

COMPARATIVE STUDY OF THE EFFECTS OF GASTROINTESTINAL PARASITES ON DIFFERENTIAL LEUKOCYTE PROFILE OF DJALLONKÉ SHEEP KEPT UNDER EXTENSIVE AND SEMI-INTENSIVE MANAGEMENT SYSTEMS IN NORTHERN GHANA

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SUMMARY

The effects of gastrointestinal parasites on the differential leukocyte profile of Djallonké sheep managed under farmer-conditions in the extensive system or on-station under the semi-intensive system of management were investigated for six months during the dry season (November, 2006-April, 2007). A total of 461 faecal and blood samples each were collected from 40 sheep stratified into young (6-9 months) and adults (>9 months). The system of management significantly affected the faecal egg count for *Strongyloides* spp. and oocyst counts of *Eimeria* spp. with higher loads in sheep managed extensively than those managed semi-intensively ($P<0.05$). The count of faecal eggs for *Strongyle* spp. in both management systems was however similar. The levels of lymphocytes and eosinophils in sheep managed extensively were also significantly ($P<0.05$) higher than those observed in the extensive system. The number of neutrophils, monocytes and basophils did not differ significantly ($P>0.05$) in the two management systems. Eosinophil counts in both management systems was positive and linearly correlated with eggs/oocysts of all the three intestinal parasites and became significant with the eggs of *Strongyloides* spp. ($r=0.32$; $P<0.05$) and *Strongyle* spp. ($r=0.54$; $P<0.05$) under the extensive and again with *Strongyle* spp. ($r=0.40$; $P<0.05$) under the semi-intensive system. The relationship between the count of eosinophils, and the egg and oocyst count of *Strongyloides* spp. ($r=0.13$; $P>0.05$) and *Eimeria* spp. ($r=0.25$; $P>0.05$), respectively was also positive but not significant under the semi-intensive system of management. Younger animals in the extensive system were found to have significantly higher ($P<0.05$) egg and oocysts counts of *Strongyloides* spp. and *Eimeria* spp. respectively than those raised under semi-intensive management. *Strongyle* spp. showed resistance to anthelmintic use while extensive eosinophilia was consistently associated with gastrointestinal parasitism.

KEYWORDS: *Eimeria* spp., Eosinophils, Gastrointestinal parasitism, Management system, *Strongyle* spp.

INTRODUCTION

The Djallonké also called West African Dwarf (WAD) sheep is widely distributed throughout the West and Central zones of Africa. It is believed to have evolved from the ancient Egyptian sheep *Ovis longipes palaeoaegypticus* (Yapi-Gnaore' *et al.*, 1997). It is generally smaller in size but physically and sexually vigorous with a high reproductive potential. It is also stress and disease resistant especially to unfavourable climate and trypanosomosis. Climate is therefore not likely to have any significant effects on its performance although variations in forage supply could influence physical and physiological maturation. The coat colour varies from spotted black and white to solid black, white or brown. The presence of a mane or neck ruff on males is a typical characteristic of the breed. Males are horned while females are polled. Apart from serving as a source of meat and income, its most obvious role is in religious ceremonies where its commercial value is placed far beyond its value in terms of carcass weight (Charray *et al.*, 1992).

It is estimated that 97% of all small ruminants in Africa are carriers of parasites of the digestive system (Charry *et al.*, 1992) which cause mortality and morbidity each estimated at US\$2 billion annually (Putt and Hanks, 1993). In Ghana, gastrointestinal parasitism account for 27% of all deaths and constitute the probable limitation to the multiplication of sheep (Buadu and Osafo, 1994). The effects of morbidity on sheep production include expunge weight loss, poor growth rate, infertility, abortions, lower milk yield, increased cost of drugs and vaccines, and extended age in the attainment of puberty. These are however often overlooked because their effects are not apparent, but the economic effects of morbidity on production are estimated to be higher than those of mortality since they increase the cost of production and also lower productivity (Charry *et al.*, 1992). Gastrointestinal parasites therefore cause serious economic losses and are considered the most serious health problem of sheep and goats (Perry *et al.*, 2005).

The extensive system of rearing sheep commonly practised by rural farmers predisposes them to parasites of the gut, especially in the dry season when they graze stubble very close to the ground, thereby ingesting encysted larvae. The semi-intensive and intensive systems are practised by only a few large-scale farmers, research and higher educational institutions. Gastrointestinal parasites build up and become endemic in these systems if the pen is not routinely cleaned, disinfected and the animals dewormed, and even when all these practices are observed, the animals are still liable to becoming rapidly reinfested because deworming often unarms the natural immunity of the host subsequently rendering them more susceptible to higher infestation (von Kaumann and Fitzhugh, 1993; Skyes, 1994).

A strong positive correlation between egg/oocyst counts and the prevalence of the parasites themselves in livestock has already been established with 100% of the eggs of *Strongyle* spp. and *Strongyloides* spp. developing into viable infective larvae (Ibrahim *et al.*, 2006). The count of eggs/oocysts can therefore be best used to estimate the prevalence of parasitic infestation. Gastrointestinal parasites act as antigens and their presence often evokes immune responses that cause changes in the normal physiology of the animal. The presence and degree of infestation of gastrointestinal parasites in sheep can therefore cause changes in the normal leukocyte differential. This study investigated the relationship between egg/oocyst counts as a measure of gastrointestinal load and leukocyte profile of Djallonké sheep kept extensively or semi-intensively.

MATERIALS AND METHODS

Study area

The study was conducted at Nyankpala in the Tolon-Kumbungu district of the Northern region of Ghana. It is located on latitude 9.5° N. The area is in the guinea savannah zone characterised by a unimodal rainfall pattern.

Rains begin in April, rising to a peak in August - September and ending in October or November. The dry season spans from November to March. Rainfall averages 1060 mm and temperatures usually range from 15°C in January when the weather is under the influence of the North easterly wind, to 42°C towards the end of the dry season in March (SARI, 2006).

Animal management and experimental design

A total of 40 Djallonké sheep, 20 from the extensive system managed by farmers in Dundo, a rural community, and 20 from the semi-intensive system managed by the Department of Animal Science of the University for Development Studies (UDS), Ghana, were used for the study. Those managed by the UDS were allowed to graze on natural pasture during the day but confined in the evening in a pen. The pen was routinely cleaned and disinfected while the animals were dewormed with Albendazole® (7 mg/kg), medicated (Oxytetracycline®) and supplemented with whole cotton seed. Those managed by farmers under the extensive system were also provided with pens which were hardly cleaned and/or disinfected. They were not given any medication and often slept outside the pens. The animals in each management system were stratified by age into young (6-9 months) and adults (9 months and above). Age estimation was based on dentition (Charry *et al.*, 1992). Each management system consisted of 10 young ones and 10 adults. Data was collected for a period of 6 months (November, 2006-April, 2007). The general male: female sex ratio was 2:3 and 3:7 in the semi-intensive and extensive systems, respectively.

Faecal worm egg and oocyst counts

A total of 461 faecal and blood samples were collected fortnightly from the experimental animals for analysis. Three lambs died during the course of the study. The McMaster floatation technique of faecal egg count was used. About 3g of the faecal material obtained from the rectum of each animal was macerated in a mortar with about 15 mls of distilled water. The content was then poured into a plastic test tube and centrifuged at 1,233 gm. The supernatant fluid was poured off and distilled tap water added. The process was repeated and saturated sodium

chloride (NaCl) solution added and centrifuged again at the same speed and time. The saturated NaCl solution enables the eggs to float (except eggs of flukes). The fluid was then drawn from the surface of the mixture of NaCl and faeces and the McMaster counting chamber filled. The counting chamber was then mounted on a microscope and examined under 40 (x40) objective lens. Under the microscope, eggs of *Strongyle* spp. are oval (rugby ball-like) with a thin shell and a dark content. A major distinction between the eggs of *Strongyle* spp. and *Strongyloides* spp. is that larval (L₁) movements are typically observed in the eggs of freshly collected faeces in the latter but the egg content of the former consists of intact dark cells. The number of eggs were counted and multiplied by a scale factor of 100 (Adu *et al.*, 2006) to obtain total eggs per gram (epg). Oocyst counts were determined through the modified McMaster technique by methods described by MAFF (1977).

Blood sampling and leukocyte count analysis

A total of 461 peripheral blood samples were similarly collected at the time of sampling the faeces. Smears were made from samples taken from the ear vein of each animal and fixed with absolute ethanol, and stained with Giemsa stain. The different types of leukocytes (neutrophils, basophils, eosinophils, lymphocytes and monocytes) were identified and counted with a microscope with 100/1.25 oil immersion objective lens and a haemocytometer using the straight edge method (Addah *et al.*, 2007).

Data analysis

Data from the two management systems were compared using t-test and the relationship between the worm egg/oocyst and the differential leukocyte count determined by linear correlation matrix using GenStat (version 6.0) software.

RESULTS

The effects of management system and age of host on faecal egg/oocyst and differential leukocyte counts are shown in Table I. Regardless of the management system, faecal egg/oocyst counts were highest for *Strongyle* spp. (64%), lower for *Eimeria* spp. (27%) and lowest for *Strongyloides* spp. (9%). The infestation of *Eimeria* spp. and *Strongyloides* spp. in the extensive system was significantly higher ($P < 0.01$) than in the semi-

intensive system, however, the prevalence of *Strongyle* spp. between the two management systems was similar ($P>0.05$) (Table I) but significantly higher than the other parasites within each management system ($P<0.01$) (Fig. 1).

The level of faecal worm eggs and oocysts in younger animals did not differ significantly from adult ones ($P>0.05$) though both younger and adult animals in the extensive system had higher counts than their counterparts in the semi-intensive system ($P<0.05$). All losses due to mortality (7.5%) during the study occurred in the extensively managed flock. Faecal samples recovered from the dead lambs showed mixed and heavy infestations of *Eimeria* spp. (1,412 opg), *Strongyle* spp. (1,902epg) and *Strongyloides* spp. (623 epg).

Eosinophils counts were significantly higher ($P<0.01$) in extensively managed sheep while lymphocyte counts were higher in those kept semi-intensively but the system of management did not significantly affect ($P>0.05$) the counts of neutrophils, monocytes and basophils. In either age

groups, strongyloidosis and coccidiosis induced eosinophilia in both management systems and lymphopenia in only semi-intensively managed sheep ($P<0.05$). As indicated in Table II, the leukocyte differential, especially eosinophils, of sheep in either age group of the extensively managed flock varied greatly from normal.

The matrix correlation coefficients between the three gastrointestinal parasites and differential leukocyte counts are shown in Tables III and IV. The level of eosinophils in the two management systems was positively correlated with all the three intestinal parasites under consideration and became significant with strongyloidosis ($r = 0.32$; $P<0.05$) and strongylosis ($r = 0.54$; $P<0.05$) in the extensive system and also with strongylosis ($r = 0.40$; $P<0.05$) only in the semi-intensive system. The relationships between the counts of eosinophils, and the egg and oocyst counts of *Strongyloides* spp. ($r = 0.13$; $P>0.05$) and *Eimeria* spp. ($r = 0.25$; $P>0.05$) respectively were also positive but not significant under the semi-intensive system of management.

TABLE I: Effects of type of management system and age of animals on faecal egg/oocyst count and leukocyte profile (mean \pm S.E) of Djallonké sheep.

	Faecal worm egg count			Leukocyte profile (%)				
	<i>Strongyle</i> spp. (epg)	<i>Strongyloides</i> spp. (epg)	<i>Eimeria</i> spp. (opg)	Neutrophils	Lymphocytes	Eosinophils	Monocytes	Basophils
Type of mgt. system								
Extensive	1460 \pm 194.92	307.2 \pm 61.36	799.5 \pm 177.71	39.03 \pm 0.38	51.87 \pm 0.39	7.32 \pm 0.21	1.11 \pm 0.08	0.45 \pm 0.04
Semi-intensive	1192 \pm 325.73	71.33 \pm 21.91	310.4 \pm 60.24	38.62 \pm 0.39	54.02 \pm 0.31	5.98 \pm 0.20	1.03 \pm 0.06	0.45 \pm 0.04
Significance	Ns	**	**	Ns	**	**	Ns	ns
Effects of Age								
Young(6-9 mo)								
Extensive	1717 \pm 283.3	414.9 \pm 115.1	1036 \pm 291	39.28 \pm 0.6	51.17 \pm 0.7	7.55 \pm 0.3	1.16 \pm 0.1	0.48 \pm 0.6
Semi-intensive	1243 \pm 267	216.6 \pm 57.4	600.8 \pm 216.6	38.82 \pm 0.5	53.17 \pm 0.4	6.35 \pm 0.3	1.12 \pm 0.1	0.53 \pm 0.1
Significance	Ns	*	*	Ns	**	**	Ns	ns
Adult(>9 mo)								
Extensive	143.0 \pm 267.8	216.6 \pm 57.4	600.8 \pm 216.6	38.82 \pm 0.5	52.46 \pm 0.4	7.13 \pm 0.3	1.07 \pm 0.1	0.44 \pm 0.1
Semi-intensive	675.80 \pm 140.4	46.83 \pm 21.2	255.00 \pm 74.6	38.79 \pm 0.6	54.33 \pm 0.4	5.62 \pm 0.3	0.95 \pm 0.1	0.35 \pm 0.1
Significance	Ns	**	ns	Ns	**	**	Ns	ns
Ext. + Semi-int. (over all)								
Young(6-9 mo)	1713 \pm 367.07	241.6 \pm 57.45	671.9 \pm 143.97	38.82 \pm 0.37	52.55 \pm 0.39	6.90 \pm 0.21	1.14 \pm 0.08	0.46 \pm 0.04
Adult(>9 mo)	959.6 \pm 151.99	131.7 \pm 31.00	427.9 \pm 114.85	38.81 \pm 0.39	53.40 \pm 0.31	6.38 \pm 0.21	1.00 \pm 0.06	0.45 \pm 0.04
Significance	Ns	ns	ns	Ns	Ns	ns	Ns	ns

** = $P<0.01$; * = $P<0.05$; ns = not significant

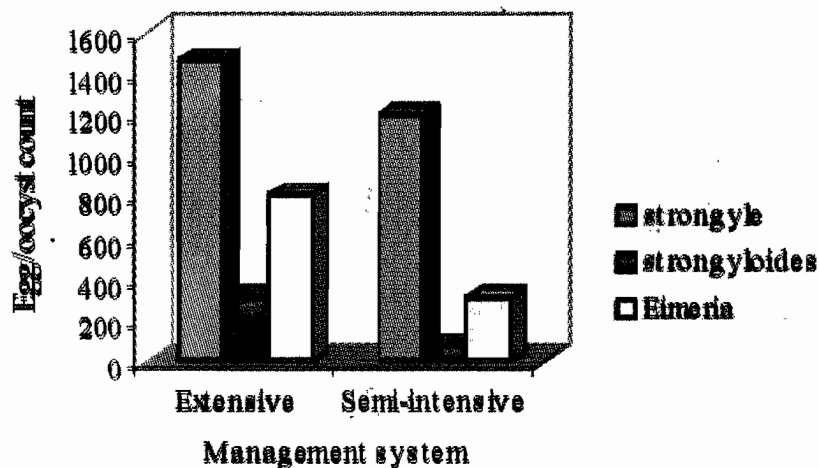


FIGURE 1: Effects of type of management on egg and oocyst counts

TABLE II: Effects of gastrointestinal parasites on percentage variation in the leukocyte from normal count

	Management system		* Normal Leukocyte count (%)	Variation from normal	
	Extensive (%)	Semi-int. (%)		Extensive (%)	Semi-int. (%)
Young (6-9 months)					
Neutrophils	39.28	38.44	42.45	7.5 ^{Lp}	9.4 ^{Lc}
Lymphocytes	51.17	53.71	50.1	2.0 ^{Lc}	7.2 ^{Lc}
Monocytes	1.16	1.12	1.85	37 ^{Lc}	39.5 ^{Lp}
Eosinophils	7.55	6.35	3.25	132.3 ^{Lc}	95.4 ^{Lc}
Basophils	0.48	0.48	2.45	80 ^{Lp}	80.4 ^{Lp}
Adults (> 9 months)					
Neutrophils	38.82	38.79	39.6	1.96 ^{Lp}	25 ^{Lp}
Lymphocytes	52.46	54.33	55.9	6.2 ^{Lp}	2.8 ^{Lp}
Monocytes	1.07	0.95	2.45	56.3 ^{Lp}	61.2 ^{Lp}
Eosinophils	7.13	5.62	1.8	296.1 ^{Lc}	212.2 ^{Lc}
Basophils	0.44	0.53	0.25	76 ^{Lc}	112 ^{Lc}

*Normal leukocyte count of healthy Djallonké sheep screened of endoparasites with anthelmintics (Ahunu and Assuoku, 1987); Lc: Leukocytosis; Lp: Leukopenia

TABLE III: Matrix correlation co-efficient of egg/oocyst and differential leukocyte counts (extensive)

Parasites	Neutrophils	Monocytes	Lymphocytes	Eosinophils	Basophils
Strongyle spp.	0.061	0.014	- 0.378	0.537	0.030
Strongyloides spp.	- 0.009	0.062	- 0.113	0.315	0.092
Eimeria spp.	- 0.138	0.088	0.003	0.223	-0.070

TABLE IV: Matrix correlation co-efficient of egg/oocyst and differential leukocyte counts (semi-intensive)

Parasites	Neutrophils	Monocytes	Lymphocytes	Eosinophils	Basophils
<i>Strongyle</i> spp.	0.007	- 0.072	- 0.252	0.404	0.130
<i>Strongyloides</i> spp.	- 0.026	- 0.009	- 0.045	0.130	-0.073
<i>Eimeria</i> spp.	- 0.095	0.066	- 0.073	0.253	0.073

DISCUSSION

Faecal egg count of *Strongyle* spp. predominated over *Strongyloides* spp. and oocyst of *Eimeria* spp. in both management systems throughout the study period. *Strongyle* spp. (cyathostomes) are highly prolific, persistent and efficient in reproduction; about 99% of *Strongyle* eggs are passed out in faeces and they take 12-24 hours to hatch into infective larvae which do not require an intermediate host. The cyst can remain up to 2 years burrowed in the gut wall; hence their predominance. Another reason for a relative dominance of *Strongyle* eggs is that, unlike *Eimeria* spp. which are species-specific and cross-infestation, for example between goats and sheep is usually not possible, *Strongyle* spp. are not species-specific and cross-infestation is common (Lindsay and Todd, 1993). Similar studies in sheep and goats in Kenya show that *Strongyle* nematodes were the most prevalent gastrointestinal parasites in both species (51%) with higher infestations in sheep than goats (Waruiru *et al.*, 2005).

Sheep under the extensive system in Ghana are usually kept in smaller household flocks, often less than 10, and of varying ages, scavenging in the dry season or foraging extensively or tethered each day, on fresh pasture in the rainy season. It was expected that this practice, unlike that of the semi-intensive system where the flock is normally kept in relatively large numbers on paddocks, often with little rotation, would have resulted in lower infestation but that was not observed. The routine deworming exercise practised in the semi-intensive system could have accounted for the lower egg counts of *Strongyloides* and oocyst of *Eimeria* than in the extensive system in this study. In intensive and semi-intensive management systems, careful routine management of grazing pastures can minimize the prevalence of L₃ of most worms but this is rarely achievable in extensive communal pastoral/grazing systems and control of gastrointestinal parasites in such management

systems is entirely dependent on the use of anthelmintics (Hunter, 1996) which unfortunately are economically inaccessible to pro-poor rural farmers and ineffective in preventing reinfestation (Restrepo and Preston, 1989). In contrast to these findings, very high faecal egg and oocyst counts of *Strongyloides* spp. (1,697 epg) and *Eimeria* spp. (21,929 opg), respectively have been observed between November and December in lambs raised semi-intensively under established pasture in the same region (Agyei, *et al.*, 2005). This may be explained by differences in management practices, especially with regard to deworming schedules.

The predominance of *Strongyle* spp. over *Eimeria* spp. and *Strongyloides* spp. in the semi-intensive system despite routine deworming (Fig. 1) may be due to their proficiency in reproduction and/or possible resistance to Albendazole[®]. High counts of *Strongyle* eggs found in semi-intensively managed goats during the dry season despite Oxytoclozanite + Levamisole (Nilzan[®]) use have severally been attributed to resistance to anthelmintics (Shavulimo, 1989 ; Ahunu and Assoku, 1987). The results of the present study suggest that routine management practices such as regular deworming, cleaning and disinfection of pens, usually carried out by research and higher educational institutions under the semi-intensive system could be effective in reducing the infestation of *Strongyloides* spp. and *Eimeria* spp. but not *Strongyle* spp.

The prevalence of gastrointestinal parasites in scavenging sheep also has public health implications, especially in rural households in Africa. This is because animal keepers in many rural communities maintain close association with their animals and in some communities, people share houses (living in the same house) ↑

with small ruminants, calves and even adult cattle where they are few, for fear of theft (Kambarage *et al.*, 2004). Under such conditions, scavenging animals pose a threat of zoonosis. *Strongyloides stercoralis* is typically a common species of *Strongyloides* that is highly zoonotic (LT, 2003).

The relationship between the prevalence of *Strongyle* spp. and *Eimeria* spp. in this study was generally indefinite and inconsistent in kids however, *Strongyle* spp. predominate only after 53 days of age when the dominance of *Eimeria* spp. (On day 39) has lowered host immunity and receded (Agyei *et al.*, 2004). Younger animals therefore tend to be more susceptible to coccidiosis leading to deaths while adults develop chronic *Strongyle* infestations that serve as a reservoir for (re)infestation of the general flock, especially in intensively managed systems with higher stocking densities (Charry *et al.*, 1992). Though ovine *Strongyles* (*Cabertia ovina*, *Oesophagostomum* spp. and *Nematodirus* spp.) are more prolific, they do not migrate through the blood vessels and hence may not cause as much damage to the host as *Eimeria* spp. and other nematodes. *E. ovinoidalis*, a dominant and pathogenic species is responsible for the mortality of most lambs in Ghana since diarrhoeic conditions in lambs have always been misconstrued as a sign of only helminth infestation and often treated with only dewormers without considering the use of coccidiocides (Agyei *et al.*, 2005).

The level of eosinophils in each management systems was positively correlated with strongylosis, strongyloidosis and coccidiosis. Rising levels of eosinophils are associated with the introduction of multicellular parasites into the body via the lungs and/or gastrointestinal tract in which they phagotosize antigen-antibody complexes while an increase in the level of neutrophils, monocytes, lymphocytes and basophils is an indication of the presence of unicellular invaders such as bacteria, fungi and viruses in the body (Thibodeau, 1987). The high egg/oocyst count which is indicative of the prevalence of the parasites could have accounted for the higher counts of eosinophils observed in the extensively managed flock.

The differential leukocyte profile reported in the

present study for sheep under the semi-intensive management system also varied greatly from those observed for the same breed of similar age range managed under similar conditions at the University of Ghana's Agricultural Research Station in the coastal region of Ghana (Ahunu and Assoku, 1987). Ovine *Strongyle* nematodes do not traverse through the blood vessels but they burrow into the gut wall and may cause irritations, diarrhoea and colic thereby eliciting a rise in the production of eosinophils. The correlation between eosinophils, and *Strongyloides* spp. and *Eimeria* spp. were not significant under the semi-intensive system of management even though these two parasites have been incriminated in 20% of lamb losses in Ghana (Agyei *et al.*, 2005).

