinea grass were allowed to wilt for a minimum of 12 hours spread under a shed. The forages were chopped into pieces of about 3 – 4cm in length with a mechanical chopper and thoroughly mixed in the ratio 70:30 (guinea grass: cassava leaves). The wilted and chopped forages were mixed with molasses without water dilution at the rate of 0, 2, 4 and 6 % of wilted materials, placed in polyethylene bags, inserted into plastic containers with 150 cm of height, 60 cm diameter and capacity to take about 200 kg of forage materials, compacted by hand and human treading, bound with a string, and pressed by placing one 5 kg bag of sand on each bag and stored for 30 and 60 days respectively for fermentation. The two plant species (guinea grass: cassava leaves) was allocated in a 2 x 4 factorial arrangement in a completely randomized design with four replicates. Before mixing the fresh and chopped tops with molasses, four samples from each species were collected for chemical analyses.

Chemical analyses

Dry matter content was determined by drying the forages and silages in an oven at 600C for 48 h. Dried samples were ground to pass through a 1-mm screen using a hammer mill and the milled samples were used to determine crude protein, neutral detergent fibre, acid detergent fibre and acid detergent lignin according to AOAC. (2000). Tannin was measured as condensed tannin using the butanol–HCl assay (Porter et al. 1986) while hydrocyanic acid (HCN) content of the silage samples was determined by the alkaline titration method (AOAC, 1995). Water soluble carbohydrates (WSC) were extracted with 800 ml 1-1 (v/v) ethanol, and the contents determined by the phenol-sulphuric acid method (Parvin et al. 2010). Silage pH was determined with JENWAY pH meter, Model 3150. Concentrations of lactic acid, acetic acid, butyric acid and propionic acid were determined by High Performance Liquid Chromatography with 60 mL silage fluid. The samples were centrifuged for 10 min at a temperature of approximately 400C to prevent loss of volatiles.

Microbiological analysis

Media

Sabouraud Dextrose agar (SDA) and Nutrient agar was used for the enumeration of mold and bacteria respectively. The Media used were prepared according to the manufacturer's instructions and autoclaved at 121oC for 15 minutes. Lactic acid bacteria were isolated on De Man Rogosa Sharpe (MRS) agar.

Isolation and enumeration of microorganisms

One gram of the sample was homogenized in 9 mls of water. Tenfold dilution

was made and aliquots of the appropriate dilutions were plated on duplicate plates using the pour plate method. The molds were plated on SDA containing 50mg/L chloramphenicol and 50mg/L chlortetracycline to inhibit bacterial growth. Incubation was at 250C for 2 to 4 days. The total aerobic viable count was done with nutrient agar and incubated at 370C for 48 hours. For LAB enumeration, samples were plated on MRS agar incubated at 370C for 48hrs under anaerobic conditions in an aerobic jar. All colonies appearing at the end of the incubation period were counted using digital illuminated colony counter and the counts were expressed in colony forming unit per gram (cfu/g) of the sample.

Identification of isolates

Representative colonies were picked from plates used for viable counts. Isolates were purified by repeated streaking on appropriate media. When pure cultures were obtained, the pure cultures were sub-cultured into agar slant containing the appropriate medium. The pure

Table 1. Dry matter (DM) content and concentrations of crude protein, fibre fractions, water soluble carbohydrates (WSC), tannin and hydrocyanide of ensiled feeds

	Feeds		
Parameters	**Molasses**	**Cassava leaves**	**Guinea grass**
DM content (g/kg)	681	328	228
Concentration (g/kg DM) of:			
CP	58	286	96
NDF	ND	573	682
ADL	ND	238	78
ADF	ND	365	374
WSC	423	56	52
Tannin	ND	84	2.4
HCN (mg/kg)	ND	635	-

CP= Crude protein; NDF= Neutral detergent fibre; ADL= Acid detergent lignin; ADF= Acid detergent fibre; WSC= Water soluble carbohydrate; HCN= Hydrocyanide; ND = Not determined

Table 2. Effects of storage periods and molasses addition on the chemical composition of mixture of guinea grass and cassava leaves silage

Molasses Addition (%)	**Storage periods (days)**	**DM**	**CP**	**NDF (g/kg)**	**ADF**	**HCN**	**Tannin (%)**
0	30	243.57	282.50	555.42	344.97	0.26	6.24
	60	231.61	274.57	522.60	348.70	0.21	5.25
2	30	231.50	265.00	508.20	357.57	0.24	4.66
	60	222.97	258.60	497.33	363.67	0.22	4.20
4	30	221.50	242.50	494.66	364.67	0.26	3.83
	60	205.20	233.40	480.00	372.10	0.26	4.49
6	30	220.57	242.50	478.38	371.00	0.26	3.25
	60	197.15	204.00	474.21	375.37	0.23	3.70
SEM		7.54	7.69	6.21	6.05	0.14	1.06
Level of Significance							
Molasses (M)		*	**	**	**	NS	NS
storage period (S)		*	*	*	*	NS	*
M x S		*	*	*	*	NS	NS

DM= Dry matter; CP= Crude protein; NDF= Neutral detergent fibre; ADF= Acid detergent fibre; HCN= Hydrocyanide

Table 3. Effects of storage period and molasses addition on fermentation products of mixture of guinea grass and cassava leaves silage

Molasses addition (%)	Storage periods (days)	pH	LA	AA	PA	L/A	BA	NH_3
0	30	4.84	26.88	38.44	3.63	0.70	10.38	3.72
	60	4.71	18.38	38.44	4.16	0.48	13.35	3.17
2	30	4.52	77.04	38.52	0.02	2.00	0.17	3.35
	60	4.41	76.96	47.48	0.05	1.62	0.20	2.69
4	30	4.29	76.30	31.15	1.26	2.45	0.09	2.50
	60	4.29	75.20	43.60	2.62	1.73	0.57	3.30
6	30	4.21	75.52	37.76	1.61	2.00	0.00	2.30
	60	4.14	75.14	45.57	1.84	1.65	0.00	2.58
SEM		1.05	5.46	3.27	0.46	0.11	2.16	1.68
Level of Significance								
Molasses (M)		**	**	**	**	*	**	*
Storage periods		**	**	**	**	**	**	*
M x S		**	*	**	**	*	**	*

LA=Lactic Acid (g/kg DM); AA=Acetic Acid (g/kg DM); PA=Propionic Acid (g/kg DM); LA=Lactic/Acetic ratio (g/kg DM); BA=Butyric Acid (g/kg DM); NH3=Ammonia Nitrogen (g/kg DM). *: $P < 0.05$, **: $P < 0.01$.

cultures were used for identification of the isolates.

Moulds

Molds were identified by using cultural and microscopic characteristics. Microscopic examination of moulds was performed by staining the molds with lactophenol-cotton blue and examining them at x40 magnification under a microscope. Moulds were classified according to Barnett and Hunter, 1987.

Bacteria

The bacteria isolated were identified using both morphological and cultural characteristics of colour, consistency, shape, size, elevation, edge and opacity. The bacterial isolates were further identified using biochemical characterization (such as gram staining, catalase, oxidase, motility test, methyl red tests and sugar fermentation) gelatin hydrolysis, voges proskauer test and production of ammonia from arginine using Collins and Lyne (1987).

Statistical Analyses

Data were subjected to two-way ANOVA in a 2 x 4 factorial arrangement, with molasses addition and storage periods as the main factors. Differences were considered significant when the probability was < 0.05. These analyses were performed using general Linear Model of Minitab Computer package (Minitab, 1998). Significant differences were separated using the Turkey Pairwise comparison in the Minitab Computer Package.

Results

The proximate, chemical and concentrations of tannin and HCN are presented in Table 2. The DM contents generally reduced across the treatments for storage periods and levels of molasses inclusion. Crude protein concentration followed a similar pattern with significant reduction from 0 to 6% molasses inclusion. The highest concentration of 282.50 g/kg DM was obtained for 0% molasses inclusion at 30 days ensiling. The proportion of NDF in the silage decreased as the ensiling periods elongate and as molasses inclusion increased. However, ADF concentration significantly increased in the silage samples and highest at 6% molasses inclusion and 60 days ensiling period. However, storage periods and molasses inclusion did not significantly affect the content of HCN in the silages.

Table 4. Effects of storage period and molasses addition on microbial counts of mixture of guinea grass and cassava leaves silage

Molasses addition (%)	Storage periods Bacteria (days)		E-coli (Log cfu/g)	Fungi
0	30	8.1	1.5	9.0
	60	7.5	1.2	8.7
2	30	6.1	6.0	2.7
	60	5.7	3.0	1.7
4	30	4.8	4.3	1.0
	60	3.3	2.1	1.3
6	30	2.6	2.7	1.2
	60	2.2	1.7	1.1
SEM		0.12	0.07	1.24
Levels of significance				
Molasses (M)		**	**	**
Storage periods (S)		NS	*	NS
M x S		**	*	**

SEM= Standard error of mean; *: P < 0.05, **: P < 0.01, NS= Not significant

Table 5. Morphological, biochemical and tentative identification of bacteria isolated from mixture of guinea grass and cassava leaves silage

Gram reaction	Citrate utilization	Catalase Test	Coagulate test	Motility test	Indole test	Glucose	Lactose	Sucrose	Identified organisms
+	+	+	+	-	-	AG	AG	AG	Staphylococcus aureus
+	+	-	-	-	-	A	A	A	Streptococcus spp.
-	+	+	-	+	-	AG	AG	AG	Enterobacter spp.
-	+	+	-	+	+	AG	NR	AG	Proteus spp.
-	-	+	-	+	+	AG	AG	AG	Escherichia coli
+	+	+	-	+	+	AG	AG	AG	Lactobacillus buchneri

AG = Acid gas production; A = Acid production; NR = No reaction; - = Negative; + = Positive

The acetic acid fermentation was intensive in the 0, 2, 4 and 6% molasses addition with values ranging from 31.15 to 47.48 g/kg DM while the lactic acid content was only 18.38 to 30.25 g/kg DM at the 0% molasses addition (Table 3). However, lactic acid fermentation increased significantly as both the storage periods and molasses addition increased from 30 to 60 days. The increase was more pronounced at the 4% molasses addition and a prolonged 60 days ensiling relative to other treatments. The decrease in lactic acid contents because of prolonged ensiling from 30 to 60 days was not observed in the 2 to 4% molasses addition as obtained in the 0% molasses addition. Small quantities of propionic was obtained only in the 2% molasses addition and butyric acid was only detected in the silage prepared without addition of molasses and this increased with prolonged period of ensiling. The butyric acid contents in the silages without molasses addition reached 13.35 g/kg DM on day 60. The NH3-N content decreased in the silages without addition of molasses. Prolonged ensiling increased the NH3-N content; however, there were no changes in the silages to which molasses was added at 2%.

Table 4 shows that the bacterial count

significantly reduced across the storage periods and addition of molasses. The highest ($P < 0.05$) bacterial count (8.1 log cfu/g) was recorded at the 0% molasses addition and this significantly reduced as molasses addition increased from 0 to 6% and as the ensiling periods elongate from 30 to 60 days. Molds were detected throughout ensiling in the silages; their numbers decrease with prolonged period of ensiling. The fungi count at 0% molasses addition was 9.0 log cfu/g; however a sharp decline in fungi count was observed with increase in the percentage of molasses added. At 6% molasses addition and 60 days storage, fungi count was 1.2 log cfu/g. The E. coli counts were highest at the 2% molasses addition and significantly reduced as molasses addition increased to 6% relative to the elongation of ensiling periods. The lowest E. coli count was obtained at the 0% molasses addition.

Table 5 presents the biochemical test of the bacterial isolated. The bacteria isolated from the silage were Staphylococcus aureus, Streptococcus spp., Enterobacter spp., Proteus spp., Escherichia coli and Lactobacillus buchneri. Among these, S. aureus and Streptococcus spp. is gram positive while Enterobacter spp., Proteus spp. and E. coli are gram negative. All the organisms except Streptococcus spp. are catalase positive while E. coli was the only organism that was citrate negative. The citrate utilization test indicated only E. coli to be negative while S. aureus and Streptococcus spp. were negative for the motility test. The sugar fermentation tests for glucose, lactose and sucrose produced an acid gas production for S. aureus and an acid production for Streptococcus spp. Acid gas production was also recorded in all the sugars for Enterobacter spp., E. coli and L. buchneri. The mould isolated was white in colour with non-septate hyphae and identified as Mucor sp.

Discussion

The reduced DM concentration obtained during the fermentation periods may be attributed to loss of nutrients occasioned by secondary fermentations, which are considered normal during the ensilage process. This phenomenon especially in tropical grasses has been reported for elephant grass silage by Peirera et al. (2007) and Mombaca grass silage (Penteado et al. 2007). The CP content of the silages drastically reduced as the ensiling period prolonged with increased addition of molasses. This could be as a result of increased proteolytic activities which are merely restricted when the pH of the fermented silage is 4.3 or lower (Carpintero et al. 1979). The reduction in the CP values could also be attributed to the low CP of the molasses used, which only contains 58 g/kg DM. The reduced NDF concentration obtained in this study could be due to the non-NDF content of molasses and the low concentration of NDF in cassava leaves. The tannin contents of the silage materials were within the range of 3.5 – 5.5 reported by Maldonado et al. (1995) within which insoluble tannin and plant leaf protein complex would be established. However, the reduced tannin concentration in the silages may not be attributed to level of molasses addition and storage periods. According to Rodriguez et al. (1998), tannin concentration decreased with increase in the duration of fermentation. A high level of acetic acid production was obtained in silages without molasses addition relative to lactic acid production. This trend had been demonstrated with guinea grass and maize silages by Kim and Adesogan (2006) and Liu et al. (2011) while enhanced lactic acid production was reported with wheat and maize silages by Ashbell et al. (2002). According to Mendieta-Araica et al (2009), high quality silage is likely to be achieved when lactic acid is the predominant acid produced, as it is the most efficient fermentation acid and reduces silage pH more efficiently than other fermentation products. According to the authors, in dealing with mixture experiment model, a predicted lactic acid concentration of 106 g/kg DM was given for Moringa and molasses. This value was higher than the highest value of 77 g/kg DM obtained in this study. It was also lower than the value of 105 g/kg DM obtained by Mendieta-Araica et al. (2009) in Moringa/elephant grass silages. However, the higher concentrations of fermentation acids obtained in this study could be attributed to low DM content of the silages as reported by Cardenas et al (2003) and Phiri et al. (2007). Lactic acid bacteria is known for the

suppression of the growth of clostridia bacteria that would normally hasten the production and proliferation of butyrate and other putrefying processes by lowering the pH and oxidation-reduction potential, competing for essential nutrients and producing inhibitory compounds (Holzer et al. 2003). In this study, little or no butyrate production was observed in the silages, even though the pH decline of some of the silages were not sufficient enough to inhibit clostridia growth. In silage made from tropical grasses, a pH value of 4.2 has been reported as the maximum to consider silage to be well preserved (McDonald et al. 2002; Cardenas et al. 2003). In this experiment, a steady decline in pH with prolonged ensiling and molasses addition was obtained in the silages. However, the pH profile of the silages might be affected by different factors. The inclusion of cassava leaves which may be a potential source of nitrogen might have increased lactate accumulation and lowered pH and further ensure high nitrate concentration which might act as a buffer against pH decline. According to Namihira et al. (2010), this decrease might be due to the fact that lactate might be acting as substrate for different fermentation processes such as acetate fermentation as evidenced by the increased acetic acid formation accompanying the decreased lactic acid concentration. The NH3-N content decreased with prolonged ensiling and molasses addition suggesting that acetic acid could have been decreased because of the deaminase activity of certain silage bacteria. The NH3-N values obtained in this study was however, similar to values obtained by Namihira et al. (2010) in nitrogen fertilized guinea grass silage after 60 days of ensiling.

Fungi are the microorganisms that are considered to be implicated in aerobic determination of silage (Woolford, 1990). The reduced fungi counts with increased storage periods and molasses addition are suggestive of stable improvement in the silage quality during aeration. According to Weinberg et al. (1993), lactic acid is associated with aerobic fungal growth. However, propionic, butyric and acetic acids are inhibitory. The increased lactic acid in this experiment did not encourage fungi growth. According to Mendieta-Araica et al. (2009), many micro-organisms are found in fresh forages and under the anaerobic conditions that characterize the ensiling process. The preservation of high-moisture forage would depend on the activity of bacteria especially the lactic acid bacteria. This however, is subject to an immediate, fast and constant institution of anaerobic conditions throughout the ensiling period for bacterial multiplication. Pedroso et al. (2005) noted that for silages made from tropical grasses, lactic acid bacteria (LAB) levels of at least 3.9 log cfu/g are desirable for a good fermentation process but lower values have been reported for as normal in silages from tropical grasses. In this experiment, the high bacteria counts obtained at 0, 2 and 4% of molasses addition across the storage periods coupled with low concentrations of lactic acids could suggest a high depletion of lactic acids produced in the silages due to minor air leakage that encouraged the increased aerobic fungi. However, the bacteria counts obtained in this study were in accordance with McDonald et al. (1991) that the number of lactic acid bacteria necessary to reduce abruptly silage pH is around 8.0 log cfu/g of silage. However, many authors evaluating silages made from different crops reported initial populations from 3.7 to 6.3 log cfu/g of silage with good preservation of the ensiled forage (Whiter & Kung Jr., 2001). The identified lactic acid bacteria; L. buchneri and Streptococcus sp. are basically heterofermenters and have been reported to show improved aerobic stability in silage and capable of being used as commercial inoculants (Kung and Ranjit, 2001). Proteus sp. and Escherichia coli however, may be implicated in the production of characteristics odours which are offensive at the end of fermentation.

Conclusion

The results obtained in this study suggested that the CP concentration of the silage with cassava leaves significantly increased relative to when guinea grass was to be ensiled alone. Lactic acid concentrations also increased with the addition of molasses. However, ensiling mixture of guinea grass and cassava leaves with molasses suppressed the production of acetic and butyric acids. The experiment also showed that a drastic reduction was obtained in the

bacterial and fungal population which could also have assisted in the decline recorded in the pH of the silage.

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OUTBREAKS OF MAREK'S DISEASE IN LAYER CHICKENS FARMS IN KHARTOUM AND GEZIRA STATE IN SUDAN: CLINICAL AND PATHOLOGICAL ASPECTS.

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Abstract

Outbreaks of Marek's disease were investigated in five commercial poultry farms in Khartoum and Gezira States during the period 2009-2012. All the affected birds were of layers aged 4-7 months. The mortality rate ranged from 0.3-2.8%, the clinical signs observed were diarrhea (60%), paralysis (60%) and drop in egg production (40%). Enlargement in visceral organs, and nerves were the main postmortem lesion in all examined birds , 100% enlargement was noticed in liver and spleen ,sciatic nerve(80 %), whereas lesion in eyes (20%), ovaries (20%), heart (40%) and lungs (20%) were not common. Infiltration of lymphoid cells was observed in livers, kidneys, spleen and nerves. The most remarkable finding in the present investigation was the excessive losses from MD in adult laying flocks over the age of 40 weeks suggesting that infection of MD in vaccinated adult commercial type chickens might be due to de novo infection (Super infection) with highly virulent strains despite existing considerable levels of vaccine immunity and age resistance, also vaccination failure may perhaps considered one of the important causes of disease outbreaks. These results may assist in disease control policies and planning research priorities.

Key words: Marek's disease,field cases, clinical signs, postmortem lesions, histopathology,Sudan

DES FOYERS DE LA MALADIE DE MAREK DANS DES FERMES DE POULES PONDEUSES DES ÉTATS DE KHARTOUM ET GEZIRA AU SOUDAN : ASPECTS CLINIQUES ET PATHOLOGIQUES

Résumé

Des foyers de la maladie de Marek (MD : Marek Disease) ont été étudiés dans cinq fermes avicoles commerciales dans les États de Khartoum et Gezira au cours de la période 2009-2012. Tous les oiseaux affectés étaient des pondeuses âgées de 4 à 7 mois. Le taux de mortalité variait entre 0,3 et 2,8 %, et les signes cliniques observés étaient la diarrhée (60 %), la paralysie (60 %) et la baisse de production d'œufs (40 %). L'hypertrophie des organes viscéraux et des nerfs était la principale lésion observée chez tous les oiseaux soumis à un examen post-mortem ; un grossissement de 100 % a été remarqué pour le foie et la rate et de 80% pour le nerf sciatique, tandis qu'une lésion des yeux (20 %), des ovaires (20%), du cœur (40 %) et des poumons (20 %) n'était pas courante. L'infiltration des cellules lymphoïdes a été observée dans le foie, les reins, la rate et les nerfs. Le résultat le plus remarquable dans la présente étude a été l'identification de pertes excessives dues à la MD parmi les troupeaux de pondeuses adultes âgées de plus de 40 semaines, ce qui porte à croire que l'infection de MD chez les poulets adultes vaccinés de type commercial pourrait être due à une infection de novo (Super infection) par des souches très virulentes malgré les niveaux considérables existants d'immunité vaccinale et de la résistance due à l'âge. En outre, l'échec vaccinal peut être considéré comme l'une des causes importantes des foyers de la maladie. Ces résultats peuvent être utiles dans les politiques de lutte contre la maladie et la planification des priorités de recherche.

Mots-clés : Maladie de Marek ; Cas de terrain ; Signes cliniques ; Lésions post-mortem ; Histopathologie ; Soudan

Introduction

In Sudan, poultry products are among the major sources of animal protein. Now-a day there is an increase in the number of poultry farms as they are considered an important economic source to increase individual income. Diseases are a symbol of one of the problems that faced poultry industry in Sudan.

Marek's disease (MD) is a lymphoproliferative disease of chickens induced by Marek's disease virus (MDV), an alpha-herpesvirus belonging to the Mardivirus genus (Schat & Nair, 2008). Three serotypes are known serotype 1, 2 and 3. Among these only viruses of serotype1 have pathogenic potential causing contagious neoplastic disease of the immune system in susceptible chickens (Susan and Davison, 1999)

MD is characterized by multiple T-cell lymphoma formation in visceral organs, muscles, skin and lesions in peripheral nerves (Calnek and Witter,1991).Clinical signs observed for MD vary from mild depression followed by ataxia and paralysis of wings or legs with enlargement of peripheral nerves ,skin nodular lesions, stunting and mortality (Santin*et al.,* 2006 , OIE,2010).

The transmission of MDV occurs by direct or indirect contact, apparently by the airborne route. However, no evidence exists for vertical transmission of MDV through the egg (Payne & Venugopal, 2000).The annual losses due to this disease world over have been estimated at more than 1billion US dollars (Nair, 2005). Diagnosis of MD is based on clinical signs, gross and microscopic lesions and sometimes chickens may become persistently infected with MD virus (MDV) without developing clinical disease (OIE, 2010).

In the Sudan the disease was first reported in 1956 among imported flocks (Anon, 1956-57). Since then it has remained as one of the serious disease problems facing the poultry industry in Sudan. Kheir et al (1992) illustrated that the incidence of the disease during the years 1960-1978 was moderate suddenly, very high incidence of the disease outbreaks occurred during the years 1986-1988 and it was diminished gradually due to the usage of HVT vaccine, but it started to rise again in 1992-1996 (Awatif, 1999). Serological studies which were conducted in both foreign and local type of breed indicate wide spread infection of MD viruses (Awatif, 1999). Three years before the investigated period, no cases of MD were reported compared to period from 2009-2012 where 5 farms suffered from high morbidity and mortality and low egg production and this was the reason of our study.

