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A RETROSPECTIVE STUDY ON THE PREVALENCE OF PLASTIC MATERIALS IN THE RUMEN OF SHEEP AND GOATS IN NAIROBI, KENYA.

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Abstract

A retrospective study of small ruminant necropsy cases between January 1982 and December 2012 was conducted at the Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Nairobi, Kenya. The objective of the study was to assess the prevalence of plastic materials in the rumen of sheep and goats examined at post mortem over the period. A total of 770 carcasses (367 sheep and 403 goats) presented for post mortem were studied. The overall prevalence of rumen plastic materials in the rumen was 4.2%. The prevalence was higher in goats (7.2%) than sheep (0.8%) and also was significantly ($p<0.05$) higher in older animals (5.5%) than in younger ones (2.4%). There were increases in the cases of plastic materials in the rumen in recent years (2002-2012) compared to the earlier years (1982-2001), though more cases were examined at post mortem in the earlier years. A significantly higher ($p<0.05$) prevalence rate (15.0%) of plastic materials in the rumen was observed in the period between 2003 and 2007. More cases of plastic materials in the rumen were observed in the dry seasons than the wet seasons. Observations in the current study may be a reflection of increased indiscriminate disposal of waste plastic materials in the urban and peri-urban areas of Nairobi. Good waste management and sound animal husbandry practices are required to prevent mortalities related to ingestion of plastic materials in sheep and goats in Nairobi, Kenya.

Key words: Plastic materials, rumen, necropsy, sheep and goats

ETUDE RÉTROSPECTIVE SUR LA PRÉVALENCE DE MATIÈRES PLASTIQUES DANS LE RUMEN D'OVINS ET CAPRINS À NAIROBI (KENYA)

Résumé

Le Département de Pathologie vétérinaire, Microbiologie et Parasitologie de la Faculté de Médecine vétérinaire de l'Université de Nairobi (Kenya) a réalisé une étude rétrospective des résultats d'autopsies de petits ruminants effectuées entre janvier 1982 et décembre 2012, dans l'objectif d'évaluer la prévalence de matières plastiques dans le rumen d'ovins et caprins soumis à l'examen post-mortem durant cette période. Au total, 770 carcasses (367 ovins et 403 caprins) présentées pour examen post-mortem ont été étudiées. La prévalence globale des matières plastiques dans le rumen était de 4,2%. La prévalence était plus importante chez les caprins (7,2%) par rapport aux ovins (0,8%) ; et elle était considérablement plus élevée ($p <0,05$) chez les animaux plus âgés (5,5%) par rapport aux jeunes animaux (2,4%). L'étude a relevé une augmentation de la prévalence de matières plastiques dans le rumen au cours des dernières années (2002-2012) par rapport aux années précédentes (1982-2001), bien qu'un nombre plus élevé de cas ait été soumis à l'examen post-mortem au cours des années antérieures. L'étude a également noté un taux de prévalence (15,0%) significativement plus élevé ($p <0,05$) de matières plastiques dans le rumen durant la période 2003 - 2007. De plus, une prévalence plus élevée de matières plastiques dans le rumen a été observée pendant les saisons sèches par rapport aux saisons humides. Les observations de cette étude peuvent être une conséquence de l'augmentation de l'élimination sans tri de déchets en

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matières plastiques dans les zones urbaines et péri-urbaines de Nairobi. La bonne gestion des déchets et les pratiques d'élevage optimales sont nécessaires pour prévenir les mortalités liées à l'ingestion de matières plastiques par les ovins et caprins à Nairobi (Kenya).

Mots-clés : matières plastiques, rumen, autopsie, ovins et caprins

Introduction

The importance of sheep and goats in household food security, income generation, poverty reduction and soil fertility for crop production is well documented (Wilson, 1988; Devendra, 1999 and 2000; Njanga *et al.,* 2003; Lebbie, 2004). Sheep and goat production is a major component of the livestock sub-sector in Kenya and plays an important role in the social and economic life of many citizens (Njanga *et al.,* 2003). Nationally, sheep and goats contribute about 30% of the total red meat (estimated at 211,000 metric tons) consumed in the country annually (GoK, 2012).