A consistent eosinophilia with increasing gastrointestinal parasitism was observed in both management systems and age groups. The level of eosinophils in younger animals in the extensive system was 17 times (132 %) higher than the normal count for sheep in that age group compared to a 15 fold rise (95 %) in the semi-intensive system. A similar trend of leukocytosis in response to gastrointestinal parasitism was observed in adult animals under the extensive system where the percentage variation in eosinophil count reached 296% compared to 212% in the semi-intensive (Table II). In healthy animals devoid of endoparasites, the normal percentage variation in eosinophil count ranges from 1015 % (Ahunu and Assoku, 1987).

A drastic deviation in leukocyte levels from normal as observed in this study has experimentally been shown to stimulate increased adrenal secretion of hydrocortisone and the physiological implications of such a surge include decreased utilization of glucose, decreased amino acid transportation to muscle cells and increased rate of glucogenesis (Chandrawathani, 2004). Studies by Coop and Holmes (1996) have further shown that gastrointestinal nematodes reduce voluntary feed intake and efficiency of feed utilization, by increasing endogenous loss of protein into the gastrointestinal tract. There is also a re-allocation of protein from productive processes into repair of the gastrointestinal tract, synthesis of plasma proteins and mucoprotein

production. The extended effects of these processes are anaemia, general unthriftiness and poor growth performance especially in young animals. The percent deviation in the counts of the rest of the other leukocytes from normal was however generally inconsistent between the two management systems and age groups except lymphocytes which decreased (lymphopenia) with increasing egg count. The correlation between lymphocytosis and gastrointestinal parasitism was therefore consistently negative in both management systems except with oocysts of *Eimeria* spp. (Tables III and IV). Lymphocytosis is a rare physiological phenomenon and would likely occur only under chronic lymphatic disorders or severe inflammation (Walter, 1982).

Gastro-intestinal parasitism is ranked globally and in West Africa with the highest index as an animal health constraint to resource-poor farmers due to their wide geographic distribution, host species range, and high economic implications in all production systems particularly in sheep, goats, poultry and camels (Perry *et al.*, 2005).

CONCLUSION

Gastrointestinal parasites cause significant deviation in the normal leukocyte physiology of sheep in rural extensive management systems that can have implications on productivity of rural sheep. The high prevalence of *Strongyles* even in the semi-intensive management despite routine anthelmintic administration may also suggest drug resistance while the consistent increase in the egg count of *Strongyloides* and oocysts of *Eimeria* spp. in the extensively managed flock could suggest the need for good husbandry practices including routine administration of coccidiocides and nematode anthelmintics in extensively managed sheep. The principal physiological index for assessing the degree of gastrointestinal infestation in the Djallonké sheep could probably be the degree of eosinophilia.

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THE EFFECTS OF MIXED *LEUCAENA* AND *GLIRICIDIA* BROWSE AS SUPPLEMENTS IN THE DIET OF WEST AFRICAN DWARF (WAD) SHEEP ON SOME ASPECTS OF REPRODUCTION

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SUMMARY

An investigation on the effects of mixed browse of *Leucaena* and *Gliricidia* on some reproductive parameters of West African Dwarf sheep is reported. *Leucaena leucocephala* and *Gliricidia sepium* browse mixed in a 1:1 ratio by weight were fed as supplements at three levels: low, medium and high to 15 rams and 16 ewes aged six months for 48 and 24 weeks respectively. In the rams, scrotal circumference and volume showed significant ($P < 0.05$) differences between feeding levels. There were also significant ($P < 0.05$) differences in the peripheral plasma testosterone titres between rams on medium and low as well as between those on the high and low feeding levels. Though the testosterone titre in the high feeding level (14.8 ± 0.98) was lower than that in the medium level (16.1 ± 0.47), the difference was not significant ($P > 0.05$). Prolonged feeding of mixed browse improved lambing performance in the ewe up to the medium feeding level. The high feeding level was associated with the delivery of a stillbirth. There were no significant ($P > 0.05$) differences in the mean lamb birth weights and litter sizes among the three feeding groups. Peripheral plasma progesterone profiles indicated that by sixty days post-partum, one (1) of the three ewes in the low, all three (3) from medium and only two (2) out of four in the high feeding level that were examined had resumed ovarian activity. It was observed also that increasing levels of *Leucaena* consumption increases the length of ovarian cycle. Peripheral plasma profiles of oestradiol 17^{β} failed to reveal any reliable sequence and titres observed were too low.

KEYWORDS: Mixed browse, Supplements, Sheep, Reproduction.

INTRODUCTION

Nigeria has a large population of domestic ruminants of which sheep and goats are the most numerous with an estimated population of 56.6 million (Bourn *et al.*, 1994) and their numerical superiority is believed to be due to their relative trypanotolerance (Porter, 1996). One of the most important constraints to their biological and economic efficiency is nutrition (Otchere and Kallah, 1990). The sheep scavenge around the huts, feeding off land lying fallow near villages or kitchen waste such as cassava, yam, potato and plantain peels, cowpea, maize and rice husks, rice bran and pawpaw leaves (Charray *et al.*, 1992). These are of low nutritive values especially with regards to crude protein content and their availability is inadequate for most part of the year (Otesile and Oduye, 1996). Tropical grasses are also deficient in protein for most year and do not meet the maintenance and production requirements for small ruminants (Charray *et al.*, 1992). Sheep feed on a wide range of feedstuff including rough browse and shrubs. They also have the ability to adapt easily to various environments (Shaker *et al.*, 2004).

The leguminous plant *Leucaena leucocephala* also known as *ipil-ipil* is acceptable as animal feed (Charray *et al.*, 1992) but has the disadvantage of being toxic at high intake levels because of the presence of the toxic amino acid-mimosine. In the rumen, this is converted to dihydroxypyridine (DHP), a compound with goitrogenic properties of the thiouracil type (Allison *et al.*, 1990; McDonald *et al.*, 1995)

Gliricidia sepium is also acceptable and contains high level of protein, which is highly degradable in the rumen, but its content of coumarin does not make it as palatable to animals as *Leucaena* (Ademosun *et al.*, 1985). This is because the coumarin content is normally converted to dicoumarol by molds during storage or ensiling (Radostitis *et al.*, 1995).

The combination of *leucaena* and *gliricidia* in a ratio of 1:1 reduces the risk of *leucaena* toxicity and

the non-palatability of *Gliricidia* while maintaining the high protein quality of the feed (Reynolds and Adeoye, 1985).

It has been reported that survival rates of lambs, birth weights and productivity of sheep would be increased for each additional 400mg of browse dry matter (DM) consumed per day (Reynolds and Adeniran, 1988).

This study was aimed at investigating the effects of *Leucaena leucocephala* and *Gliricidia sepium* browse mixed in ratio 1:1 by weight as supplements in the diet of WAD sheep on body weight, scrotal circumference and volume, testosterone, progesterone titres, litter size, birth weight and lambing performance.

MATERIALS AND METHODS

Sixteen (16) female and fifteen (15) male West African Dwarf (WAD) sheep lambs maintained on 100gm DM of *Leucaena leucocephala* and *Gliricidia sepium* browse mixed in 1:1 ratio as supplement to a basal diet of *Panicum maximum*. The animals after weaning at 12 weeks of age, they were kept in individual pens. They were later randomly allotted into three feeding groups after balancing for weights at the age of six months viz: There were 6 females in Group 1 and 5 in Groups 2 and 3 while there were 5 males in Groups 1-3.

The animals were fed a basal diet of *Panicum maximum ad libitum* and 14gm of dried cassava peels as energy source. This was supplemented with *Leucaena leucocephala* and *Gliricidia sepium* browse mixed in 1:1 ratio by weight at 3 levels: low, medium and high. The supplementation was in two phases, each lasting 24 weeks in the rams but only the first phase in ewes. In phase I, Group 1 (low feeding level) had 300gm/head/day, group 2 (medium feeding level), had 600gm/head/day and group 3 (high feeding level), had 900gm/head/day, while in phase II, group I had 400gm/head/day, group 2 had 800gm/head/day and group 3 had 1,200gm/head/day.

The ewes received the first phase of supplementation (i.e. for 24 weeks) only: after

which they were introduced into the breeding pens with two rams each for six weeks. From the time the ewes were put into the breeding pen through gestation, parturition and lactation in individual pens, they were placed on the same feeding regime containing *Panicum maximum ad libitum*, 50gm per head per day of dried cassava peel and 800gm/head/ day mixed leguminous browse. Water was available to all the animals *ad libitum* and all animals had access to commercial mineral block (salt licks).

The daily feed intake was obtained from total offered minus left over. The scrotal circumference was estimated fortnightly over a period of twelve weeks for each phase and on each ram at the point of greatest diameter using a measuring tape. The scrotal length of each testicle was estimated and the average of both testicles was used as the scrotal length.

The scrotal volume was calculated using this formula:

$$SV = r^2h$$

r = the radius calculated from the scrotal circumference
H = scrotal length

Jugular venous blood was taken from the rams fortnightly and the ewes were bled twice weekly between day 31-59 postpartum. The blood samples were centrifuged at 2000rpm for 10 minutes in a cold centrifuge at 4°C. Plasma was stored at 20°C until assayed for testosterone, progesterone and oestradiol 17^B via radioimmunoassay (Edqvist and Stabenfeldt, 1989).

Data collected were analysed using one-way analysis of variance (ANOVA) and double-checked with student T-test to establish significance (Snedecor and Cochran, 1980).

RESULTS

Table I shows the differences in the mean scrotal circumference and scrotal volume between the rams in groups 1, 2 and 3 at the end of the study (i.e. 48 weeks). The feeding group 1 (low) had

mean scrotal circumference of 22.8 ± 0.44cm, while the medium and the high feeding groups had 24.6 ± 0.15cm and 26.7 ± 0.17cm respectively. The differences among these three groups were found to be significant (p < 0.05). Similarly, the mean scrotal volume for the feeding groups 1, 2 and 3 (i.e. 232.0 ± 4.15, 339.14 ± 18.99 and 425.87 ± 17.32 respectively) were also significant (p < 0.05). Correlation of scrotal circumference with body weight was positive for feeding group 2 (i.e. r = +0.774, p < 0.05); +0.039; p < 0.05 and -0.655; p > 0.05 respectively for groups 3 and 1.

Table II shows the differences in mean testosterone titres during the study. The differences between the high (group 3) and the medium (group 2) as well as between the low and the high groups were significant (p < 0.05). The titre obtained in the high feeding group 3 was lower than that for the medium group (p > 0.05).

Table III shows that the number of lambs born alive is highest for the medium feeding group though not significantly compared with values obtained for other groups (p > 0.05). A still born lamb weighing 1.55kg was also found in the high feeding group. This weight was less than the least mean birth weight in all the three feeding groups but also higher than the lowest birth weight recorded (1.5kg) in its feeding level which was born alive and survived.

Figure 1 shows the progesterone profile for the low feeding group 1. The Figure reveals that within 29 days postpartum, ovarian cyclicity evidenced by progesterone peaks was observed in only one dam. The progesterone peak occurred twice, first at 41st day (0.44ng/ml) and secondly at 56th day (0.47ng/ml).

Figure 2 shows that ovarian activity was observed in three dams in the medium group with progesterone peaks noticed at 2.00ng/ml for ewe A, 1.4ng/ml for B and 0.74ng/ml for C. Figure 3 similarly shows that ovarian activity was observed in only two ewes with peak titres occurring at 1.3ng/ml for ewe A and 0.8ng/ml for ewe B.

In all the groups, progesterone titres were not detected at some points. This occurred at day 45 in

the low feeding group as shown in Figure 1. In the medium feeding group, it occurred on days 41 and

TABLE I: Scrotal circumference (SC), volume and final body weights of rams and their correlation at the end of the feeding experiment.

Group	Mean SC (cm)	Body Weights (kg)		R	Scrotal Volume
		Initial (Kg)	Final (Kg)		
1	22.8 ± 0.044 ^a	11.1 ± 0.73	22.1 ± 0.039	+0.04	232.0 ± 4.148 ^a
2	24.6 ± 0.152 ^b	10.05 ± 0.74	24.7 ± 0.358	+0.73	339.14 ± 18.99 ^b
3	26.6 ± 0.168 ^c	11.10 ± 1.06	27.9 ± 0.286	-0.66	425.87 ± 17.32 ^c

r = Correlation Coefficient
a, b, c = means with unidentical superscripts are significantly different from each other.

TABLE II: Testosterone levels in the plasma of WAD rams between weeks 38 and 48 of the feeding experiment

Group	Mean Testosterone titres (ng/ml)
1	9.1 ± 0.67 ^a
2	16.1 ± 0.47 ^b
3	14.8 ± 0.98 ^b

a, b = means along the same column with identical subscripts are significantly different from each other.

TABLE III: Lambing Parameters in the three feeding groups

Group	No of Dams	No of lambs born alive	Mean litter Size	Mean birth weight (kg)	Stillborn Lambs
1	5	5	1.00 ± 0.00 ^a	1.91 ± 0.35 ^a	
2	5	6	1.20 ± 0.06 ^a	1.92 ± 0.28 ^a	
3	6	5	0.83 ± 0.23 ^a	1.87 ± 0.30 ^a	1

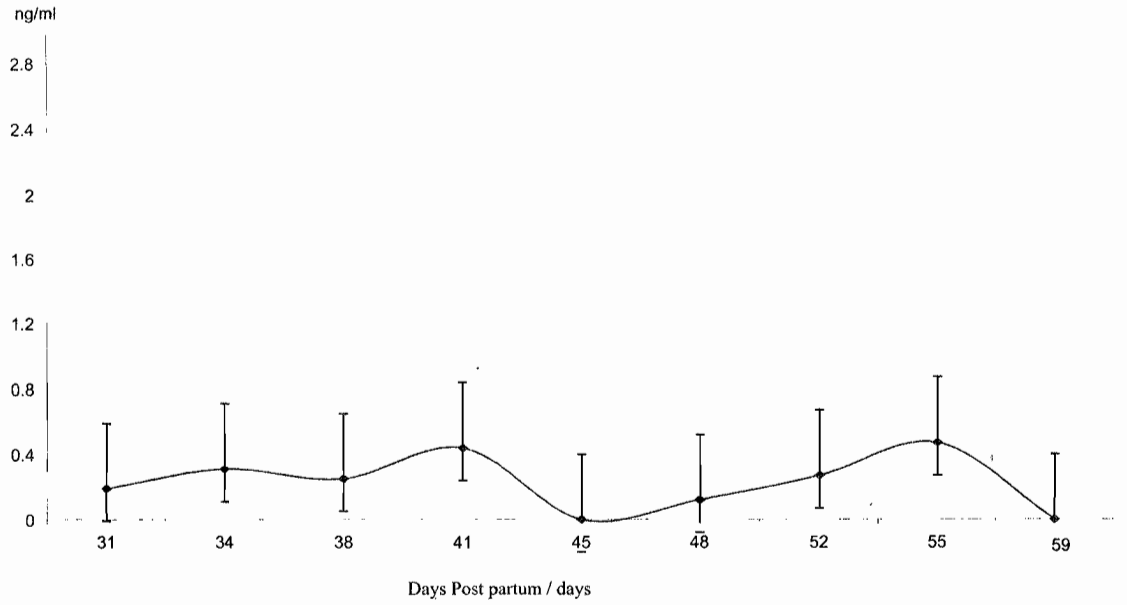


Fig. 1: Peripheral plasma progesterone profiles in group i ewes from day 31 - 59 post-partum

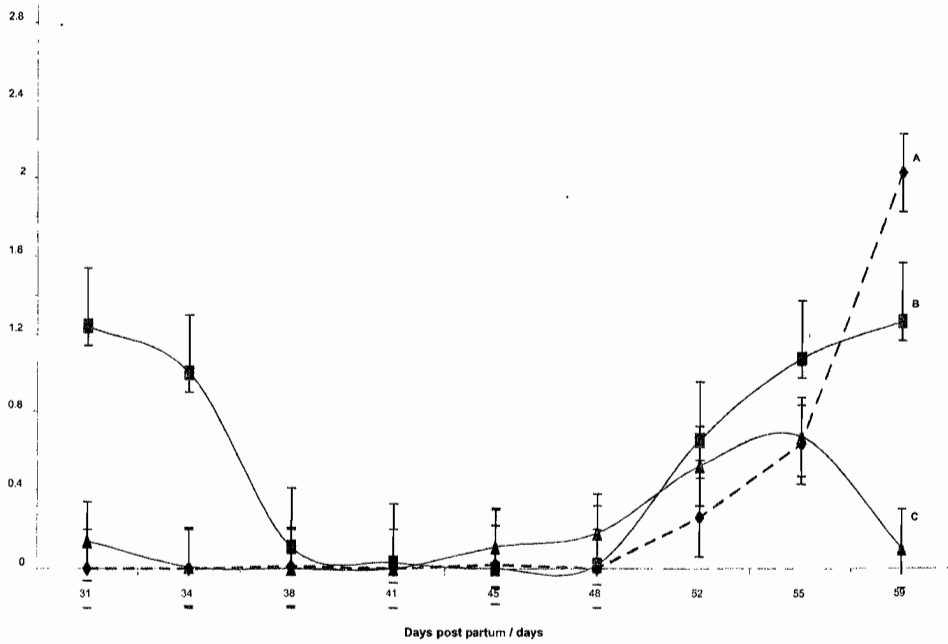


Fig. 2: Peripheral plasma progesterone profiles in group 2 ewes from day 31 - 59 post partum

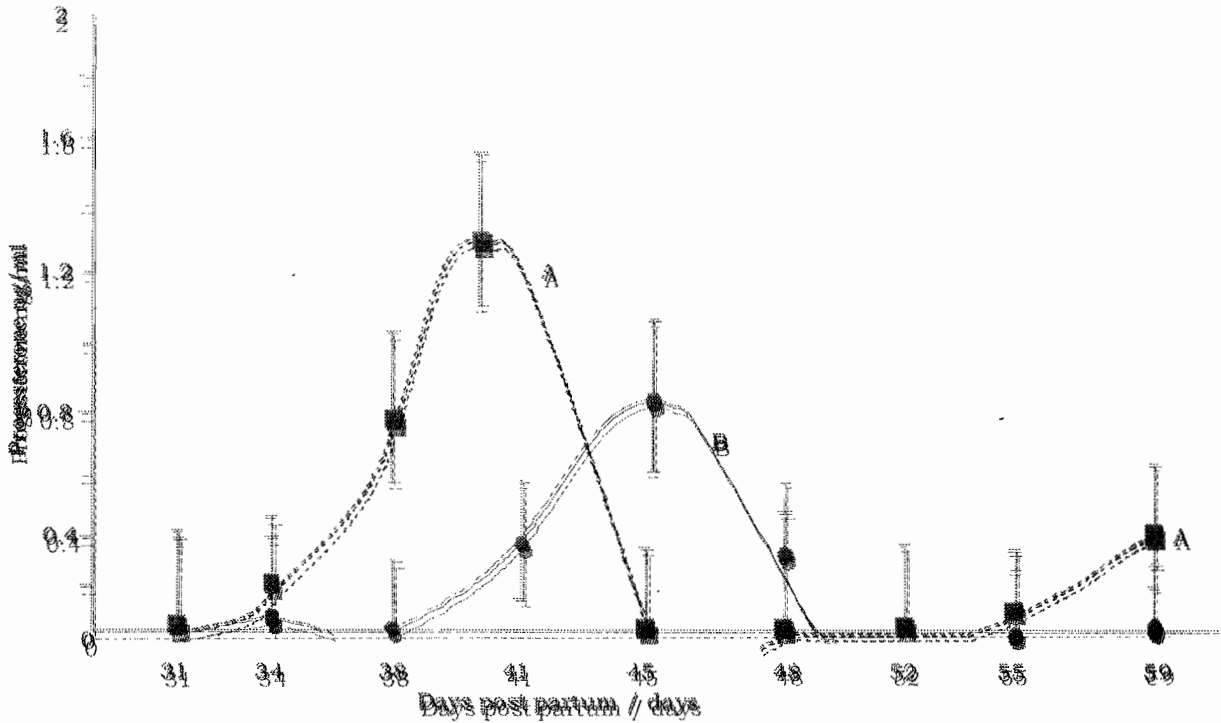


Fig 3: Peripheral Progesterone profiles in Group 3 Ewes From Day 31-59 Post Partum

DISCUSSION

Testicular development in rams is associated with growth rate and age and could be used as basis for sexual maturity (Das and Sarkar, 2004). The observations in scrotal circumference and its correlation with body weights between the low and medium feeding groups are in agreement with findings in bulls (Elmore *et al.*, 1976; Coulter and Foote, 1977), goats (Bongso *et al.*, 1982) and sheep (Braun *et al.*, 1980; Offs and Menon, 1980). The significant correlation of scrotal circumference with body weights in the medium feeding group could result in earlier attainment of puberty if supplementation started shortly after weaning with subsequent use for breeding at an earlier age. This will result in longer reproductive lifespan, increased reproductive efficiency, increased off take and more income from sheep rearing. The negative correlation observed in the high feeding group may be explained based on the finding of Lapwood (1986) that levels of nutrition affects

reproduction through the output of Luteinizing hormone (LH) from the pituitary gland. This is supported by the lower daily mean total feed intake in this group.

The lower mean titres of testosterone in the high feeding group may be connected with reduced LH stimulation. Levels of Testosterone are reported to be positively correlated with those of LH in the testes of rams (Pineda and Faulkner, 1980b). The slight decrease in scrotal size in the high feeding group towards the end of treatment confirms some adverse effects of Leucaena on scrotal growth and function. The still born lamb encountered in the high feeding group may be connected with the high Leucaena content of their feed and this is similar to reports by Heqarthy *et al.* (1964) in sheep and Hamilton *et al.* (1971) in cattle. This view is supported by the fact that no pathology was found in the stillborn lamb.

The lambing performance of the medium was better than that of the high feeding group (Table III). This may be explained by the higher protein available to the medium group (Knight *et al.*, 1975). The best individual performers were from the medium while the least performers were from the high feeding groups. This observation together with others between the middle and highest feeding groups could have resulted from the reaction of the animals to mimosine toxicity which is in agreement with reports by Hegarthy *et al.* (1964) in sheep.

Physiological changes occurring during the puerperium include uterine involution and resumption of ovarian cyclic activity. Several factors also affect postpartum- first oestrus interval, and these include season, suckling intensity, environmental temperature, nature of parturition, perinatal infection, and nutritional status.

In both test groups, the mixed browse enhanced resumption of ovarian cyclic activity due to the level of available protein in their diets and this agrees with previous report by Rattray (1977). Also, the ewe with stillbirth failed to commence ovarian cyclic activity fifty-nine days post partum, which again agrees with earlier report by Jainudeen and Hafez (1982). The ewe with twins in the medium feeding group also resumed ovarian cyclic activity 48 days post partum despite intense lactation and suckling. The enhancement of resumption of ovarian activity by increased mixed browse intake from low to high feeding groups confirm their usefulness as protein supplements capable of reducing the interval to first oestrus and conception post partum (Reynolds and Adeoye, 1985).