Materials and Methods

Five farms were investigated in this study from different areas designated as A,B,C,D and E , 5-10 birds were submitted from each infected farm for diagnosis, birds were of different ages, breeds and rearing system (Table 1). All birds had been vaccinated at day old. The flock history, clinical signs and Postmortem lesions were recorded. Tissues for histopathological examination were taken from organs which showed enlargement including liver, spleen, proventiculus, kidneys, lungs, heart, ovaries and nerves. These tissues were fixed in 10% neutral buffered formalin, processed for paraffin embedding, sectioned, and stained with haematoxylin and eosin (HE) and examined by light microscope using 10X, 40X and 100X objectives for histopathological changes.

Results

Five farms were infected with MD during the period 2009-2012, three of them located in Khartoum state two farms were of hisex breed while the third of lohman breed, all flocks in the same age 7 months number of flocks were 40,000 – 50,000 and 9,000.The remaining two farms were in Gezira state of hisex and lohman breed aged 7 and 4 months, number of flocks were 11,400 and 3,500.

The affected chicken flocks had a history of MD vaccination with HVT at day old of age. The morbidity, mortality, clinical signs and postmortem lesions exhibited by diseased birds varied among the five studied farms. In all farms examined; diarrhea, paralysis, drop in production were reported as 60%, 60% and 40% respectively. One hundred percent (100%) enlargement was noticed in liver and spleen,

80% in nerve, 40 % kidney and heart, and 20% in skin, ovary and lung (figures 1- 4).

The mortality ranged between 0.3-2.8% as showed in Table 2. Regarding the breed of chickens it was found that 60% of the flocks examined belongs to Hisex while 40% of Lohman breed.

Histopathological results indicate intense infiltration of lymphoid cells in visceral organs and nerve figures 5-7.

Discussion

Marek's disease (MD) is currently one of the important contagious diseases affecting the poultry industry. It is not only causes the death of birds directly, but also causes immunosuppression of infected chickens which are more sensitive to other pathogens such as Escherichia. coli (Mingxing etal,2011).

In this study, all the MD affected farms had a history of vaccination but it was uncertain whether the cases represented improper vaccination procedures or possibly infected by highly virulent strains of MDV which are capable of over-riding vaccine -induced protection. Occurrence of the disease in vaccinated flocks warrants more attention to vaccination failure and this may be due to many factors such as brand of vaccine and vaccine preparation, handling, administration and stress (Dudnikov and Witters, 2001).

In the present investigation, MD has been recorded from 20 to 28 weeks of age. Witter (2001) stated that MD has been thought to be a disease for young chickens (4 to 20 weeks old), but recently excessive losses from MD have been noted in adult laying flocks .

Although all the five farms examined were infected with MDV, the levels of mortality from MD varied from one farm to other (0.3 %-2.8%) , the assessment is based on the history taken from the farm owner on one-time visit. The magnitude of the problem and resulting economic losses including the cost of losses due to deaths, decreased egg production and medication estimated as 9,500 US dollars. The recorded mortality rate is considered low as reported by Panneerselvam et al. (1990) who reported that the percentage of mortality due to Marek's disease was higher in the younger age group (9-20 weeks) than that of older birds (above 20 weeks).Similarly Kamaldeep et al(2007) found a mortality rate 0.24-1.0% in MD affected farms in India. Labgo(2004) explain that the difference in rate and duration

Table 1: Field data of Marek's disease affected Farms.

Farms	Location	No.of Birds at risk	Breed	Age	Date of outbreaks
A	Khartoum	40,000	Hisex	7 months	2009
B	Khartoum	50,000	Lohman	7months	2011
C	Gezira	11,400	L ohman	4months	2011
D	Gezira	3,500	Hisex	7months	2012
E	Khartoum	9,000	Hisex	7months	2012

Table 2: Mortality, Clinical signs and Postmortem lesions of Marek's disease on affected Farms.

Farm no	Mortality	Clinical signs			Organ (Postmortem lesion)								
		Diarrhoea	Paralysis	Drop in production	Heart	Lung	Liver	Proventiculus	Spleen	Kidney	Nerve	Skin	ovary
A	*0.3%*	+	+	-	-	-	+	-	+	+	+	-	+
B	*1%*	_	+	+	+	-	+	-	+	-	+	-	-
C	*2.4%*	+	+	-	-	-	+	-	+	_	+	-	-
D	*2.8%*	+	-	-	-	-	+	-	+	+	+	-	-
E	*2%*	_	-	+	+	+	+	-	+	_	+	+	-

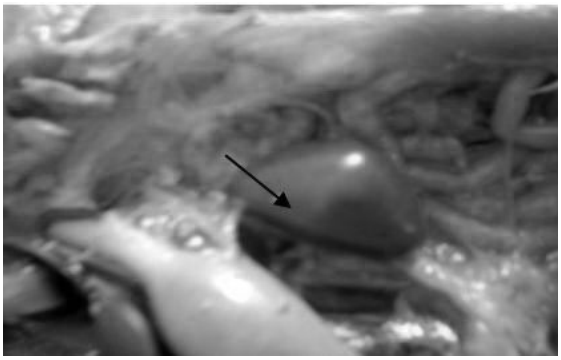

Figure 1: Enlargement of spleen

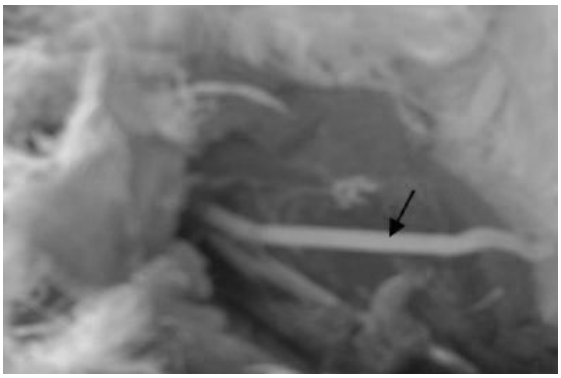

Figure 2: Enlargement of the sciatic plexus

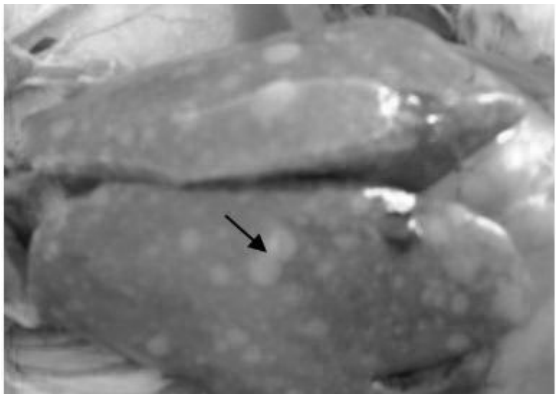

Figure 3: Enlargement of liver With grayish tumor nodules.

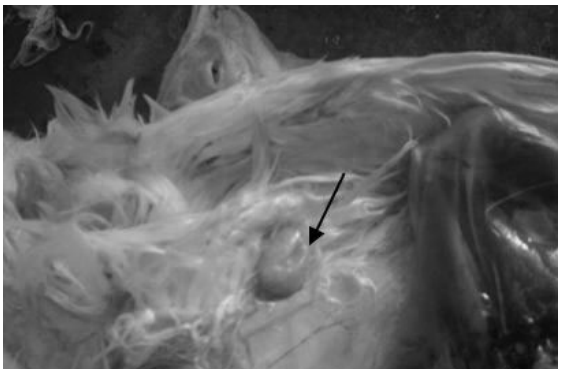

Figure 4: Subcutaneous tumor

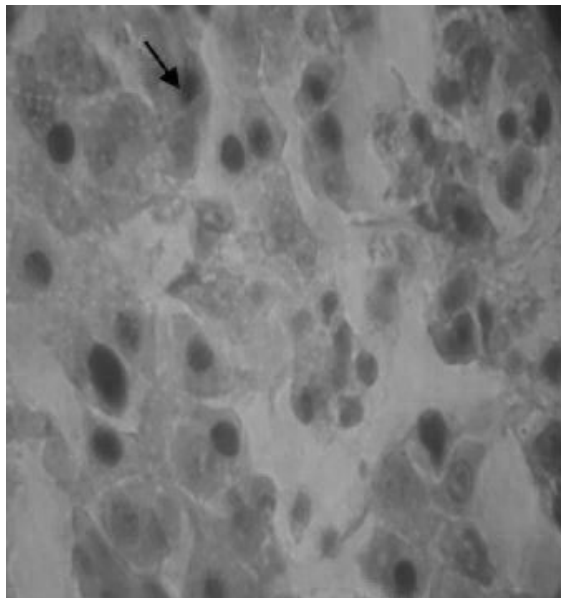

Figure 5: liver- Pleomorphic lymphoid cell infiltration.

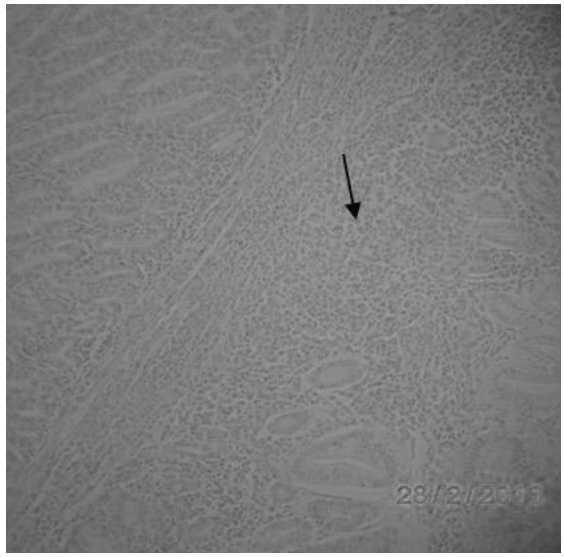

Figure 6: kidney-Pleomorphic lymphoid cell infiltration H&E X10. H&E H&EX40.

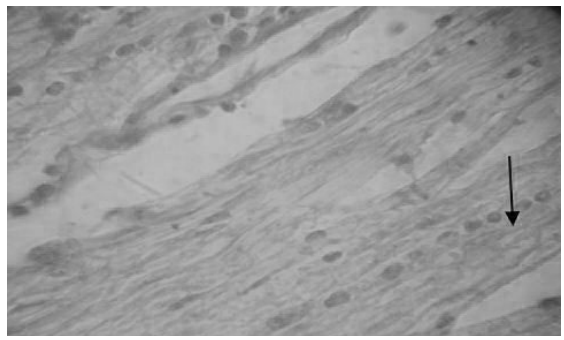

Figure 7: Sciatic nerve - Pleomorphic lymphoid cell infiltration H&EX40.

of mortality in a flock and the age at which an outbreak of MD occurs are influenced by a number of factors. These are related to either the infective agent (virus strain, dosage and route of exposure) or host factor (genetic constitution, age, sex and immune status.

The diagnosis for presence of MDV in these farms has been based on clinical symptoms, postmortem lesions and histopathological changes. Among these include:enlargement in liver,spleen,heart,kidneys ,lungs, ovaries and nerve were the main postmortem lesion , tumor nodules grayish-white in colour in both liver and spleen .MD lymphomas in visceral organs have been reported by several workers (Purchase and Biggs, 1967; Rathore etal ; 1985;Narang etal ;2003). Infiltration of lymphocytes in section of the affected birds organs were consistent with other reports (Frazier, 1974., Lobago and Woldemeskel, 2004.,Goyal etal.,2006).

Conclusion: Nowadays, MD continues to be a serious threat to poultry industry, isolation and characterization of MDVs are essential for monitoring changes of viruses and evaluating the effectiveness of existing vaccines, so we can develop better vaccines and control program of MD.

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THE IMMUNE RESPONSE OF MATERNALLY IMMUNE CHICKSTOVACCINATION WITH NEWCASTLE DISEASE VIRUS

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Summary

This study was conducted to determine the persistence of maternally derived antibodies (MDA) to Newcastle disease virus (NDV) in newly hatched chicks and the effect of MDA on vaccination with a very potent vaccine (Avinew® (VG/GA)). Individual variations of chicks in acquiring and maintaining MDA and in their response to vaccination were also investigated.

In order to study the persistence of MDA, 50 one-day-old Hisex breed chicks were divided into five groups based on their age at serum collection (one-day-old, seven days-old, fourteen-days-old, twenty-days-old and twenty eight-days-old), respectively. To assess interference of MDA with vaccination, 30 chicks were divided into three groups based on time of vaccination (days 1, 14, and 28).

Haemagglutination inhibition test was used to measure antibody titer in sera. Chicks at 1, 7, 14, 21, and 28 days of age showed MDA titers of 9, 8.7, 5.8, 4.2, and 1.6 (log2) respectively. Vaccination at day 1, 14, and 28 of age elicited titers of 2.6, 3.98, and 4.75 respectively. It was noticed that among each group there was variations in titer.

The minimum titer that interfered with vaccination was 4.2 (log2). This titer was obtained in day 18 of age which was considered the optimum time for vaccination with Avinew ®(VG/GA) strain.

LA RÉPONSE IMMUNITAIRE DES POUSSINS AYANT ACQUIS UNE IMMUNITÉ D'ORIGINE MATERNELLE À LA VACCINATION AU VIRUS DE LA MALADIE DE NEWCASTLE

Résumé

Cette étude a été réalisée dans le but de déterminer la persistance des anticorps d'origine maternelle (AOM) contre le virus de la maladie de Newcastle (MNC) chez les poussins fraîchement éclos et l'effet des AOM sur la vaccination avec un vaccin très puissant (Avinew® (VG/GA). Les variations individuelles entre poussins au niveau de l'acquisition et du maintien des AOM et de leur réaction à la vaccination ont également été étudiées.

Dans la perspective d'étudier la persistance des AOM, 50 poussins d'un jour, de race Hisex, ont été répartis en cinq groupes en fonction de leur âge au moment du prélèvement du sérum (respectivement 1 jour, sept jours, quatorze jours, vingt jours et vingt-huit jours). Pour évaluer l'interférence des AOM avec la vaccination, 30 poussins ont été répartis en trois groupes en fonction de l'époque de vaccination (jours 1, 14 et 28).

Le test d'inhibition de l'hémagglutination a été utilisé pour mesurer le titre des anticorps dans les sérums.

Les poussins âgés de 1 ; 7 ; 14 ; 21 ; et 28 jours ont montré des titres d'AOM respectivement de 9 ; 8,7 ; 5,8 ; 4,2 ; et 1,6 (log2). La vaccination des poussins âgés de 1 ; 14 ; et 28 jours a provoqué respectivement des titres de 2,6 ; 3,98 ; et 4,75. On a remarqué au sein de chaque groupe des variations au niveau des titres.

Le titre minimum ayant interféré avec la vaccination était 4,2 (log2). Ce titre a été obtenu au jour 18 d'âge qui a été considéré comme le meilleur moment pour la vaccination avec la souche Avinew ® (VG/GA).

Introduction

Newcastle disease is a highly contagious viral disease that attacks many species of domestic and wild birds and causes devastating losses to the poultry industries. There are many vaccines which are of considerable value in reducing losses (Mozaffor *et al.,* 2010). However, and despite the extensive use of vaccines, outbreaks of ND continue to occur in both vaccinated and unvaccinated flocks (Sa'idu and Abdu, 2008). A crucial unsolved problem for all vaccines is the interference with the maternally derived antibodies (Dhohyung, 2012).

Maternal antibodies to avian paramyxoviruses type-1 (APMV-1) are transferred from vaccinated dams to their chicks, the amount of transferred antibodies varies between individuals to a degree that some chicks may be at risk to (APMV-1) infection in due time before scheduled vaccination (Thomas *et al.,* 1998).

Variation in efficacy of ND vaccines in eliciting high antibody titers to be transferred through eggs to chicks, interference of MDA with vaccination and production of different breeds of chicken necessitate the determination of acquisition and maintenance of MDA against each NDV vaccine.

Materials and Methods

Chicks:

For the purpose of determining maintenance and decline of maternally derived antibodies to Newcastle disease virus in newly hatched chicks, a total of 50 one-day-old Hisex breed chicks from parents vaccinated with AVINEW (VG/GA) strain were obtained from Coral Company (Khartoum, Sudan). Chicks were divided into five groups and Serum was collection as in table (1).

Haemagglutination Inhibition (HI) was used for determining antibody levels in sera of these birds.

In order to determine the effect of maternally derived antibodies on vaccination a total number of 30 one-day-old chicks, were concurrently obtained from the same flock of ND vaccinated parents from Coral Company (Khartoum, Sudan). These chicks were divided into three equal groups and were vaccinated with AVINEW® (VG/GA) vaccine as shown in table (2).

ND vaccine:

The AVINEW (VG/GA strain) live vaccine was developed exclusively by MERIAL*. This vaccine was obtained from Coral Company in a form of freeze-dried vaccine stored at controlled temperature environment, between + 2 ˚C and + 8 ˚C, and away from light. This vaccine is stable under laboratory condition for a minimum of 2 hour at 25 ˚C after reconstitution in phosphate buffer saline (PBS) (Merial, 1998).

Vaccination:

The AVINEW (VG/GA strain) live viral vaccine was dissolved in 30 ml (PBS) according to the manufacture instructions, and was used immediately after reconstitution (with in 2 hour). Approximately 100µl was administered intranasally to each chick individually. No post-vaccinal reaction was observed.

*Merial select, INC., (1998). Avinew USA: Gainesville, GA 30503, U.S. vet. Lic. No. 279

Serum collection:

A Hundred microlitter of blood was collected by heart punture of chicks using 1ml syringe containing 0.3ml of normal saline (NS) to make 1:4 dilution, in order to increase the volume for centrifugation purpose; the diluted blood was centrifuged at (1000 rpm for 5 minute), the serum was separated in another tube and preserved at -20°C (Ismail, 1987).

Determination of the effect of maternal antibodies on vaccination: Haemagglutination

(HA) test:

Haemagglutination and Haemagglutination inhibition tests were performed according to (Brian and Hiller, 1996). Haemagglutination test was done to determine the four Haemagglutination units (4HAU) of the virus, (AVINEW (VG/GA strain)). Two folds serial dilution along the rows was carried out by adding 100µl of (PBS) into U-shaped wells of a micro titer plate, and then 100µl of virus

suspension were added to the first well and mixed, 100µl from this well were transferred to the second well and the mixing process was repeated, 100µl of (0.5% v/v) washed chicken RBCs were added to all wells and incubated at room temperature for 1 hour. The reciprocal of the highest dilution that produced positive HA was considered as the virus titer (one HAU).

Haemagglutination inhibition (HI) test:

The haemagglutination inhibition test was done to determine the titer of antibodies .100µl of (PBS) were added to each of the 96 U-shaped wells of a micro titer plate; then 100µl of chicks' serum were added to the first well and 100µl from this well were transferred to the second well and two-fold serial dilution was carried out along the row, this serial dilution process was done to each serum sample, then 100µl of 4HAU of the virus preparation were added to each well. The micro titer plate was incubated at room temperature for 1 hour, then 100µl of 0.5% washed chickens' RBCs were added to each well and incubated for 1 hour at room temperature. The result was observed and recorded. The mean titers of sera collected at each date were figured out.

Determination of individual variations:

Individual variations in the immune response among chicks of the same group were assessed using the concept of flock profile adopted by (**Biogal-Galed Labs, 2004. Catalog No: 50PTV203/50PTV230); the results of individual birds were plotted as a bar graph.

Results

Maintenance and decline of maternally derived antibodies in chicks of different age:

Maternally derived antibodies were measured weekly starting from day 1 to day 28 and the titers are presented in Table (3) and figure (1).

Individual variations of maternally derived antibodies in chicks:

One day-old chicks showed four different titers; eight, nine, eleven and twelve (log_2) given by five, three, one and one chicks respectively figure (2).

Figure (3) summarizes the individual variations of seven days-old chicks that showed four different titers; seven, eight, nine and twelve (log2) given by two, two, five and one chicks respectively with an average of (8.7).

With an average of 5.6, the flock profile of the fourteen days-old chicks showed five different titers; four, five, six, seven and eight (log2) given by two, four, one, two and one chicks respectively figure (4).

Figure (5) summarizes individual variation of chicks at day twenty one when they showed three, four, five and six (log2) given by two, five, two and one chicks respectively.

The MDA flock profile of twenty eight days-old chicks is shown in figure (6).

The effect of maternal antibodies on vaccination:

The mean of pre-vaccination and post-vaccination titers of chicks at different times

Table 1: Age at which serum was collected:

Group	Age at which serum was collected	Number of samples
A	One day-old	10
B	Seven day-old	10
C	Fourteen day-old	10
D	Twenty one day-old	10
E	Twenty eight day-old	10

Table 2: Schedule of vaccination and serum collection:

Group	Number of samples	Age of vaccination	Age at serum collection
A	10	One day-old	Twenty two day-old
B	10	Fourteen day-old	Thirty five day-old
C	10	Twenty eight day-old	Forty nine day-old

Table 3: Mean titer of MDA of chicks at different ages:

Group	Group Age of chicks	Mean titer (log2)
A	One day-old	9
B	Seven days-old	8.7
C	Fourteen days-old	5.6
D	Twenty one days-old	4.2
E	Twenty eight days-old	1.5

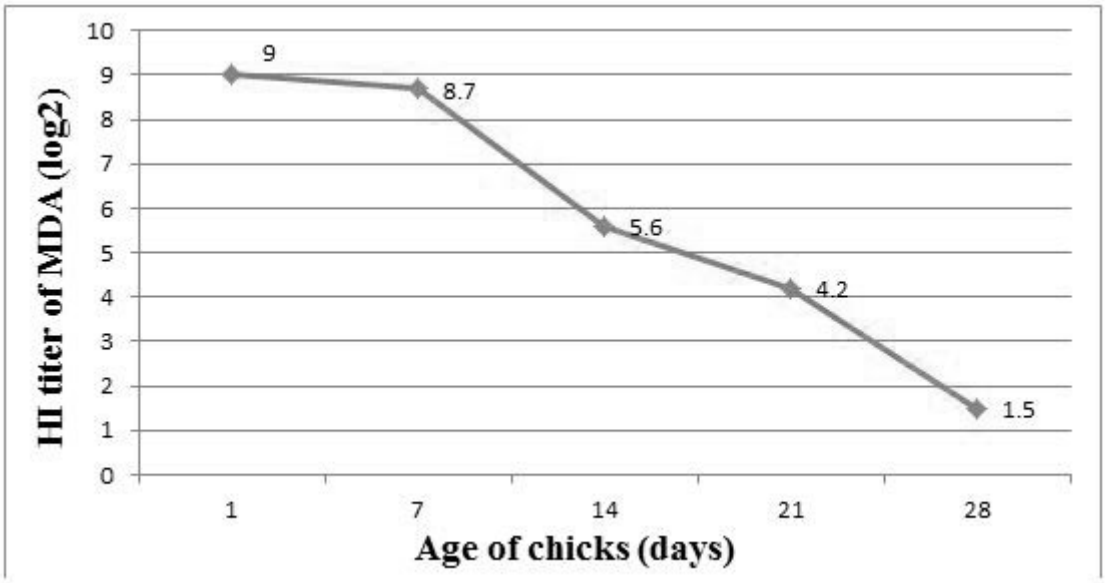

Figure 1: Mean titer of MDA of chicks at different ages

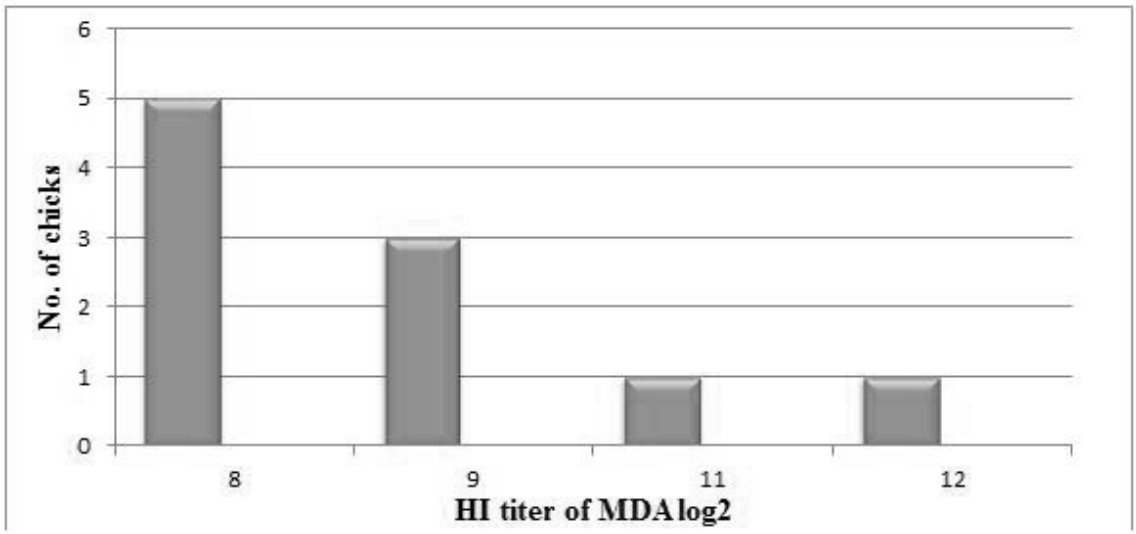

Figure 2: MDA profile of day-old chicks.