The monitoring of peripheral plasma progesterone reveals the fact that higher protein intakes such as obtained in the medium and high feeding groups favoured an earlier resumption of ovarian activity. The peak titres recorded (0.41ng 2.02ng) which was followed by non-detectable levels about three days after is in agreement to the observation of Baird and

Scaramuzzi (1976), as well as Baird and McNelly (1981).

CONCLUSION

From the results of this research, *Leucaena leucocephala* and *Gliricidia sepium* when mixed in equal proportions and fed as supplements to WAD sheep have beneficial effects on steroidogenic capacity, lambing performance and resumption of ovarian cyclic activity post partum. At higher levels of supplementation where the *Leucaena* content of total feed is more than 30%, toxicity due to mimosine and certain undesirable effects may be observed.

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THE PREVALENCE OF BOVINE CUTANEOUS ONCHOCERCIASIS IN RELATION TO SKIN LESIONS IN KADUNA STATE, NIGERIA

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SUMMARY

A total of five hundred and eighteen White Fulani cattle of ages 5-9 years were examined at the point of slaughter in Zaria abattoir, for gross lesions of skin diseases, from November, 2001 to October, 2002. Tick infestations in association with scabs and rough hair coat observed in 130(25.1%) were highest in occurrence, while abscesses were fewest 3(0.6%) among the cattle. Firm nodules of variable sizes (0.5 to 1.0cm in diameter) were found on 39 (7.5%) cattle and were suspected as cases of onchocerciasis. After slaughter, 195 skin specimens were collected from the neck regions. These were fixed in 10% buffered neutral formalin, later processed and stained using Haematoxylin and Eosin (H & E) technique. Histopathology revealed sections of *Onchocerca spp* in the dermis of 30 (15.4%) cattle. The prevalence of onchocerciasis was highest (22.2%) in cattle from Anchau, while those from Sheme had the lowest (9.1%). There was no significant difference ($p>0.05$) between the prevalence of onchocerciasis in cows and in bulls. There was also no significant difference ($p>0.05$) between the prevalence of the disease in cattle in dry and rainy seasons. It was

KEYWORDS: Onchocerciasis, Prevalence, Cattle, Kaduna State, Nigeria

INTRODUCTION

Microfilariasis is caused by worms of the Filarioidea superfamily, while onchocerciasis refers to the same disease where only members of the genus *Onchocerca* are involved and clearly identified (Soulsby, 1982; Georgi and Georgi, 1990). *Onchocerca species* are found in the skin, living in the connective tissues of their hosts, often giving rise to firm nodules in which they lie coiled up. They may be found in subcutaneous tissues of the hump, back, neck, ear, or ventral abdomen, depending on the feeding habits of the Simuliid intermediate hosts in different geographic areas (Soulsby, 1982). *Onchocerca gutturosa* may also be encountered in the nuchal ligament and *O. lienalis* in the connective tissues between the rumen and

spleen (Georgi and Georgi, 1990). The predilection of *O. ochengi* is in the subcutaneous and intradermal nodules of the ventral regions of the abdomen, including the udder or scrotum (Bwangamoi, 1968), while *O. dukei* inhabits subcutaneous and perimuscular tissues of the thorax, abdomen and thighs (Soulsby, 1982).

Animal onchocerciasis is endemic in Africa, Australia and North America (Soulsby, 1982; Georgi and Georgi, 1990; Achikwi *et al.*, 2004). It has the same vectors with the human blinding onchocerciasis (Anosike and Onwuliri, 1995; Mario *et al.*, 1995; Achikwi *et al.*, 2004). The prevalence of the disease could be reduced by mass treatment with ivermectin (Gilbert, 1995) and the control of vector populations (Bissan *et al.*, 1995).

A study of aortic onchocerciasis due to *O. armillata* in cattle slaughtered at Zaria abattoir revealed a high prevalence of 84.5% in the rainy season and 79.2% in the dry season (Schillhorn and Robl, 1975). Some few years later, Ogunrinade (1980) reported a lower prevalence (27.5%) of bovine onchocerciasis in Nigeria. The present study investigated the prevalence of onchocerciasis using skin sections of cattle sampled at the point of slaughter within Kaduna State, to determine whether the prevalence of onchocerciasis has changed since it was last studied more than three decades ago by Schillhorn and Robl (1975).

MATERIALS AND METHODS

Five hundred and eighteen adult White Fulani cattle (293 males and 225 females) presented for slaughter at Zaria abattoir, and slaughter slabs at Anchau, Giwa and Soba were examined for skin lesions. The study was between November, 2001 and October, 2002. The period of the experiment (May to October and November to April) corresponds to the rainy and dry seasons, respectively in the Northern Guinea Savannah zone of Nigeria.

Out of the total number of cattle examined, skin samples were collected from the neck regions of 195 cattle (83 males and 112 females; 53 apparently

normal and 142 clinically sick) at postmortem and fixed in 10% buffered neutral formalin. The samples were processed, sectioned at 5µm thickness and stained with Haematoxylin and Eosin as described by Luna (1968). The sections were then examined for *Onchocerca spp.*

Data were summarized as percentages of population samples and differences between the percentages were assessed by Chi-square test. Values of $p < 0.05$ were considered significant. The percentages of *Onchocerca* positive cases were calculated based on the 195 cattle skin samples examined microscopically.

RESULTS

The post mortem examinations of skins revealed that gross lesions, such as thick scabs with purulent exudates 40(7.7%), nodules with cheesy exudates 37(7.1%) and the wrinkling of skin 8 (1.5%) were found among the cattle examined (Tables I).

Under the light microscope, sections of *Onchocerca spp* were found in the dermis of 30(15.4%) cattle. The prevalence of cutaneous onchocerciasis was found to be highest in cattle from Anchau (22.2%) and lowest among those from SHEME (9.1%) (Table II).

TABLE 1: Prevalence of gross skin lesions in cattle slaughtered at Zaria abattoir (November, 2001 October, 2002).

Skin lesions	Number	%
Rough hair coat	103	19.9
Thick scabs with purulent exudates	40	7.7
Firm nodules	39	7.5
Nodules with cheesy exudates	37	7.1
Tick infestation, thick scabs and rough hair coat	130	25.1
Open wounds (traumatic)	38	7.3
Tick infestations alone	20	3.9
Wrinkling	8	1.5
Subcutaneous abscess	3	0.6
Skin lesions (total)	418	80.7
Normal	100	19.3
Total	518	100.0

TABLE II: The prevalence of intradermal onchocerciasis in cattle according to location

Locations	Number sampled	Number positive	%
Anchau	45	10	22.2
Charanchi	16	2	12.5
Kafur	23	4	17.4
Kano	8	1	12.5
Katsina	19	4	21.0
Makarfi	22	4	18.2
Sheme	11	1	9.1
Soba	7	1	14.3
Zaria	28	3	10.7
Birnin-Gwari	15	0	0
Total	195	30	15.4

There was no significant difference ($p > 0.05$) in prevalence between the cows and the bulls (Table III). Similarly, there was no significant difference ($p > 0.05$) between the dry and rainy seasons' prevalence of onchocerciasis during the study (Table IV). It was observed that the presence of the *Onchocerca spp* did not stimulate infiltration of any inflammatory cells into the dermis of cattle (Fig. 1).

TABLE III: The prevalence of intradermal onchocerciasis in cattle according to sex

Sex	Number sampled	Number positive	%
Males	83	10	12.0
Females	112	20	17.9
	195	30	15.4

$\chi^2 = 1.24$ ($P > 0.05$)

TABLE IV: The seasonal prevalence of intradermal onchocerciasis in cattle

Season	Number sampled	Number positive	%
Dry	172	27	15.7
Rainy	23	3	13.0
Total	195	30	15.4

$\chi^2 = 0.1098$ ($P > 0.05$)

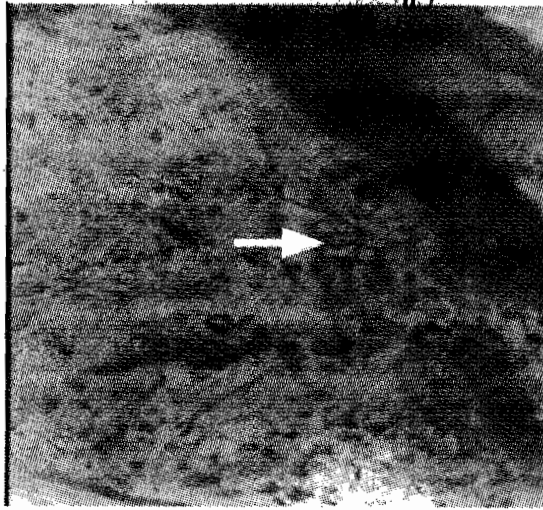


Fig. 1: Scanned colored photomicrograph of a skin section from a bull. Note the coiled *Onchocerca* spp (arrow) in the dermis. H & E stain. X250

The gross skin lesions such as firm nodules associated with onchocerciasis were found in 39(7.5%) cattle. Of these 20(10.3%) cattle were found positive for *Onchocerca*, but there were also 10(5.1%) positive cases among the 53 cattle with apparently normal skins.

DISCUSSION

During the investigation, 20(10.3%) of the cattle found with firm nodules in their skins were confirmed to have had *Onchocerca* spp within the dermis. The observed gross lesion associated with the disease was similar to what was earlier described by Bwangamoi (1968). However, *Onchocerca* spp were also found in skin sections of apparently normal cattle. This variation in gross manifestation of the disease may be as a result of differences in the duration of infection or host reaction to the presence of *Onchocerca* spp.

The present finding of 15.4% prevalence of bovine onchocerciasis in Kaduna State was significantly lower than the results recorded in earlier studies (Schillhorn and Robl, 1975; Ogunrinade, 1980). The lower prevalence observed in this study contradicts the pattern recorded in Australia, in which Ottley and Moorhouse (1978) reported 100% infection rate by *O.gutturosa* among cattle, and later a range of 59-79% prevalence of the

disease was found (Ladds et al., 1979). The decline in prevalence of onchocerciasis in Kaduna state, in the present report, may be due to frequent clinical use of ivermectin. Ivermectin treatment in humans is known to decrease the severity of *Onchocerca* lesions (Gilbert, 1995), and thereby reduce the transmission of *Onchocerca* species and prevalence of onchocerciasis (Tripis et al., 1990; Mario et al., 1995) by preventing embryogenesis and steady attrition of the adult worms (Brian et al., 1992; Rao et al., 1992; Kassa et al., 1994). The variation in prevalence may have also resulted from the difference in predilections of the *Onchocerca* spp involved, and the methods of examination. Our sampling protocol was different, since we preferred the neck skin.

The previous investigators (Ottley and Moorhouse, 1978; Ladds et al., 1979) observed that *Onchocerca gutturosa* infestations were predominantly in the nuchael ligament, while *O. gibsoni* affected the brisket, stifle and hips and *O. lienalis* invaded the gastrosplenic ligament (Ottley and Moorhouse, 1978), but *O. armillata* (Chodnik, 1957) and *O. ochengi* (Achukwi et al., 2004) affected the aorta and cutaneous tissues, respectively. Bwangamoi (1968) also reported *O. armillata* in the skin sections of cattle. The currently lower prevalence of onchocerciasis may also be due to the fact that all of the cattle examined in our study were adults. This is in line with the results of Ladds et al. (1979) who found that the occurrence of onchocerciasis tended to decline as animals reached maturity.

Prevalence of bovine onchocerciasis may indicate the occurrence of the disease in humans who live in the environment where the cattle are reared. For example, in Ningi area, Bauchi state of Nigeria, there was a high prevalence (71.0%) of onchocerciasis among cattle rearers, while farmers and other herdsmen had 49.8% and 40.0%, respectively (Anosike and Onwuliri, 1995). Such high prevalence of onchocerciasis in the population was referable to the presence of the vector (*Simulium* species) in the environment (Bissan et al., 1995; Achukwi et al., 2004) and indicates the need for the treatment of the population with ivermectin. The

finding of 15.4% prevalence of bovine onchocerciasis in this study may similarly indicate that the human population is at the risk of being infected in Kaduna State.

The observed sex and seasonal prevalence of cutaneous onchocerciasis had no significant difference. These results were in agreement with the previous report by Schillhorn and Robl (1975). The observations were also similar to the report of Ladds *et al.* (1979), in which the observed difference was not significant in Australian cattle. Therefore, it seems reasonable to speculate that differences in sex may not affect the occurrence of cutaneous onchocerciasis in cattle.

CONCLUSION

It was concluded that the prevalence of onchocerciasis has reduced drastically in cattle reared in Kaduna State when compared with the previous findings (Schillhorn and Robl, 1975). The lower prevalence may be due to increasing use of ivermectin among humans and animals in the state.

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PREVALENCE OF PPR CASES AMONG SAHEL GOATS PRESENTED AT THE BORNO STATE VETERINARY CLINIC MAIDUGURI NIGERIA FROM 1996-2005

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SUMMARY

A study of the prevalence of PPR among Sahelian goats of the semi-arid region of North-eastern Nigeria, presented at the Borno State Veterinary Clinic over a period of 10 years (1996-2005) was carried out using clinical reports. The results revealed that the number of cases rose to a peak (36.5%) in 1999 and later showed a continuous decline in the subsequent years. The study also showed seasonality, gender and age distribution of the disease among Sahel goats.

KEYWORDS: PPR, Sahel goats, Semi-arid region, Nigeria

INTRODUCTION

Small ruminants are a major component of the livestock sub-sector in most parts of the world including Nigeria (Odo, 2003). Nigeria is blessed with abundant livestock resources with most of the animals concentrated in the northern parts of the country (Egwu *et al.*, 1995). The semi-arid zone of north-eastern Nigeria is reported to account for about 25% of the ruminant population in Nigeria that is put at 13.3 million cattle (Ngere *et al.*, 1984), 20.1 million sheep and 34.5 million goats (Shamaki *et al.*, 2004). Small ruminants accounts for a substantial share of the total household income in most parts of the world and plays a significant role in the religious, social, traditional and cultural rites as well as the provision of high quality animal protein for most developing countries (Saliki *et al.*, 1987). However, the major constraint to a successful

development of this industry is the menace of infectious diseases, often associated with high morbidity and mortality and decline in productive and reproductive performances and even public health concern (Odo, 2003). Principal among these diseases is the peste des petits ruminants (PPR) (Diallo, 2003; Odo, 2003). This is a trans boundary animal disease characterised by fever, erosive stomatitis, nasal and ocular discharges, pneumonia and diarrhoea (Ozkul *et al.*, 2002; Couacy-Hymann *et al.*, 2005). This disease is a contagious trans-boundary disease with significant impact on rural poor farmers, whose control should be considered in the programmes that aim at alleviating poverty in developing countries (Diallo, 2006). However, scanty information exists on the prevalence of this disease among the small ruminant population of the semi-arid region of north-eastern Nigeria.

This study is therefore designed to initiate a preliminary investigation of the prevalence of PPR among the goat population of the semi-arid region of north-eastern Nigeria.

MATERIALS AND METHODS

Study area

This study was carried out in Maiduguri, the capital of Borno state Nigeria. The area is characterised by shrubs and thorny trees with grasses on the low-land areas. The inhabitants of the area are predominantly farmers engaged in crop cultivation and/or animal rearing. The climate of the area is divided into rainy season (June September), cold dusty harmattan season (October February) and the hot dry season (March May) (El-Yuguda *et al.*, 2005).

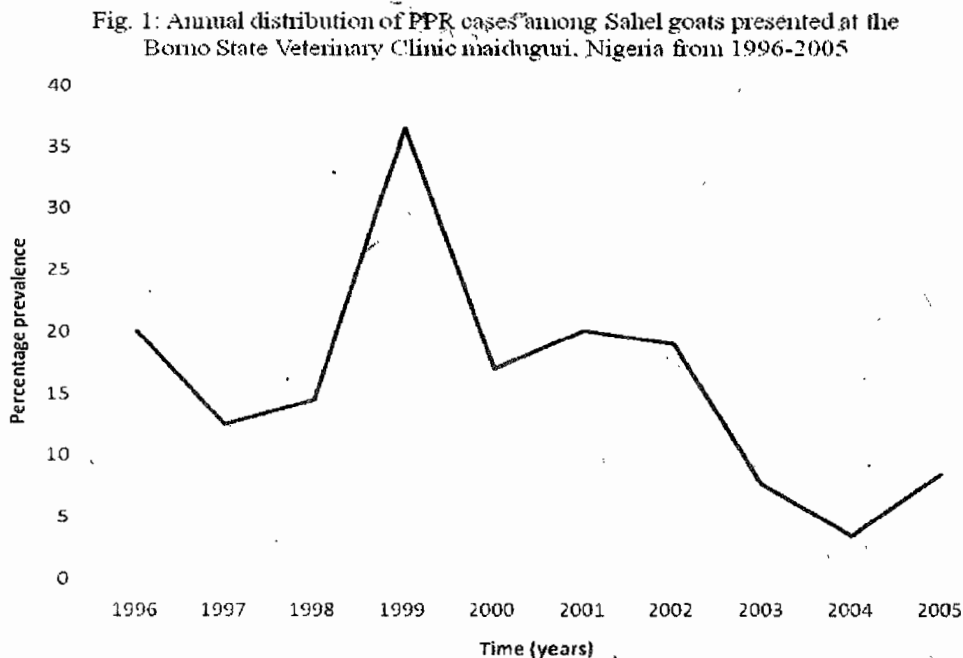
Data collection

The data presented in this report were collected from clinic records of cases involving goats presented at the Borno state Veterinary clinic from 1996 to 2005. The data was subjected to annual, seasonal, age and sex distribution. However,

because of lack of proper aging records we restricted ourselves to using the terms kids (<1 year) and adults (=1 year).

RESULTS

The results of the retrospective survey for the prevalence of PPR among the Sahelian goats presented at the Borno State Veterinary Clinic Maiduguri from 1996 to 2005 showed a gradual increase in percentage prevalence that peaked in 1999 with 36.5% and thereafter exhibited a gradual decline in the subsequent years (Fig. 1). The seasonal distribution of the PPR cases showed the rainy season (June September) to have recorded the highest cumulative prevalence 47.7%, followed by the cold dusty harmattan season (October February) with 28.2% and then the hot dry season (March May) with 24.1% (Fig. 2). The gender distribution of the disease showed higher cumulative prevalence for all the years among the females (63.9%) than the males (36.1%) (Fig.3). The age distribution of the cases showed the adults to be more infected (81.2%) than the kids (18.8%) (Fig.4).



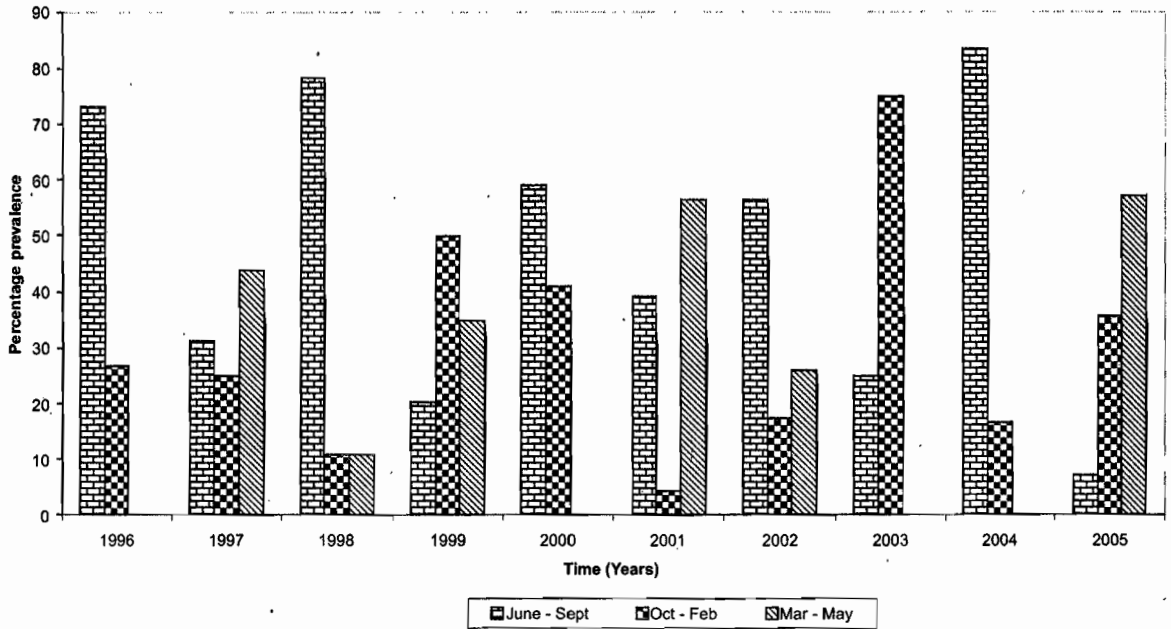


Fig. 2: Seasonal distribution of PPR among sahel goats presented at Borno state Veterinary clinic Maiduguri Nigeria from 1996 to 2005

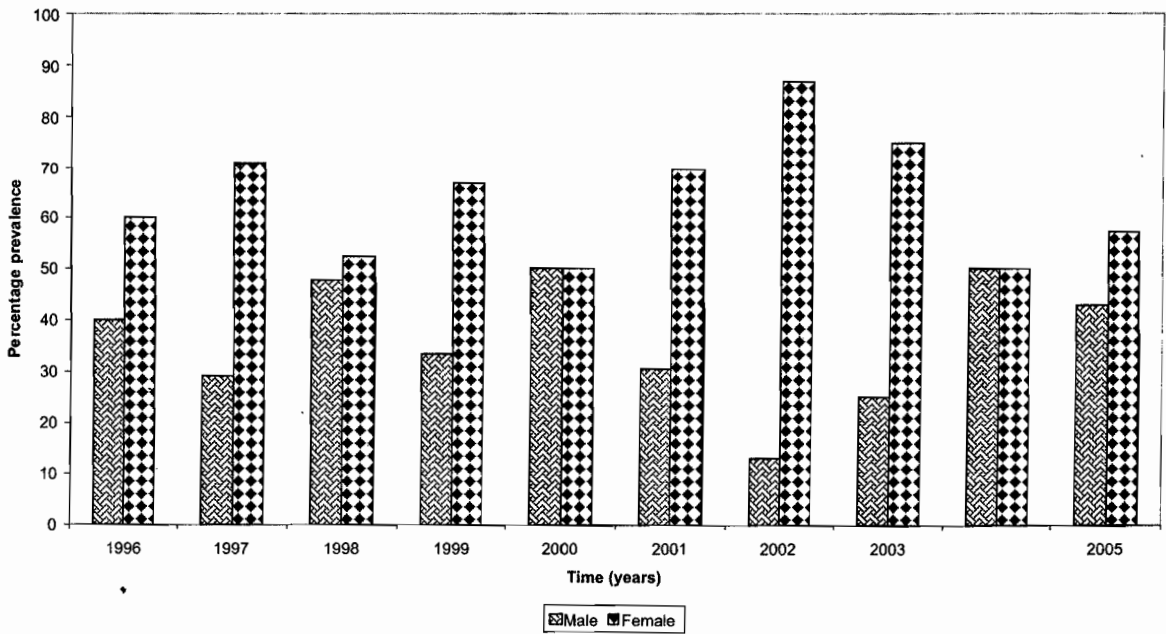


Fig. 3: Sex distribution of PPR cases among Sahel goats presented at the Borno state Veterinary clinic Maiduguri Nigeria from 1996 to 2005

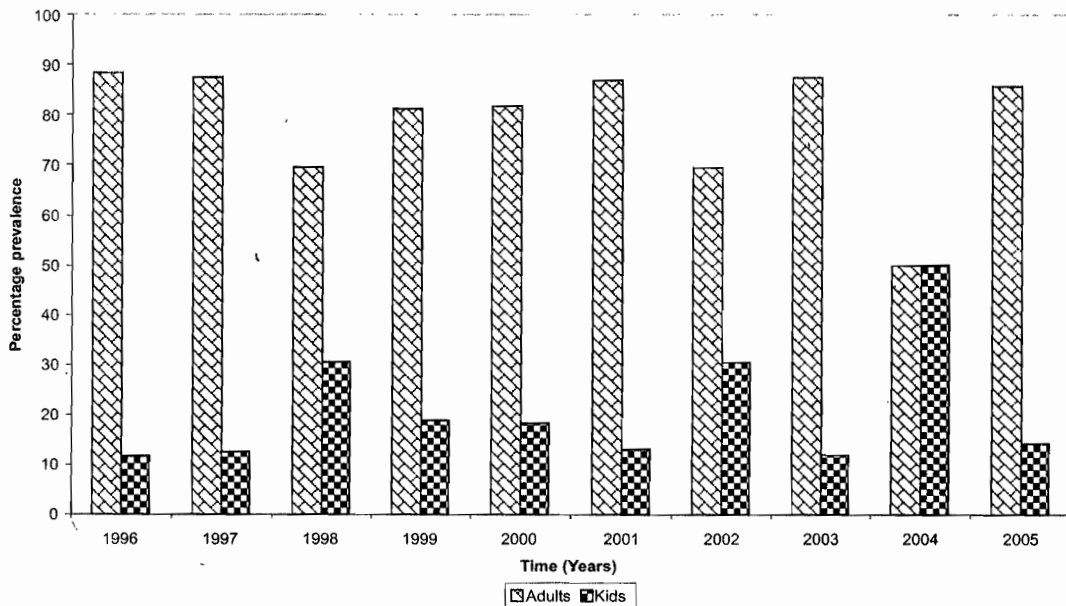


Fig. 4: Age distribution of PPR cases among sahel goats presented at the Borno state Veterinary clinic Maiduguri Nigeria from 1996 to 2005

DISCUSSION

The outbreaks of PPR are recorded regularly by field Veterinarians in many parts of the world using the characteristics of the disease; fever, erosive stomatitis, nasal and ocular discharges, pneumonia and diarrhoea (Couacy-Hymann *et al.*, 2007). The occurrence of a fast spreading fatal disease with the above signs affecting mainly small ruminants should arouse the suspicion of PPR (Obi *et al.*, 1988).

The prevalence of the disease reported in this study was also diagnosed on the basis of these characteristic clinical signs and sometimes post mortem examinations. No plausible explanation could be given for the gradual decline in the cases observed in this report. It was observed in this report that PPR occurred all year round with peak periods in the rainy season followed by cold harmattan season and lowest in the hot dry season. This tally with other reports that showed PPR to occur all year round with tendency to peak during the rainy and cold dusty harmattan seasons (Obi *et al.*, 1988). The difference in sex distribution

observed in the report varies from other reports that indicated no difference in the sex distribution of PPR. The difference reported in this study could be associated with the fact that most farmers sell off their bucks early in life. The difference could also be attributed to reduction in immunity due to pregnancy among the females. More adult goats were also observed to be infected than the kids. This observation does not conform to other reports that showed kids to be more infected than the adult goats (Obi *et al.*, 1988; Ezeibe, 2000). Further studies of the active disease and serosurvey may be required for a better understanding of the epidemiology of the disease among Sahel goats.

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ZOONOTIC RISKS AND TRANSMISSION OF *MYCOBACTERIA* SPECIES FROM COWS' MILK AND SLAUGHTERED CATTLE TO MAN IN IBADAN: ROLE OF BUTCHERS

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SUMMARY

To ascertain the zoonotic risks associated with the handling, processing and consumption of milk and meat products in respect to bovine tuberculosis in Ibadan. This study was conducted by simultaneous screening of 105 unpasteurised cows' milk samples and 587 slaughtered cattle some of which showed gross lesions suggestive of tuberculosis. Samples from the milk and suspected tuberculous lesions were cultured on Lowenstein-Jensen media while nitrate and niacin tests were carried out to classify the isolated *Mycobacteria* species. Prevalence rates of 5.7% and 4.3% were confirmed from the milk and cattle samples screened respectively. Based on the biochemical tests, three isolates of *Mycobacterium tuberculosis*, one of *M. bovis* and one of *M. africanum* were identified from the milk samples; while six *M. tuberculosis*, fourteen *M. bovis*, two *M. africanum* and three unclassified *Mycobacteria* species were obtained from the tuberculous cattle. The unhygienic handling and processing of these animal products by butchers may lead to the zoonotic transmission of *M. tuberculosis* complex to the public and a source of occupational exposures to the butchers.

KEYWORDS: Zoonoses, Food-products, *Mycobacterium tuberculosis*, Butchers, Nigeria

INTRODUCTION

Tuberculosis is an endemic problem in the human and cattle populations in Nigeria (Cadmus *et al.*, 2004; 2006; WHO, 2004). Evidence of zoonotic transmission of *Mycobacteria* species between humans and cattle have been reported in Nigeria (Idrisu and Shnurrenberger, 1977; Idigbe *et al.*, 1986; Cadmus *et al.*, 2006). The pulmonary form of zoonotic tuberculosis (TB) caused by *Mycobacterium bovis* is indistinguishable from that caused by *M. tuberculosis* (Dankner *et al.*,

1993; Cosivi *et al.*, 1998), however, both species belong to the *M. tuberculosis* complex group, which also includes *M. africanum*, *M. microti*, *M. caprae* and *M. canettii* (Brosch *et al.*, 2003; Smith *et al.*, 2006). Cattle derived tuberculosis in man is attributed to *M. bovis* and occasionally *M. tuberculosis* and *M. africanum* (Kazwala, 1998; Cadmus *et al.*, 2006).

Mycobacterium bovis has the widest host range including animals and humans (Acha and

Syrups, 1987; Sreevatsan *et al.*, 1997). *M. bovis* has been a historical source of TB in humans infected through drinking of contaminated unpasteurised milk or inhaling aerosols produced by diseased farm animals (Kleeberg, 1984; Cosivi *et al.*, 1998), and to some extent through consumption of improperly cooked infected meat and meat products.

In countries with a relatively high prevalence of bovine TB in cattle, abattoir and farm workers are the professional groups mostly exposed to infection (Ayele *et al.*, 2004). In Nigeria, the degree of zoonotic transmission of tuberculosis from animals to humans is not fully known, however, cultural practices exist that could facilitate transmission between cattle and humans. For example, prior to sale, cattle are raised and fattened in close proximity to farmers' home. After being sold at markets, cattle are often slaughtered in nearby abattoirs, where the butchers wear minimal protective clothing and process offal from diseased carcasses with bare hands. The close association between farmers and cattle is exemplified by the Fulani herdsmen, who live their entire lives with their cattle, offering ample opportunity for zoonotic transmission of infection (Cadmus *et al.*, 2006).

Cows meant for sale and slaughter at the cattle markets and abattoirs are sometimes milked; the milk samples are consumed by some of the Fulanis'/Hausas' believing that these serve as nutritional or medicinal supplements. However, recent study from a local setting in Ibadan through molecular characterization of *Mycobacteria* species from human isolates revealed that approximately 13% of the disease in humans was caused by strains of *M. africanum* and *M. bovis* rather than *M. tuberculosis* (Cadmus *et al.*, 2006).

The purpose of this study was therefore to establish whether *Mycobacteria* species were being secreted in milk of cows' slaughtered at the abattoir and if the cattle slaughtered were

also infected with these organisms. This is with a view to ascertain the zoonotic risks involved in handling and consuming these food products.

MATERIALS AND METHODS

Study site

The field work was carried out at the Bodija Municipal Abattoir, Ibadan, Oyo State in South-Western Nigeria. It is the largest abattoir in the state and most of the cattle slaughtered here came from the northern parts of the country as well as the neighboring African countries of Benin Republic, Burkina Faso, Cameroon, Chad and Niger. In addition, a few of the cattle bred within the premises of the abattoir were also slaughtered in the abattoir. Like many other abattoirs in the country, there was minimal facility and personnel for proper ante-mortem and post-mortem inspection. This was compounded by a deplorable water supply system, lack of a functional effluent disposal system and heap of wastes from human and animals left over the years.

Duration of study

The entire study spanned a period of two months from June 1st to July 31st 2006. During this period, collections of samples were made on week days (Monday-Friday) between 8.00 a.m. and 12.00 noon (i.e. the peak period of slaughtering).

Milk sample collection

Milk samples were collected from 105 different breeds of cows awaiting slaughter in Bodija abattoir. The choice of cows from which milk was collected was based on the cooperation of animal owners. Milk samples were collected aseptically from udders into 50 ml sterile universal containers and then placed in clean cooler packs. Samples were later refrigerated at 4°C in the laboratory prior to culturing.

Collection and storage of suspected tuberculous lesions

The animals inspected were identified based on sex, age and breed (Tables I, II and III). Post-

mortem examination was carried out on 587 breeds of slaughtered cattle with different tissue samples, organs and lymph nodes inspected for suspected lesions of TB. From these, only 25 animals had suspected lesions of TB. About 50g to 150g of the infected tissues were collected aseptically and kept individually in well labeled and sealed sampling bags in cooler packs in the abattoir before being transported to a -4C freezer in the laboratory prior to processing. The samples collected included lungs (n=17), mediastinal lymph node (LN) (n=2), mesenteric LN (n=2), other LN (n=3), parenchymatous organs (n=2), spleen (n=2), aorta (n=1) heart (n=1), muscles (n=3) (Table I).

Laboratory work

All the laboratory work was done in the Tuberculosis Laboratory of the Department of Veterinary Public Health & Preventive Medicine, University of Ibadan, Ibadan, Nigeria.

i) Processing of samples for detection of

Mycobacteria: The processing of milk samples and lesions was based on the Becton Dickinson digestion and decontamination procedure (Anonymous, 1999). The same procedure was carried out for processing both the milk and suspected lesions (for the lesions, grinding with pestle and mortar was first done with the addition of sterile distilled water before the procedure). The digestion and decontamination procedure entailed using a sterile 15 ml centrifuge tube; equal amounts of specimen and activated NALC (N-acetyl-L-cysteine)-NaOH of about 5 ml each were added. The centrifuge tube was capped and mixed on a vortex-type mixer until the specimen was liquefied. The mixture was allowed to stand at room temperature for 15 min with occasional gentle shaking. Prepared phosphate buffer was added to the 15 ml mark on the centrifuge tube and mixed, followed by centrifugation for 15 to 20 min at 3,000 x g. The supernatant was decanted, and 2 ml of phosphate buffer of pH 6.8 was added to re-suspend the pellet.

ii) Microscopic examination: In all, sediments from 33 suspected tuberculous lesions (i.e. tissues,

organs and lymph nodes) from 25 animals and 105 milk samples (Table I and II) were examined microscopically using the Ziehl Neelsen staining technique for the detection of acid fast bacilli.

iii) Cultural examination: The Lowenstein-Jensen (L-J) medium was used to culture sediments from 33 suspected tuberculous lesions from 25 animals and 105 milk samples after the decontamination and digestion method according to the Becton Dickinson procedure. Samples were cultured at 37°C for 8 to 12 weeks on paired L-J media enriched with pyruvate (L-J-P medium) or with glycerol (L-J-G medium) as earlier described by Cadmus *et al.* (2004).

iv) Biochemical tests

Niacin test: An extract from 3-4 weeks old *Mycobacterium* culture was prepared by adding 1.5 ml of sterile distilled water to the culture. The surface growth was gently scraped off using a pipette and a stab was made through the growth into the medium to permit extraction of the niacin employing the use of a 1ml sterile pipette. The tube was slanted such that the medium was covered with the liquid and was left in this position for about 25 minutes. Then, approximately 0.6ml of the extract was removed with a sterile capillary pipette fitted with a bulb and transferred into a test tube and was covered with a stopper. A negative control was prepared by adding 0.6ml of distilled water in place of the extract. Using a pair of flamed forceps, a BBL Taxo TB Niacin Test Strip (Becton, Dickinson and Company, Sparks, Maryland 21152 USA) with arrow downward was dropped into each tube and covered with a stopper immediately. The tubes were shaken gently to mix the fluid with the reagent on the bottom of the strip. This was repeated after 5-10 minutes. The colour of the extracts was then compared after 12-15 minutes. Yellow colour in the extract was considered positive for *Mycobacterium tuberculosis*; suggestive of *M. africanum*,

while no colour change is suggestive of *M. bovis*.

Nitrate test: 0.5ml of distilled water was added to a clean screw-cap tube into which two clumps of growth was added using a 1 ml pipette. The growth was dispersed. Using a pair of flamed forceps, a BBL Taxo TB Nitrate Test Strip (Becton, Dickinson and Company, Sparks, Maryland 21152 USA) with arrow downward was dropped into each tube and the tubes held vertically. The tubes were then capped and incubated at 37°C for 2 hours. The tubes were gently shaken at the end of the first and second hours of incubation. After the 2 hours of incubation, the tubes were carefully tilted back and forth six times to wet the entire strip and were then slanted at room temperature to cover the strip with the liquid and were left to remain in this position for 10 minutes. A change of colour at the top portion of the strip to light or dark blue indicated nitrate reduction; thus implying *Mycobacterium tuberculosis* while no colour change indicated a negative reaction suggestive of *M. africanum* or *M. Bovis*.

RESULTS

Cultural examination

From the milk samples, isolations were made from two (4.35%) White Fulani, one (7.14%) Red Bororo and three (7.9%) Sokoto Gudali breeds of cattle giving a prevalence rate of 5.7% (Table II). However, all the 33 suspected tuberculous lesions from the 25 cattle were culture positive, giving an overall prevalence rate of 4.3%. The highest prevalence rate of 8.4% was recorded among the White Fulani breed followed by 3.0% in the Red Bororo breed and 1.5% among the Sokoto Gudali breed (Table II). The adult cattle were the most affected (5.1%) (Table III).

Microscopic examination

All the isolates had typical microscopic appearances of *Mycobacteria* upon acid-fast staining.

Biochemical tests

Results from the biochemical tests revealed three isolates of *Mycobacterium tuberculosis* (presence of nitrate reduction and positive niacin production), one of *M. bovis* (absence of both nitrate reduction and niacin production) and one of *M. africanum* (absence of nitrate reduction and slightly positive niacin production) from the milk samples based on the related interpretations used by Kallenius et al. (1999) and Niobe-Eyangoh et al. (2003); while six *Mycobacterium tuberculosis*, fourteen *M. bovis*, two *M. africanum* and three unclassified *Mycobacteria* species were identified from the isolates of the slaughtered cattle (Table IV).

TABLE I: Predilection sites of suspected tuberculous lesions collected

Animal no	Breed	Sex	Age	Lesions collected							Total lesions collected per animal			
				Lungs	Mediastinal ln	Mesenteric ln	Hepatic ln	Liver	Spleen	Aorta		Heart	Muscles	
I	WF	F	A	*		*								2
II	WF	F	A	*										1
III	WF	F	Y.A	*						*				2
IV	WF	F	A	*			*						*	3
V	WF	F	A										*	1
VI	WF	M	A	*										1
VII	WF	F	A				*							1
VIII	WF	F	A	*						*				2
IX	WF	F	Y.A								*			1
X	WF	F	A	*										1
XI	WF	F	Y.A	*						*				2
XII	WF	M	A	*										1
XIII	WF	F	A		*									1
XIV	WF	F	A					*						1
XV	RB	M	A	*										1
XVI	WF	F	A				*							1
XVII	WF	F	A	*										1
XVIII	WF	F	A	*									*	2
XIX	WF	M	A	*										1
XX	WF	F	A	*	*									2
XXI	WF	F	A			*								1
XXII	RB	F	A	*										1
XXIII	SG	M	A					*						1
XXIV	RB	F	A	*										1
XXV	WF	F	Y.A	*										1

KEYS:

WF: White Fulani; RB: Red Bororo; SG: Sokoto Gudali

M: Male; F: Female

A: Adult; YA: Young Adult

*: Predilection site from which lesion was collected

TABLE II: Breed distribution of culture positive milk samples and slaughtered cattle lesions

BREED	MILKING COWS			SALUGHTERED CATTLE		
	TOTAL EXAMINED	CULTURE POSITIVE	% POSITIVE	TOTAL EXAMINED	CULTURE POSITIVE	% POSITIVE
WHITE FULANI	46	2	4.35	251	21	8.40
RED BORORO	14	1	7.14	101	3	3.00
SOKOTO GUDALI	38	3	7.90	66	1	1.50
KURI	-	-	-	38	-	-
MIXED	7	0	0.00	64	-	-
BOKOLO	-	-	-	67	-	-
TOTAL	105	6	5.7	587	25	4.3

TABLE III: Sex and age distribution of culture positive slaughtered cattle

		TOTAL	CULTURE	% POSITIVE
		EXAMINED	POSITIVE	
SEX	MALE	138	5	3.6
	FEMALE	449	20	4.5
AGE	<1YR	4	-	-
	1-3YRS	174	4	2.3
	>3YRS	409	21	5.1
TOTAL		587	25	4.3

TABLE IV: Results of the biochemical tests

ISOLATES FROM MILK SAMPLES				ISOLATES FROM CATTLE LESIONS			
No of Samples	Nitrate Test	Niacin Test	Interpretation	No of Samples	Nitrate Test	Niacin Test	Interpretation
3	Dark blue	Yellow	<i>M. tuberculosis</i>	6	Dark blue	Yellow	<i>M. tuberculosis</i>
1	NVR	NVR	<i>M. bovis</i>	14	NVR	NVR	<i>M. bovis</i>
2	NVR	Yellow	<i>M. africanum</i>	2	NVR	Yellow	<i>M. africanum</i>
0	Dark blue	NVR	Unclassified	3	Dark blue	NVR	Unclassified

NVR: No Visible Reaction

DISCUSSION

The prevalence rate of 5.7% obtained from the milk samples is lower than the previous work by Cadmus and Adesokan (2007) in which 11.3% was obtained from 53 unpasteurised cows' milk samples in the same abattoir. Another major difference between the two studies is that unlike in the previous work where *M. bovis* was the only species isolated; *M. tuberculosis* and *M. africanum* were also identified in this study based on the available biochemical tests. Since there was no molecular typing of these isolates when compared to the studies by Kallenius *et al.* (1999) and Niobe-Eyangoh *et al.* (2003) particularly regarding the identification of *M. africanum*, conclusions made were based on extrapolations of biochemical analyses carried out in these two previous studies.

Compared to the work done by Alhaji (1976), our result was lower than the 54.5% he obtained from 11 pooled milk samples from markets in the northern states of Nigeria. However, he also isolated *M. bovis*, *M. tuberculosis* and other unclassified *Mycobacteria* species. The reason that could be given for the lower prevalence rate in our present work when compared to other works cited may not be unconnected with the larger sample size in our present study.

As regards the results from the slaughtered cattle, the prevalence rate obtained was lower than the 8.8% by Cadmus *et al.* (unpublished data) in 2004 in the same abattoir. In the same vein our result is also lower than the 8.2% and 10.5% prevalence obtained in a private beef cattle herd in Ibadan by Cadmus *et al.* (2004) and by Wekhe and Berepubo (1989) in the eastern abattoir of the country respectively. However, one important finding from this work is the isolation of different *Mycobacteria* species; results which are similar to published work by Cadmus *et al.* (2006).

The results from both the milk and cattle screened can be summarized as follows: **i.** Different *Mycobacteria* species were incriminated in the

occurrence of bovine tuberculosis in this abattoir **ii.** The disease was found mostly in the White Fulani breed of cattle which were also the majority of cattle slaughtered. **iii.** The most affected age group was the over three year olds (adult). **iv.** All the suspected animals with tuberculous lesions were confirmed positive by culture.

Judging from the summary above, there are some zoonotic risks the cattle marketers, meat inspectors, butchers and other people directly involved in the cattle industry in this abattoir are exposed to. This submission is drawn from the following realities:

i. About 50% -70% of animals examined at this abattoir are emaciated, weak and unthrifty. **ii.** There are no proper infrastructural facilities to separate diseased and healthy animals. **iii.** The setting in this abattoir does not allow for the opportunity to carry out detailed meat inspection. **iv.** Cows brought for slaughter are sometimes milked for human consumption. **v.** There are no protective wares used while carrying out postmortem examination despite the cases of bovine tuberculosis recorded in the abattoir. **vi.** Most butchers use their bare hands to process carcasses, even those showing TB suspect lesions. **vii.** Butchers who have cuts on their hands still go ahead to process infected carcasses. **viii.** Improperly washed hands are also used by butchers to eat while slaughtering is going on. **ix.** Children and food sellers are always present in the slaughter slabs while slaughtering and meat processing is going on. **x.** The slaughter slabs are always over congested with humans and cattle. **xi.** Gutters meant for easy passage of effluents within the slabs are sometimes used for urinating. **xii.** Drainages inside the slabs are often blocked through stuffing of hidden infected tuberculous tissues and other diseased organs. **xiii.** Water supply is grossly inadequate and often non-potable water is used for meat processing. **xiv.** The slaughter slabs are always in an un-hygienic state and slaughtering is done on bare floors. **xv.** The same knives and other processing materials are used to handle healthy and

infected/contaminated carcasses. **xvi.** Condemned meat and offal due to TB are also regularly smuggled out of the slabs by butchers and other meat processors to be sold to unsuspecting buyers.

The consequences of the above findings and practices in the abattoir therefore support the transmission of bovine tuberculosis to most workers involved in meat processing in this abattoir, together with the general consuming public. Therefore, our observations and findings support the assertion made by Ayele *et al.* (2004) that in the countries with a relatively high prevalence of bovine TB in cattle, abattoir and farm workers are the most exposed to infection.

As earlier confirmed, in countries where animal TB is uncontrolled, most human cases occur in young persons and results from the drinking or handling of contaminated milk or milk products (Acha and Syzres, 1987; Cosivi *et al.*, 1998) and close association with infected livestock (Cadmus *et al.*, 2005). The increasing trend of pulmonary and extra-pulmonary TB in children in Ibadan has been confirmed by Akang *et al.* (1993) and Osinusi (1998) and this may not be unconnected with the endemicity of bovine TB in both farm and slaughtered cattle in Ibadan (Cadmus *et al.*, 2004, 2006).

The isolation of *M. tuberculosis* from the milk samples and the lesions of the slaughtered cattle further confirm the zoonotic nature of tuberculosis. In an earlier work by Cadmus *et al.* (2006), *M. tuberculosis* was also isolated from cattle in this abattoir. This confirms that due to the close co-habitation of humans and cattle and the endemicity of tuberculosis due to *M. tuberculosis* in the human population, man has also been found to infect cattle and other animals.