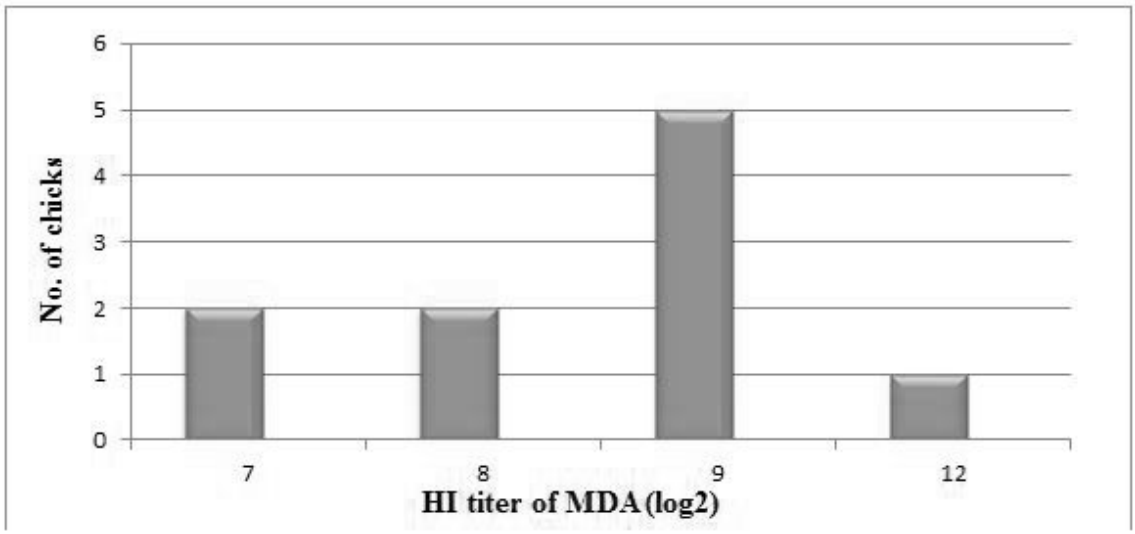

Figure 3: MDA profile of seven days-old chicks.

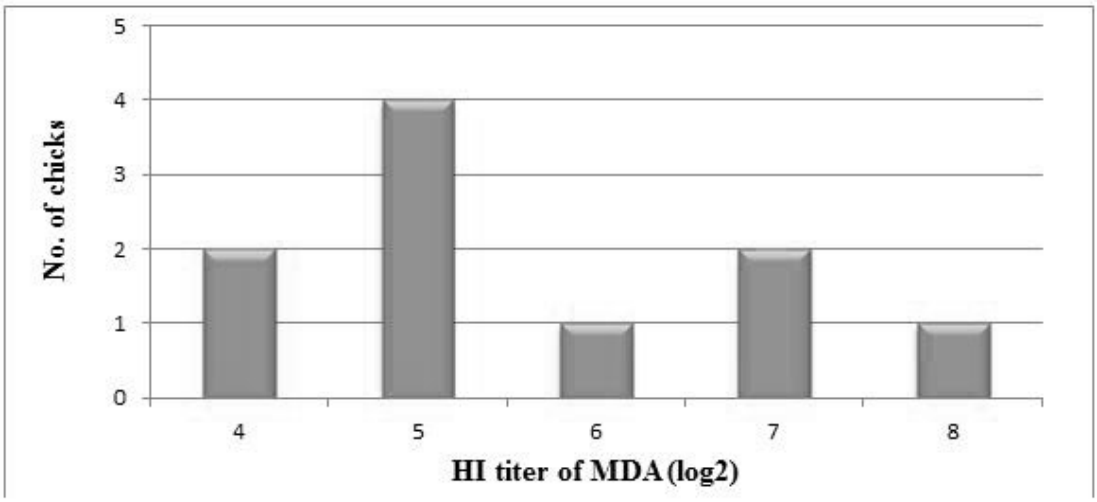

Figure 4: MDA profile of fourteen days-old chicks.

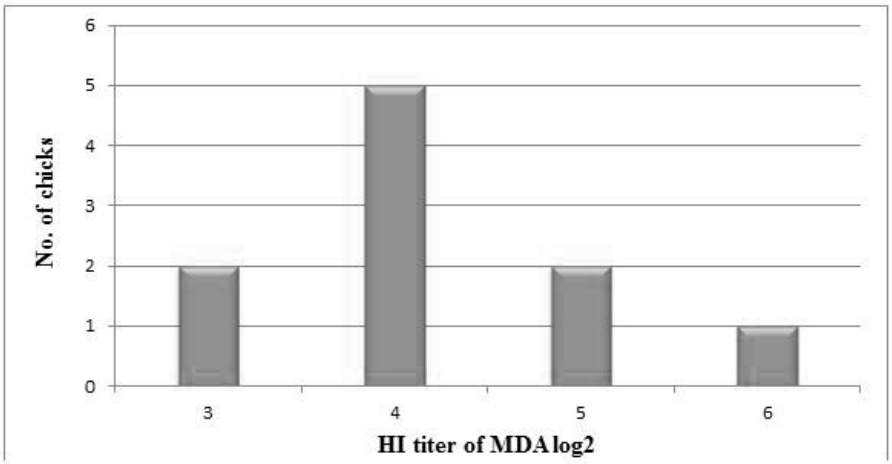

Figure 5: MDA profile of twenty one days-old chicks.

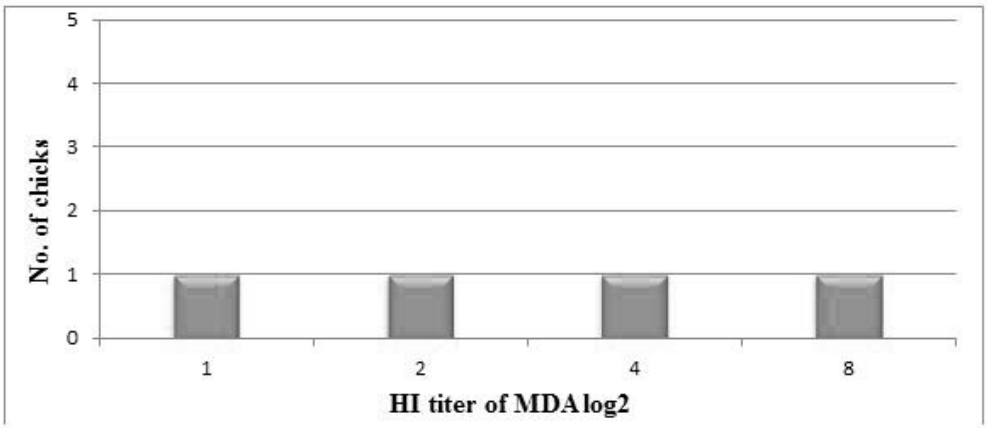

Figure 6: MDA profile of twenty eight days-old chicks.

Table 4: Pre-vaccination and post-vaccination titers of chicks at different ages.

Groups	Mean of pre-vaccination titer(log2)	Mean of 21 days post-vaccination titer
A	9.00	2.67
B	8.7	ND.
C	5.6	3.98
D	4.2	ND.
E	1.5	5.4

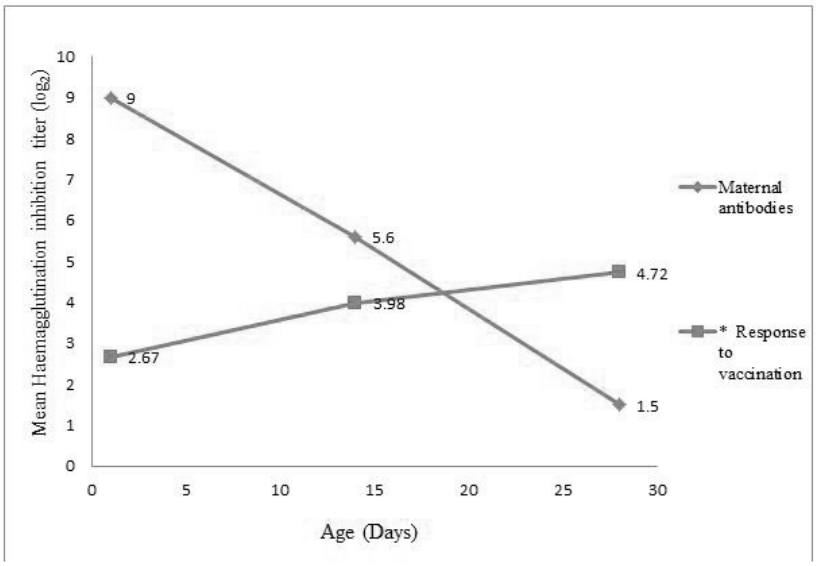

Figure 7: Pre-vaccination and post-vaccination titers of chicks at different ages.

are shown in table (4) and Figure (7).

*Age in graph was the age at which vaccination process was done; the response to vaccination was measured 21 days later.

When the MDA decline curve was overlapped with the vaccination response curve, the point at which they crossed (4.2 log2, day 18) was considered the minimum titer that interfered with vaccination. It was also considered the best day of vaccination because after it almost all chicks gave positive response.

Individual variation of maternally immune chicks to vaccination:

Flock profiles of post-vaccinated chicks are shown in figure 8, 9, and 10.

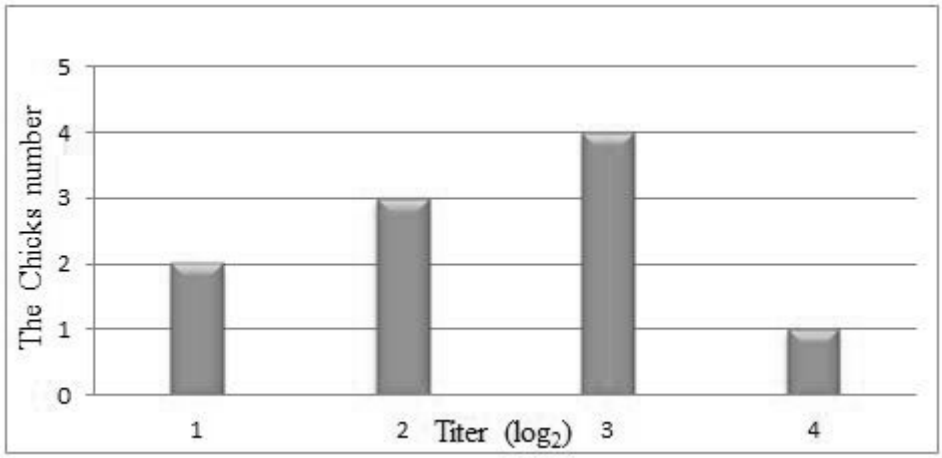

Figure 8: Individual variation in Immune response to vaccination at day

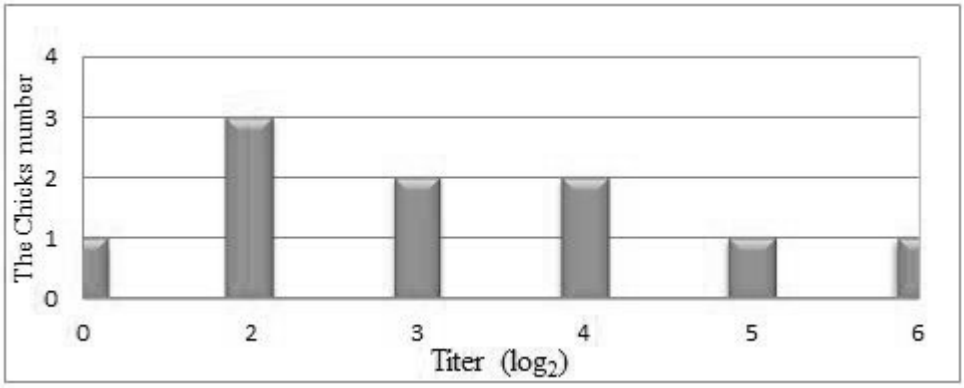

Figure 9: Individual variation in Immune response to vaccination at day 14 of age.

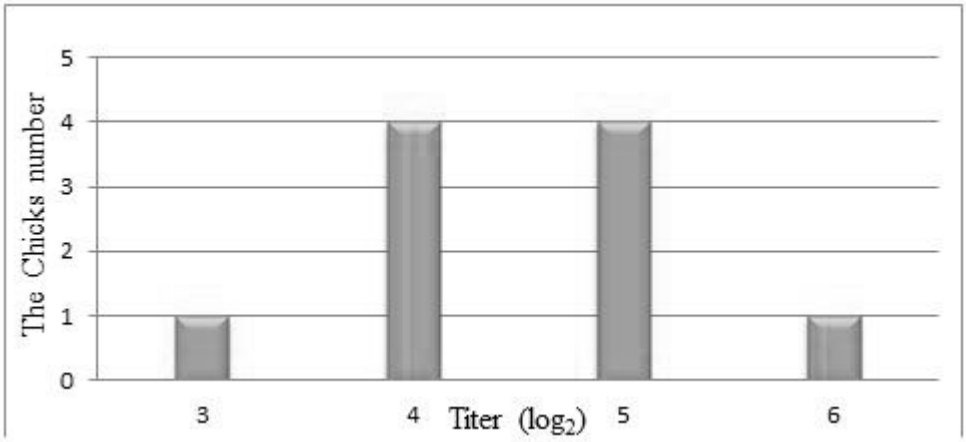

Figure 10: Individual variation in Immune response to vaccination at day 28 of age.

Discussion

Decline of maternally derived antibodies in chicks with age:

The fact that maternally derived antibodies confirm the transfer of MDA from vaccinated parents to offsprings was stated by many researchers (Gharaibeh *et al.,* 2008; Hamal *et al.,* 2006). It was noticed that the initial titer 9 (log2) at day one, was higher than any previously reported levels by:(Banu *et al.,* 2008; Jalil *et al.,* 2009; Shil *et al.,* 2011). A limited decline of HI titers was recognized from the first week when the titer decreased from 9 (log2) in day-old chicks to 8.7 (log2) in seven days-old chicks. This initial small drop

is in agreement with that observed by Ismail, (1987), who reported a limited decrease in titer from 3.52 in day-old chicks to 3.39 at one week of age. This limited decline differed from that reported by Banu *et al.,* (2008) when they detected a decline from 7.5 at day one to 6.5 at day seven, and from that observed by Begum et al (2006) when they measured a higher decline from 8 at day one to 6.6 at day seven. These relative differences in MDA levels may be due to variations in experimental conditions such as; type of vaccine and vaccination procedure. MDA decreased rapidly from day seven reaching 5.4 (log2) at day fourteen. This result reaffirmed the finding of (Ismail, 1987; Begum *et al.,* 2006; Jalil *et al.,* 2009; Msoffe *et al.,* 2006; Shil *et al.,* 2011) when they observed that MDA declined rapidly from day seven to day fourteen. Rao *et al.,* (1987) however, could not detect any antibodies in 15-day-old chicks; this difference may be due to variations in the initial titer at day one or due to experimental conditions. Mean titer decreased from 4.2(log2) at day twenty one to 1.5(log2) at day twenty eight. This low level of titer at day 28 is in agreement with many previous findings (Msoffe *et al.,* 2006; Begum *et al.,* 2006; Mahmud *et al.,* 2007; Jalil *et al.,* 2009; Banu *et al.,* 2009 and Shil *et al.,* 2011).

Our results agreed with the general trend that MDA decline starts from the first week of age with a limited drop, it continues to decrease towards day 14, and the decline rate increases dramatically from the 14th day to the 21th day, and undetectable level of MDA was reached by the end of the 4th week of age.

Results of the flock profile showed that there were individual variations among chicks in acquiring MDA specially those of day fourteen when five chicks showed different five titers. These results reaffirmed results of leandro *et al.,* (2011).

In a parallel experiment we studied the effect of MDA on vaccination. We employed chicks from the same flock and used the same vaccine AVINEW® (VG/GA strain).

We found out that the decrease in MDA with time corresponded with an increase in the HI antibodies after vaccination with AVINEW (VG/GA strain) which gave means of 2.67, 3.98, and 4.72 (log2) at days 1, 14, and 28 respectively. This reciprocal relationship between MDA and response to vaccination agreed with Lancaster *et al.,* (1960) who found that congenital passive immunity interfered with the development of active immunity in response to Newcastle disease virus given intramuscularly or subcutaneously A similar result was also obtained by Ismail, (1987) who observed that chicks vaccinated at day 1, 7, and 14 of age and had maternal antibodies above 3 (log2) did not respond to vaccination. That could be due to neutralization of the vaccine virus by these antibodies; similar findings were also obtained by Giamborne and Closser, (1990) when they found that higher antibodies titer in one day-old broilers resulted in fewer vaccine-induced reactions, less vaccine virus shed, and decreased duration of vaccine-induced immunity from coarse-spray vaccination. Jalil et al. (2009) observed that HI antibodies titer decreased significantly after 7 days of single vaccination with Baby Chick Ranikhet Disease Vaccine (BCRDV) and could not protect the birds which were vaccinated at day 1 and day 7 separately. They speculated that was due to the neutralization of vaccine virus with (MDA).

It could be easily deduced that, in spite of individual variations, the best time of vaccination is the end of the third week particularly at day 18 of age. This result agreed with Begum et al. (2006) when they reported that maternally derived antibody passed over from the parents to progeny chicks remained protective for the chicks hatched from vaccinated parents until 18 days of age, although they found that the minimum titer that interfered with the vaccine was 3.6 (log2).

Chicks vaccinated at day 1 and day 14 with MDA above 4.2 (log2) did not respond to vaccination, this result agreed with Ismail (1987) who reported that chicks vaccinated at day 1, 7, and 14 did not respond to vaccination; although he found that the titer of MDA that interfered with the vaccine was above 3 (log2),. This could be due to the difference in the NDV vaccine strain on one hand and the poultry breed on the other hand.

The individual variation in the immune response of vaccinated chicks might be due to their initial acquisition of different amounts of MDA from parents, that variably interferes with the vaccine at different ages of chicks; a

similar explanation was given by Rhman et al. (2004) who stated that individual variations in the production of HI antibody response might be due to the presence of variable passive immunity in chicks or to varying degree of sensitivity of immune mechanism to antigen.

The wide range of individual variations in acquiring MDA (from 1 to 12 log2), together with the wider range of response of individuals with deferent levels of MDA to vaccination, may explain some of the vaccination failures, encounter with many different NDV vaccine strains and different poultry breeds.

This study shows that the best time of vaccination of chicks from immunized parents should be done no earlier and no later than day 18 of age.

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SEROPREVALENCE OF PESTE DES PETITS RUMINANTS AMONG GOATS AND SHEEP IN ENUGU STATE OF NIGERIA.

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Abstract

Prevalence of Peste des petits ruminants (PPR), a viral disease of small ruminants was studied in Enugu State. A total of six hundred and fifty five (655) serum samples (429 from goats and 226 from sheep) were collected between May, 2010 and April, 2011 from various farms that had no history of vaccination against the disease. The samples were screened for antibodies against PPR virus, using the competitive enzyme-linked immunosorbent assay (cELISA). The prevalence rate of PPR varied across local government areas 38.9% in Enugu-East to 57% in Enugu-Ezike, sexes from to 33% in male to 56% in females and age groups from 5.3% in those <1 to 78% in those >3 years of age, with a state specific prevalence rate of 47% ($p \leq 0.01$). This prevalence estimate compares with 50% recorded in some Weredas goats and sheep in Ethiopia. Sex, species and age as well as location are of epidemiologic importance. Therefore, vaccination of goats and sheep against PPR virus is important in this part of the country.

Key words: Prevalence, Peste des petits ruminants

SEROPREVALENCE DE LA PESTE DES PETITS RUMINANTS CHEZ LES CAPRINS ET OVINS DE L'ETAT D'ENUGU AU NIGERIA

Résumé

La prévalence de la peste des petits ruminants (PPR), une maladie virale des petits ruminants a été étudiée dans l'État d'Enugu. Au total, six cents cinquante-cinq (655) échantillons sériques (429 de chèvres et 226 de moutons) ont été recueillis entre mai 2010 et avril 2011 dans diverses fermes n'ayant pas d'antécédents de vaccination contre la maladie. Les échantillons ont été examinés pour rechercher la présence d'anticorps contre le virus de la PPR, en utilisant la méthode immuno-enzymatique de compétition (cELISA). Le taux de prévalence de la PPR variait selon les collectivités locales - entre 38,9 % à Enugu-Est et 57% à Enugu – Ezike ; selon les sexes - de 33% chez les mâles à 56 % chez les femelles ; et selon les groupes d'âge, de 5,3 % chez ceux âgés de < 1 à 78 % chez ceux âgés > 3 ans, avec un taux de prévalence spécifique à l'État de 47 % ($p \leq 0,01$). Cette estimation de la prévalence est du même ordre que les 50 % enregistrés chez des chèvres et moutons de certains Weredas en Éthiopie. Le sexe, l'espèce et l'âge ainsi que la localisation ont une importance épidémiologique. Par conséquent, la vaccination des ovins et caprins contre le virus de la PPR est importante dans cette partie du pays.