From the above scenarios, more cases of *M. tuberculosis*, *M. bovis* and *M. africanum* infection may spread to the larger society through the food chain and close contact with

abattoir workers since they are highly exposed to the *M. tuberculosis* complex. The human to human transmission of *M. bovis* is therefore likely to be facilitated through the deplorable lifestyles and living conditions of most butchers in Ibadan. Majority of the butchers are known to have multiple sexual partners, hence vulnerability to human immunodeficiency virus (HIV). Some of them are also involved in drug abuse coupled with the habit of heavy drinking of alcohol while working within the abattoir premises. Due to these risk factors, the butchers are more at risk of being infected with these *Mycobacteria* species either through the pulmonary or extrapulmonary route. Hence, once infected through the pulmonary route, they are therefore more likely to infect others through contacts made over long or short periods.

Based on all the above, vis-à-vis the unwholesome activities in meat processing and risk factors the butchers are exposed to, coupled with their lifestyles, it therefore becomes evident that they are a special group that is highly at risk with the *Mycobacterium tuberculosis* complex infection. Hence, the butchers are a group that needs to be studied in the epidemiology of bovine tuberculosis in Nigeria.

CONCLUSION

Bovine tuberculosis remains a major zoonotic problem in animals slaughtered in Nigerian abattoirs. Evidences have shown that *M. bovis*, *M. tuberculosis* and *M. africanum* are found in milk and organs of slaughtered cattle; hence humans are exposed to these pathogens through the food chain. However, there is a need to complement biochemical tests with molecular typing to conclusively characterize species and strains of *Mycobacteria* from infected cattle in order to better understand the epidemiology of the disease in the country. The butchers and all those involved in the trade cattle industry are at high risk of exposure to tubercle bacilli and could therefore serve as sources of spread to other humans. In conclusion, government should step

up bovine TB control programmes in the country as well as incorporate stakeholders in the livestock industry in the national control of tuberculosis in Nigeria.

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THE ROLE OF NEURAMINIDASE IN THE PATHOGENICITY OF NEWCASTLE DISEASE: A REVIEW

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SUMMARY

The enzyme neuraminidase (mucopolysaccharide N-acetylneuraminyl hydrolase EC 3.2.1.18) is part of haemagglutininneuraminidase protein present in Newcastle disease virus (NDV) and all members of paramyxovirus genus. Neuraminidases are known to play an important role in the pathogenicity of many diseases by enzymatic removal of sialic acids from carbohydratecontaining molecules, such as erythrocytes of chickens and other animal species. It is also believed that neuraminidases could facilitate the production of infectious particles *in vitro* by removing sialic acid residues, and exposing appropriate cleavage site in cell culture. This special feature will enable neuraminidases to fulfill important pathological role during infection or disease. Because of the presumed role of neuraminidases in pathogenicity of diseases, it is important to critically examine the neuraminidase of NDV in relation to its intimate connection with the structure and function of the host cells, and the often serious consequences that result during and after NDV infection in poultry.

KEY WORDS: Neuraminidase, Newcastle disease, Pathogenicity, Sialic acids, Review

INTRODUCTION

Newcastle disease virus (NDV) is the causative agent of a major poultry disease in the world. The virus has a wide range of susceptible avian hosts. About eight thousand species from twenty-seven of the fifty orders of birds are apparently susceptible to NDV (Kaleta and Baldauf, 1988). The susceptibility of different avian hosts to NDV has been demonstrated in both naturally occurring and experimentally induced infections (Roy *et al.*, 2000; Wehmann *et al.*, 2003).

Newcastle disease (ND) is still dreaded and causes serious economic losses in poultry industry in many parts of the world, owing to its high

mortality and morbidity rates (Oladele *et al.*, 2003; Saidu *et al.*, 2006). For example, infection of susceptible birds with virulent strains of NDV could result into morbidity and mortality of about 50% and 100%, respectively. Egg production may be reduced drastically or complete loss of egg production may be experienced in infected flock following infection with virulent strains of the virus (Alexander, 1997; Aldous *et al.*, 2003; Al-Garib *et al.*, 2003). Reports from many parts of Nigerian rate ND as one of the greatest constraints to the development of rural poultry production (Adene, 1990) Also among the

diseases of poultry, ND constitutes the most important epizootic disease in most developing countries, causing serious economic threat to poultry (Shamaki *et al.*, 1989; Oladele *et al.*, 2003).

DISTRIBUTION OF NEWCASTLE DISEASE VIRUS HAEMAGGLUTININ-NEURAMINIDASE (HN) PROTEIN

Newcastle disease virus contains six proteins (Samson, 1988; Gould *et al.*, 2003). One of the most important of these proteins is the haemagglutinin-neuraminidase (HN) protein, which is responsible for haemagglutinin and neuraminidase activities of the virus (McGinnes and Morrison, 1986; Lin *et al.*, 2003). It is known that both the haemagglutinin and neuraminidase activities are found on larger NDV glycoprotein in contrast to the distribution of these activities on two separate glycoproteins in orthomyxoviruses (Gould *et al.*, 2003; Kommers *et al.*, 2003). By analogy with orthomyxoviruses, the haemagglutination activity is a consequence of the adsorption of virus to cell via the virus glycoprotein and cell surface receptors (Lipkind and Shimanter, 1986). These receptors contain sialic (neuraminic) acid. One of the presumed roles of the neuraminidase activity is to aid elution of budding virions from the host cell by destroying local receptors. Sialic acid residues are not found on glycoproteins from virions which contain neuraminidase and this is thought to be significant in preventing dissemination (Alexander *et al.*, 1999).

In some avirulent NDV strains, such as Queensland V4 and Ulster 2C, the HN protein is synthesized as an inactive precursor HN₀ (Gould *et al.*, 2003). The NDV HN gene sequence studies have revealed that there is a single open reading frame coding for 577 amino acids for both NDV Beaudette C and Hitchner B1 strains, with predicted unglycosylated molecular weight of 63,149 and 63,250 daltons, respectively. Both strains contain a highly hydrophobic sequence close to the N terminus, which is highly

conserved between the two strains (Russell *et al.*, 1990; Gould *et al.*, 2003).

Neuraminidases cleave the O-glycosidic linkages between the terminal sialic acids and the sub-terminal sugars of the free and glycoconjugates-bound oligosaccharides as one of the first steps in sialoglycoconjugate degradation. Neuraminidases are also present in metazoan animals and in diverse microorganisms, such as viruses, fungi, bacteria and protozoan parasites (Guzman *et al.*, 1990; Engstler *et al.*, 1993). In view of their ability to cleave O-glycosidic linkages, it is believed that these enzymes play a major role in spreading infection or acting as virulence factor in invasive infections (Godoy *et al.*, 1993; Kommers *et al.*, 2003).

THE DISTRIBUTION OF SIALIC ACIDS RECEPTORS IN NEWCASTLE DISEASE VIRUS AND OTHER ORGANISMS

In general, sialic acids have great chemical and biological diversity. They are ubiquitous, relatively large, hydrophilic and acidic molecules that exert physicochemical effects on glycoconjugates to which they are bound, and on the environmental molecules *in situ*; for example, in cell membrane (Schauer *et al.*, 1995; Koketsu *et al.*, 2003).

The analysis of Hitchner B1 strain of NDV HN sequence showed that the sialic acid binding analogue to that of the influenza neuraminidase activity protein is the sequence: asn arg lys ser cys ser, between amino acid positions 234 and 239 in NDV HN (Sakaguchi *et al.*, 1989; Gould *et al.*, 2003). This sequence is well conserved among other paramyxoviruses that have been analysed (parainfluenza 3, Sendai virus) and exactly the same amino acids are predicted at the same position in the HN of Beaudette C strain of NDV that have been sequenced (Schaper *et al.*, 1988). The conserved region between NDV and sendai virus is: gly ala glu gly arg leu at amino acid positions 399 to 404 in NDV shows similarity to influenza A sialic acid receptor binding site. This sequence is also found in B1 strain of NDV

(Gould *et al.*, 2003).

Sialic acids act as masks to prevent biological recognition, thus playing the role of maintaining the life span of molecules and cells which they protect (Schauer, 1982; 1985). However, it is known that viruses, bacteria and protozoan parasites recognize sialic acids receptor sites and bind to them on cell surfaces via haemagglutinin, and consequently, exert deleterious effects on their hosts (Traving and Schauer, 1998; Christensen and Bisgaard, 2000).

It has been established that during the life span of red blood cells (RBCs), sialic acids are also removed stepwise from the surface of the cells by action of serum neuraminidases, and by spontaneous chemical hydrolysis (Durocher *et al.*, 1975; Schauer and Kamerling, 1997), thereby exposing the desialylated RBCs to destruction by reticulo-endothelial system.

POSSIBLE ROLE OF NEURAMINIDASE IN PATHOGENICITY OF NEWCASTLE DISEASE

The Paramyxovirus haemagglutininneuraminidase (HN) protein from NDV is a multifunctional protein which is responsible for binding to cellular sialylglycoconjugate receptors, promotion of fusion through interaction with the second viral surface fusion (F) glycoprotein, and processing progeny virions by removal of sialic acid from newly synthesized viral coat protein (Crennell *et al.*, 2000; Connaris *et al.*, 2002). This process of sialic acid removal is vital in the pathogenicity of many diseases, affecting both man and animals.

Neuraminidases are key enzymes of sialic acids catabolism, hydrolyzing the glycosidic linkage between sialic acid molecules, and the penultimate sugar of the carbohydrates chains of oligosaccharide and glycoconjugates (Nagai *et al.*, 1976).

The role of neuraminidases in pathogenesis of disease is controversial. However, certain assumptions have been made. For example, some microbial pathogens' neuraminidases are believed to act as virulence factors, allowing successful competition with the host, by alleviating their spread in host tissue (Godoy *et al.*, 1993). It is also believed that neuraminidases unmask the sub-terminal host cell structures, which then serve as receptors for the parasites and toxins, as in the case of cholera (Gallen *et al.*, 1992). Neuraminidases enable the release of viral progeny by the cleavage of host sialic acid (Wehmann *et al.*, 2003).

The action of neuraminidases on erythrocytes' sialic acids could result in anaemia in animals (Figure 1). This is because it is believed that neuraminidases can remove the sialic acids, which cover the RBCs. As a result, the galactose residues are demasked on the RBCs surfaces, thus presenting a signal for degradation by liver hepatocytes (Durocher *et al.*, 1975; Esiebo *et al.*, 1982; Schauer, 1982; Wen *et al.*, 2000).

Chickens inoculated with NDV Kudu 113 strain was observed to develop anaemia which was pronounced during the period of high neuraminidase activity. This was coupled with negative and significant correlations between neuraminidase activity and erythrocytes surface sialic acid concentrations ($r = -0.764$, $P < 0.001$), and between neuraminidase activity and packed cell volume (PCV) ($r = -0.792$, $P < 0.001$). These results became presumptive evidence of a close relationship between circulating NDV Kudu 113 strain, the production of neuraminidase and accelerated erythrocytes destruction. Therefore, the acute anaemia observed in the infected chickens was attributed to the activities of the circulating NDV Kudu 113 strain, which produced neuraminidase, and in turn cleaved off erythrocytes surface sialic acid from RBCs, thus rendering them more prone to erythrophagocytosis (Oladele *et al.*, 2002b;

Oladele, 2005). This could be responsible for the scanty phenomenon of erythrophagocytosis observed histopathologically in the liver of infected chickens as a result of desialylation of erythrocytes by neuraminidase (Oladele, 2005).

Durocher *et al.* (1975) found that following injection of desialylated ⁵¹Cr-labelled erythrocytes into rats and rabbits, there was a rapid clearance of desialylated erythrocytes from circulation, with sequestration in the liver. Kaptzan *et al.* (2000) and Shibuya (2001) also found that reduction in erythrocytes sialic acid contents rendered the RBCs more vulnerable to phagocytosis by macrophages. Also in the mice, it was found that apoptotic cells were recognized and phagocytosed by macrophages, and the molecular property of these cells, recognized by macrophages was the loss of cell surface sialic acids (Itzhaki *et al.*, 2000).

In previous studies by Oladele (2005) the reduced erythrocytes surface sialic acid concentrations observed during the period of

acute anaemia probably contributed to the reduction in infected chickens' erythrocytes half-life. Similar assumption was made in bovine trypanosomosis, that significant reduction in erythrocytes surface sialic acid concentrations in infected animals, during the period of anaemia, might be contributing, at least in part (Magaji, 1975; Esievo *et al.*, 1982; Lipkind and Shimanter, 1986), to the reduced erythrocytes half-life observed in trypanosomosis. Also, studies on human erythropoietin have shown that direct relationship exists between sialic acid-containing carbohydrate and its serum half-life (Egrie and Browne, 2001).

Although Cheville and Beard (1972) and Cheville *et al.* (1972) attributed the frequent anaemia in NDV infection to be due, at least in part, to replication of the virus in the host cells and lysis of erythrocytes, the *in vivo* removal of erythrocytes surface sialic acid by NDV Kudu 113 strain neuraminidase which consequently, resulted in erythrophagocytosis by macrophages, has added another mechanism to the pathogenesis of NDV (Oladele, 2005).

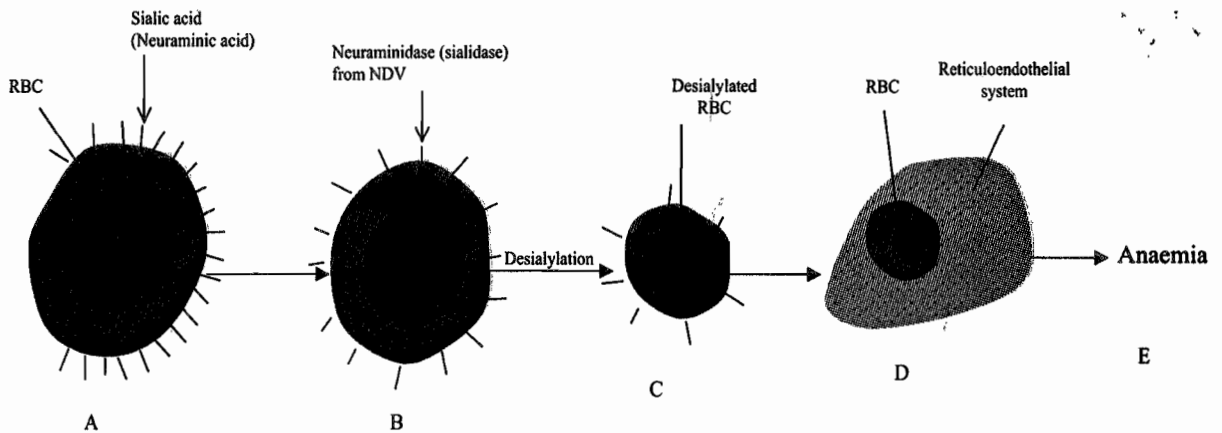


Figure 1: Schematic diagram showing the role of neuraminidase in inducing anaemia in poultry in Newcastle disease

- A:** Normal RBC masked by sialic acids (Neuraminic acids)
- B:** NDV neuraminidase attacks the RBC and cleaves off sialic acids
- C:** Desialylated RBC as a result of cleavage of sialic acids by neuraminidase
- D:** Desialylated RBC is engulfed by reticuloendothelial system
- E:** Anaemia ensues as a result of reduced number of circulating RBC

Also in previous studies in infected chickens, NDV Kudu 113 strain induced necrosis and depletion of reticulo-endothelial cells of the spleen, intestine, caecum and other intestinal lymphoid tissues (Oladele, 2005). These findings were in line with the results of Cheville *et al.* (1972), Lam and Hao (1987), Lam and Vasioncelos (1994), and Lam (1996) who found that virulent NDV strains induced the disappearance of lymphoid tissues, necrosis of spleen, vacuolation of lymphoid tissues, destruction of lymphocytes and lymphopaenia. The exact mechanism of lymphoid depletion in NDV infection is still unknown. However, from histopathological findings, Cheville and Beard (1972) postulated that NDV could be lymphocidal. Furthermore, Woodruff and Woodruff (1972) postulated that after NDV infection, the lymphocyte surface receptors could be altered and their migration patterns changed, so as to cause seeding of lymphocytes in the lymphoid organs. It is therefore, reasonable to surmise that the neuraminidase produced by the NDV Kudu 113 strain as reported by Oladele (2005) might have cleaved sialic acid off lymphocytes too, thus altering their surface receptors and migrating patterns.

During the studies of the effects of neuraminidase on RBCs of chickens naturally infected with NDV, the erythrocytes surface sialic acid concentration obtained from chicken naturally infected with NDV was significantly lower ($P < 0.001$) than mean values obtained from apparently healthy chickens (Oladele *et al.*, 2002b). This suggests that the reduction in erythrocytes surface sialic acid of chickens that were naturally infected with NDV was probably a mechanism of erythrocytes destruction as previously observed by Durocher *et al.* (1975).

Furthermore, Oladele (2005) found negative and significant correlations between neuraminidase activity and erythrocytes surface sialic acid concentration ($r = -0.447$, $P < 0.001$) and between neuraminidase activity and PCV ($r = -0.698$, $P < 0.001$) in chickens that were naturally infected with NDV. These results suggest that the presence

of NDV might have caused increased neuraminidase activity in circulation, drastic cleavage of erythrocytes surface sialic acids, and hence increased erythrocytic senescence and removal from circulation, with reduction in the PCV value of chickens naturally infected with NDV (Durocher *et al.*, 1975; Oladele *et al.*, 2002b).

It was also observed that chickens vaccinated with NDV Komorov vaccine had higher daily mean values of neuraminidase, free serum sialic acid and haemagglutination inhibition antibodies than their counterparts that were vaccinated with NDV La Sota vaccine (Oladele *et al.*, 2006). This result suggests that the level of neuraminidase and free serum sialic acid concentrations in chickens vaccinated with NDV vaccines will depend among other things, on the pathogenicity and or virulence of the viruses from which the NDV vaccines were produced.

CONCLUSION

The role of neuraminidase in the pathogenicity of ND (in *in vitro* and *in vivo* studies; in naturally occurring NDV infections and in chicken vaccinated with NDV vaccines) has been reviewed. The precise intra and extracellular pathological roles of this enzyme during NDV infection, to some extent, remain obscure. Further elucidations of the role(s) of neuraminidase in the pathogenicity of ND is required for better understanding of the pathogenesis of the disease, and consequently, assist in the management, control and eradication of ND in poultry.

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THE EFFECTS OF SINGLE AND COMBINED CHEMOTHERAPY OF DL- α -
DIFLUOROMETHYLORNITHINE AND DIMINAZENE ACETURATE IN EXPERIMENTAL
TRYPANOSOMA BRUCEI GAMBIENSE INFECTION IN MICE

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SUMMARY

The chemotherapeutic effects of DL- α -difluoromethylornithine (DFMO) and diminazene aceturate in *Trypanosoma brucei gambiense* infected mice were studied. All the infected mice developed parasitaemia 4 days post infection (P.I.). Weakness, increased respiration, rough hair coat, pallor of pinnae, snout and footpads were the major clinical signs observed in the acute phase of the disease. Terminally, there was progressive weight loss and hypersomnia (somnia) which occurred from day 22 P.I. Hepatomegaly, splenomegaly and atrophy of body fats were the major necropsy findings. All treatments commenced at the onset of parasitaemia by day 4 P.I. DFMO at 400mg/kg body weight was administered orally as a 4% concentration in Group B or in combination with diminazene aceturate at a single standard dosage of 3.5mg/kg body weight in Group C, caused a significant amelioration of all the clinical signs. There was a significant ($P < 0.05$) decline in mean packed cell volume (PCV) but the decline in mean white blood cell counts (WBC) was not significant ($P > 0.05$). The combined therapy of 4% DFMO and diminazene aceturate resulted in a faster attainment of PCV, WBC pre-infection values and the disappearance of the parasites from circulation than the other treatment regimes.

KEY WORDS: Chemotherapy, DL- α -difluoromethylornithine, Diminazene aceturate, *Trypanosoma brucei gambiense*, Mice.

MATERIALS AND METHODS

Experimental animals

Thirty (30) adult (Balb/c) albino mice of both sexes weighing between 32-50g and obtained from the Department of Biochemistry, University of Maiduguri were used for the study. The mice were maintained on a standard diet (ECWA Feeds Ltd., Jos) and housed in clean plastic cages maintained at room temperature in the Laboratory of the Department of Veterinary Microbiology and Parasitology, University of Maiduguri. Clean

water was provided *ad libitum*. They were allowed to acclimatize to their new environment for 14 days before the commencement of the experiment.

Source of trypanosomes and experimental drugs

Trypanosoma brucei gambiense strain (NITR/Abraka) was obtained from the Nigeria Institute for Trypanosomiasis Research (NITR), Vom, Nigeria. The isolates were confirmed to be *T.b. gambiense* after they were subjected to the

negative Serum Incubation and Infectivity Test (SIIT) (Kaguraka *et al.*, 1988; Owen and Gillet, 1992). The parasites were passaged serially in donor rats. The experimental mice were infected with blood from the donor rats containing $1.5 \times 10^6/\mu\text{l}$ of *T.b. gambiense*. Blood samples were diluted with phosphate-buffered glucose saline (pH 7.2). Twenty-five mice (25) were infected with the parasite intraperitoneally while the remaining five served as uninfected control. DL- α -difluoromethylornithine (DFMO) was obtained from Merrill Dow Research Institute Ohio, USA, in a white crystalline form, while diminazene aceturate (Berenil[®]) was obtained from Hoechst, Farbwerk, Germany.

Experimental protocol

The mice were randomly divided into 6 groups (A, B, C, D, E and F) of 5 mice each. Group A was treated with 2% solution of DFMO orally for 4 consecutive days starting from day 4 P.I. starting at the onset of parasitaemia. Group B was treated with 4% solution of DFMO for the same number of days starting at the onset of parasitaemia. Group C was treated with a combination of 4% solution of DFMO orally for 4 consecutive days and 3.5mg/kg of diminazene aceturate as a single standard dose intraperitoneally from day 4 P.I. Group D was treated with 3.5mg/kg of diminazene aceturate alone as a single standard dose intraperitoneally starting at the onset of parasitaemia from day 4 P.I. Groups E and F on the other hand served as infected and uninfected controls, respectively.

Estimation of parasitaemia, haematology and necropsy Parasitaemia was detected by the wet mount and buffy coat microscopy, thereafter it was estimated every 4 days by the rapid matching technique (Herbert and Lumsden, 1976). The packed cell volume (PCV) of the tail blood of the mice was determined by the microhaematocrit method, while the white blood cell counts (WBC) was determined by the Neubauer counting method every 4 days (Schalm *et al.*, 1995). Dead mice and those routinely sacrificed at the end of the study were subjected to necropsy while the liver and spleen were carefully removed, washed and

weighed using Metler's electronic weighing balance (Hawksley, England).

Statistical analysis

The data obtained were analyzed using two-way analysis of variance (ANOVA) to detect significant differences between groups in tested parameters at 95% confidence limit (Maed and Curnow, 1983).

RESULTS

The clinical signs observed were weakness, rough hair coat, pallor of ears, snout and foot pads. These were, however, more pronounced during the first wave of parasitaemia for groups B, C, D and E, while group A experienced the same in the first wave and during relapsed parasitaemia. Hypersomnia (somnia) was characteristically observed from day 22 P.I. These symptoms however, became less pronounced in Group C, treated with a combination of 4% DFMO and 3.5 mg/kg of diminazene aceturate. The pre-patent period for the infected groups ranged from 4 - 6 days.

The parasite scores of the mice treated with either DFMO or diminazene aceturate or its combinations is presented in Figure 1. The maximum survival period for the infected and untreated controls was 20 days, while those in the other groups did not manifest any death during the study period. A relapsed parasitaemia was however noticed by day 26 P.I. with a count of $5.5 \times 10^3/\mu\text{l}$ in the group treated with 2% DFMO. Detectable parasitaemia for all groups was $5.5 \times 10^3/\mu\text{l}$ and it rose sharply to a peak of $25.5 \times 10^3/\mu\text{l}$ by day 10 P.I. but later declined sharply in all treatment groups. Parasitaemia reached a mean peak value of $500.0 \times 10^3/\mu\text{l}$ by day 12 and was maintained till day 16 P.I. and in the infected but untreated group it led to the death of all mice.

Following infection, the PCV values showed

sharp decline in comparison to that of the uninfected controls, corresponding to the first wave of parasitaemia (Fig.2). However, those treated with either single or combined treatment experienced increase to pre-infection levels of their mean PCV values by day 28 P.I. Meanwhile, the infected but untreated control group which had a significant ($P<0.05$) drop in PCV value by day 18 P.I. with all the mice dead within the period. The decline in mean PCV between groups was most pronounced in the group treated with 2% DFMO due to relapsed parasitaemia encountered by day 20 P.I. The PCV of the healthy control remained relatively constant throughout the study period. All groups experienced minor fluctuations in WBC

values which were not statistically ($P>0.05$) significant, except in the infected but untreated group, which experienced a significant ($P<0.05$) decline in mean WBC values. The WBC counts of the uninfected control, however, remained relatively constant throughout the period of the study (Fig.3). The weights of the liver and spleen of mice that died as a result of the infection or those routinely sacrificed were more in the infected but untreated control than in those treated. Among the treated groups, however, the mean weights of the liver and spleen of those treated with a combination of both drugs was much lighter, followed by those treated with 4% and 2% DFMO respectively (Table 1).

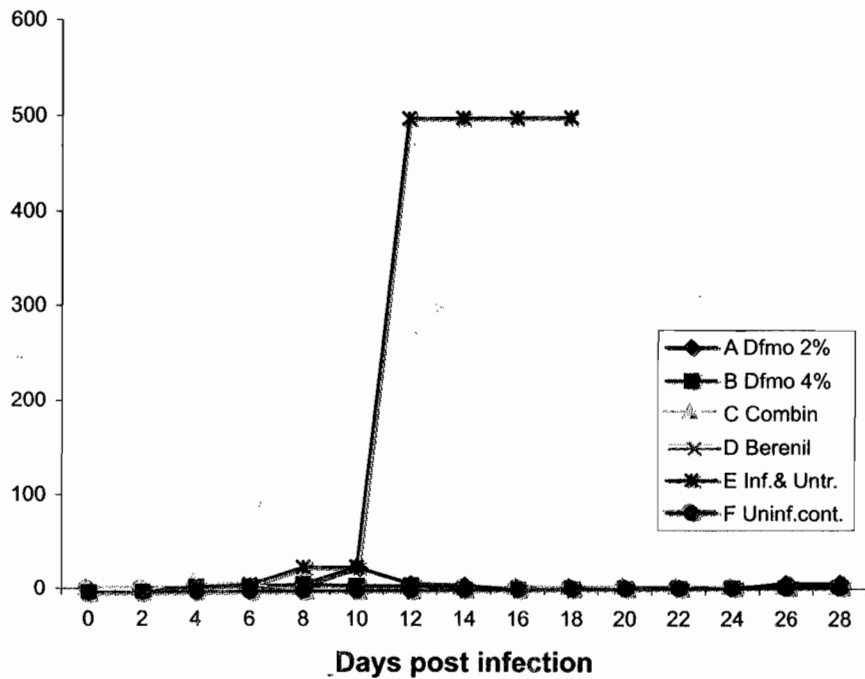


Fig. 1:

Keys:

- DFMO = DL- α -difluoromethylornithine
- Combin. = Combination of 4% DFMO and Berenil[®]
- Inf. & Untr. = Infected and Untreated control
- Uninf. Cont. = Uninfected control

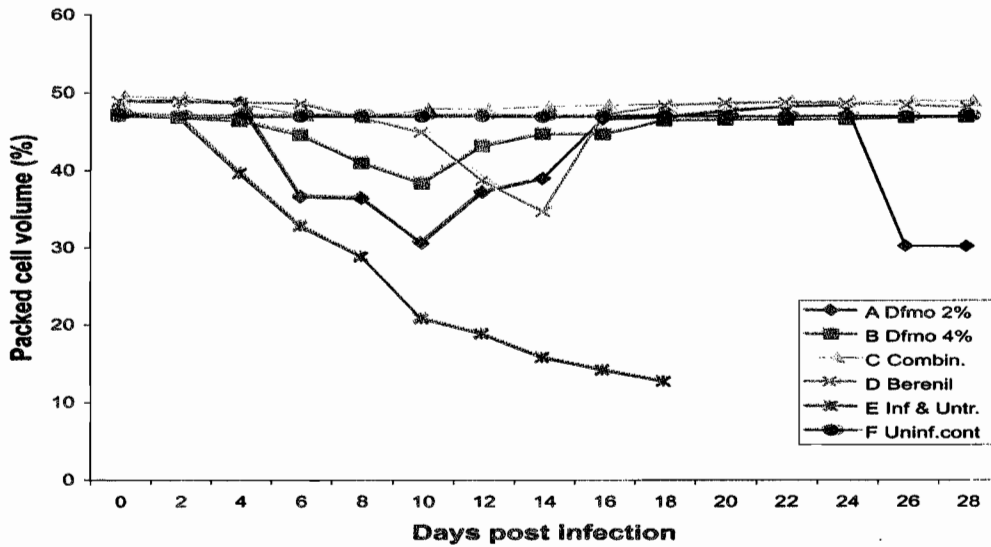


FIGURE 2: Packed cell volume (%) of mice infected with *Trypanosoma brucei gambiense* and treated with either DFMO, Berenil® or their combination

Keys:

- DFMO = Di-4-difluoromethylornithine
- Combin. = Combination of 4% DFMO and Berenil®
- Inf. & Untr. = Infected and Untreated control
- Uninf. Cont. = Uninfected control

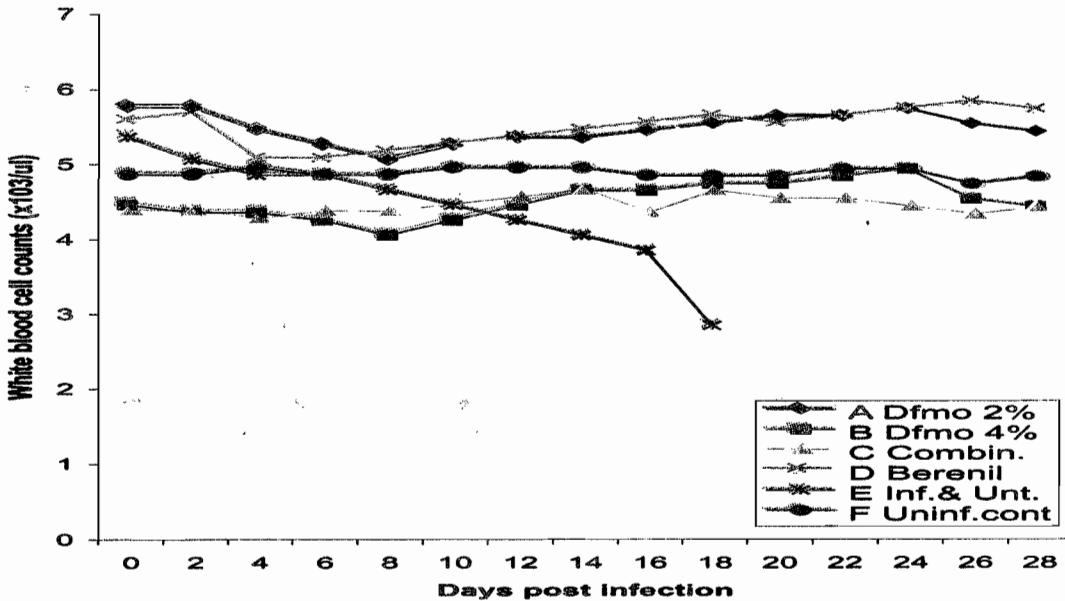


FIGURE 3: White blood cell count ($\times 10^3/\mu\text{l}$) of mice infected with *Trypanosoma brucei gambiense* and treated with either DFMO, Berenil® or their combination

Keys:

- DFMO = Di-4-difluoromethylornithine
- Combin. = Combination of 4% DFMO and Berenil®
- Inf. & Untr. = Infected and Untreated control
- Uninf. Cont. = Uninfected control

TABLE I: Mean (\pm S.D) liver and spleen weights (g/100g body weight) of mice infected with *Trypanosoma brucei gambiense* and treated singly with various concentrations of DL- α -difluoromethylornithine or in combination with diminazene aceturate with their controls.

Groups	Treatments		Liver weight (g/100g body weight)	Spleen weight (g/100g body weight)
A	DFMO 2%	(n = 5)	6.4 \pm 0.7 ^a (3.2)*	4.0 \pm 0.3 ^a (2.2)*
B	DFMO 4%	(n = 5)	4.6 \pm 0.3 ^b (2.2)*	2.0 \pm 0.3 ^b (1.0)*
C	Combination	(n = 5)	3.0 \pm 0.2 ^c (1.0)*	1.2 \pm 0.2 ^c
D	Berenil [®]	(n = 5)	4.7 \pm 0.3 ^b (2.2)*	2.2 \pm 0.3 ^b (1.0)*
E	Infected control	(n = 5)	10.1 \pm 0.8 ^d (4.4)*	4.8 \pm 0.3 ^a (2.3)*
F	Uninfected control	(n = 5)	2.8 \pm 0.3	1.2 \pm 0.2 ^c

n = number of rats in each group; * = number of times organ weights increased
^{a,b,c,d} Values in columns with different superscripts differed significantly ($P < 0.05$)
 DFMO = difluoromethylornithine

DISCUSSION

Experimental infection of albino mice with virulent strain of *Trypanosoma brucei gambiense* led to anaemia demonstrated by low packed cell volume (PCV) and pallor of ears, snout and which feet were the predominant symptoms encountered. Anaemia is a consistent feature of human trypanosomiasis due to *T. brucei gambiense* (Damian *et al.*, 1994; Radomski and Buguet, 1995; Hepburn *et al.*, 1995), which is mainly haemolytic (Anosa, 1988). The virulent course of the *T.b. gambiense* strain in the mice differed from the typically chronic nature of the disease in man (Scott, 1970), but mimicked the course of an experimental *T.b. brucei* in animals (Losos and Ikede, 1972). The observation, however, confirmed the existence of a typical type II of *T.b. gambiense* with the resultant *T.b. rhodiense*-like syndrome in man (WHO, 1998; Abenga and Anosa, 2004). Secondly, the virulence of this strain might have been further enhanced by serial

passages prior to sub-inoculation.

Serial passages have been shown to enhance virulence of the *T. brucei* sub-group (Mbaya *et al.*, 2007). Such virulent course by the parasite leading to human sleeping sickness had been reported in Gboko, Nigeria (Emiribe, 1988) and has been described in an outbreak in Abraka, Nigeria (Enwezor and Ukah, 2000).

Treatment of the mice with 2% concentration of DFMO experienced relapse parasitaemia in contrast to those treated with 4% concentration. This shows that DFMO is dose - dependent as mice given the higher concentration of the compound singly and in combinations with Berenil[®] demonstrated greater and faster parasite clearance and subsequent reversal of clinical signs. Graded dose response has been reported as a consistent feature of DFMO in the treatment of coccidiosis of chickens (Jibike *et al.*, 2002) and in animal trypanosomiasis (Jibike *et al.*, 1995). *T.b. gambiense* metabolizes glucose

to produce 4-hydroxy-4-methyl a-ketoglutarate, which is inhibitory to the tricarboxylic acid cycle in the mitochondria and also destroys blood glucose due to aerobic glycolysis (Igbokwe, 1994). These might have been responsible for the profound body weakness encountered in the infected mice. The relapse parasitaemia encountered with 2% concentration of the compound is an indication of central nervous system involvement in the disease process. Trypanosomes sometimes evade the action of trypanocidal agents because the drug molecules are too large to cross the blood brain barrier in sufficient quantity to be curative (Jennings, 1991).

Hypersomnia encountered in the mice is the cardinal sign encountered in human African trypanosomosis. This occurrence in humans (Damian et al., 1994; Hepburn et al., 1995) and in primates experimentally infected with *T.b. gambiense* (Abenga and Anosa, 2004) has been attributed to the meningitis, which occurs during early infections.

The final stage in the pathogenesis is the break down of the choroids plexus thereby compromising the blood brain barrier (Pantreath, 1995) with a subsequent movement of the parasites into localized areas of the brain. This was authenticated during the course of the infection when brain homogenates of a relapsed albino mouse injected intraperitoneally into a recipient albino rats produced parasitaemia after 4-8 days post- inoculation. This suggests that the brain in the later stages of the infection harboured the trypanosomes. Homogenates of the spleen, liver, kidney, heart and lymph nodes at this stage were however, non-infective. This is consistent with earlier reports in dogs infected with *T. brucei* (Chukwu et al., 1990).

The liver and spleen were enlarged in the positive control. This observation agrees with the findings of Anosa (1988). The degree of enlargement of these organs might have contributed to the more severe anaemia experienced by this group than their counterparts. Enlarged liver and spleen have been reported to contribute significantly to the level of the anaemia in African animal trypanosomosis (Anosa, 1988; Hepburn et al., 1995), through increased erythrophagocytosis in the spleen and liver (Anosa, 1988) and also due to the formation and enlargement of germinal centres, proliferation of plasma cells and macrophages and accumulation of oedema fluid (Ikede, 1981). The combined therapy of 4% DFMO and diminazene aceturate was found to be more effective in the treatment of *T. brucei gambiense* with a faster attainment of pre-infection levels of haematological indices than all the other treatment regimes.

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Short Communication

AGGLUTINATION OF RED BLOOD CELLS BY CANINE DISTEMPER VIRUS

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INTRODUCTION

Canine distemper is a disease of dogs and other carnivores. However Gordon *et al.* (1991) has reported association of canine distemper virus in cases of Pagets disease of humans. This suggests that the disease is of zoonotic importance. Canine distemper disease is caused by a morbillivirus which possesses the haemagglutinin antigen (Gibbs *et al.*, 1979). Despite this, haemagglutination test has not been developed for diagnosis of canine distemper. Current methods of diagnosing canine distemper disease include, the use of clinical signs and gross lesions (Hagan and Brunner, 1961), histopathological examination of tissues (Frisk *et al.*, 1999), virus isolation in cell cultures or in embryonated chicken eggs (Evans *et al.*, 1991, Kai *et al.*, 1993), serum neutralization (Frjolic *et al.*, 2000), Enzyme Linked Immunosorbent Assay (ELISA) (Welsh *et al.*, 1992) and Florescent Antibody Technique (FAT) (Yoshida *et al.*, 1999). Use of clinical signs and gross lesions alone are not reliable because there are other diseases which show similar manifestations (Horst, 1975). Histopathology is reliable only when both cytoplasmic and intranuclear inclusion bodies are found (Pare *et al.*, 1999). In addition, this method is time consuming. ELISA and FAT require sophisticated and expensive equipment often not available in developing countries. Virus isolation in cell culture and in embryonated chicken eggs take days before results can be obtained. Haemagglutination and the corresponding haemagglutination - inhibition tests are very simple, valid, cheap and rapid techniques (Johnson, 1971). Haemagglutination of red blood cells by morbilliviruses such as measles virus and peste des petits ruminants (PPR) virus has already been reported (Wosu, 1985, Ramarchandran *et al.*, 1993). This work reports a successful attempt to demonstrate haemagglutination technique for diagnosis of canine distemper.

KEY WORDS: Canine Distemper, Haemagglutination test, Dogs, Body fluids, Organs

MATERIALS AND METHODS

Sample Collection

Tissues of Seven 12-week old puppies infected with 0.1 ml of chorioallantoic membrane (CAM) of chicken eggs in which *Canine distemper* virus was isolated were used for the test. The virus was isolated from clinical cases got from an outbreak in Nsukka in South - East Nigeria. The 0.1ml of the CAM had fifty percent egg infectivity dose (EID₅₀) of 10⁵.

Samples collected from the infected dogs include cerebrospinal fluid (CSF), bile and extracts of liver, spleen, lungs, kidney, and lymphnodes. The samples were confirmed positive for *canine distemper* via histopathology by demonstration of both intranuclear and intracytoplasmic inclusion bodies in different cell types (Frisk *et al.*, 1999) and by Agar gel precipitation test using measles antiserum prepared in rats (Mori *et al.*, 1994).

Haemagglutination (HA) Test

HA test was done for each of the cerebrospinal fluid, bile and extracts of the infected dog organs with chicken red blood cells (RBC), pig RBC, human group "O" RBC and goat RBC by the method described for PPR by Wosu (1985). The HA test was done using 0.05ml of PBS (pH 6.8)

deposited in each of the wells in a microtitre plate. Then 0.05ml of the *canine distemper* antigen was added to the first well in a row and double diluted serially over the wells. Following 0.05ml of the 0.6% RBC was added to each well. For RBC control, only 0.05ml of RBC was added to the 0.05ml of PBS. For the virus control, in a row containing the distemper virus diluted in PBS as described above, 0.05ml of measles positive serum was added to each well and incubated for 45 minutes before 0.05ml of the RBC was added. The set up was incubated at room temperature (25°C) for 30 minutes when the RBC in the RBC control wells settled. Haemagglutination titre of the tested samples was read as reciprocals of the highest dilutions which gave a complete haemagglutination.

RESULTS AND DISCUSSION

Results of HA test with extracts of brain and Cerebrospinal fluid (CSF) of three of the seven dogs were HA positive with titres of 32 to 4096 and 32 to 2048 respectively. Liver, lymph nodes and spleen were HA negative. Lungs and kidney extracts were consistently positive but had low titre of between 8 and 64. The HA results of the extracts are shown on Table I. In each case, the RBC control was good. Also the measles positive serum inhibited agglutination of red blood cells by the fluids and tissue extracts.

TABLE 1: Distribution of *Canine distemper* haemagglutinin (HA positive) in infected dog tissues

CSF/Organ Extract	HA Titre						
	1	2	3	4	5	6	7
CFS	2048	1024	32	—	—	—	—
Brain	4096	1024	32	—	—	—	—
Liver	—	—	—	—	—	—	—
Kidney	32	32	16	64	64	8	8
Lung	64	16	16	32	32	8	64
Lymph node	—	—	—	—	—	—	—
Spleen	—	—	—	—	—	—	—

With CSF as a source of antigen and PBS of pH 6.8, HA titre varied among the species red blood cells used in the study. Human group "O" RBC was the most sensitive followed by chicken RBC (Table II). Haemagglutination by the fluids and extracts of organs from the dogs which was inhibited by known measles positive serum shows that the haemagglutination was due to *Canine distemper* virus because antigenic relationship exists between the morbilliviruses (Johnson *et al.*, 1968; Hamdy *et al.*, 1976). Also cross protection between measles and *canine distemper* has been recorded (Horst, 1975). Vaccines made from Rinderpest H and F antigens were able to protect Ferrets against lethal *Canine distemper* virus (Jones *et al.*, 1997). Sixt *et al.* (1998), have reported that protection by measles vaccines against *Canine distemper* is due to the presence of the anti-H, and anti-F and or anti-N antibodies in the sera of the vaccinated dogs. This information on protection by measles vaccine justifies use of measles serum in place of *Canine distemper* specific serum for this work.

TABLE II: Sensitivity of different RBCS to *Canine distemper* haemagglutinin

Rbc	HA Titre
Man "O"	2048
Chicken	1024
Porcine	512
Dog	zero

Measles nonspecific vaccine is available even in the remote places of the developing countries. So measles serum can be prepared by small laboratories for use in confirming *Canine distemper* by the HA technique. On the other hand, *Canine distemper* vaccines usually come in combination with other vaccines. So use of measles serum for this work appears to have some advantage over use of *Canine distemper* specific serum.

Wosu (1985) who demonstrated for the first time that PPR virus could cause haemagglutination further observed that cultured PPR viruses obtained from reference laboratories could not produce agglutination of any RBC. Durojaiye *et al.* (1983) had earlier reported that cultured PPR viruses obtained from a reference laboratory (National Veterinary Research institute, Vom, Nigeria) were unable to produce clinical PPR disease in experimentally infected sheep. So, in this work homogenate antigens were used for the attempt to develop a haemagglutination test for *Canine distemper*.

The clinical signs and gross lesions observed in the dogs used in this work agree with the clinical disease described for *Canine distemper* by Horst (1975). The histopathologic lesions observed in the infected dogs are also similar to those described for *Canine distemper* by Frisk *et al.* (1999). In addition, production of line of precipitation between the antigens and the measles serum in agar gel precipitation test is a further confirmation of our diagnosis of *Canine distemper*.

Results of this experiment suggest that HA technique has been developed for confirmation of diagnosis of *Canine distemper*. The specimens to use for diagnosis of *Canine distemper* by HA technique appear to be the kidney and the lungs which were consistently positive in the seven dogs. The cerebrospinal fluid and the brain can also be tested. These findings appear to be supported by report of Alldinger *et al.* (2000) who reported that inflammatory processes clear *Canine distemper* virus from the blood and other organs but not from the brain. In the

presence of humoral immunity Canine distemper virus often gets to the brain by migrating through the cells without getting into the blood or other body fluids (Zurbriggen et al., 1995). This migration to the brain through the cells may be responsible for the high HA titre obtained from the brain extracts and from the closely associating cerebrospinal fluid in three of the cases examined. It is possible that the other four dogs where the brain and CSF were HA negative the virus did not get to the brain.

The neutralizing antibodies against Canine distemper virus include anti-haemagglutinin (Hirayama et al., 1991). So failure in this work to get haemagglutination with extracts of the liver, spleen and lymph nodes in the dogs tested may be due to neutralization of the Canine distemper haemagglutinins in these organs of the dogs used. The low HA titre recorded with kidneys and lungs may be due to partial clearance of the haemagglutinin antigens from these organs in the dogs.

Demonstration of a haemagglutination test for Canine distemper virus will facilitate laboratory confirmation of the disease in the developing countries where hitherto diagnosis was based on only clinical signs due to lack of facilities for the other sophisticated and expensive techniques.