Mots-clés : Prévalence ; Peste des petits ruminants

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Introduction

In South-Eastern Nigeria including Enugu State, small ruminant farming contributes significantly to the agrarian economy, being second to poultry as the major source of income to local farmers. These animals provide high quality meat and skin in addition to milk and wool (Osuagwuh and Akpokodje, 1981) in a locality where large scale cattle farming is uncommon.

However, the two principal constraints to the optimal performance of these species are diseases and inadequate food supply (Mack, 1982; Wilson, 1982). The most serious single cause of production losses ascribable to disease among small ruminants in West Africa is PPR (ILCA, 1979; Lamorde, 1980). The Morbilivirus disease which was described first in Cote'd'Ivore in West Africa in 1942 is transmitted primarily by inhalation of aerosols from infected animals (Gibbs *et al.,* 1979; Rowland *et al.,* 1971). Clinical signs include fever, erosive stomatitis, gastroenteritis, pneumonia with sneezing and coughing (Barret and Rossitor, 1999). Though primarily a disease of sheep and goats, natural cases have been reported in wild ungulate (Barret and Rossitor, 1999).

Pneumonia and diarrhoea are prevalent in South-eastern Nigeria but the involvement of PPR virus in the epizootiology of these diseases is largely unknown (Furley *et al.,* 1987). There is paucity of information about the distribution of PPR in Enugu State with respect to animal hosts, place and time. The possible risk factors associated with the occurrence of the disease remain unclear. These information are relevant guides for designing control measures against the disease. This study focuses on the prevalence of PPR in sheep and goats in Enugu State, Nigeria and the relationship between occurrence of infection and possible risk factors.

Materials and Methods

One hundred microlitres of blocking buffer - 0.5% negative lamb serum in PBST (PBS + 0.05% Tween 20) was added into N-PPR antigen precoated wells of microtitre plates. This was incubated at room temperature (28oC) for two hours and washed thrice at three minutes interval with PBST.

Also 100µl of either serum samples or negative or positive controls was added into the blocked antigen precoated wells, followed by 100 µl of conjugate (rabbit anti-N-PPR monoclonal antibody conjugated with horse-raddish peroxidase). These were gently mixed, incubated at 28C for one hour and also washed thrice with PBST. Then 100 µl of chromogen/ substrate (Orthophenylenediamine/hydrogen peroxide) was added to each well, similarly incubated for 10 minutes and reaction stopped by the addition of 100µl of stop solution (1M sulphuric acid) per well. Results were read spectrophotometrically in an ELISA reader (Immunoskan, England) at 492 nm wavelength.

Results

PPR infection rate varied from 30.4% in Enugu East to 64.4% in Enugu-Ezike in goats and from 27.3% in Aninri to 43.2% in Enugu-Ezike in sheep. Overall small ruminant prevalence rate also varied from 38.9% in Enugu East to 57.35 in Enugu-Ezike. There was no significant relationship between location and occurrence of PPR in goats but infection in sheep was significantly related to local government areas in Aninri, Oji River and Igbo-Etiti ($p \leq 0.05$). Occurrence of PPR was also related to species of small ruminants being significantly lower in sheep (34.5%) than in goats (53.6%) ($p \leq 0.05$). With respect sex, both species showed significantly higher prevalence in females (64.6% in goats, 41.9% in sheep) than in males (37.8% in goats, 20.5% in sheep) ($p \leq 0.05$). PPR infection rate was also found to significantly increase with the age of both species from 8.4% in goats under one year to 78.0% in those above three years and from 0.0% in sheep below one year to 76.9% in those above three years of age ($p \leq 0.05$).

Discussion

This study shows that PPR is highly prevalent in small ruminants in Enugu State, Nigeria. A variation in infection rate across local government areas may be as a result of

Table 1: Prevalence of PPR in goats and sheep in various Local Government Areas of Enugu State.

Local Govt. Areas	Goats			Sheep			Total
	No Tested	No positive (%)	*p- value*	No Tested	No positive (%)	*p- value*	No positive(%)
Nkanu-West	64	37 (57.8)	0.211	35	12 (34.3)	0.063	49 (49.5)
Enugu-East	33	13 (39.4)	0.223	21	8 (38.1)	0.275	21 (38.9)
Aninri	52	24 (46.2)	0.579	22	6 (27.3)	0.033	30 (40.5)
Oji-River	66	40 (60.6)	0.085	41	13 (31.7)	0.019	53 (49.5)
Igbo-Etiti	59	28 (47.5)	0.696	36	12 (33.3)	0.046	40 (42.1)
Udi	68	32 (47.1)	0.157	27	8 (29.6)	0.034	40 (42.1)
Enugu-Ezike	87	56 (64.4)	0.07	44	19 (43.2)	0.366	75 (57.3)
Total	**429**	**230(53.6)**	**0.134**	**226**	**78 (34.5)**	**< 0.01**	**308 (47.0)**

Table 2: Distribution of PPR in species of small domestic ruminants

Small ruminants	No. tested	No. positive (%)	p-value
Goats (Capra hirtus)	429	230 (53.61)	0.134
Sheep (Ovis aries)	226	78 (34.51)	< 0.01
Total	**655**	**308 (47.02)**	**0.128**

Table 3: Sex distribution of PPR goats and sheep in Enugu State

Sex	Goats			Sheep			Total
	No. tested	No. positive (%)	*p-value*	No. tested	No. positive (%)	*p-value*	No. positive
Males	180	68(37.78)	0.01	78	16(20.50)	< 0.01	84 (32.56%)
Females	249	162(64.62)	< 0.01	148	62(41.89)	0.049	224 (56.42%)
Total	**429**	**230(53.61)**	**0.134**	**226**	**78(34.51)**	**< 0.01**	**308 (47.02%)**

Table 4: Age distribution of PPR in goats and sheep in Enugu State

Age (yr)	Goats			Sheep			Overall total
	No Tested	No positive (%)	*p- value*	No Tested	No positive (%)	*p- value*	
<1	*83*	*7(8.4)*	*< 0.01*	*48*	0	*<0.01*	*131(5.3)*
1<2	*106*	*46(43.4)*	*0.174*	*60*	*13(21.7)*	*< 0.01*	*166(35.5)*
2<3	*117*	*81(69.2)*	*< 0.01*	66	*25(37.9)*	*0.049*	*183(57.9)*
>3	*123*	*96(78.0)*	*< 0.01*	52	*40(76.9)*	*<0.01*	*175(77.7)*
Total	**429**	**230(53.6)**	**0.134**	**226**	**78(34.5)**	**<0.01**	**655(47.0)**

differences in production systems and degree of biosecurity in various localities (Chah *et al.,* 2009). A similar prevalence rate of 50% was also reported among small ruminants in Ethopia (Shaila *et al.,* 1996). Occurrence of PPR was found to be higher in females than in males. Households with only female animals usually borrow males from others for reproductive service when there female animals are on heat. Such practices may lead to more frequent introduction of PPR virus in female animal population. The infection probably varies with practices in various environments. Whereas a higher prevalence was reported in female animals in Gambia (Singh *et al.,* 2004), the contrary was observed in Pakistan in which the prevalence was higher in male goats (Anderson and McKay, 2007).

Higher prevalence of PPR was observed in goats than sheep in this study. The reason for this was not clear. Since in Enugu State, both species are confined and zero-grazed in households, they should therefore be equally exposed. However, similar results have been reported in Pakistan, Turkey and Saudi Arabia (Atta-ur-Rahman *et al.,* 2004; Ozkul *et al.,* 2002; Al-Dubaib, 2010). Specie differences in resistance to PPR may account for this. Infection rate was found to increase with age of small ruminants. It is possible that among the West African Dwarf breed of sheep and goats, younger animals are more resistant to PPR infection than older ones. PPR virus may also be so highly immunogenic that naturally infected animals remain serologically positive for very long periods. Similar finding was reported in Ethiopia (Waret-Szkuta, 2008). The high prevalence and widespread distribution of PPR in Enugu State, Nigeria calls for adequate control measure because small ruminant farm contributes significantly to economy of the population. Vaccination campaign is necessary to protect all animals at risk of infection. Further studies may be required to determine long-term trends and epidemiological patterns which may guide future interventions.

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A REVIEW OF THE PUBLISHED ANATOMICAL RESEARCH ON THE AFRICAN GIANT RAT (Cricetomys gambianusWaterhouse)

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Abstract

Retrospective study of published anatomical research available online was carried out on the African giant rat (AGR) to determine the pattern, frequency and field of study that have received attention or a lack of it. The AGR (Cricetomys gambianus Waterhouse) has been recommended as a micro-livestock of Africa to supplement dietary animal protein with progressive use as landmine and tuberculosis detectors and pet animals. The applied usefulness of the pouched rats can be expanded with a thorough knowledge of their anatomy and morphophysiology however, the scientific bases for these functions are largely unknown. Various authors have provided insight into the anatomy of parts of the body system which reveal information and data upon which relevant inferences can be drawn for further research and determining the potentials and adaptive features of this animal. Scientific publications found on Google and Pubmed were used to access online International journals. Records of all anatomically inclined publications were collated and analyzed excluding abstracts, conference proceedings and unpublished research dissertations and thesis. All data were dated till December 2012. A total of 62 publications were found with reproductive anatomy ranking highest (33.9%) with 21 publications followed by, blood studies and angiology, osteology and renal studies (12.9%) 8 publications apiece then, neuroanatomy (11.3%) with 7 publications. Myology, arthrology and endocrine studies had no research finding. The authors propose a need for research focus on Africa's 2nd largest micro-livestock and rare rodents (AGR) with policies and funding from government and the private sector in order to evolve an African model of rodent for scientific research.

Keywords: African giant rat, anatomical research, review, African model, research focus

UN EXAMEN DES RECHERCHES ANATOMIQUES PUBLIEES SUR LE RAT GEANT D'AFRIQUE (Cricetomys gambianus Waterhouse)

Resume

Une étude rétrospective des recherches anatomiques publiées en ligne a été réalisée sur le rat géant d'Afrique (AGR) dans le but de déterminer le modèle, la fréquence et le champ d'études qui ont reçu ou non une attention particulière. L'AGR (Cricetomys gambianus Waterhouse) a été recommandé comme un mini-élevage en Afrique pour compléter les protéines animales alimentaires, avec une utilisation progressive comme détecteur de mines et de tuberculose et comme animal de compagnie. L'utilité appliquée des rats à poche peut être étendue si on a une connaissance approfondie de leur anatomie et morpho-physiologie, mais les bases scientifiques de ces fonctions sont largement méconnues. Divers auteurs ont donné un aperçu de l'anatomie des parties du corps qui contient des informations et données sur lesquelles des conclusions pertinentes peuvent être tirées pour la recherche et la détermination des potentialités et des fonctions adaptatives de cet animal. Les publications scientifiques trouvées sur Google et Pubmed ont été utilisées pour accéder aux revues internationales en ligne. Les données de toutes les publications sur des formes anatomiques inclinées ont été rassemblées et analysées, à l'exclusion des résumés, des actes de conférences et des mémoires et thèses de recherche non publiés. La date limite pour toutes les données analysées était décembre 2012. Au total, 62 publications ont été trouvées, l'anatomie de la reproduction occupant le premier rang (33,9) avec 21 publications, suivie par les études hématologiques et angiologiques, ostéologiques et rénales (12,9) avec 8 publications chacune, ensuite la neuroanatomie (11,3) avec 7 publications. Les études myologiques, arthrologiques et endocriniennes ne comportaient aucune conclusion de recherche. Les auteurs évoquent le besoin d'un axe de recherche sur les 2èmes grands et

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rares rongeurs de mini-élevage d'Afrique (AGR) avec des politiques et un financement du gouvernement et du secteur privé afin de développer un modèle de rongeur africain pour la recherche scientifique.

Mots-cles : Rat géant d'Afrique ; Recherche anatomique ; Aperçu ; Modèle africain ; Axe de recherche

Introduction

The African giant rat (AGR) also known as the giant pouched rat is one of Africa's largest rodents. In what can be regarded as a most authoritative reference and checklist for mammals, Musser & Carleton (2005) recognized four species of giant pouched rat: Cricetomys gambianus, Cricetomys emini, Cricetomys ansorgei, and Cricetomys kivuensis. Olayemi et al, 2012 noted altogether six species (Cricetomys gambianus, Cricetomys ansorgei, Cricetomys emini, and three undescribed taxa) that can be distinguished on the basis of their mitochondrial DNA sequences and craniometry. Most influential publications however noting the presence of several forms across the geographical range of Cricetomys recognize only two species (Genest-Villard 1967; Rosevear 1969 and Kingdon 1997). The two main species are: Cricetomys gambianus, which lives chiefly in Savannahs and around the regions of forests and human settlements; and Cricetomys emini, which occur mainly in rain forests. They are found in Central Africa, in regions south of the Sahara desert as far south as Zululand; this includes countries such as Nigeria (Ajayi, et. al., 1978; Happlod, 1987). In the indigenous African population, these rats are considered a delicacy and are often hunted for food (Ajayi, 1977a). They are known to be high in protein and are savoured in West Africa and have been a mammal of increasingly diverse interest (Ajayi et. al., 1978).

Several anatomical research efforts on the AGR have been undertaken subsequent to the pilot studies (Ajayi 1974a; 1974b; 1975, Ajayi *et al.,* 1978), to make data available for the successful description of form and structures, utilization, domestication and husbandry of this rodent especially in Nigeria with provision of baseline research data that might interest the research world.This drive may be because of the scientific paucity of the AGR when compared to other rodents found in temperate regions. Also, the rodent has not been fully explored for useful purposes and research despite currently generating a lot of interest in the scientific world.

Rodents are often quoted as mini-livestock species with great potentials, due to their supposedly high rate of reproduction and widespread popularity in certain areas of Africa. As a result, some rodents popular for meat consumption have been studied over the last decades in different African countries; these are basically the cane rat or grasscutter (Thryonomys swinderianus), the brush-tailed porcupine (Atherurus africanus) and the giant rats (Cricetomys gambianus and C. emini). The AGR (Cricetomys gambianus, Waterhouse) has been recommended for supplementation of dietary animal protein intake because of its sheer body size, vast population and availability (FAO, 1970) and is one of the most commonly consumed wildlife especially in Nigeria (Martin, 1985;Anadu *et al.,* 1988). Earlier studies in Africa gave annual wild animal consumption figures ranging from 20% of the animal protein among rural people living in Nigeria's rain forest areas as compared with 13% in the whole country, to 75 % in rural Ghana compared to 9.2% nationally, 70-80 % in Cameroon's forest zone compared with 2.8 % for the entire country and as much as 80-90 % in Liberia (Asibey, 1974).

In recent times in developed countries, the AGR is progressively being recognized as a pet animal (Cooper, 2008).For these populations living in urban, temperate regions, where it is not naturally found, it is scarce and as a result, sold at higher prices being an import pet (Chardonnet *et al.,* 1995; Steel, 1994; Jori, 1997; Wilkie and Carpenter, 1999). However, these rats with their rather large sizes have been found to be sensitive to temperature changes which impact on their metabolism, resulting in a need for high maintenance. Scientific basis for this metabolic pattern is generally unknown. Also, the AGR has been documented as carriers of disease pathogens, especially incriminated in small pox outbreaks

in 2003 (Machang'u*et al.,* 2004). Recent reports show them as potential pest species, as invasive populations have been discovered in the Florida Keys in the USA (Engeman*et al.,* 2007; Perry *et al.,* 2006; Peterson *et al.,* 2006).

APOPO, a Dutch acronym for Anti-PersoonsmijnenOntmijnende Product Ontwikkeling (English: Anti-Personnel Landmines Detection Product Development), is a registered Belgian NGO which trains African giant pouched rats to detect landmines and tuberculosis (Wikipedia, 2011). APOPO's mission is to develop detection rat's technology to provide solutions for global problems and inspire positive social change. This training outfit has opened up a world of possibilities as the rats have been trained to detect landmines in war torn areas and diagnosis of tuberculosis (Verhagen et. al., 2003; Weetjens *et al.,* 2009). This applied usefulness of the pouched rats can be expanded with a thorough knowledge of their anatomy and morpho-physiology.

Anatomy is the branch of basic sciences that deals with the study of the form and function of living organisms (Sisson, 1975). In the Veterinary world, it is divided into different aspects which include gross or macroscopic anatomy, histology or microscopic anatomy, embryology, comparative anatomy and applied anatomy (Sisson, 1975). All these fields provide insight into the anatomy of parts of the body and reveal an immense deal of information and data upon which relevant references can be drawn for further research and or conclusion on the adaptive features of an animal.

This paper therefore seeks to review by highlighting the importance of anatomical studies to the present understanding of the forms and functions of this rodent and to focus on the anatomical research undertaken thus far in a period of over 5 decades, the impact on the increasing knowledge of this animal, and highlight areas receiving insufficient attention in order to promote new fields and topics of research.

Materials and Methods

Data collection

Research design is a database search of Google and Pubmed at the University of Witwatersrand Johannesburg, to access online International journals. Records of all anatomically inclined publications were collated and analyzed excluding abstracts, conference proceedings and unpublished research dissertations and thesis. All data were dated till December 2012. The research publications were described systemically (osteology, arthrology, myology, and splanchnology: digestive, respiratory, urinary, genital, angiology, neuroanatomy, and anaesthesiology). Data distribution is shown using a pie chat.

Results

A total of sixty two scholarly research topics and articles were found published from a combined search of pubmed and google scholar and library resource in the field of anatomy (see tables 1and 2) with reproduction having the greater share with 33.9% while myology, endocrinology and arthrology had no representation. Major advancements in this field began in the early 1980s with morphometric analysis of male accessory glands and other parts of the male reproductive organs including the bulbo urethral gland (Oke and Aire, 1989), prostate gland (Oke and Aire, 1995), vesicular gland (Oke and Aire, 1997), epididymis (Oke et al.1987, Oke, 1995) and the coagulating gland (Oke and Aire, 1996). Oke *et al.,* (1987) divided the epididymis into 5 histological zones and described their structure and histochemical properties. Seasonal effect on the reproductive anatomy was also examined in males (Oke, 1985). It was not until 1989 that ultrastructural studies began with the work of (Oke, 1988; Oke *et al.,* 1989; Oke and Aire, 1990) on the epididymis and other components of the male system. Two sub zones were further elucidated from zone 5 of the epididymis as a further work from pilot studies. They concluded that, the epididymis of the AGR was capable of synthesising abilities as shown by its highly developed endoplasmic reticulum content further corroborating the all year round sexually active status of this rodent. Anatomical investigations of the female reproductive tract commenced with vaginal cytology and seasonal changes (Oke and Oke, 1999, Oke *et al.,* 2000), morphometric and seasonal morphometric evaluations of the

female tract (Akinloye and Oke, 2009b) which revealed the AGR as having a duplex uterus and two cervixes, 8 mammary glands (4 in the lateral inguinal region and 4 in the lateral thoracic region arranged in cranial and caudal rows). Further studies include ultrastractural and hormonal patterns (Akinloye and Oke, 2009a). The most recent internationally recognised publications utilised immunohistochemistry to detect oestrogen, progesterone receptors (where the intensity was tagged to the seasonal variations) and smooth muscle activity in the ovary (Madekurozwa*et al.,* 2010); similarities were noted with wistar rats with some species specific dependency at various stages of the season (Selstam et al 1993; Callebaut and Van Nassauw, 1987). Ali *et al.,* 2010, utilised morphometry to generate data on the non-gravid reproductive organs of the AGR.

Studies on the kidneys have demonstrated calbindin localization in AGR kidney (Moutairou *et al.,* 1996). Other studies have been morphometric (Onyeanusi *et al.,* 2007; 2009) who measured and compared the urinary system of the AGR and the wistar rat taking into account the sex differences and the functional morphology of the kidneys. The earliest published record of any article involving the AGR is the histochemical study of the adrenal cortex (Quenum and Camain 1959). The authors did several related works also on the adrenal cortex, determined the effects of castration on the adrenal cortex and the problem of compensatory hypertrophy of the adrenal cortex (Camain and Quenum, 1961, Quenum, 1962a; Quenum and Camain, 1962 and Quenum, 1962b).

In the field of Osteology, 8 publications were observed as at the time of this review with advancements in the study of the skull. Studies by Olayemi and Akinpelu (2008) and Olude et al (2009a) characterized and classified this rodent on the basis of skull morphometry and showed significant sexually dimorphic parameters in the skull. In addition, other neurocraniometric studies revealed shape variations in the occipital region particularly in the foramen magnum and in the multiplicity of the hypoglossal foramina; the authors also highlighted the possibility of the estimation of the brain density and the use of the African giant rat for cranial pressure experiments (Olude *et al.,* 2009b). Four parameters were statistically significant between both sexes including the intercondylar width, temporal bone height, external auditory pore height and the sub arcuate fossa height in skull typology studies (Olude and Olopade, 2010). Among the morphologic findings was the presence of a ventral mandibular foramen, a complete jugal arch and presence of jugal foramen. The appendicular skeleton has also been described (Olude et.al. 2009c, 2010) with similar studies repeated by Salami et. al.(2011a, b).The pelvic limb studies resulted in a copyright finding on the distal portion of the tibia hitherto not reported in literature which the authors named the tibial depression of Olude(Olude 2009c Nigerian Copyright Commission notification number: CN/L/2560).The forelimb also revealed some prominent features in the forelimb bones of the AGR that typifies them as fast running, burrowing and shoveling rodents due to the presence of the supracondylar foramen which is typically observed only in cats and the well developed ridges and tuberosities which are better developed in all burrowing animals (Olude et. al., 2010). Morphometric studies of the bony orbit and ocular dimensions were also documented (Olude *et al.,* 2011),

Articles on the digestive system began with investigations on the microstereological

Table 1: Broad classification of the published articles on the AGR found in various aspects of the field of anatomy.

Field of study	Published Article
Osteology	8
Myology	-
Arthrology	-
Angiology	8
Respiratory	4
Endocrine	-
Neuroanatomy	7
Reproduction	21
Urinary and Adrenals	8
Digestive	5
Anesthesiology	1
TOTAL	**62**

Table 2: Distribution pattern of publications on the AGR in the past six decades in various fields of anatomy.