Use of 0.6% RBC concentration at 25o and acidic pH of 6.8 to produce positive HA result with Canine distemper is similar to the conditions used for PPR virus by Wosu (1985). Wosu (1985) had reported that PPR homogenate virus was able to agglutinate only piglet RBC. However, a conference on Morbilliviruses in Edinburgh confirmed that PPR virus agglutinates pig RBC and also other red blood cells including those of human group "O", monkeys and chickens

(Ramachadran et al., 1993). The result of this work also shows that human RBC is the most sensitive to canine distemper haemagglutinins. Chicken RBC follows the human RBC in sensitivity to canine distemper haemagglutinins.

CONCLUSION

It has therefore been concluded that haemagglutination test can be adopted for confirmation of diagnosis of canine distemper by using PBS at pH of 6.8 and with either human group "O" RBC or chicken RBC.

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Short Communication

CRUELTY TO DOGS: A SURVEY OF RESPONSES IN BUKURU METROPOLIS, JOS-NIGERIA

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INTRODUCTION

The word dog refers to the domestic pet, *Canis lupus familiaris*. Dogs are in the order *Carnivora*. Recent genetic and DNA evidence (Lindblad, 2005) has shown that they were domesticated from wolves 100,000 years ago. Dogs like humans are highly gregarious (Wiki-Dog, 2006), the similarity in their overall behavioural pattern accounts for their trainability, playfulness and the ability to adapt into human households and social situations (Villa, 1997). Dogs are not included as food animals (Gracey, 1989). Since domestication they have lived and worked loyally with humans, thus earning them the unique sobriquet "man's best friend". Despite the good attribute of these animals a number of countries in the world breed and slaughter them for meat (Villa, 1997; Wiki-dog, 2006). Cruelty is defined as the readiness to cause pain or suffering to others (Advanced Learners Dictionary). Cruelty to animals is further defined as the treatment that causes unacceptable suffering or harm to animals (Munro, 1999). The term unacceptable suffering varies: a school of thought considers only suffering inflicted for sadistic reasons as cruelty, whereas the other school of thought included suffering inflicted for reasons such as, fur production, meat and animal testing or vivisection (Munro, 1999). Jurisdictions around the world have enacted statute prohibiting cruelty to animals. These statutes provide minimal care of animals, but do not require optimal or mandate kindness or love. They require that animals be provided with shelter, food, water and medical treatment (Arnold, 2004). Most people keep dogs not as pets, but for security reasons (Tafaderma, 2006) and in Nigeria, their meat is popularly referred to as '404' or 'dogmycin'. The meat is a special delicacy amongst some tribes of Plateau State and other tribes in Nigeria (Tafaderma, 2006) and regarded as "the tastiest of all meat" by the Chinese philosopher Mencius, 372 - 289 (Transition on-line, 2006). This study was conducted to assess handling and killing of dog at dog markets in Bukuru metropolis, Plateau State, Nigeria. Respondents' view on legislation on inhumane treatment and banning of dog killing for meat and why people eat dog meat was also assessed.

KEYWORDS: Cruelty, Dogs, Survey, Bukuru, Nigeria

MATERIALS AND METHODS

Survey Site

Bukuru is located at latitude 9° 47' N and longitude 8° 51' E, and about 10 miles from Jos. It is the oldest and most densely populated settlements of Jos South Local Government area of Plateau State, Nigeria. The dog market

in Angwan kare, along the Jos-Bukuru express road was visited. Agwan kare serves as the central distribution point of dog meat to neighbouring villages and Jos metropolis. About 2 kilometres from Agwan kare is the major dog meat spot called Kugiya, where hundreds of people come to buy boiled or roasted dog meat.

Oral Interview

A survey was carried out to assess methods of handling and killing of dog. Respondents' view on legislation on inhumane treatment and banning dog killing for meat and why people eat dog meat was also assessed. One hundred (100) people were randomly selected and assessed by the use of questionnaires, while twenty (20) dog meat sellers at the various dog meat spots in Kugiya, Angwan Doki and Angwan Kare respectively in Bukuru metropolis were orally interviewed. Parameters assessed were

RESULTS AND DISCUSSION

Ninety percent of the respondents were against inhumane handling of dogs. Seventy-six percent (76%) of the respondents were not comfortable with the handling and execution of dogs, 74% would support any legislation banning cruelty to dogs while 52% will not support legislation banning eating of dog meat (Table. I). This is an indication that there is a lot wrong with the treatment of "man's best friend".

It was also observed that women trek long distances with sick and emaciated dogs tagging behind from different parts of Jos to Angwan kare. On arrival these dogs are kept without adequate food and water in confinement in very dirty environments for an average of 4-7 days before being displayed in the market for sale (Fig. 1 and 2). Majority of dogs killed for meat are either stolen or sold by their owners to meet financial needs.

The methods by which dogs are killed are cruel and inhumane. Some of the dogs are clubbed to death or strangled using wire strings tied around the neck (Fig. 3). Others are hung on trees or on iron pillar until they die (Fig. 4). Strong and very aggressive dogs are held by the hind limbs and their head smashed against a wall or tree, while others are pinned to the ground before their throats are brutally slit open with a knife. Dogs are usually prepared and processed by burning their skin with kerosene in very dirty environment (Fig. 5) or dropped into boiling water. The methods of

killing observed in the study were similar to those reported in China and South Korea (Captive Animals, 2006; In defence of Animals, 2006; and Seousearch.com, 2006).

The survey revealed that 66% of the respondents did not see anything wrong with eating dog meat and 56% indicated that they will oppose any legislation prohibiting the eating of dog meat since it is culturally acceptable and individuals have the right to eat the type of meat they desire however, only 38% of the people interviewed affirmed that they eat dog meat.

Dog meat is eaten for reasons such as: its taste; its medicinal effect as a cure for malaria fever; a source of protection against the attack of evils spirits/witchcraft and aphrodisiac effect. The reasons were similar with those given in China and South Korea (Ceeol.com, 2006; Lebanolinks.com, 2006; Transition on-line, 2006).

All dog meat sellers stated that the male genital parts of the dog are exclusively reserved for special people and sold at an exorbitant price; it is believed that it increases sex drive and cures impotence. The survey further revealed that averages of 200 dogs are killed daily at the various dog markets, the number increase during festive periods. It was also gathered that consumers of dog meat cuts across all ages, gender and educational background. The practice is culturally acceptable and tolerated by most of tribes in Plateau State and other parts of Nigeria.

Nineteen out of the twenty, representing 95% of the dog meat sellers orally interviewed in Kugiya and Agwan Kare in Bukuru were women (Fig. 6), while men are involved only in scouting, killing and processing of the carcasses of dogs. This is consistent with the findings of Tafaderma (2006) and Viet Nam (2006).

TABLE 1: Response of people to cruelty to dogs.

Question	No	Yes	Indifferent	Total	%No	%Yes	%indifferent
Do you eat dog Meat?	62	38	0	100	62	38	0
Do you think eating Dog meat is wrong?	66	14	20	100	66	14	20
Should eating dog Dog meat be Prohibited?	56	26	18	100	56	26	18
Are you comfortable With the way dogs are Handled and Slaughtered?	76	16	8	100	76	16	8
Will you support Legislation banning Cruelty to dogs?	8	74	18	100	8	74	18
Will you support Legislation banning Eating of dog meat?	52	24	24	100	52	24	24
Do you think It is right for dogs To be maltreated and Killed inhumanely?	90	4	6	100	90	4	6



FIGURE 1: Sickly dogs tied to a tree in a dirty environment waiting for prospective buyers.



FIGURE 2: Buyers and sellers in one of the dog markets bargaining on dogs.



FIGURE 3: Dog strangulated to death by application of pressure around the neck by a string.

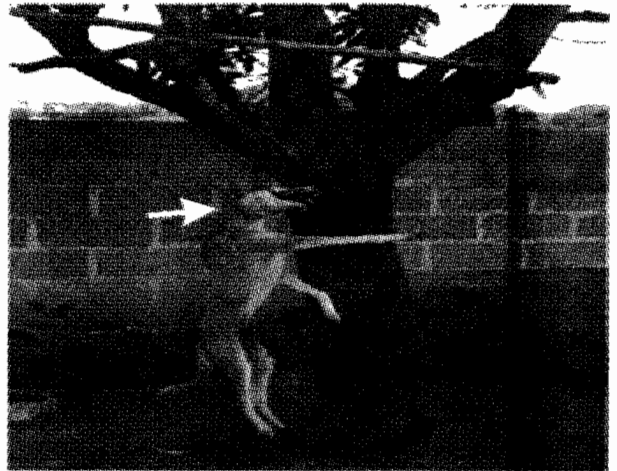


FIGURE4: Dog brutally hanged on a tree.



FIGURE.5. Executed dogs burnt or dropped in buckets containing hot water before being prepared for meat.



FIGURE 6: Women selling prepared dog meat in one of the dog meat markets in Bukuru metropolis.

CONCLUSION

Cruelty to dogs will be very difficult to abolish. However, there is an urgent need for advocacy and outright campaign to stop cruel practices. The act of eating dogs will be a very difficult task to stop. Enlightenment campaign and the use of various media to educate people will go a long way in checking these cruel and gruesome practices. Tradition and culture plays a significant role in the number of people eating dog meat. A law banning the eating of dog meat will be an almost impossible task, as 52% of the respondents indicated that they will not support any law

banning the eating of dog meat.

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Case Report

PYOMETRA IN A GREAT DANE: A CASE REPORT

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INTRODUCTION

Pyometra is a condition mainly of middle-aged female dogs that have not been spayed. It is a hormonally mediated, diestrual disorder that results in abnormal uterine endometrium (Venugopalan, 1997, Amstutz *et al.*, 1998). Infection of the lining of the uterus is established as a result of the hormonal changes in the presence of bacteria. Following estrus (heat), progesterone levels remain elevated for 8-10 weeks thereby promoting endometrial growth while decreasing myometrial activity in preparation for pregnancy (Amstutz *et al.*, 1998, Chinaroad, 2006). If pregnancy does not occur for several oestrus cycles, the lining continues to increase in thickness until cysts form within it (Chinaroad, 2006). These cysts contain numerous secretory cells producing large quantities of fluids released into the interior of the uterus. This fluid along with the thickening of the walls of the uterus brings about drastic increase in the overall size of this organ (Foster and Smith, 2006). With this, the uterus becomes an enlarged, sac-like pouch of about 12-18 inches long (Foster and Smith, 2006). As the disease progresses, fluid spills out of the vagina causing the animal to lick the area in an attempt to keep itself clean. Through the cervix, bacteria enter the uterus and this produces even greater response by the body which produces additional fluid and white blood cells infiltrate into the affected organ (Foster and Smith, 2006). This may subsequently result in toxemia (Venugopalan, 1997). In an attempt to flush out the built-up endotoxins in the bloodstream, there is polydipsia and polyuria (Foster and Smith, 2006; Anon, 2006). There will be discharge of white fluid if it is an open cervix pyometra. The animal may also run a low-grade fever. As the condition deteriorates, kidney failure may occur and the animal becomes very lethargic. Because of the acute toxemia involved, urgent management is always advocated. Intravenous fluid infusion with antibiotic therapy is instituted for several days. Prostaglandin may be administered for 5-7 days, although restlessness, panting, vomiting, increased heart rate, fever and defecation may occur as side effects (Foster and Smith, 2006). In cows with postpartum pyometra injection of Fenprostalence has resulted in expulsion of large amount of pus within 3 days (Okada *et al.*, 1994). The treatment of choice in most cases is ovariohysterectomy unless the reproductive potential of the bitch is to be salvaged (Venugopalan, 1997). With the growing interest in exotic breeds of dogs in Nigeria in recent time, it becomes very imperative to present this case and its successful management for the benefit of pet owners and veterinarians.

KEYWORDS: Pyometra, Great Dane, Ovariohysterectomy, Nsukka, Nigeria

CASE HISTORY

A five-year old female Great Dane was presented at the University of Nigeria Veterinary Teaching Hospital (UNVTH) on 25th February 2002 with the history of anorexia and bloody discharge from the vulva few days after service. Prior to the referral, the dog had been given Vitamin K, Gentamycin, Vitamin B complex and fluid therapy. The dog had a history of vaccination against canine distemper, hepatitis and leptospirosis.

CLINICAL EXAMINATION

The dog weighed 46.5kg. The integument and the musculoskeletal systems were normal. There was bloody discharge from the genital tract. The dog was weak and unable to stand or walk. The rectal temperature was 39°C, pulse rate - 180/min, respiratory rate - 25/min. The dog drank a lot of water. There was polyuria. Vaginal swab was collected for culture and sensitivity. The culture yielded a heavy growth of *Escherichia coli* and *Staphylococcus species* both of which were sensitive to ciprofloxacin. A plain radiograph of the abdomen revealed a loop of soft tissue mass (uterus) in caudal and mid abdominal areas. Blood was collected for hematology.

The result of the haematological test was as follows:

Packed Cell Volume	17%
Red Blood Cell Count	$1.15 \times 10^6/\text{mm}^3$
Total Leucocyte Count	$= 237,300/\text{mm}^3$
Differential Leucocyte Count:	
Neutrophil	- 9 0
	(30% are band cells)
Lymphocytes	- 4
Monocytes	- 6
Basophils	- Nil
Eosinophil	- Nil

Based on the age, the history, physical manifestation of the condition as well as the laboratory and radiological findings a clinical diagnosis of open cervix pyometra was made.

MANAGEMENT

Ovariohysterectomy was recommended. Prior to the surgery, the dog was maintained with intravenous fluid (750 ml of Dextrose-Saline solution). Atropine sulphate and xylazine were used as premedicants at 0.02mg/kg and 0.03mg/kg body weight respectively. The surgery was aseptically carried out under general anaesthesia using Pentobarbital Sodium at 30mg/kg body weight. The pus-filled uterus was carefully exteriorized through a laparotomy incision avoiding spillage. The ovaries, ligaments and blood vessels were carefully identified, ligated and removed. The Laparotomy incision was then closed routinely.

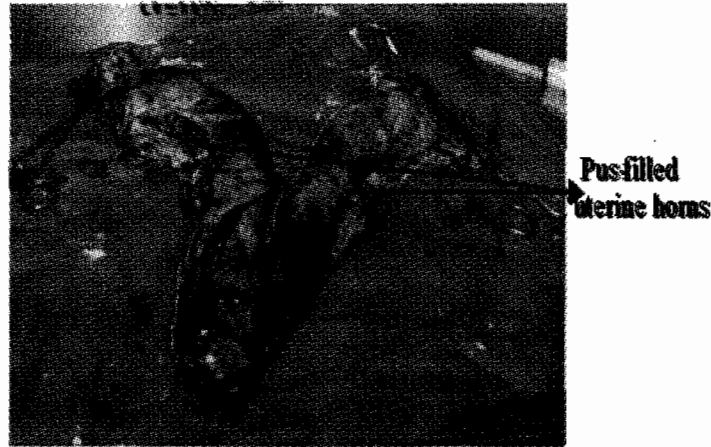


Plate 1

The following postoperative treatment was given: Dextrose-Saline, 500ml IV daily for 2 weeks, Tab. Ciprofloxacin 500mg per Os x 5/7 and Inj. Vitamin B complex 2ml im x 5/7

The dog was hospitalized and hematology was carried out weekly to monitor the response of the animal to treatment. By the 14th day post-surgery when the cutaneous stitches were removed, the bitch generally appeared strong with good appetite and no vulva discharges.

DISCUSSION

Ovariohysterectomy was used to manage the condition. Although the incriminating organisms (*Escherichia coli* and *Staphylococcus spp.*) were sensitive to Ciprofloxacin, the case was not managed conservatively because of the severity of the condition. Ovariohysterectomy was performed to prevent recurrence and avoid the problems associated with heat periods such as messing up of the environment with bloody discharges and waywardness (Noakes *et al.*, 2001).

The clinical, laboratory as well as the radiological features of this case are consistent with the previously described features of open cervix pyometra (Foster and Smith, 2006; Anon, 2006; Amstutz *et al.*, 1998; Arthur *et al.*, 1998). These features ruled out transmissible venereal tumor and oestrus due to bloody discharge. Haematological result was characterized by neutrophilia. This neutrophilia is consistent with the findings of other workers except for that of Seielius *et al.* (1990) where there was no significant increase in the white blood cell count in their study of 103 cases of pyometra in dogs. The leukocytosis characterized by neutrophilia and marked leucopenia was an indication of severe infection. The low packed cell volume of the patient was an indication of anaemia. It is believed that the success of the treatment was as a result of meticulous surgery, the use of the right antibiotic sensitive to the bacterial infection and adequate fluid therapy.

The choice of ovariohysterectomy was to stop the endotoxaemia and avert probable kidney failure (Foster and Smith, 2006). Ovariohysterectomy also prevents unwanted breeding and the nuisance associated with heat periods in pet animals (Noakes *et al.*, 2001).

The surgery involved the removal of both ovaries and the uterus. This is always more complicated and carries a higher risk than routine spaying because of infections. This goes to justify the sensitivity test carried out and the use of

ciprofloxacin and fluid therapy in management of the patient. Blood tests were carried out weekly because this was useful in monitoring the prognosis of the case.

The isolation of *E. coli* and *Staphylococcus spp.* from the vagina was not surprising as these have always been the predominant isolates in dog pyometra (Arthur *et al.*, 1998). Amstutz *et al.* (1998) also discovered that the progesterone-sensitized endometrium and myometrium had affinity for *E. coli*, *Staphylococcus*, *Streptococcus*, *Pseudomonas* and *Proteus spp.* It was because of these organisms isolated that ciprofloxacin was administered.

Prostaglandin was not administered to the patient considering the severity of this particular case at the time of presentation to the UNVTH. Moreover, the side effects might outweigh the benefits, since the dog was already recumbent and may not have survived within the next 48 hours during which the drug was expected to manifest its beneficial effects (Anon, 2006).

It has been reported that the best prevention for pyometra is to have all female dogs that are not meant for breeding spayed before six months of age (Foster and Smith, 2006). This information is quite important to pet owners in Nigeria who have little or no knowledge of the pathogenesis of pyometra.

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Short Communication

A SURVEY OF THE GASTRO- INTESTINAL HELMINTHS OF CHICKENS IN SOKOTO
METROPOLIS, NIGERIA

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INTRODUCTION

A survey of the gastro-intestinal parasites of local chickens was carried out in Sokoto metropolis between June and September 1998, one hundred and fifty alimentary tract were observed: of which 139(92.6%) had helminths. The genera of gastro-intestinal helminths encountered were *Ascaridia* (18.66%), *Heterakis* (28.66%), *Capillaria* (4.00%), *Tetrameres* (9.33%), *Trichostrongylus*, (1.33%), *Raillietina* (74.66%), *Chaonotaenia* (5.33%), *Davainea* (0.66%) and *Amoebotaenia* (2%). The progenitor of the domestic fowl was the Red jungle fowl (*Gallus gallus*), modern forms of which are found in central and South India (*Gallus gallus sonerati*), East India (*Gg. murghi*), Burma and Malaysia (*Gg. spadiceus*) and Thailand and Cambodia (*Gg. gallus*). It is a smaller bird than most domestic varieties—an adult female weight about 800g and it is a tropical species (Michael *et al.*, 1992). Verminosis of the digestive tract of birds is a helminthosis due to the presence and development in the digestive tract of one or several species of pathogenic worms that belong to the class of Nematoda, Trematoda, Cestoda, or Acanthocephala (Serres, 1989). The poultry industry is one of the most promising and progressive of livestock industries and offers an important source of income for producers, even when they are operating on a small scale. Although the need for more eggs and poultry meat is obvious and the availability of these products can go along way to meet the protein needs of the several populaces, there are several constraints to the future development of the poultry industry (Daghir, 1995). Disease in poultry can be summed up as the changes in health, morbidity, mortality and productivity resulting from the effects of invading infectious, parasitic or non infectious agents. In Nigeria, indigenous chicken constitute about 92.7% of the total chicken population of 134 million (Nawathe and Lamorde, 1982). These local chickens are kept under extensive system roaming freely and scavenging for food. These birds are therefore very exposed to parasitic infections. It is believed that these free wandering chickens act as potential reservoirs and carriers of infection to themselves and the more susceptible exotic breeds in commercial enterprises (Adu, 1982). This study was thus carried out to determine the incidence of gastro-intestinal helminths parasites in local chickens slaughtered in Sokoto metropolis, Nigeria.

KEY WORDS: Helminthosis, Gastro- intestinal parasites, Local chickens, Sokoto, Nigeria.

MATERIALS AND METHODS

The survey of the gastro-intestinal helminths parasites of domestic chickens was carried out in Sokoto from June to September 1998. One hundred and fifty alimentary tracts were collected from markets and other slaughter houses in Sokoto metropolis.

The chickens were of the local breed and were mainly adults although their specific ages could not be determined. The alimentary tracts were collected on weekly basis. The digestive tracts were extracted intact and the various sections separated in Petri dishes. The oesophagus and the crop were slit open and each was emptied of its contents. Both were then washed and examined under light for embedded helminths. The contents of the proventriculus and gizzard were washed separately into Petri dishes and examined for worms. After peeling off the mucosa of the proventriculus, the brightened red patches of *Tetrameres* were teased to collect the embedded parasites. The duodenum, jejunum, ileum, caecum and rectum were examined for parasites separately. They were opened on a sieve, and parasites in the lumen were picked up, after which the contents were washed thoroughly under running tap water. The mucosal surfaces were rubbed carefully between fingers to remove adhering parasites. The mucosae were then scrapped into Petri dishes which were which were examined for parasites.

The parasites were fixed and preserved in a labeled sample bottle containing 10% buffered formalin. The detection of parasites in the sample involved the identification of eggs as well as preserved adult worms. Identification of each worm was done by thorough examination of the different morphological characteristics of the anterior mid gut and posterior region of the worms, using the x10 and x40 objectives of a light microscope (Fatihu *et al.*, 1991).

RESULTS AND DISCUSSION

Of the one hundred and fifty chickens examined, 139 (92.66%) were infected with helminths parasites. The most common helminths observed were cestodes, followed by nematodes, but trematode infection was not recorded (Table I and II). It means that there is a high prevalence of helminth parasites in domestic chickens in Sokoto metropolis. This result agrees with the results of previous surveys in other parts of the country. Prevalence rates of 90% (Fabiyyi, 1972), 93.3% (Okon and Enyenihi, 1980), 100% (Gadzama and Srivastava, 1986) and 91.2% (Dayo, 1993; Fatihu, *et al.*, 1991) were recorded in Vom, Oron, Borno and Zaria respectively. The complete absence of trematodes is a feature of this survey. Fabiyyi (1972) found no trematodes in a survey carried out in Vom. This might imply that chickens generally do not harbour trematodes. Schillhorn Van Veen *et al.* (1974) found many species of helminth parasites in a survey carried out in Zaria. Some of these helminths were not recorded in this study, which could be due to geographical location or seasonal variation that may account for the presence or absence of intermediate hosts of these helminthes. It could also be due to the type of management system practiced. Traditional production systems are conducive for parasitism, but because they are extensive systems the infections are rarely severe. The amphibious habits of the Anseriformes birds expose them to more parasites than the Galliformes birds. In intensive systems certain parasites with an indirect cycle can be eliminated but conditions are favourable for the rapid spread of helminths with a direct cycle. This is valid for *Ascaridia*, *Hetarakis* and certain *Capillaria spp.* (Serres, 1989).