Field of study	1951-1960	1961-1970	1971-1980	1981-1990	1991-2000	2001-2010	2011-2012	Total	Percentage (%)
Reproduction	-	-	-	7	6	8	-	21	33.9
Digestive	-	-	-	2	1	2	-	5	8.1
Neuroanatomy	-	2	-	-	3	1	1	7	11.3
Osteology	-	-	-	-	1	4	3	8	12.9
Respiratory	-	-	-	-	1	1	2	4	6.5
Myology	-	-	-	-	-	-	-	-	-
Angiology	-	-	1	1	4	2	-	8	12.9
Urinary	1	4	-	-		3	-	8	12.9
Endocrinology			-	-	-	-			-
Anesthesiology	-	-	-	-	-	1	-	1	1.6
Arthrology	-	-	-	-	-	-	-	-	-
TOTAL	1 (1.6%)	6 (9.7%)	1 (1.6%)	10 (16.1%)	15 (24.2%)	22 (35.5%)	6 (9.7%)	62	100%

and histochemical studies (Asojo and Aire, 1983) describing the structure of the three salivary glands and comparing it with laboratory rodents. Knight M.H. who obtained a PhD in 1988 studying the digestive tract of the AGR had co-published an extensive pilot article on the morphological, anatomical and physiological aspects of the digestive tract of the AGR, to test the relevance of two hypotheses relating structure to dietary habits (Knight and Knight-Ellof, 1987). The authors stated that the gut as a whole appears intermediate on a scale from omnivory to herbivory. The unilocular stomach with its papillated corpus and fornix ventriculi and dense bacterial mass, important in starch, glucose and nitrate reduction, and the fully glandular antrum are not indicative of cellulose digestion. The long small intestine emphasizes its importance in the digestion and absorption of protein and other metabolites; while the rather voluminous, differentiated caecum, with its folds and ridges designed to direct and retard digesta flow, is suggested to have considerable nutritional value. The nnutritional value of the AGR meat has been analysed to contain over 65% Moisture, 20% Crude protein, 11% Fat, 1% Carbohydrates and Ash making up the rest per 100grams of muscle meat (Oyarekua and Ketiku, 2010). The complex gut, with its characteristically long passage rates, is suggested to be a product of many selective forces. Paneth cell studies were done in the stomach and compared with the colon (Satoh *et al.,* 1994). Ali *et al.,* (2008) worked again on the gross morphometric aspects of the gastrointestinal tract of the African giant rat comparing it to the wistar rat, dog, ruminants and equines. Morphometric studies also have been carried out on the accessory digestive organs (Nzalak *et al.,* 2010).

In neuroanatomy, earliest advancements also date back to the work of Quenum(1961;1962c) who studied the various cell components of the pituitary gland. This was later followed by investigations by Piechl and Moutairou (1998) who noted the absence of S-cones retinae of the two species of AGR using immunohistochemical methods. Bastianeli *et al.,* (1999) published their work on the pineal gland where they demonstrated the presence of true neurons in the rodent pineal gland by using immunohistochemistry with five antibodies against calcium-binding proteins (calbindin-D28k, calretinin, calmodulin, neurocalcin and S-100/3) and thus postulated their functions. Ever since then, other publications have focussed on morphometric studies (Nzalak et. al., 2005, Ibe et al, 2010a) and histochemical studies (Ibe *et al.,* 2011a) of different parts of the brain. Presently, immunohistochemical

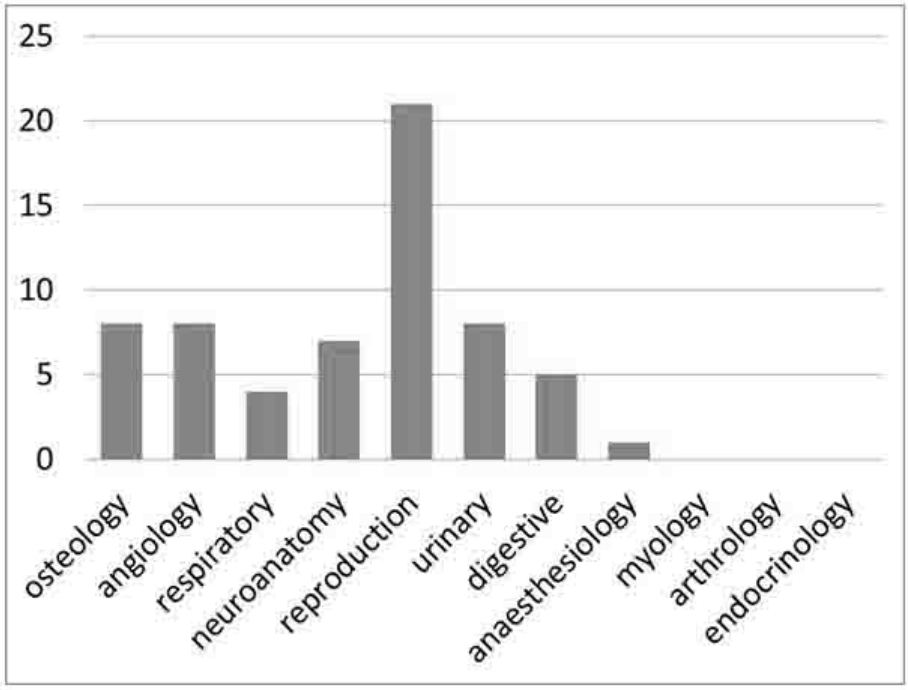

Figure 1: Bar Chart showing distribution of published articles on the AGR in different fields of Veterinary Anatomy in over 6 decades.

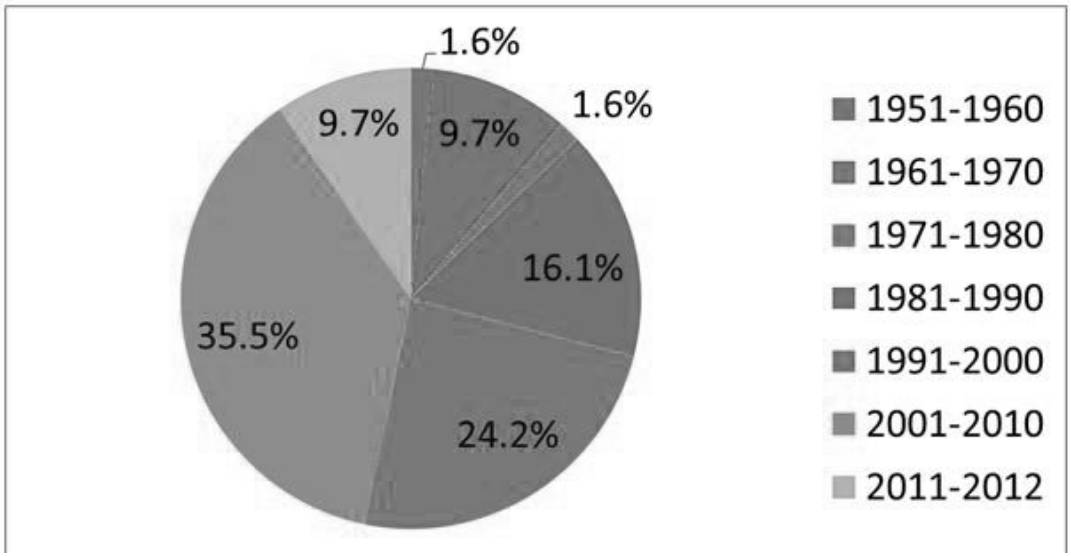

Figure 2: Pie chart showing distribution of published papers on the AGR in each of the decades.

studies of neurogenesis are on-going in our laboratory as a way of advancing this field using the AGR model to study adult neurogenesis (unpublished article). This is in part driven by the quest for domestication and a much recent use of the African giant rat as a research model because of its sheer size, strong olfactory powers and big sized brain for neuroanatomical research.

The earliest published information on the respiratory system was by Valerius (1996) who worked on the comparative morphology and structural patterns in the conductive bronchial tree of four species of myomorph rodents of different body weights. This was determined by lung casts which were inflated to 20 cm H_20, frozen, freeze-dried, hardened, and filled with silicone rubber. The casts were

pruned, and branching pattern, diameter, and volume of the conductive bronchial tree were determined using a binocular magnifier. The AGR in particular was described to have four lobes on the right lung and an undivided left lung, and the central bronchial tree on either side shows an identical monopodial branching pattern. The diameter of the left main bronchus equalled 0.6% of body length in Cricetomys, and the conductive bronchial tree made up 6% of the total lung volume. He concluded that relatively wider airways and a decline in airway resistance with declining body mass in small mammals compared to large ones resulted in a high ventilatory dead space, which is compensated for by a higher breathing frequency. Further work on the respiratory system has been on the vomeronasal organas present in the lateral wall and on the dorso-lateral region between the sensory and non-sensory epithelia; suggesting that the organ is important in sexual behaviours (Igbokwe and Nwaogu, 2009). Most recent publications were on microscopicand macroscopic anatomy of the lower respiratory system (Ibe *et al.,* 2011b, c).

Angiology was limited to blood studies (Oyewale et. al., 1998, Oke et. al., 2000, Olayemi *et al.,* 2001) and recently the spleen with conclusions that the high spleen size is indicative of effective blood conservation in compensation for their subterranean habitat characterised by low oxygen (Ibe *et al.,* 2010c).

Anaesthesiology was low in research with only one article published on the adaptive vibrissae (Ibe *et al.,* 2010b). Our search showed no form of publications in the fields of myology, endocrinology and arthrology

Discussion

Sixty two (62) publications were reviewed of which 50 (about 80%) were authored solely by or in conjunction with Nigerian researchers. The African giant rat is found in various countries of sub Saharan Africa but particular interest of Nigerian authors to the animal can likely be attributed to the early domestication drive by Ajayi in the 1970s. This list is by no means exhaustive considering the methodology which excludes abstracts and unpublished projects, dissertations and theses.

Anatomical articles were largely descriptive entailing morphometric measurements and often did not reflect research specialization by many authors who wrote on virtually any and all systems. This however, could be attributed to the drive to offer baseline research data in all available areas on the rodent; though certain fields of study have been selectively neglected. More so, morphometry is a recognised tool for scientific study of living animals. In the past, morphological studies were restricted to qualitative description of tissue structures and therefore, they are essentially subjective. In recent years, the applications of morphometric and stereological techniques have increased in biomedical research and have been well recognized as a new approach in morphological study (Mukerjee and Rajan, 2006).

It was also noted that some research topics were repeated without due credence or citation of initial authors as in digestive system where Knight and Knight had done a comprehensive pilot study on the digestive system which included morphometric and histologic work in the 80s which were not reflected in the articles of Ali et al. (2008) and Nzalak et al. (2010) who actually did portions of the initial study. A likely cause is a lack of in-depth literature review on the subject matter. It is in our view that though over 40% of anatomic publications on the African giant rat were published in the last 12years, there had been several investigations dating back to the 1950s; and to this end, authors must carry out extensive literature search before conducting their research to avoid repetition. Other possible cause of this repetition is the apparent lack of a unified research focus and continuity in certain fields of study which can be directly linked to a dearth in research grants in sub Saharan Africa; inadequate specialized and advanced equipment for research and the accompanying technical know-how in most local institutions as reflected by the data over fifty years.

There is thus, a need for research focus on Africa's 2nd largest micro-livestock (Asibey and Addo, 2000) and rare rodents (AGR) with policies and funding from government and

the private sector because the vast applied potentials of the rodent have started yielding results for science and scientists all over the world with particular usefulness in African settings e.g. in tuberculosis diagnosis, detection of landmines, their small pox carrier status and usefulness in research as models due to their large size and unexplored anatomy.

Anatomy and indeed Veterinary anatomy as a basic clinical science is most useful in the gathering of research data on the form, structure and function of living organisms; and thus, is a very important tool in the study of this unique rodent of Africa to a logical point where its full usefulness can be utilized to the benefit of mankind. In conclusion, it can be said that a dearth of data exists on the AGR in the field of Veterinary anatomy with many areas lacking a single research article on the internet or pubmed. The authors use this paper as a clarion call to the Nigerian and indeed African governments, private funding organizations, professionals and stakeholders to set up a national, continental or International task force to fully profile the potentials of the AGR and indeed the unique rodents and animals of Africa using the tool of anatomy and other relevant studies to evolve an African rodent model. A model is the work of Olayemi et. al., (2012) which involved International collaborators. This will be greatly facilitated by the establishment of African giant rat colonies after the initial, successful but unsustained efforts of Ajayi (1974b). From such colonies, AGR stem cells can be developed alongside various research topics undertaken in understudied areas.

Impact

Many features make the African giant rat (Cricetomys gambianus, Waterhouse) unique; it is Africa's second largest micro livestock, it has been successfully trained to sniff out landmines and tuberculosis positive samples, its uniqueness to Africa among many others. These features have generated scientific investigations which have been on the increase in recent years. This manuscript thus serves to review the pattern of anatomical research over the decades and analyse the advancements or setbacks in the ongoing anatomical research of the African giant rat.

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FOREIGN BODY RUMEN IMPACTION WITH INDIGESTIBLE MATERIALS IN RUMINANTS IN NIGERIA: A REVIEW

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Abstract

Rumen impaction due to foreign indigestible materials has become one of the major gastro-intestinal disorders in ruminant livestock causing severe loss of production and high mortality rates. Foreign bodies affect the health of animals and cause economic loss to the owner. Among many factors, the current trend of widespread use of polythene bags and other non-biodegradable materials with improper waste disposal constitute major predisposing factors to the development of this condition. In this paper, the Nigerian situation with regard to the prevalence, reported fatalities, composition of recovered foreign materials, risk factors and clinical signs of foreign body rumen impaction have been reviewed. With a view to providing information on the clinical management approaches to the condition, the hemato-biochemical, trace element profile and pathology of the rumen in affected animals, as well as approaches to diagnosis and treatment have been discussed. The review also provides information on the waste disposal situation in Nigeria as it affects the development of this condition with highlights on its economic implications and recommendations on preventive strategies to counteract the problem of rumen impaction.

Keywords: Rumen impaction, Nigeria, foreign body, cattle, sheep, goats.

SURCHARGE DU RUMEN PAR DES MATIERES INDIGESTES ETRANGERES CHEZ LES RUMINANTS AU NIGERIA : UN APERCU

Resume

La surcharge du rumen par des matières indigestes étrangères est devenue l'un des principaux troubles gastro-intestinaux chez les ruminants, et est à l'origine de pertes de production considérables et de taux de mortalité élevés. La présence de corps étrangers dans le rumen affecte la santé des animaux et engendre une perte économique pour le propriétaire. Parmi les principaux facteurs prédisposant au développement de cet état sanitaire figure la tendance actuelle à l'utilisation généralisée de sachets en plastique et d'autres matériaux non biodégradables qui sont à l'origine d'une élimination inadéquate des déchets. Le présent article s'est penché sur la situation au Nigeria en ce qui concerne la prévalence, les mortalités signalées, la composition des matières étrangères récupérées, les facteurs de risque et les signes cliniques de surcharge du rumen par des corps étrangers. Dans la perspective de fournir des informations sur les approches de prise en charge clinique de cette affection, le profil hémato-biochimique et oligo-éléments et la pathologie du rumen chez les animaux affectés, ainsi que les approches de diagnostic et de traitement ont été discutés. L'aperçu fournit également des informations sur la situation de l'élimination des déchets au Nigeria car celle-ci contribue au développement de cette affection, les implications économiques et les recommandations sur les stratégies de prévention afin de contrer le problème de surcharge du rumen.

Mots-clés : Surcharge du rumen ; Nigeria ; Corps étrangers ; Bovins ; Ovins ; Caprins

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Introduction

The indigenous ruminant livestock industry in Nigeria represents a very important national resource, contributing immensely to national health and wealth through supply of protein and industrial raw materials (Alayande and Olorede, 1999). However, efforts at improving livestock productivity in the tropics and subtropics particularly in Nigeria continue to be unrewarding because of near ubiquitous shortage of good quality livestock feeds, rapidly diminishing forage and poor husbandry practices (ILCA, 1986). A high proportion of this livestock population are reared under the extensive system of animal husbandry characterized by uncontrolled movement over a large expanse of land, grossly inadequate feed intake, poor nutrition and high disease prevalence. In Nigeria and West Africa, the ruminant population consists of different breeds of domestic cattle, sheep and goats.
Sheep and goats are reared traditionally at subsistence level. They are usually left to scavenge and cater for their own nourishment (Adeloye, 1985).

Rumen impaction has been described as a condition which results from the accumulation of the indigestible materials in the rumen which interferes with the flow of ingesta leading to distension of the rumen and passing of scanty or no feces (Abdullahi *et al.,* 1984). Gastrointestinal foreign bodies are becoming one of the most common surgical emergencies in veterinary medicine (Tesfaye et al, 2012a). Ingestion of foreign body in cattle was reported to be a condition of great economic importance and causes severe loss of production and high mortality rates (Radostitis *et al.,* 2000). On the other hand, sheep and goats are highly selective feeders and ingest significantly less amount of foreign bodies as compared to cattle (Hailat *et al.,* 1996). However, the ingestion of indigestible materials may occur during period of food scarcity (Igbokwe *et al.,* 2003).

Ingestion of indigestible materials leading to ruminal impaction has been widely believed to be peculiar to the sub-Saharan geographical zone of the northern part of Nigeria because of its heavy livestock population and poor fodder resources (Igbokwe et al, 2003; Remi-Adewumi et al, 2004; Mohammed and Mohammad, 2007). However, a phenomenal increase in the incidence of foreign body rumen impaction proportional to increase in demand and use of polythene materials in urban and rural areas has also been reported by in the southern geographic zone (Akinrinmade and Akinrinde, 2012a, Akinrinmade and Oluwagbemigun, 2011).

Prevalence of Foreign Body Rumen Impaction

Several workers have reported the prevalence of foreign body rumen impaction in ruminants in different parts of the country and in other developing countries. Rumen foreign body occurred less frequently in goats than in sheep due to the selective nature of goats while grazing (Remi-Adewunmi *et al.,* 2004; Murray, 1980).Reports in small ruminants appear to be much more than those in cattle.

Sheep and Goats

An abattoir survey by Remi-Adewumi et al (2004) in Zaria, northern Nigeria, revealed that of 800 small ruminants slaughtered over a 3-month period, 77% (144 out of 187) of sheep and 20.7% (127 out of 613) of goats had indigestible garbage in their rumen. Plastic bag was found to be the most prevalent material as observed in 85% of cases. In a similar abattoir survey carried out in Ibadan, southwest sub-tropic humid zone, over a period of 4 months, Akinrinmade and Akinrinde (2012a) reported a lower prevalence of 9.61% in goats. In both studies, sex and age were found to have significant interaction with rumen impaction, with the condition being more prevalent in female animals than in males, while older animals exhibited higher prevalence than younger ones. Akinrinmade and Akinrinde (2012a) suggested that the difference in prevalence rates from their study and that of Remi-Adewumi et al (2004) could have resulted from differences in ecological/geographic zone and period of studied/examined (October-December compared to May-June) and also the sample size (127 out of 613 for Remi- Adewumi et al as against 432 out 4488 for the study by Akinrinmade and Akinrinde).

Furthermore, Igbokwe et al (2003) studied 540 sheep in Maiduguri, a town in

the semi-arid region of Nigeria, and reported a prevalence of 19.3% in sheep slaughtered at the Maiduguri abattoir. Among a variety of indigestible foreign materials including polythene/cellophane materials, ropes, dry seeds, caked sand, metallic objects, paper, fiber and hair balls, polythene/cellophane materials occurred in 81.6% of the sheep. Again, more females were affected than males.

Elsewhere, several reports have emanated on cases of foreign body rumen impaction in small ruminants. Tiruneh and Yesuwork (2010) reported an overall prevalence of 23.2% (162 out of 697) of rumen foreign bodies in sheep and goats slaughtered at the Addis Ababa municipality abattoir in Ethiopia, over a 5-month period (November to March, 2008). Plastic bags were recovered as the most common foreign bodies, while other foreign bodies retrieved included leather 30 (4.3%), rope 3 (0.4%), hair ball 2 (0.3%), and paper 2 (0.3). Also in Ethiopia, a cross-sectional study of 768 slaughtered sheep and goats at the Luna Export abattoir, East Shoa, Central Ethiopia, revealed a much lower prevalence of 6.1% over a comparable 5-month period (November to March) (Fromsa and Mohammed, 2011). A recent report by Tesfaye et al (2012b) observed a prevalence of 9.2% (53 out of 576) small ruminants slaughtered at Jimma municipal abattoir, Ethiopia from November 2010 to March 2011.

Hailat et al (1996) in a study of foreign bodies in 1453 Awassi sheep presented to a Veterinary Clinic in Jordan during a 27 month period observed that of the 1453 sheep, 130 (20.64%) had rumen impaction by plastic, while also reporting that plastics were the most common materials recovered. Also in Jordan, a study by Hailat et al (1998) in goats revealed that out of 347 rumens examined in the summer of 1996, 39 (11%); 10/136 (7%) rumens at Ajloun and 29/311 (7%) at Irbid slaughterhouses contained plastics. Out of the 888 goats brought to the Veterinary Health Centre (VHC) from January 1993 to September 1997 for treatment of different conditions, 32 (3.6%) had plastic impaction and were treated by rumenotomy.

A retrospective study by Mohammed et al (2006) at the Omdurman Veterinary Hospital in Sudan revealed an incidence of 44.4% in goats over a 5-year period (1998-2002).It was observed that the incidence of rumen foreign body was season-related as the prevalence rate was higher in summer and autumn, while it declined in winter seasons of the year. The abundance of green forage was thought to reduce the incidence during winter.

Cattle

Most reports of foreign body rumen impaction in cattle in Nigeria have focused on case reports of fatalities rather than surveys on the prevalence. Mohammed and Muhammad (2007), in a retrospective study of 45 cattle necropsied over a five-year period (2000-2004) in Zaria, Nigeria, reported as much as 23 (51.2%) cattle that had masses of non-degradable polythene bags in the rumen. A further 40.3% of 67 slaughtered cattle in a meat laboratory within the same period had rumen impaction due to ingestion of polythene materials. However, Akinrinmade and Oluwagbemigun (2011) reported an overall prevalence of 10.77% in 3031 cattle slaughtered over 3 months (March-May) at the municipal abattoir in Ibadan, Southwest Nigeria.

Recent reports by Tesfaye et al (2012a) in Ethiopia observed that out of a total of 384 cattle examined, 92 (23.9%) were found positive for different types of foreign bodies in their rumen and/or reticulum. The study was conducted at the Hirna municipal abattoir in Ethiopia from November 2011 to March 2012.

Fatalities due to Foreign Body Rumen Impaction

Case reports of animal mortalities due to foreign body rumen impaction have been documented (Akinrinmade *et al.,* 1988, Otesile and Akpokodje, 1991, Elsa et al, 1995; Mohammed and Mohammad, 2007). Deaths of animals due to foreign body impaction may occur acutely as in those associated with the occurrence of acute bloat due to blockage of the rumino-reticular orifice and subsequent accumulation of gas from rumen fermentation. In some cases, the animals are lost if management of the bloat is not handled promptly.

Composition of Foreign Body Materials

Several reports, in Nigeria, on the condition of foreign body rumen impaction indicated the presence of indigestible, non-biodegradable materials which include Plastic/ polythene bags, pieces of cloth and leather, ropes, shreds of twine and ropes, hairballs, paper, dry seeds, caked sand, fiber and sometimes, metallic objects (Abdullahi and Mohammed, 1981; Abdullahi et al, 1984; Otesile and Akpokodje, 1991; Igbokwe et al, 2003; Remi-Adewumi et al, 2004; Mohammed and Mohammad; 2007; Akinrinmade and Akinrinde, 2012a, Akinrinmade and Oluwagbemigun, 2011). This is also similar to observations from elsewhere outside Nigeria (Hailat et al, 1996; Tiruneh and Yesuwork, 2010; Fromsa and Mohammed, 2011). Plastic/polythene bags have reportedly been the most common materials observed in the affected animals. Widespread use of polythene bags, lack of adequate and proper legislations on waste disposal predisposes animals to ingestion of these materials. Inadequate availability of feed for animals with dwindling fodder resources especially in the urban areas where small ruminants are left to roam and seek their own food have greatly contributed to the nature of materials ingested by these animals (Garba et al, 1994; Sanni et al, 1998).

Similar natures of impacted materials have been discovered in affected animals in other countries. Plastics, rope, hair, paper, leather were recovered from the rumen of animals studied in Addis Ababa, Ethiopia (Tiruneh and Yesuwork, 2010). At Luna export abattoir, Ethiopia, Polyethylene plastic bags, hairballs and leather were the frequently encountered impacted materials (Fromsa and Mohammed, 2011).

The weights of impacted masses of foreign materials recovered from ruminants usually vary from 0.2-6.0kg in small ruminants (Remi-Adewumi et al, 2004; Igbokwe et al, 2003). In cattle, the weights of impacted masses were between 2.5-4.3kg (Mohammed and Muhammad, 2007). Elsewhere, in an exceptional case of Kankrej cattle being managed surgically for rumen impaction, masses weighing between 15-37kg were recovered from the animals (Suthar et al, 2011).

Risk Factors for Development of Foreign Body Rumen Impaction

Foreign body rumen impaction has been associated with a variety of factors. It has been reported that ingestion of foreign bodies is associated with shortage of forage and increased pollution of grazing land with indigestible foreign bodies (Abdullahi et al, 1984; Otesile and Akpokodje, 1991; Igbokwe et al, 2003; Remi-Adewumi et al, 2004). Similar conclusions were also echoed by Akinrinmade and Akinrinde (2012a) who suggested that foreign body rumen impaction in goat is mainly associated with declining fodder resources that transcend geographical boundaries, suggesting in addition that nutritional inadequacy in terms of quality and quantity appears to be a common denominator. They also highlighted the environmental hazard posed to animals by improper waste disposal and the urgent need for proper legislation.

The challenge of urbanization with improper waste disposal poses great risk to animals that usually are left to roam and find their own food. Pasture and supplementary concentrate feed for intensive livestock management are limited and expensive. As a result, most livestock farmers adopt free-range management system in the urban and semi-urban communities where their animals, mostly sheep and goats, scavenge for food, often going into refuse dumps, which are around the towns (Igbokwe et al, 2003).

In Nigeria, prevalence of foreign bodies was observed to be higher in animals originating from urban settings than from rural areas revealing that management of animals plays a crucial role for the occurrence of foreign bodies. In urban areas, the land available for grazing is generally smaller and animals that are kept under extensive system in urban areas are to graze the refused dumps generated from households and factory wastes (Tesfaye et al, 2012). Polythene plastic bags constitute the most frequently encountered foreign material in cases of impaction. Wide spread use and improper disposal of plastics, which are used for packing of goods could contribute greatly to the occurrence of foreign bodies in the rumen and reticulum. Polythene materials were usually discarded in the environment from packages

for food, water and household wares (Igbokwe et al, 2003).

Shortage of feed during the long dry season increase the likelihood of ingestion of plastic foreign bodies which is also associated with a shortage of feed specifically of minerals and vitamins origin (Tiruneh and Yesuwork, 2010). It was suggested by (Rossow and Horvath, 1985). Water deprivation combined with shortage of feed during the dry season in arid/semi-arid areas of Northern Nigeria has been identified as a major risk factor for the ingestion of foreign bodies (Sanni et a, 1998). It is possible that goats ingest foreign bodies because of shortage of feeds and perhaps specifically, shortage of minerals and vitamins. In goats, insufficient supply of sodium chloride may lead to depraved appetite and chewing of soil and debris (Haenlein, 1994). Copper-, phosphorus- and cobalt-deficient cows have been shown to eat foreign bodies (Spaeis, 1975).

Abnormal appetite or pica, predisposing animals to foreign body impaction, has been associated with phosphorus deficiency (Fraser and Broom, 1990). However, pica may sometimes not be associated with phosphorus deficiency, but rather related to poor nutrition, anemia, iron and cobalt deficiencies and other unknown causes (Fraser and Broom, 1990; Radostits *et al.,* 1994). Deficiency of phosphorus is regarded as the most widespread and economically important of all the mineral disabilities affecting grazing livestock (McDonald et al, 1998). Most livestock grazing areas of tropical countries contain soils and plants low in phosphorus (McDowell, 1992). Sowande et al (2008) reported a significant decrease in phosphorus concentration in the blood of WAD goats and sheep grazing natural pastures in southwest Nigeria and ascribed this to climatic fluctuations. High incidence of foreign body rumen impaction reported by Remi-Adewumi et al (2004) in their study in sheep and goats during the dry season also supported seasonal variation in phosphorus levels. Significantly low phosphorus levels obtained in studies of goats with foreign body impaction may have clinical and economic implications with respect to its probable role in the etio-pathogenesis of foreign body impaction and depraved appetite related disorders (Akinrinmade and Akinrinde, 2012b).

The impact of age and sex as risk factors for foreign body rumen impaction has also been evaluated in several studies. Tiruneh and Yesuwork (2010) reported that animals aged between 3-4 years were found frequently to have rumen foreign bodies. Animals in this age group had more foreign bodies than the young ones because of gradual accumulation of these types of foreign bodies in the rumen. Sanni et al (1995) also earlier suggested that the higher prevalence of foreign body rumen impaction in older than younger ones observed in this study, suggest that the process of formation is slow, gradual and progressive. Reports of higher prevalence of foreign body rumen impaction in older animals have also been observed in other studies (Akinrinmade and Akinrinde, 2012a, 2012b; Remi-Adewumi et al, 2004; Fromsa and Mohammed, 2011).

Generally, rumen impaction with foreign indigestible materials occurs more frequently in females than the males. This is thought to be due to the fact that the females might have increased appetite due to the nutritional demands of estrus, pregnancy and lactation.

Mechanism/Etiopathogenesis of Foreign Body Rumen Impaction

Stray animals are generally seen on the roadsides or refuse eating away the plastic bags and their contents in search of food items. The ingested material e.g. polythene, hinders the process of fermentation and mixing of contents in the rumen leading to indigestion. They also obstruct the orifice between reticulum and omasum. If not removed though surgery, polythenes may become fatal. The plastic bags cannot be digested or passed as such through faeces by an animal. The accumulation of the indigestible materials in the rumen interferes with the flow of ingesta leading to distension of the rumen and passing of scanty or no feces (Abdullahi *et al.,* 1984). They, therefore stay in the gut causing pain and death.

Lambs often lick their hair coat especially after early weaning (Fraser and Broom, 1990), thereby ingesting hair, which eventually form hair balls or trichobezoars

(Radostits *et al.,* 1994). In the young animals, caked sand and paper balls in the rumen might be associated with licking of sandy materials and eating of papers, respectively. Fiber balls or phytobezoars have been reported to be common in areas where fibrous feeds form a large part of the diet (Radostits *et al.,* 1994) like in the sahel region during the dry season. The other materials such as metallic objects, dry seeds and ropes might be indiscriminately ingested when imbedded in other available palatable foods.

Consequences and Clinical Signs of Rumen Impaction

Rumen impaction by foreign bodies is more often asymptomatic in nature and only diagnosed in live animals if the material is accumulated in large amounts. As such, most cases are usually discovered at necropsy or during slaughter of animals at abattoirs (Abdullahi et al, 1984; Remi-Adewumi et al, 2004; Akinrinmade and Akinrinde 2012a,b). Very acute cases have resulted in fatalities with little or no clinical signs. Adequate attention has not been given to the grave consequences of foreign body ingestion due to the fact that the effects of impaction many times, go unnoticed or wrongly diagnosed as it presents similarities to other conditions (Akinrinmade et al, 1988; Garba and Abdullahi, 1995).

However, rumen impaction is known to interfere with flow of ingesta leading to distension of the rumen and passing of scanty or no feces (Abdullahi *et al.,* 1984). The presence of these foreign materials in the rumen and reticulum also hampers the absorption of volatile fatty acids and consequently reduces the rate of animal fattening (Igbokwe *et al.,* 2003).

The various pathological conditions that are encountered due to ingestion of plastic and polythene materials in animals are indigestion, impaction, tympany, polybezoars, and immunosuppression (Singh, 2005). The most common symptoms observed in the affected animal include bloat and may be exhibited by the abnormal bulging of the paralumbar fossa on the left side of the abdominal wall. Acute bloat associated with rumen impaction may be due to the complete obstruction of the rumino-reticular orifice, preventing ingesta from being moved to the next compartment and the stasis resulted in accumulation of the fermented gas in the rumen. The other clinical symptoms may include depression, complete or partial anorexia followed by loss of weight, reduction of milk yield, and suspended rumination. Milk and weight reduction in the affected animals are variable according to the stage of lactation, quantities of foreign bodies ingested and severity of the bloat (Ramaswamy and Sharma, 2011)

Haematological Profile of Animals with Foreign Body Rumen Impaction

The relatively insidious nature of development of rumen impaction more often precludes the establishment of obvious clinical signs. However, alterations in haematology in various disease conditions usually would give an indication of abnormalities occurring in the animal. Previous studies have shown that changes in hematologic and biochemical indices are pointers to various disease conditions, even at sub-clinical levels and have been used to provide diagnostic and prognostic aids in some disease conditions (Jain, 1986; Taiwo and Anosa, 1988).

In the report of Akinrinmade and Akinrinde (2012c) in West African Dwarf goats, it was observed that although packed cell volume, erythrocyte counts, hemoglobin values and white blood cell counts of goats with impaction were within the range of normal values, they were significantly lower than those of goats without foreign body impaction. The findings were attributed to gastrointestinal disease and malnutrition as suggested in previous reports (Akinrinmade et al, 1988, Otesile and Akpokodje, 1991, Remi-Adewumi et al, 2004), as well as an overwhelming of ruminal physiology by the continuous ingestion and accumulation of foreign materials. Abdullahi et al. (1984) also reported that the haematological parameters of sheep with rumen impaction were within the normal range.

In a similar study by Akinrinmade and Akinrinde (2012d) in cattle, significant decreases in mean values of PCV, total erythrocyte count and hemoglobin concentration were observed in cattle with foreign body rumen impaction.

The observation corroborates earlier reports in cattle with foreign body rumen impaction (Vanitha et al, 2010; Mayer et al, 1992). Studies on rumen pathology in Sudanese sheep with rumen impaction showed that sheep with foreign bodies had high white blood cell (WBC) counts and low RBC count, PCV and Hb concentration. The MCV, MCH and MCHC values were high (Bakhiet, 2008). The finding that sheep with plastics had low RBC, PCV, Hb and, after calculation, MCV, MCH and MCHC were increased, is suggesting a microcytic hypochromic type of anemia (Coles, 1986).

Biochemical Profile of Animals with Foreign Body Rumen Impaction

The biochemical changes identified in the clinical cases might be due to poor nutrition and interference with the onward flow of ingesta in the gastrointestinal tract.

Igbokwe et al (2003) reported hypoproteinaemia, hypoalbuminaemia, hyponatraemia and hypochloridaemia in some clinical cases of foreign body impaction in sheep and these are thought to be due to decreased availability of dietary protein and salt. Hypoproteinemia, Hypocalcemia and Hypophosphatemia have also been documented in cattle (Akinrinmade and Akinrinde, 2012c).

Hypoglycaemia in animals with rumen impaction had been earlier observed by Llewellyn (1976) in Friesian cows. Hypoglycaemia might be due to inadequate intake of feed (Ramakrishna, 1994). Similar observations by Akinrinmade and Akinrinde (2012c) reports that Mean serum glucose value in cattle with foreign body impaction was significantly lower than in animals without foreign body impaction and other reference values. Low level of glucose in animals with foreign body rumen impaction was ascribed to high levels of free fatty acids and cholesterol associated with reduced energy intake, decreased water deprivation and decreased glucose synthesis (McDowell, 1992). However, Igbokwe et al (2003) observed hyperglycemia in sheep with foreign body rumen impaction. This was attributed to a response to the systemic stress induced by clinical rumen impaction (Duncan and Prasse, 1977).

Zilva and Pannall, 1984 suggested that Hypoalbuminaemia is often accompanied by hypocalcaemia because plasma albumin binds Ca2+. However, other causes of hypocalcaemia like dietary deficiency might also have existed. Hypocalcaemia might also be due to failure of calcium absorption due to reduced ruminal motility (Vanitha et al, 2010).

Trace Element Profile of Animals with Foreign Body Rumen Impaction

Trace element assessment is normally done to determine the presence or prevalence of nutrient deficiencies and evaluate the efficacy of dietary supplementation or to compare available supplement. Physiological functions are progressively affected by deficiencies. Economically important effects on performance and health of animals can be affected by trace element deficiencies even before clinical signs are evident (Kincaid, 1999).

The precise role of individual trace element in the etio-pathogenesis of foreign body rumen impaction has not been ascertained. Akinrinmade and Akinrinde (2012c) reported significant decreases in the mean values of copper, zinc, manganese, selenium and cobalt in cattle with foreign body impaction. The observed decrease in the mean values of these trace elements could be ascribed to the presence of foreign bodies in the rumen. In ruminants, efficiency of absorption of many trace minerals and dietary factors that affect

Figure 1: Cow feeding on a refuse dump (Courtsey Reddy and Sasikala, 2012).

Figure 2: Scavenging cattle at a grazing point littered with sed polythene materials (Mohammed and Mohammad, 2007)

Figure 3: Animals eating plastic bags (Ghurashi et al, 2009).

bioavailability of minerals differ greatly. Because ruminant diets are usually high in fiber and considerable digestion of fiber occurs via microbial fermentation in the rumen, this process may be hampered by the presence of foreign body materials. Association of minerals with fiber fractions in feedstuff (Whitehead et al, 1985) and/or binding of minerals to indigestible constituents may alter bioavailability of some trace minerals in ruminants (Kabaija and Smith, 1988). This probably explains the observed decrease in the mean values of these minerals

Pathology of the Rumen In Relation To Impaction

The main pathological lesions encountered in sheep with plastics in their rumens are rumenitis, erosion and hyperplasia (Hailat *et al.,* 1995). Pathological hyperplasia may be a precursor to neoplastic proliferation (Robbins *et al.,* 1984). It is, however, unclear whether these hyperplasic changes were due to the mechanical irritation induced by the ingested plastic or to some chemical substances released from the plastic irritating the proliferation of the ruminal epithelium (Hailat *et al.,* 1998).

Bakhiet (2008) provided considerable insight into the nature of pathological changes in affected sheep as the study observed signs of rumenitis, haemorrhage, and presence of foreign body on gross examination with majority of plastic foreign body cases and impaction. These were confirmed with microscopic examination that showed that rumenitis papillae damage was present. On necropsy, areas of congestion, haemorrhages and stunting of the papillae, thickening of the wall, erosion, ulceration and scar formation were seen in the rumen. In sparsed papillae, there were shortening. In other areas complete loss of patches of papillae was evident. Sloughed mucosa was also observed. Histopathological lesions concerned with foreign body include hydropic degeneration, cellular vacuolation, submucosal oedema and disruption of stratified epithelium with dilated lymphatics in sub mucosa. Focal hyperplasia of the ruminal epithelium in different regions was also prominent.

The sloughing, stunting, erosions, inflammatory response, and the hyperplasia are most likely to be due to the pressure on the wall of the rumen caused by the foreign body. This may also be due to the chronic irritation of the fore-stomach wall by the sloughing, stunting, erosions, inflammatory response, and the hyperplasia were thought to be most likely due to the pressure on the wall of the rumen caused by the foreign body and also the chronic irritation of the fore-stomach wall by the foreign body leaving the wall exposed to secondary infection which resulted in both inflammatory and hyperplasic changes.

Histopathological examinations by Hailat et al (1996) revealed that lesions in the rumen and/or reticulum of sheep with plastics were made up of focal to extensive mostly superficial coagulative necrosis affecting the mucosa and submucosa, and erosive but occasionally ulcerative type lesions were evident. Marked hyperemia and inflammatory cellular infiltration with mainly neutrophilic granulocytes were evident in the mucosa and sometimes extending into the submucosa. Focal hyperplasia of the mucosal epithelium in different regions was also prominent. It had the

appearance of fingerlike projections of variable length and was growing towards the lamina propria and the underlying submucosa and in some cases reaching the muscularis. Several mitotic figures and cells with more than one nucleolus were seen in hyperplastic foci.

Diagnosis of Foreign Body Rumen Impaction

Field diagnosis of the presence of ruminal foreign bodies is naturally a difficult one, except where exploratory surgery is involved. The diagnosis of rumen impaction based on abdominal ballotment in small ruminants may be inconclusive (Abdullahi et al 1, 984) because masses of impacted foreign materials may be mistaken for a fetus during ballotment (Akinrinmade et al, 1988; Garba and Abdullahi, 1995).

Affected animals with the clinical condition often present with signs indicative of primary rumen dysfunction. In coming to a diagnosis of rumen impaction, appropriate history including duration of observed problems, appetite, number of animals affected in the herd, type of feed, use of additives/ mineral supplements, recent changes in diet and husbandry practices are obtained from the owner (Abdullahi et al, 1984). This is often combined with thorough physical examination including determination of rectal temperatures, respiratory and pulse rates, examination of mucous membranes, systematic examination of the gastro-intestinal tract, rumen motility through the abdominal wall of the left flank, symmetry of the abdomen and rectal palpation. Wither test by pinching withers to cause depression of back and eliciting grunt is effective diagnostic tool usually heard 2-3 seconds before primary ruminal contraction can be felt through the left flank (William, 1955).

Boodur et al (2010) reported that main diagnostic sign noticed was bilateral sunken flank region with doughy on hard impaction of rumen (Kohli et al 1998). Boodur et al (2010) reported that alkaline pH can be important diagnostic tool and field condition for early detection of plastic indigestion cases. Tripati et al (2010) observed low pitched reticular sounds audible on auscultation at 7th to 8th rib on left side with severe distention in left para lumbar fossa and slight distention in right flank for diagnosis in foreign body associated with plastics in 4year old crossbred cow.

The most reliable non-invasive diagnostic method in cattle is by rectal palpation (Abdullahi et al, 1984). This technique has been described by Grymer and Ames (1981) as very useful in the diagnosis of diseases of the digestive tract, particularly those due to primary rumen dysfunctions. Definitive diagnosis of rumen impaction has been achieved through exploratory rumentomy (Akinrinmade et al, 1988; Elsa et al, 1995; Igbokwe and Kolo, 2005). Radiography (Akinrinmade et al, 1988) and Ultrasonography (Arthar et al, 2010) have proved to be very reliable tools in the diagnosis of foreign body impaction.

Clinical Management of Rumen Impaction

Emptying the rumen by rumenotomy is considered as the only method for the management of foreign body rumen impaction (Boodur et al, 2010). Suthal et al, 2011 also regarded surgery as the only effective method of treatment of plastic foreign bodies but that an early diagnosis is essential for a favorable outcome. Surgical removal of foreign body resulted in progressive increase in body weight attributed to increase in apettite (Igbokwe and Kolo, 2005). Rumenotomy along with transplantation of fresh ruminal cud have been found satisfactory in restoration of normal ruminal function (Suthal et al, 2011) at the field level for treatment of chronic impaction due to plastics/polythene foreign bodies. Post-operatively, ample amount of fluid therapy along with antibiotics, analgesics and antihistaminics should be administered. Pre-biotic oral administration has proven to be rewarding in reviving normal fermentation process in the rumen following surgery (Suthal et al, 2011). Restoration of ruminal ecology and function after surgical manipulations ensure maximum benefit from surgery (Purohit, 2010).

Clinical cases of rumen impaction due to dietary error were successfully managed in bovines using the prokinetic effect of metclopramide in combination with fluids and vitamin B complex injection (Vijayalakshmi et al, 2010). An effective prokinetic drug induces forestomach motility in a co-ordinated sequence of contractions and relaxation of

sphincter. Metoclopramide increases the rate of ruminal contraction and might be beneficial in rumen hypomotility associated with foreign body rumen impaction (Radostits et al 2009) El-Khodery and Sato 2008 suggested the use of metoclopramide at 0.3mg/kg to produce mild and transient prokinetic effect.

Economic Implications of Rumen Impaction

This condition is economically important because of the severe loss of production. It causes high mortality rate and many cases go unrecognized. Among the clinically affected animals, about 25% develop incurable condition while other 75% can be expected to recover completely with conservative treatment or routine surgical intervention (Radostits *et al.,* 1994). Diseases of the rumen and reticulum are of great economic importance because of severe losses on the productivity of the animals, sometimes leading to death of the animals.

Reduction in milk yield may be consequence of rumen impaction. As noted earlier, milk and weight reduction in the affected animals are variable according to the stage of lactation, quantities of foreign bodies ingested and severity of the bloat (Ramaswamy and Sharma, 2011).

In monetary terms, a recent survey by Remi-Adewumi et al (2011) concluded that an estimated annual financial loss of over thirty-eight billion (N38,315,689,716= $247,197,997) Naira due to rumen impaction is incurred in Northern Nigeria alone. These were reported to be estimates from surgical treatment (N8,005,270,451= $51,646,906), poor live animals prices (N1,574,094,119= $10,155,446), poor carcass weight prices (N3,521,755,346= $22,721,002), premature culling (N22,969,286,052= $148,188,942) and mortalities (N2,245,283,748= $14,485,701).

In Jordan, it was estimated that losses in sheep production and health, due to plastic impaction was around fifteen million dollars in 1993 (Al-Dwery, 1994).

It may be predicted that foreign body impaction would be a growing problem for grazing animals in Nigeria, as grazing lands become more and more polluted with plastics and other non-biodegradable materials.

Prevention of Foreign Body Rumen Impaction

In view of the prevailing environmental issues that pre-dispose animals to the ingestion of indigestible foreign materials, it is essential that preventive measures be taken to at least minimize the ever-increasing incidence of the condition. A variety of measures have been advocated for prevention by several workers:

- Government should enforce strict regulations on waste disposal with animal confinement in towns and cities (Remi-Adewumi et al; Akinrinmade and Akinrinde, 2012a,b)
- Enforcement of recycling of plastic bags and other environmental pollutants so that threat to environment and life can be reduced (Ramaswamy and Sharma, 2011)
- Public education through mass media on careless disposal of plastic bags as well as periodical cleaning of these wastes in the grazing area (Remi-Adewumi et al, 2011; Ramaswamy and Sharma, 2011)
- Production of biodegradable packaging materials e.g. papers and cardboards, which would not persist in the environment or bottles and tins which can be readily recycled (Remi-Adewumi et al, 2011)
- Good husbandry practices. i.e. Intensive or semi-intensive system is encouraged. Cattle owners are advised not to allow their animals to freely wander in streets, especially in cities (Ramaswamy and Sharma, 2011)
- The municipal authorities in cities and towns and peri-urban areas should provide covered disposal bins for polythene materials separately to avoid ingestion by the animals (Reddy and Sasikala, 2012).
- Establishment of grazing reserves across the entire geopolitical/geographic/ecologic zones of Nigeria.