Amongst the nematodes, the most common was *Hetarakis spp* occurring in 28.66% cases. The incidences of *Ascaridia spp* and *Tetrameres spp* were 18.66% and 9.33% respectively. While those for *Capillaria spp* and *Trichostongylus spp* were 4% and 1.33% respectively. The helminth species that appear to be known world wide which were also recorded in this study are *Hetarakis spp*. They have been found in Dahomey (1911), Brazil

(1929), Puerto Rico (1964), Uganda (1968), Chad (1969), and Ghana (1969) *Ascaridia* spp were recorded in Gambia (1904), South Africa (1929) and Chad (1959,1969) (Fabiyyi, 1972).

The most common cestode parasite was *Raillietina* spp, occurring in 74.66% of the birds (Table II). *Chaonotaenia* spp. and *Amoebotaenia* spp. were recorded in 5.33% and 2% of the birds respectively, while *Davainea* spp. was only found in 0.66% of the birds. *Davainea proglottina* a common tape worm in many parts of the world was recorded in this study only in one bird. Their very low prevalence might suggest that the intermediate hosts of this worm do not exist in this part of Nigeria.

Helminths occurring most frequently are *Heterakis* spp., *Ascaridia* spp. and *Raillietina* spp. These helminths have been encountered in some West African Countries with which Nigeria shares some common geographical features. Hodasi (1969) recorded that *Ascaridia galli* occurred in 29.6% of native and 32.4% of exotic birds. This author also reported the prevalence of *Raillietina tetragona* in local and exotic birds to be 53.3%, and 32.4% respectively. While the rates recorded for *R. echinobothrida* were 85.2% and 45.4% respectively.

TABLE I: Survey of gastro intestinal heminiths in local chickens

Types of Helminths	Number of Chickens Infected	Percentage Prevalence
<i>Acaridia</i>	28	18.66%
<i>Heterakis</i>	43	28.66%
<i>Capillaria</i>	6	4.00%
<i>Tetrameres</i>	14	9.33%
<i>Trichostrongylus</i>	2	1.33%
<i>Raillietina</i>	112	74.66%
<i>Chaonotaenia</i>	8	5.33%
<i>Davainea</i>	1	0.66%
<i>Amoebotaenia</i>	3	2.00%

TABLE I: Types of helminths based on classes

A. Nematodes	Number of Chickens infected	Percentage prevalence
1. <i>Ascaridia</i> Spp	28	18.66
2. <i>Heterakis</i> Spp	43	28.66
3. <i>Capillaria</i> Spp	6	4.00
4. <i>Tetrameres</i> Spp	14	9.33
5. <i>Trichostrongylus</i> Spp	2	1.33
B. Cestodes	Number of chickens infected	Percentage prevalence
1. <i>Raillietina</i>	112	74.66
2. <i>Chaonotaenia</i>	8	5.33
3. <i>Davainea</i>	1	0.66
4. <i>Amoebotaenia</i>	3	2.00

CONCLUSION

It is clear from the results obtained that verminosis of the digestive tract is one of the disease conditions present in local chickens within Sokoto metropolis. Worm burden is associated with the management system practised and extensive system of management is very common in Sokoto metropolis. This extensive system exposes the birds to infections with a variety of helminthes. This is because of high contact with a larger area of land and different intermediate hosts while feeding.

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A RETROSPECTIVE (2004-2006) STUDY OF POULTRY DISEASES DIAGNOSED IN BENIN, EDO STATE, NIGERIA

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SUMMARY

A retrospective study of records of the outstation diagnostic and investigation laboratory, National Veterinary Research Institute Vom and State Veterinary Clinic, Benin City was carried out to establish the occurrence and distribution of Poultry diseases over a period of three years (2004-2006). Out of the 730 cases, 216 (29.6%) were Newcastle disease, 104 (14.2%) was Coccidiosis, while 83(11.4%) and 82(11.2%) were Infectious bursal disease and Fowl typhoid respectively. The least included malnutrition/starvation, cannibalism and the highly pathogenic avian influenza with 31 (4.2%), 23 (3.2%) and 2 (0.3%) cases respectively. However, viral diseases of poultry indicated the highest prevalence of 45.3% (331 cases). Dry season (November-April) represented the period of increased disease occurrence of with 67.4% (492cases), which revealed statistical significance ($P<0.05$) by chi square analysis. The year 2005 also recorded the highest disease occurrence of 311cases (42.7%). Poor vaccine handling, management and quack practices amongst poultry farmers, in conjunction, with lack of facilities and awareness on laboratory diagnoses may be associated with the distribution pattern of cases recorded in the clinic.

KEYWORDS: Prevalence, Poultry diseases, Edo State, Nigeria

INTRODUCTION

Chickens originated from several wild species of jungle fowl from South East Asia, which were domesticated as early as 200BC and have been subjected to breeding practice to increase the productivity of meat and eggs (Bhatti, 1989). They are generally described as a genus of the avian species that are grown and domesticated throughout the world. In Nigeria, chickens are the most important of the poultry species in terms of number and development. The exotic breeds are managed intensively using either battery cages or

deep litter, while the local breeds are managed extensively. The major constraint in raising these chickens is the substantial economic losses (David-West, 1972), due to diseases of which viral diseases account for the highest percentage of mortality in chicken because of their contagious nature (Adeboyega, 1999). Although, analysis of poultry diseases has been conducted in some part of the country (Abdu *et al.*, 1985; Saidu *et al.*, 1994), complete information on the prevalence of poultry diseases in Edo state is scanty, hence the need for this study.

MATERIALS AND METHODS

Data Collection

Data on cases of diseases of poultry presented to both the state Veterinary Clinic and Diagnostic & Investigation Laboratory, National Veterinary Research Institute (N.V.R.I), Vom in Benin City for three years (2004-2006) were considered for this study. The cases recorded in this period of study were obtained from casebooks, files and post mortem records in the clinic.

Diseases were diagnosed based on flock history, clinical signs, and post mortem findings. In addition, some of the cases were confirmed by laboratory analysis. However, the Viral Research Department of the N.V.R.I, Vom, confirmed the two suspected cases of the Highly Pathogenic Avian Influenza. The distribution pattern of the poultry diseases reported to the clinic in Benin City, was analyzed using proportional (percentage) data presentation.

Statistical Analysis

The level of significance between the occurrence of diseases in the dry season (November April) and rainy season (May October) was determined using chi square.

RESULTS AND DISCUSSION

A total 730 cases of poultry diseases were recorded during the period of study. This gives an average of about 243 cases annually. The year 2005 recorded the highest number of cases (42.7%), while the year 2006 recorded the lowest with 25.3% cases, (Table I). This may be due to increased establishment of recent backyard poultry farms in Edo state, while the drop in number of cases and poultry activities in year 2006 could be attributed to the upsurge of the highly pathogenic avian influenza outbreaks in the country.

TABLE I: Prevalent poultry diseases in Edo state (2004-2006)

Diseases	2004	2005	2006	Total
IBD	27	40	16	83(11.4%)
Newcastle disease	77	95	44	216(29.6%)
Coccidiosis	41	49	14	104(14.2%)
Fowl pox	7	17	6	30(4.1%)
Fowl typhoid	27	34	21	82(11.2%)
Fowl cholera	6	16	3	25(3.4%)
CRD	15	19	11	45(6.2%)
Cannibalism	5	4	14	23(3.2%)
Helminthiasis and Ectoparasitism	9	17	21	47(6.4%)
Heat stress	17	20	5	42(5.8%)
Malnutrition and Starvation	3	-	28	31(4.2%)
HPAI	-	-	2	2(0.3%)
Total	234(32.1%)	311(42.7%)	185(25.3%)	730

The monthly distribution of cases indicates a steady rise from November through January to peak in April, as shown in Table II. This represents the dry season when the weather is stressful. It also coincides with the periods of increased poultry activities such as preparation and sales of birds during the festive periods, increased demand for

eggs and re-stocking and brooding of day old chicks thus, an increase rate of spread of diseases. The seasonal prevalence revealed statistical significance ($P < 0.05$) by chi-square (Table III), which correlate with the findings of Abdu, *et al*; 1992.

TABLE II: Monthly distribution of diseases during the period 2004-2006

Diseases	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec	Total
IBD	15	1	18	26	-	1	-	1	6	5	5	5	83
Newcastle disease	34	18	30	37	12	6	1	-	1	3	43	31	216
Coccidiosis	8	-	11	7	16	22	20	19	-	1	-	-	104
Fowl pox	7	2	7	3	1	-	1	-	-	-	2	7	30
Fowl typhoid	24	14	13	8	12	-	4	1	1	-	3	2	82
Fowl cholera	1	-	4	-	8	2	-	-	-	3	2	5	25
CRD	8	6	4	1	-	1	-	-	-	3	11	11	45
Cannibalism	1	3	-	-	-	6	8	2	-	-	2	1	23
Helminthiasis and Ecotoparasitism	-	-	-	5	13	11	15	2	1	-	-	-	47
Heat stress	-	6	12	21	2	-	-	-	-	-	-	1	42
Malnutrition and Starvation	1	-	-	3	9	11	6	-	-	1	-	-	31
HPAI	-	-	-	-	-	-	-	-	-	-	1	1	2
Total	99	50	99	111	73	60	55	25	9	16	69	64	730

TABLE III: The distribution of diseases by season

Season	No. of cases	Percentage (%)
Dry season (Nov-April)	492	67.4
Rainy season (May- October)	238	32.6
Total	730	100

Out of the 730 cases, 216 (29.6%) were Newcastle disease, 104 (14.2%) was Coccidiosis, while 83(11.4%) and 82(11.2%) were Infectious bursal disease and Fowl typhoid respectively. The least included malnutrition/starvation, cannibalism and the highly pathogenic avian influenza with 31 (4.2%), 23 (3.2%) and 2 (0.3%) cases respectively, as shown in Table I. However, viral diseases of poultry indicated the highest

prevalence of 45.3% (331 cases) as shown in Table IV, with Newcastle disease recording the highest occurrence rate. This is in line with observations of other studies in other parts of Nigeria (Abdu *et al.*, 1985; Adeboyega, 1999; Saidu *et al.*, 1994). The high records of these viral diseases could be associated with complications arising from poor vaccine handling and vaccinations by farmers, which result in viral environmental contamination.

TABLE IV: The distribution of diseases by aetiologic agent

Aetiologic Agent	No. of cases	Percentage (%)
Viral	331	45.34
Bacterial	152	20.82
Protozoan	104	14.24
Helminthiasis and Ectoparasitism	47	6.43
Other	96	13.15
Total	730	100

The pattern of disease distribution so observed is suggestive of the demand for veterinary services and management practices amongst farmers where indiscriminate and unprescribed use of antibiotics is a routine thus making diagnosis difficult when cases are presented to the clinic. Hence, the need to enlighten poultry farmers in Edo and its environs on the need to avoid quackery, improve farm biosecurity and personal safety measures, in addition, to the need for an adequate laboratory back up as a diagnostic tool for an effective control of infectious poultry diseases.

CONCLUSION

In conclusion, this study provides preliminary information on the pattern of poultry diseases prevalent around Benin-city, Nigeria.

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Letter to the Editor

Climate Change Challenge To Veterinary Practice In Nigeria



Global warming has become a new reality with deleterious effects where tremendous disruption of seasonal cycles occur, leading to changes in ecosystems. Extreme climate conditions such as high wind, heavy rainfall, heat, cold can result in wide-ranging scenarios like tropical storms, floods, draughts, landslides, and sea level rise. The consequence of these climatic catastrophes are environmental degradation (decay), loss of biodiversity, water and air environmental pollution thereby inducing population displacement (this in turn can lead to conflict/civil unrest), public infrastructures would be eroded (government resources are diverted to disaster recovery, to individuals for property damage or loss, unemployment, clean up as well as to reduce the socioeconomic effect on the affected communities).

There is new and strong evidence that most of the warming observed in the globe over the last 50 years is attributable to human activities. This warming is attributed to the increased concentration of a number of gases such as methane, carbon dioxide, and chlorofluorocarbons referred to as greenhouse gases. (EPA/ UNEP, 1986; NAS, 1983). These gases are called greenhouse gases because they allow incoming solar radiation to pass through the earth's atmosphere but absorb outgoing infrared radiation. This then results in warming within the confines of the atmosphere much as is seen within a greenhouse.

Scientists have known about the greenhouse effect since 1824, when Joseph Fourier calculated that the earth would be colder if it had no atmosphere. This greenhouse effect is what keeps the earth's climate habitable. In 1895, the Swedish Chemist

Svante Arrhenius discovered that humans could enhance the greenhouse effect by making carbon dioxide a greenhouse gas. He kicked off 100 years of climate research that has given us a sophisticated understanding of global warming.

Levels of greenhouse gases have gone up and down over the earth's history, but they have been fairly constant for the past few thousand years. Global average temperatures have stayed fairly constant over that time as well until recently. Through the burning of fossil fuels and other greenhouse gases emission humans are enhancing the greenhouse effect and warming earth.

Scientists often use the term 'climate change' instead of global warming. This is because as the earth's average temperature climbs, winds and ocean currents move heat around the globe in ways that can cool some areas, warm others, and changing the amount of rain and snow falling. As a result, the overall net global warming is not the same throughout all regions of the world. Changes in temperature, rainfall, humidity length of day, average daily solar radiation, and or storm patterns as well as changes in the frequency of floods, drought and other climate elements vary considerably from one region to another. Based on global deductions and paleoclimate data, the Northern hemisphere summer temperature in recent decades appear to be the warmest since at least about 1000AD and the warming since the late 19th century is unprecedented over the last 1000 years. These large and rapid climatic changes affects the atmospheric and oceanic circulation as a result, temperature change of 1.2 to 4°C over the next century would be unprecedented in comparison with the best available records from the last several thousand years. Global mean sea

level has been rising at an average rate of 1 to 2mm per year over the past 100 years, which is significantly larger than the rate averaged over the last several thousand years. The projected increase from 1990 to 2100 is between 0.09 to 0.38 meters, based on which greenhouse gas scenario used as well as the many physical uncertainties in contributions to sea-level rise from a variety of frozen and unfrozen water sources

The rapid rise in greenhouse gases is a problem because it is changing the climate faster than some living things may be able to adapt. Also a new and more unpredictable climate poses unique challenges to all life historically; earth's climate has regularly shifted back and forth between temperatures like those we see today and temperatures cold enough that large sheets of ice covered much of North America and Europe. The difference between average global temperatures today and during those ice ages is only about 5°C, and these swings happen slowly, over hundreds of thousands of years. As a result of the present increase in earth's temperature due to increase in concentration of greenhouse gases, the remaining ice sheets (such as Greenland and Antarctica) are starting to melt too the extra water is what is probably seen as raise in sea levels. As temperature rises the change in climate can be quite unpredictable. Coupled with the rising sea levels, Weather can become more extreme. These may be seen as intense major storms, heat wave more rain or late arrival of the rains which may be followed by flooding or longer and drier droughts. The change in weather pattern results in enormous challenge for growing crops changes in the ranges in which plants and animal can live, and loss of water supplies as observed in the disappearance of the glaciers.

Nigeria (as a country of focus): With these, it should be expected that the Sahara desert would move further south increasing the threats of desertification in Northern Nigeria this would be further complicated due to direct human activities

(such as over grazing, deforestation and over cultivation) in arid, semi-arid and dry sub humid areas resulting in the development of patches of degenerated land. These patches can expand and join together further complicating an already bad situation. The desert encroaching at a fast rate will see the complete elimination of communities as a result of shifting sand dunes. These usually result in the loss of pasture causing malnutrition in grazing animals increase susceptibility to disease outbreaks and loss of livestock. These reductions in pasture also results in increase in frequency of contact between wild animals, which are usually reservoir host for many diseases of domestic animals (Rinderpest, Anthrax, Foot and Mouth Diseases) as a result, increasing the chances of disease outbreak in livestock.

Flooding along the coastal and low line areas of big rivers resulting in land slides and submerging of entire communities is another expected result warmer, wetter weather will see the emergence of insects to new territories increasing the spread of disease conditions (vectors).

Environmental temperature is considered an important factor in the transmission of bacterial agents causing enteritis. It is therefore plausible that an increase in warmer weather may facilitate the transmission of infectious diseases. Based on data generated from case file records in some private clinics in Jos Plateau State the increase in cases of bacteria infection is quite high during the hot humid months most especially Salmonella infection.

The changes in climate would increase the incidence and severity of skin infections such as dermatophytosis, candidiasis, streptococcal pyoderma, erythrasma, and tinea, versicolor and scabies are altered by temperature and humidity as a result it is expected that more of these skin condition which affect livestock would be seen.

The change in climate would change the insect distribution such the incursion of the *G. tachnoides* that are vectors of *Trypanosoma brucei gambiense*, zoonotic trypanosome parasites. Taking the case of the Eastern equine encephalitis, this is transmitted in a mosquito-bird cycle by *Culex melanura* in the United States. The vector *C. Melanura* does not feed on large vertebrates; as a result, infection in other animals is very rare. However, climatic changes altering the conditions of the wetlands, such as rainfall, could likely introduce new mosquito breed or types of susceptible birds. The virus might spill over to a species of mammal feeding mosquitoes most likely *Aedes* or other *Coquatiidia*, via infected birds, thus changing the host range. This scenario could be replicated anywhere on the globe.

All the implications of global climate change are not yet known; however, in Nigeria structures should be put in place to evaluate the potential impacts. Consideration should be given to how hydrology, agriculture, forestry and infrastructure will change within the country. Potential changes which would result from sea level rise along the coastal areas, changes in temperature and rainfall patterns include increased use of irrigation and impacts on wetlands and coastal, lake and river ecosystem, such as geographic shifts and changes in composition; changes in the composition and growth rate of the rain forest; and changes in crop yields, geographic distribution change in livestock migratory routes and pesticide use. All of these changes have potential for impact on livestock disease patterns.

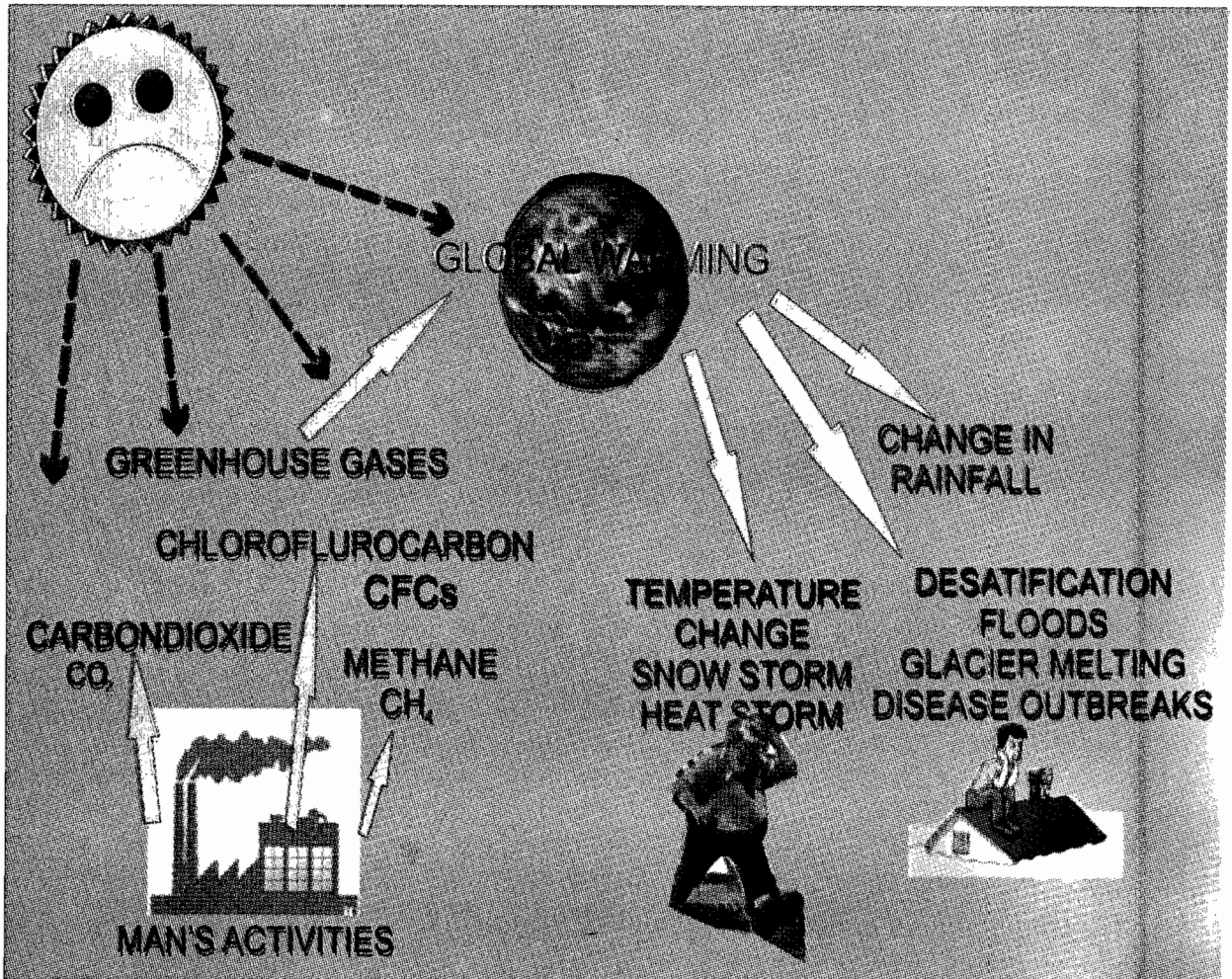
Here in Nigeria, based on the report titled Nigeria Climate Change by Nigerian Environment Study/Action Team (NEST), Nigeria and Global change Strategies International (GCSI) Canada; the expected effect of climate change can be divided into five broad groups

- 1. Human settlement and health
- 2. Water Resources, Wetlands, and Freshwater Ecology
- 3. Energy, Industry, Commerce and Financial Services
- 4. Agriculture, Food security , land Degeneration Forestry, and Biodiversity
- 5. Coastal zone and Marine Ecosystem

If this report is studied there is little report on the expected effect of climate change on the livestock/animal population and health especially as it would affect food security, and health (zoonotic diseases). We should note that the mentioned document is what policy makers in the country would be using to plan for the expected effect of climate change.

In other to be ahead of this pending danger the veterinary profession need to network with other professional such as entomologist, epidemiologist and work out possible answers to address the questions relating to animal health, which may result from climate, change? Issue, including the following: which infectious diseases will become a greater problem to our animal population (livestock and pet); what measures can be taken to reduce the potential impact of these diseases; and what research agenda should be established in regard to these issues. By so doing we would be on the road to develop programs to effectively meet the changes in veterinary disease patterns caused by climate change.

The Nigerian Veterinary Medical Association (NVMA) should also prepare a report for the highest decision making body in the country on the potential adverse effects that might be seen in the country due to the effect of climate change. It should focus on the possible morbidity or mortality of infectious diseases both those endemic in the country as well as those that may be introduced from other location. The report should also attempt to identify the necessary steps to reduce the uncertainty in these conditions.



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