Environmental Policy on Waste Disposal

As earlier observed in this review, plastic bags and polythene materials have been prominent among materials causing rumen impaction. Open dumping of residential wastes including plastic is observed commonly in almost all parts of municipalities. Dumping is commonly observed near road side, open plots, river side, in drains and public places. Residents

commonly use plastic sacks and polythene bags for storing their wastes. From the temporary and final waste disposal sites, the stray and other domestic animals like cattle, sheep, goats and donkeys engulf these plastic bags containing food materials inside.

In recent years in Nigeria, the level of public concern and response of the government about environmental waste pollution has increased. The challenge of addressing the problem of waste pollution of the environment was endorsed 'Agenda 21' of the 1992 Summit on environment and development by the United Nations Conference on Environment and Development (UNCED, 1992; Adedayo, 2000). The goals, as stated, seek the achievement of environmentally sound management through increased safe disposal, recovery of materials and sustainable patterns of production and consumption of goods and services. The targets of these goals were to decouple economic growth from ultimate waste yields, prevent and minimize hazardous waste generation.

However, as in other policy areas, the Nigerian Government particularly at the local level has displayed weakness in the implementation of environmental sanitation and waste management policies. According to Adedayo (2000), this implementation gap in policy is a function of a number of constraints, including inadequate and unco-ordinated enforcement of legislation, institutional problems (e.g. shortage of manpower and financial resources, inadequate technology), inadequate public awareness and participation as well as shifts in public policy which causes distortion or discontinuity of environmental programs or alterations in priorities.

Successful waste management in Nigeria will require a holistic program that will integrate all the technical, economic, social, cultural and psychological factors often ignored in solid waste programs (Agunwamba, 1998).

Conclusions

Rumen impaction due to foreign indigestible materials has become a major constraint at improving the fortunes of indigenous livestock productivity in Nigeria. The situation with regard to the prevalence, risk factors, diagnosis and management constitute a great challenge to both the clinician and livestock producer. The problem of waste disposal has become 'endemic' to tropical environments, with adverse effects exerted on both human and animal populations. The associated economic losses are enormous and overwhelming. In the light of the above, it is pertinent that the recommendations suggested in this review be given prompt consideration by both the government and the animal producers.

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RIFT VALLEY FEVER IN CAMELS IN NORTHERN BURKINA FASO

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Abstract

This study was done in three provinces located in Northern Burkina Faso, home of about 15705 camels. To investigate Rift Valley Fever (RVF) in these animals, serological examinations including Virus neutralization Test (VNT) were performed on 270 camel serum samples. Positive results were obtained in 140 (51.85%) camels thus tested. Seventy five percent of seropositive camels were adult ≥ than 4 years old and the remaining 25% were young ranging from 8 months to 4 years. The results of the questionnaires administrated during the sampling to the shepherds and owners showed that association of abortion and mortalities in young animals were often observed.

The survey revealed that high prevalence of RVFV is observed in camels in the sahelian desert zone of Burkina Faso which is not routinely diagnosed. Recommendations for systematic RVF investigation in camels and others domestic ruminants were made in order to improve the animal productivity. Habitual consumption of raw milk and close contact with infected animals signify possible zoonotic importance of RVF in the studied area. A risk assessment of the disease should be also undertaken in order to understanding the epidemiology and knowledge of the disease in the country and the sahelian region.

Keywords: Serology, Camel, Rift Valley Fever, IgG, IgM, Seroneutralisation, Public health, Northern Burkina Faso.

FIEVRE DE LA VALLEE DU RIFT CHEZ LES CHAMEAUX DANS LE NORD DU BURKINA FASO

Resume

Cette étude a été réalisée dans trois provinces situées dans le Nord du Burkina Faso, qui abrite environ 15.705 chameaux. Pour étudier la fièvre de la Vallée du Rift (FVR) chez ces animaux, les examens sérologiques dont le test de séroneutralisation (SNT) ont été effectués sur 270 échantillons de sérum des chameaux. Des résultats positifs ont été obtenus chez 140 (51,85%) chameaux ainsi examinés. Soixante-quinze pour cent des chameaux séropositifs étaient des adultes ≥ 4 ans et les 25% restants étaient des juvéniles dont l'âge variait entre 8 mois et 4 ans. Les résultats des questionnaires administrés aux bergers et aux propriétaires lors de l'échantillonnage ont révélé une association entre l'avortement et les mortalités observés chez les jeunes animaux.

L'enquête a démontré une forte prévalence du virus de la FVR chez les chameaux de la zone désertique sahélienne du Burkina Faso qui n'est pas régulièrement soumise au diagnostic. Des recommandations pour un dépistage systématique de la FVR chez les chameaux et autres ruminants domestiques ont été formulées, en vue d'améliorer la productivité des animaux. La consommation habituelle de lait cru et le contact étroit avec des animaux infectés signifient une importance zoonotique possible de la FVR dans la zone étudiée. Une évaluation des risques de la maladie doit être également menée afin de

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mieux comprendre l'épidémiologie et connaître la maladie dans le pays et dans la région sahélienne.

Mots-clés : Sérologie ; Chameau ; Fièvre de la Vallée du Rift, IgG, IgM, Séroneutralisation ; Santé publique ; Nord du Burkina Faso

Introduction

Camels (camelus dromedarius) are vital domestic animal species that are known to be best adapted to harsh environments and fluctuating nutritional conditions of arid and extreme arid zones (Bekele, 2004). In Burkina Faso, camels are mainly reared in the sahelian region, the desert part of the country. Camel population was estimated at 15,401 heads with annual growth and operating rates of 2% and 8% respectively (MRA, 2010). Camel husbandry is mainly practiced by specific socio-ethnic groups, Touareg and Sonrai. Its plays a paramount role in the life of the populations of this dry zone. Subsistence camel production is practiced in the sahelian region where cattle and small ruminants (sheep and goats) are also intensively reared. The region is also known to be the heartland of camel production as around 84% of population is produced in this region.

The increasing demand of animal products (milk and meat including hides) inherent to human population growth, camels' meat and milk are also consumed not only by Touareg and Sonrai in sahelian region but also all over the country mainly in urban areas. Despite the low production capacity, the camel milk has the potential to permanently change the livelihoods of poor communities living in arid and semi-arid lands (Musiga *et al.,* 2008). In addition, they also serve as a draught animal for agriculture and most intensively for transport of people as well as goods (Swartz and Dioli, 1992). Indeed, despite the arrival of motorized transport, camels are the sole means of transport in this arid and semi-arid zone as well as water lifting.

In spite of its vital importance particularly to the marginalized communities in these dry zones of the country, studies about camels are almost nonexistent. Due to the fact that camel production is usually a migratory system in remote areas with harsh living conditions and poor infrastructures, the animals are presumed to be inaccessible to research (Bekele, 2004). In many African countries, camels are considered as neglected animals, because, from global perspective, their economic production seems minimal. In Burkina Faso, they are subset of huge livestock resource when considered from national economic point of view (MRA; 2010) and no development project features camels. Generally there is negligence towards the promotion of camel health and production in most African countries (Kane et al; 2002). It is only recently that camels became the subject of more intensive and systematic interest (Baumann et al, 1992) because of the susceptibility to many diseases both parasitic (trypanosomiasis) (Demeke, 1998) and infectious (poxvirus) (Kane et al.; 2002) and most recently Peste des petits ruminants (PPR) (Abraham et al. 2005; Khalafalla et al. 2010) as well as Rift valley fever (Ould El Mamy et al. 2011; El-Harrak et al. 2011). RVF is one the infectious diseases that affect both camels and camels owners (Eisa, 1977; Iman *et al.,* 1979; Eisa, 1981). Although, the prevalence of RVFV in domestic ruminant is known (Some, 1989), the status of the disease in camels has not been investigated in Burkina Faso. The disease has been reported recently in 2010 in camels with heavy losses in Mauritania coupled with human cases (Ould El Mamy et al. 2011). The aim of the present study was to investigate the presence of RVF virus in camels in Burkina Faso and its impacts in livestock rearing system and public health.

Materials and Methods

Study areas

The study was conducted in two provinces (Seno and Oudalan) of the sahelian region of Burkina Faso bordering with Niger and Mali from November 2006 to March 2007). The region has high (84%) concentrated populations of camels of the country. It has several dams, swamps and ponds. The climate is arid with average rainfall ranging from 300 to 500 mm per annum. Surface water is a

paramount serious problem in the region. Traditional deep wells, sinking, natural and artificial damps, swamps, ephemeral ponds and shallow wells are water sources for both human and livestock.

Livestock rearing is mainly characterized by extensive pastoral production system and seasonal mobility. Cattle are dominating livestock species 20.6% followed by goats16.78%, and sheep 14.02% in addition to camels 84%. Cattle and camel herd are divided into seasonal migration (transhumance) and home-based as a strategy to mitigate forage and water scarcity (Desta, 2000) but also as provide milk to the family.

Sampling and analysis methods

Serum samples were collected from camels throughout the sahelian region of Burkina Faso mainly in Seno and Oudalan provinces from November 2006 to March 2007. Herd, age, sex, clinical story and date and place of sampling were systematically recorded in a structured data collection form. Blood samples were drawn from 270 camels and three serological tests were performed. These tests included indirect ELSA for IgG, competitive ELISA (cELISA) for IgM and virus neutralization test (VNT) (Paweska *et al.,* 2005).

Results

Positive results were recorded in 80 (29.62%) camels for IgG and 140 (51.85%) camels for VNT. All the samples were found negative for IgM. RFV virus IgG antibodies were found positive in 25 (19.23%) and 45 (32.14%) camels in Oudalan and Seno provinces while respectively 50 (38.46%) and 90 (64.28%) camel samples were positive to VNT test. The distribution of RVFV antibodies varies significantly to the province Oudalan and Seno were there natural seasonal swamps favourable the multiplication of the disease vector. Seventy per cent of the positive camels (56) were adults older than 5 years and the remaining 30% were younger (24) from 9 months to 5 years for IgG. One hundred (78.57%) positive camels were adults and 30 (21.43%) were young for VNT test. Sex has not influence the RVFV antibodies distribution. Sixty five (28.88%) positive camels were females and 15 (33.33%) males for IgG and 25 (55.55%) males and 115 females (51.11%) were positive to VNT test.

In infected herds, abortion cases, stillborn and stillbirths were reported by herders and camels owners.

Discussions

The results of the study indicated the serological evidence of RFV virus in camels in the Sahelian region of Burkina Faso. These results corroborate with the previous study conducted 30 years ago that RVFV was circulating among the domestic ruminants (Some, 1988; Akakpo *et al.,* 1994) despite the absence of clinical disease (swanepoel *et al.,* 2004). High seroprevalence of RVFV antibodies have been described in different sub-Saharan countries and sahelian countries (Davies et al, 1985; Formenty *et al.,* 1992Mariner et al, 1995; Olaleye *et al.,* 1996, Abd el-rahim et al, 1997), either by epidemics outbreaks (Senegal and Mauritania), virus isolation or serological evidence in Cote-Ivoire, Mali, Niger, Nigeria, Burkina Faso and Central African region (Zeller et al. 1995; Ringot *et al.,* 2003; Lebreton *et al.,* 2006; Martin et al, 2008). Camels have been involved in the spread of the disease in some instances (Scott et al. 1963; Abd el-rahim et al, 1997, Labeaud *et al.,* 2008; Linthicum et al.1999; Ould El Mamy *et al.,* 2011; Schwartz *et al.,* 1992).

The results clearly indicate high seroprevalence of RVFV antibodies in older animals than younger camels, showing that could have occurred some years ago. Previous study also support that camels moving across the Sahara have contact with RVFV (eisa *et al.,* 1977; Mehdi al-Haralk et al, 2011). In particular semi-arid and arid areas such as the Sahel and desert such as the Sahelian region of Burkina where the study was conducted, particular attention should be paid to singular wet areas such as the oases (Chevalier *et al.,* 2003). Indeed, in our study, the highest seroprevalence of RFVV antibodies was found in Seno province where there semi-permanent large swamp which is the grazing and water points of almost 80% of the province livestock population during the dry season. The presence

of water and pasture makes this environment favourable to the multiplication of the disease vectors and hosts and could be considered as breeding site for mosquitoes (Chevalier et al. 2003; Sissoko et al 2009, El-Harrak et al, 2011). In addition, local climatic parameters can play a central role in determining the distribution and abundance of these vector organisms, either directly or indirectly, through the effects of such parameters on the host animals (Martin *et al.,* 2008). The population of camels of the region should be monitored for RFVF and others vectors borne diseases. The impact both artificial ponds and temporary natural swamps on human and animal health should be investigated to identify advantages and drawbacks of a possible alternative to their use for livestock in the region and the country (Thiongane et al. 2000; Chevalier *et al.,* 2003).

In most of the Saharan region of Africa, camels are valuable livestock appreciated as source of meat, milk and as main means for transportation for both human and goods (Desta. 2000; Bekele, 2004; Medhi Al-Harrak, 2011). The movement of camels across the Sahara desert could carry the disease from North Africa to West Africa desert where the study was carried out. It should be noted that RFVV was previously isolated from blood samples from healthy, naturally infected camels in Egypt and Sudan (Iman *et al.,* 1979; Eisa *et al.,* 1981;El-Harrak et al. 2011)). Furthermore, camels are suspected of playing major role in RFV propagation from northern Sudan to southern Egypt (Eisa *et al.,* 1977). This could explain the seroprevalence of camels in Oudalan province bordering Niger and Mali desert with same populations living across the countries and also sharing the same desert areas from western desert to northern desert in Sudan and Egypt (Meegan et al. 1977).

The results of the VNT test showed high seroprevalence of RFV-specific antibodies of 51.85% in camels in the region with 38.46% and 64.28% respectively for Oudalan and Seno province. This confirmed the evidence of RFVV circulation in the region without any clinical outbreaks (Zeller et al. 1995; Thiongane et al. 2000; Martin *et al.,* 2008). The presence of RVFV-specific antibodies indicates that the infection starts in early life probably through sucking and the younger animals may have inherited through maternal immunity (Medhi El-Harrak *et al.,* 2011). Similar patterns were reported in cattle, sheep, goats in Chad (Ringot *et al.,* 2003) in Senegal (Chevalier et al. 2005) and camels in Kenya (Labeaud et al. 2008) that positive animals were younger 3 years.

The recent unexpected occurrence of RVF outbreak in Mauritania has heavily impacted camels with human cases (Ould El Mamy *et al.,* 2011) has introduced a new dimension to the epidemiology of the disease. This study recommended that camels could be among livestock species that could function as particularly sensitive indicators of RVF activity, because of their longevity and specific range of movement. The national veterinary authorities need to consider camels as risk species for the disease and undertake RVF risk assessment in the country (Consultative group for RVF Decision-Support; 2010). An active laboratory-based surveillance system for RFV should be implemented for early detection the occurrence of the disease and early response in order to undertake preventive activities as well as the use of existing early warning systems and risk assessment tools for better forecasting of RVF in the sahelian region (Linthicum et al. 1999; Martin et al. 2008). Sero-surveillance programs should be carried out across a range of environments throughout the sahelian region targeting different animal species that occupy this ecological niche. Despite the fact that, RVF infection in humans can be acquired through mosquito bites; the primary risk factors are contact with infected domestic animals or animal parts, or consumption of raw meat, blood, or milk (Britch et al.; 2013). With the increase demand of camel milk for human consumption, a strong public sensitization and awareness should be undertaken coupled with camel owner's education on these risk factors in close collaboration with public health.

The results of the study establish the need to continue and expand sero-surveillance of domestic animal species including wildlife species in the studies areas and elsewhere in the sahelian region to further research and improve the RVF epidemiology knowledge, and better understand the dynamics of RVFV transmission.

Conclusion

This survey confirmed the serological evidence of RVF virus specific antibodies in camels in the sahelian region of Burkina Faso showing a significant prevalence of 29.62% for IgG and 51.85% to VNT. The results of this study support that camels moving across the Sahara desert have contact with RVFV.

Intervention strategies should include safe breeding systems, lab-based surveillance system with regular serological testing in order to alert veterinary services, health care providers, epidemiologists as well as policy makers. Furthermore, particular attention should be paid to singular wet areas such as oases, natural swamps and artificial ponds. Thus, it is recommended that regular serosurveillance study to be carried out through sentinel herds of both camels and domestic ruminants. Further studies are also needed to assess the influence of ecologic factors on Aedes abundance and their relationships to the risk for RVF transmission around the temporary ponds and swamps. It is also recommended that the use of existing knowledge and generate new data to develop systems for anticipating, preventing and controlling changes in the occurrence and distribution of certain climate-associated transboundary animal diseases and zoonoses mainly RFV and its potential outbreaks in the sahelian region as well as the other vector-borne diseases.

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THE IMMUNOLOGICAL RELATIONSHIP BETWEEN TYPANOSOMA EVANSI AND TRYPANOSOMA VIVAX: SERUM NEUTRALIZATION STUDIES FINDINGS

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Summary

Trypanosoma evansi and Trypanosoma vivax show very high cross-reactivity in serology. This suggests that there is a close immunological relationship between the two parasite species and the possibility that antiserum against one species contains lytic antibodies against the other species was investigated. T. evansi and T. vivax cell free trypanosomes were mixed with negative serum control, anti T. vivax and anti T. evansi sera respectively at room temperature for three hours. Trypanosome counts after incubation showed that anti T. evansi serum contained lytic antibodies that lysed a large proportion of T. vivax trypomastigotes but anti T. vivax serum had no effect on T. evansi trypomastigotes. And the two parasite species were still able to induce infection in sheep after incubation in the above antisera.

Key words: Immunological relationship, T. evansi, T. vivax, lytic antibodies

LA RELATION IMMUNOLOGIQUE ENTRE TYPANOSOMA EVANSI ET TRYPANOSOMA VIVAX : CONCLUSIONS DES ETUDES DE SERONEUTRALIZATION

Résumé

Trypanosoma evansi et Trypanosoma vivax montrent une forte réactivité croisée en sérologie. Ceci fait penser qu'il existe une relation immunologique étroite entre les deux espèces de parasite ; et la possibilité que l'antisérum contre une espèce contienne des anticorps lytiques contre l'autre espèce a été étudiée. Des trypanosomes acellulaires T. evansi et T. vivax ont été mélangés avec du sérum de contrôle négatif, des sérums anti T. vivax et anti T. evansi à la température ambiante pendant trois heures. Les numérations de trypanosomes après incubation ont montré que le sérum de T. evansi contenait des anticorps lytiques qui lysaient une grande proportion des trypomastigotes de T. vivax, tandis que le sérum anti T. vivax n'a eu aucun effet sur les trypomastigotes de T. evansi. Et les deux espèces de parasites étaient toujours susceptibles d'induire une infection chez le mouton après incubation dans les antisérums ci-dessus.

Mots-clés : Relation immunologique ; T. evansi ; T. vivax ; Anticorps lytiques

Introduction

Trypanosomes causing disease in livestock include Trypanosoma congolense, Trypanosoma simiae, Trypanosoma brucei brucei, Trypanosoma vivax, Trypanosoma evansi and Trypanosoma equiperdum; let alone Trypanosoma brucei gambiense, Trypanosoma brucei rhodesiense and Trypanosoma cruzi species which cause disease in humans (1). Among the species causing disease in livestock, T. evansi and T. vivax are the most widely distributed in the world, occurring in tropical Africa (1), south eastern Asia (2) Middle East and South America (3, 4 and 5). The two parasite species pose a disease threat to 500 million cattle, 100 million domestic buffaloes and 12 million camels, horses and other domestic animal species found in parts of the world where the disease is endemic (6). T. evansi and T. vivax antigens show high immunological cross reactivity in capillary agglutination, indirect enzyme linked immunosorbent assay (ELISA), western immunoblots and immunofluorescence microscopy (7). T. evansi antigens have also been used to detect antibodies produced in response to T. vivax infection (8, 9 and10). This suggests that T. evansi and T. vivax are immunologically related and it is possible that antiserum against one species contains lytic antibodies against the other species, a possibility that had not yet been investigated. Olaho-Mukani (1986) observed that camels previously exposed to T. evansi infection and deliberately exposed to tsetse challenge for one year on Galana Ranch, Kenya, where T. vivax infection was rampant in cattle, failed to contract the latter infection, but succumbed to T. congolense (pers. com.). Yet, camels are susceptible to T. vivax (11). This further suggests that the two infections probably do cross-protect against each other and antiserum against one species possibly, contains lytic antibodies against the other species, a possibility that requires to be investigated. Uzcanga et al. (2002) identified some antigens responsible for the serological cross reactivity between T. evansi and T. vivax by immunobloting, and found a series of polypeptide species ranging from 14 – 109kDa in the clarified soluble antigenic fraction of T. evansi to be common antigens for both anti T. evansi equine antibodies and anti T. vivax bovine antibodies. Among the identified T. evansi cross-reacting antigens, a 64kDa antigen was purified and immunofluorescence microscopy indicated the latter to be primarily localized on the parasite surface (10). Protective antibodies are surface specific and the host response to the parasite surface antigens plays an important role in controlling the parasite infection (12). Trypanosomes change their surface antigens through variation of surface glycoprotein antigens (13). During each peak parasitaemia, a mixture of variable antigenic types of parasites is present but the dominant antigenic type parasite population determines the specific antibody response. The specific antibodies produced in response to the infection kill off the dominant antigenic parasite population, leaving a small non-dominant antigenic parasite population to multiply and the process continues in cycles until the animal dies if not treated or the immune mechanisms catch up with the parasite and the animal recovers on its own (14). This phenomenon is responsible for the successive waves of parasitaemia in infected animals. There is also evidence that following repeated episodes of infection and recovery (with or without treatment) in an enzootic area, animals encounter a variety of antigenic types and become less susceptible to strains in that area (13).

The purpose of this study was to establish whether antiserum against the two trypanosome species contained lytic antibodies against each other before embarking on identification, isolation, characterization and determination of the immunological properties of the two Trypanosoma species proteins for possible use in serodignosis and vaccine production.

Materials and Methods

T. evansi and T vivax stabilates were sourced from Livestock Health Research Institute (LIRI) Tororo, Uganda and Trypanosomiasis Research Centre, Kenya Agricultural Research Institute (KARI) Muguga, Kenya. The stabilates were multiplied in mice and goats then cryopreserved until required. Sheep were inoculated, monitored for infection

until the fourth parasitaemic peak, treated, bled 10 days after treatment, serum harvested and frozen until required.

T. evansi and T. vivax were mixed with T. vivax and T. evansi antisera respectively, including negative serum as a control and incubated at room temperature for three hours. After the three hours incubation a wet preparation was made from each mixture, examined under the microscope using the 40X objective and the trypanosomes detected in each entire preparation counted. A portion of each incubated mixture was also inoculated into susceptible animals and the animals were monitored for infection over time by direct microscopic examination of their capillary blood after every third day.

Results

T. evansi and T. vivax were able to induce infection in susceptible animals after incubation in anti T. vivax and anti T. evansi sera, respectively. Anti T. evansi serum showed an apparent effect on T. vivax parasites, with the trypanosome counts in positive serum being far lower than those in negative serum after incubation at room temperature for three hours. The T. vivax mean trypanosome counts in anti T. evansi positive and negative sera were 7.2 ± 4.43846820423443 (Standard deviation) and 26.4 ± 10.6442472725881 (Standard deviation) respectively (Figs. 1a and 1b). There was a significant difference between the mean trypanosomes counts of the 5 separate positive and negative serum incubation tests done ($p = 0.002924 < 0.05$).

Anti T. vivax serum showed no apparent effect on T. evansi parasites after incubation at room temperature for three hours. The T. evansi mean trypanosome counts in anti T. vivax positive and negative sera mixtures were 26.8 ± 7.190271(Standard deviation) and 27.4 ± 7.162402 (Standard deviation), respectively (Figs. 1c and 1d). There was no significant difference between the positive and negative sera mean counts after incubation ($p = 0.449047 > 0.05$).

Discussion

Trypanosomes change their surface antigens through variation of surface glycoprotein antigens (13). During each peak parasitaemia, a mixture of variable antigenic types of parasites is present but the dominant antigens determine the specific antibody response. The specific antibodies produced in response to the infection eliminate the dominant antigenic parasite population, leaving a small non-dominant antigenic parasite population to multiply and the process continues in

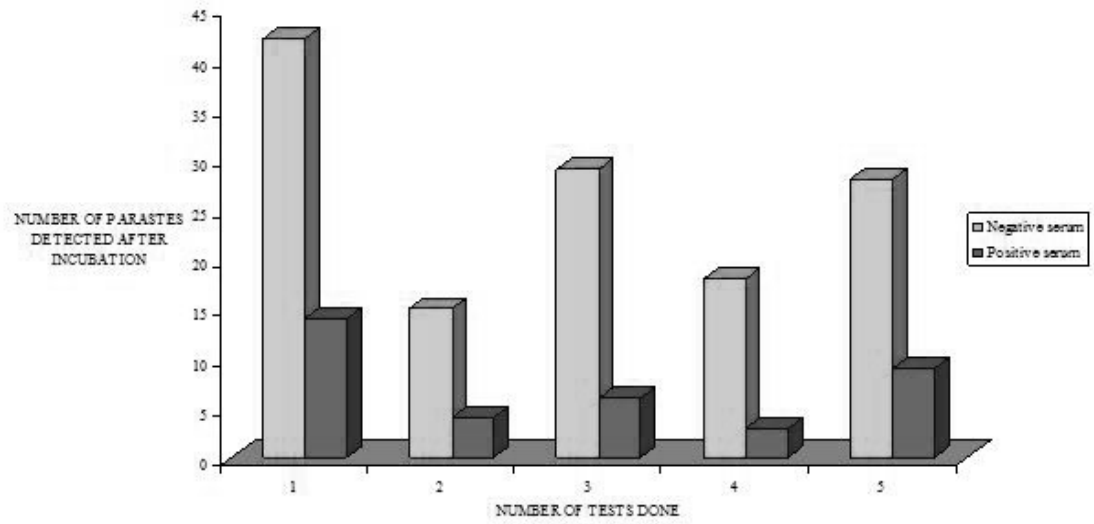

Figure. 1a: T.vivax parasites counts after three hours incubation at room temperature in positive and negative T.evansi sera.

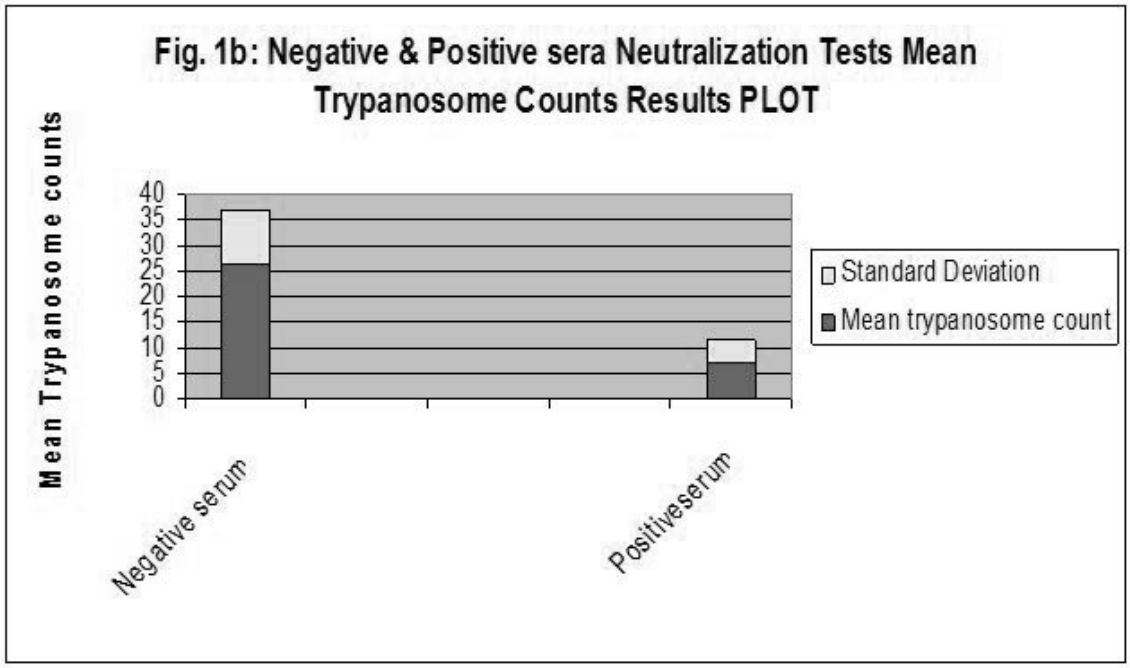

Figure 1b: T. vivax parasites mean counts after three hours incubation at room temperature in negative and positive T. evansi sera.

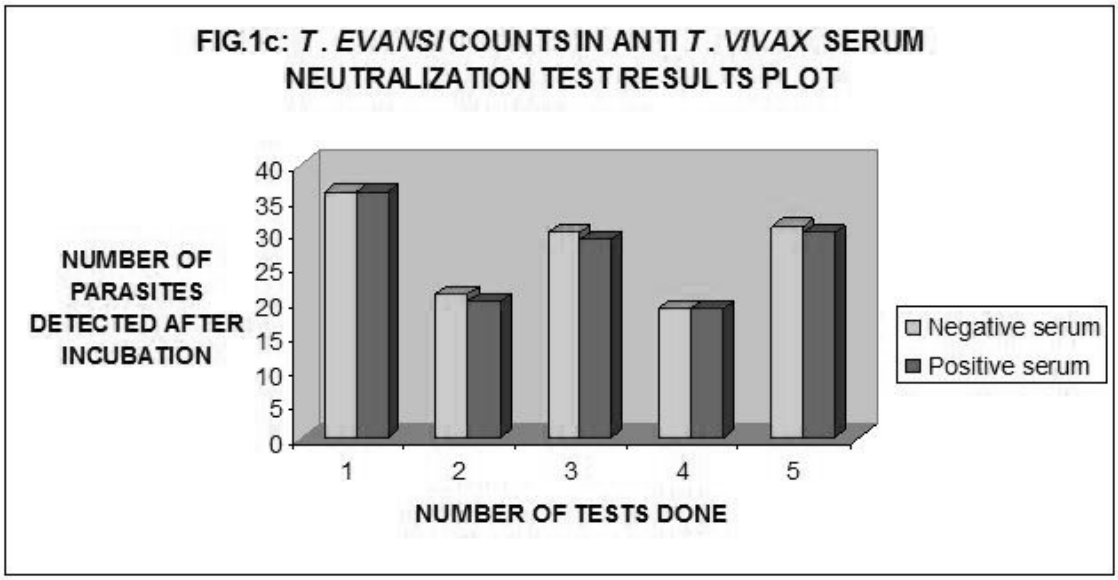

Figure 1c: T.evansi parasite counts after three hours incubation at room temperature in positive and negative T. vivax sera.

cycles until the animal dies if not treated or the immune mechanisms catch up with the parasite and the animal recovers on its own (14). Findings of this study indicated that anti T. evansi serum contained lytic antibodies which eliminated a large population of T. vivax parasites but anti T. vivax serum appeared not to contain lytic antibodies against T. evansi. This suggests that T. evansi possesses dominant cross-reacting antigens that induce production of cross-reacting antibodies that eliminated a large population of T. vivax parasites, leaving some, which were able to induce infection when inoculated in sheep. This observation is similar to what Barry and Turner observed in their study conducted in 1991 (14). However,

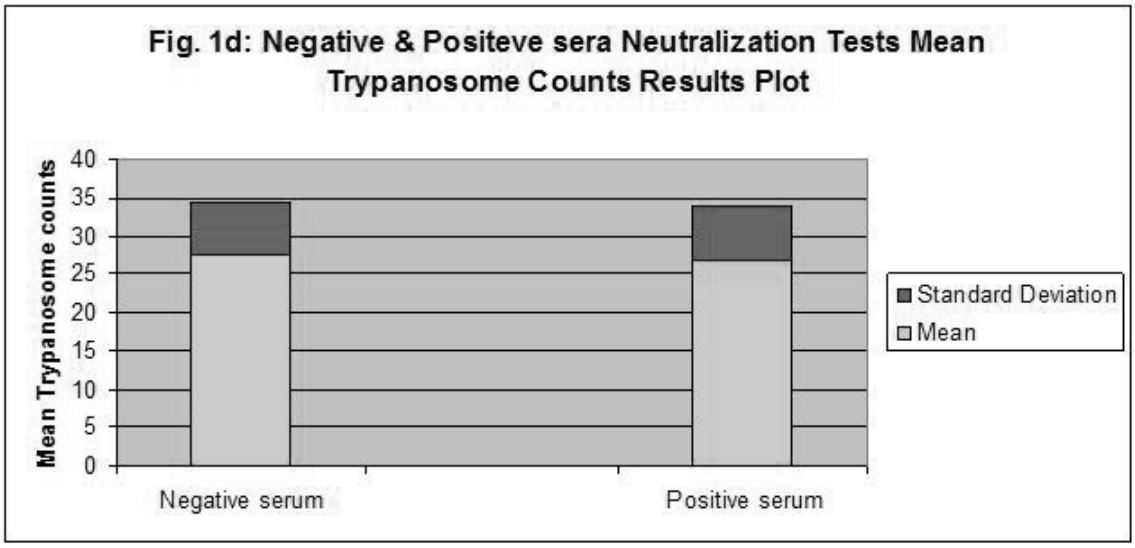

Figure 1d: T. evansi parasites mean counts after three hours incubation at room temperature in negative and positive T. vivax sera.

similar antigens in T. vivax appear not to be dominant and were overshadowed by other more dominant antigens in the same parasite in eliciting antibody production in response to infection with T. vivax as indicated by the results after incubating T. evansi in anti T. vivax serum for three hours at room temperature.

Uche and others (12) observed that anti trypanosome lytic antibodies are surface specific and play an important role in controlling trypanosome infection. The lytic anti T. vivax antibodies observed in anti T. evansi serum appear to be surface specific and played an important role in eliminating a large proportion of T. vivax parasites after incubation in anti T. evansi serum at room temperature for three hours. These results are in line with the observation of 1986 by Olaho-Mukani (personal communication) who observed that camels previously exposed to T. evansi infection did not contract T. vivax infection when moved to an area where the latter parasite species was rampant in cattle. Yet camels are known to be susceptible to T. vivax infection (11). Basing on the findings of this study, the possible explanation to Olaho-Mukani's observation is that lytic antibodies against T. vivax similar to those demonstrated to be present in anti T. evansi serum prepared in this study must have been produced in camels previously exposed to T. evansi infection. This suggests that prior exposure of camels to T. evansi infection elicited production of lytic antibodies that conferred protection against T. vivax infection to camels previously exposed to T. evansi infection or made the previously exposed animals less susceptible to natural T. vivax infection.

Conclusion

The presence of lytic antibodies against T. vivax in anti T. evansi serum together with existing evidence that repeated episodes of infection and recovery (with or without treatment) confers less susceptibility to trypanosome infection (12) suggest that T. evansi has antigenic proteins that can confer some kind of protection against T. vivax infection. And therefore there is need to carry out further investigations to identify, isolate and characterize such antigenic proteins suggested to be present in T. envansi for possible use as vaccine against T. vivax infection. And any antigenic proteins identified as unique to either Trypanosoma species during such investigations can be adopted as candidates for possible use in development of species-specific serological tests.

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HELMITH PARASITES FOUND IN GOATS SLAUGHTERED FOR MEAT IN ETIM EKPO LOCAL GOVERNMENT AREA OF AKWA IBOM STATE, NIGERIA

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Abstract

Goats are small ruminants which are reared for several purposes especially in the rural area where they serve as a ready source of cash in meeting with sudden family needs. One of the most prevalent diseases militating against them is worm parasites. The aim of the study was to find out the major types of worms that are prevalent in Etim Ekpo local Government Area of Akwa Ibom State which parasitize goats, thus confirm the economic importance of the worm parasites. 250 samples were randomly collected from the goats slaughtered slabs scattered all over the local government Area using sterile swaps. Saturated salt solution was prepared and used in locating the worm eggs present thus the types of worm parasites using simple floatation method. It was found out that several species of worm viz: Heterakis, Trichostrongylus, Heamonchus, Tricuris, Monienza, Oesophagustomum, Ascaridia, Ostergia etc were found in an average of 48.8% of samples collected, using simple percentage. This it was confirmed that worm parasites are a major constraint in it development of goats in the area. It was also noted that the worm was present in all goats slaughtered for meat in the local government area. Since the offal are washed into a nearby streams which help to spread it to the people who use water from the same stream to bath, wash, and other things that may require waster. It was suggested that thorough ante and post mortem should be carried out and treatment should be given where necessary to prevent the spread of the disease to the consumers.

Keywords: Goats; Helminthes; Tricuris; Ascaridia; Heterakis; Slaughter.

HELMINTHES TROUVES CHEZ LES CHEVRES ABATTUS POUR LA VIANDE DANS LA COLLECTIVITE LOCALE D'ETIM EKPO DE L'ETAT D'AKWA IBOM AU NIGERIA

Resume

Les chèvres sont de petits ruminants élevés pour plusieurs raisons, en particulier dans les zones rurales où elles constituent une source de liquidités immédiatement disponibles pour faire face aux besoins familiaux soudains. Les vers parasites constituent l'une des maladies les plus répandues qui affectent cette espèce animale. L'objectif de l'étude était de déterminer les principaux types de ver répandus dans la collectivité locale d'Etim Ekpo de l'État d'Akwa Ibom qui affectent les chèvres, et de confirmer ainsi l'importance économique des vers parasites. 250 échantillons ont été prélevés de manière aléatoire sur des chèvres abattues éparpillées dans différentes localités de la collectivité locale au moyen d'écouvillons stériles. Une solution salée saturée a été préparée et utilisée pour localiser les œufs des vers présents ainsi que les types de vers parasites en utilisant la méthode de flottation simple. Le résultat était que plusieurs espèces de ver, à savoir Heterakis, Trichostrongylus, Heamonchus, Tricuris, Monienza, Oesophagustomum, Ascaridia, Ostergia, etc. étaient présentes dans un pourcentage moyen de 48,8 % des échantillons prélevés. Ceci confirme donc que les vers parasites constituent une contrainte majeure au développement de la production de chèvres dans la région. De plus, on a noté que les vers étaient présents chez toutes les chèvres abattues pour la viande dans cette collectivité locale. Etant donné que les abats sont nettoyés dans un cours d'eau voisin utilisé par les personnes des environs pour la baignade, la lessive, et d'autres activités nécessitant l'usage de l'eau, il est proposé que des examens ante et post mortem approfondis soient effectués et le traitement accordé le cas échéant pour empêcher la transmission de la maladie aux consommateurs.

Mots-clés : Caprins ; Helminthes ; Tricuris ; Ascaridia ; Heterakis ; Abattage.
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Introduction

Worms are internal parasites. These worms are found in the gastrointestinal, lungs, tract and the muscles of the animals. Sometimes, worm maybe seen with the naked eye, but they are not easily seen in many cases. Gastrointestinal parasites are a worldwide problem for small and large animals.

Goats play a very important role in the nation's economy through protein supply and in the rural socio-economic development.

In view of these, one cannot neglect the development of such agricultural commodities like goats because the help the overall development the rural communities of the State and the Nation at large.

The breeding of ruminants in Nigeria is carried out using traditional methods, hence, animals grazing on pastures leads to parasitic infections. Soraya and Gorgani, (2011), reported that parasites are major problems in small ruminants, by causing disease, mortality and production losses. Loss of millions of farm animal products is incurred daily due to the devastating effects of the parasites, (Njoku-Tony, 20011). Tremendous losses and damage to the liver and other Organ (Okafor and Ikpeama, 1985).

Bashir et.al., (20012) in his report said that sheep develop a strong natural immunity against helminthes around 12 months of age while goats acquire a lower level of immunity to gastrointestinal parasites. This, (Macaldowie et.al., 2003) observed, can result in goats having greater population of parasites with high egg output.

The diseases they cause, cost the Nigerian meat industry millions of Naira each year due to losses in productivity and the cost associated with treatment and control. They also represent a significant animal welfare and public health concern.

The aim of this study is to identify the species and investigate the prevalence of the gastrointestinal worms of goats slaughtered in Etim Ekpo Local Government Area of Akwa Ibom State. The study also seeks to highlight the public health importance of these gastrointestinal parasites.

Materials and Methods

A total of 250 fecal samples of goats slaughtered for meat in Etim Ekpo Local Government Area of Akwa Ibom State, were collected and investigated throughout the period of the study which last for one month (1st-31 March, 2012).

The samples were collected with a sterile swap directly from the rectum of these goats before they were slaughtered at the slaughter slap and placed in a sample collecting bottle (capped) and transported to the laboratory for analysis.

Etim Ekpo Local Government Area, were this study was carried out is a group of rural villages in Ikot Ekpene Senatorial District of Akwa Ibom State. As it is the case with most rural communities, there is no central abattoir for the slaughter of small ruminants; therefore, goat meat traders slaughter their goats in their stalls within the market. The study covered 10 markets in 10 different villages within the study area.

The identification of this endoparasitic infection was carried out using simple egg floatation method with saturated salt solution. The worm eggs were fixed and examined under the microscope at (X 10 and X 100) magnification. The identification of the worm eggs or cysts was made on the basis of morphological characteristics and size of eggs as described by (Foriet, 1999; Nolte, 2011; Soulsby 19832).

Egg counting to determine the number of eggs per gram of feaces in the samples and the severity was grouped based on methods described by (Soulsby, 1982; Urquhart et. al., 1996). The data generated were subjected to simple percentage computation

Result and Discussion

The species of worm/endoparasitic identified during the study is as presented in the table below. Since the fecal sample were collected from 10 different markets in 10 separate villages, it is necessary we mention the name of the market with species of worm eggs identified accordingly.

Table 1: Showing the market name and species of worm eggs identified

S/no.	Village	Species of Helminthes
1	Urua Utu	Heterakis spp, Trichostrongylus, Haemonchus
2	Urua Oba	Heterakis Spp, Tricuris, Monienza, Oesophagostomum
3	Urua Utu	Ascaridia spp, Tricuris, Haemonchus, Heterakis , Ostertagia
4	Urua Obo	Heterakis, Ascaridia, Heamonchus
5	Edere Obo	Heamonchus, Heterakis, Strongylus, Giardia
6	Urua Obo	Paramphistomumes, Heterakis, Heamonchus, Ascaridia
7	Urua Obo	Tenia, Trichuris, Ascaridia, Heterakis
8	Urua Offiong	Ascaridia, Tricuris, ostergia, Strongylus, Heterakis
9	Edet Obo	Monienza, Heamonchus, Trichostrongylus, Ascaridia
10	Eka-offiong	Tricuris, Strongylus, Ascaridia, Heamonchus

Table 2: Prevalence or worm parasite infestation in goats slaughtered in etim ekpo l.G.A.

Village	No. of Sample Examined	Number Infected	% Prevalence
Urua Utu	25	11	44
Urua Obo	25	15	60
Urua Utu	25	7	28
Urua Obo	25	12	48
Edere Obo	25	9	36
Urua Obo	25	15	60
Urua Obo	25	10	40
Urua offiong	25	16	64
Edere Obo	25	14	56
Eka-Offiong	25	13	52
∑	250	122	488
±		12.2	48.8

The general prevalence of all the species of endoparasites identified in this study was 48.8%. Sample collected from 122 goats tested positive for one or more genera of Cestodes, Trematodes, and Nematodes. However, the prevalence of individual species of endoparasites identified in this study was irrelevant. The species of helminthes parasites is as presented in Table I. And Table II depicts the prevalence of this helminthes parasites in the goats slaughtered in the area.

The highest percentage of prevalence of the helminthes parasite was recorded from samples collected from Urua Offiong (64%), followed by two of the Obo markets, Urua Obo (60%). The lowest percentage of parasitic prevalence was recorded from Urua Utu (28%) followed by that of the Edere obo Market with (36%).

Most species of the endoparasite identified in this study has also been reported by various authors (Asnji and Williams, 1987; Gupta *et al.,* 1987; Guiomaraes and Walter, 1987; Njau, 1987; Uriarte and Valderrabno, 1989; pal and Qayyum, 1993) and (Asif, et.al, 2008).

The high prevalence of the prevalence the helminthes observed is similar to (Lone et.al., 2012; pandit et.al., 2003; Al-Saeed et.al., 1990 and Dhana Laskmi et.al, 2001).

Giardia is generally considered to be a zoonotic parasite and play a causative role in diarrheal disease, however, the clinical significance of the parasite is quite variable depending on it pathogenicity for the particular host species.

Heamonchus identified in the study is of economic importance and a common

nematode parasite and requires special attention for its control (Asif et.al, 2008).

Torres-Acosta et.al, 2003 reported that Haemonchus can acquire resistance faster than other gastrointestinal nematodes, like Trichostrongylus, because of its high biotic potential.

The high prevalence of the helminthes could result in diarrhea, emaciation, abortion and death resulting in loss or revenue that would have accrued to the farmer.

It is the intention of the author to report that most of these goats slaughtered in this locality are brought from the Northern part of the Nigeria, Niger and Chad Republic; therefore, the parasites are also brought alongside the animals, and possibly spread in the area that consume the goat meat.

These animals are taken from the animal markets straight to the slaughter with little or no ante-mortem inspection where some diseases of Veterinary and Public health importance could have been recognized and treated before the animals are slaughtered.

It is of Veterinary and public health importance because; the slaughtered animals are washed in nearby streams/ body of water thereby releasing egg/cyst of the helminthes into the environment that was initially free from these parasites.

In conclusion, various gastrointestinal parasites have been found in goats slaughtered for meat in Etim Ekpo Local government area. Preventive and control measure should be practiced to reduce the number of infected goats slaughtered for meat and well reduce the parasitic burdens in the affected areas. It is therefore advised that thorough ante mortem and post mortem inspection should be carried out before goat meat could be said to be wholesome for consumption. It was also suggested that since it is very difficult for Veterinarian who are few to cover the multi slaughter slabs for adequate meat inspection, a central abattoir should be built and butchers forced to use if for slaughtering in order to present wholesome meat for the public so as to reduce the incidence of worm parasitism in man.

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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 2013

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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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- *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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