Traditionally, many communities rear sheep and goats together with cattle (Kiptarus, 2005) and they represent about 57.8% of the total domestic grazing livestock (GoK, 2010). In the urban and peri-urban areas, sheep and goat rearing is practiced as a commercial undertaking, for food self-sufficiency (Maxwell, 1994 and 1995) and mitigation against the hardships of urban life (Rogerson, 1992; Sawio, 1994). Sheep and goats are a common sight, especially in open spaces in the outskirts of cities and towns (Mwangi and Foeken, 1996).

The city of Nairobi is reported to generate about 2,400 tons of garbage daily. The reported average household waste generated is 253 kg per year of which, 20.6% is plastics and 61.4% organic and food waste (UNEP, 2005). Only 60% of the household waste generated is collected, the rest (40%) is disposed through burning and dumping in open spaces and pits (Ikiara, 2006). In the low income areas where up to 60% of the urban population lives, local authorities provide almost no waste collection services (UNEP/NEMA, 2005). Hence, open spaces and fields in and outside the city are heavily polluted with household waste, making these materials available for ingestion by roaming and scavenging sheep and goats (Njeru, 2006).

Ingestion of indigestible foreign bodies by sheep and goats has been associated with scarcity of feed, nutritional deficiencies and poor pastures which compel animals to roam and scavenge for feed in polluted environments (Igbokwe *et al.,* 2003, Hailat *et al.,* 1997; Ramaswamy and Sharma, 2011). Case reports of mortalities due indigestible foreign body impaction in sheep and goats have been previously documented (Akinrinmade *et al.,* 1988; Otesile and Akpokodje, 1991; Elsa *et al.,* 1991). However, information associating mortality of sheep and goats to indigestible rumen plastic materials in Kenya is unavailable. In view of the increased waste pollution and the common sight of roaming and scavenging sheep and goats in the urban and peri-urban areas of Nairobi, a retrospective study of the presence of plastic materials in the rumen of carcasses of sheep and goats presented for necropsy was considered pertinent. This was in speculation that ingested plastic materials may play a role in the pathogenesis of mortality in the sheep and goats.

Materials and methods

Study location

The study was carried out in the Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Nairobi, Kenya.

Nairobi is located at the southern end of the country's central highlands and occupies an area of 696 km^2. It lies at an altitude of between 1600 and 1800 meters above sea level. Mean annual temperature is 17°C, while the mean daily maximum and minimum are 23°C and 12°C, respectively. Mean annual rainfall ranges from about 800 to 1,050 mm and most of it falls in two distinct seasons, with mid-March to June as the long rains season and mid-October to early December as the short rains season. The Faculty of Veterinary Medicine serves clients in the urban and per-urban areas of Nairobi through clinical and post mortem services. Carcasses presented for necropsy at the Faculty of Veterinary Medicine are mostly

drawn from the urban and peri-urban livestock farming households.

Study design and data collection

Necropsy records were retrieved from the record archives of the Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Nairobi. Case records between January 1982 and December 2012 were examined and relevant data associated with the presence of plastic materials in rumen of sheep and goats were extracted. These included the date of necropsy, species of animal, age, sex, presence or absence of plastic materials in the rumen and type of plastic material.

Data management and analysis

Data collected were analyzed to establish the prevalence of plastic materials found in the rumen in relation to species, age, sex, year of necropsy and season over the period of study. The prevalence of plastic materials found in the rumen of carcasses of the sheep and goats was calculated by dividing the number of positive cases by the total number of cases examined at necropsy and the result expressed as a percentage. The chi square ($\chi 2$) test was applied to determine if there were any statistically significant associations between the various factors and the occurrence of plastic materials in the rumen of sheep and goats. Significance was determined at $p \leq 0.05$.

Results

A total of 770 carcasses of small ruminants were presented to the Faculty of Veterinary Medicine for necropsy between January 1982 and December 2012. Three hundred and sixty seven (367, 47.7%) were carcasses of sheep, while 403 (52.3%) were carcasses of goats. Three hundred and twelve (312, 40.5%) of the total number of carcasses necropsied during the period were male while 458 (59.5%) were female. Out of the total number of carcasses necropsied, 32 (4.2%) were found to have plastic materials in their rumen. Out of the 367 sheep necropsied, only 3 were found with plastic materials in the rumen, while 29 of the 403 goat carcasses necropsied during the period had plastic materials in the rumen. A total of 507 (65.8%) carcasses of sheep and goats were necropsied in the first 10 years (1982-1992), while 263 (34.2%) were necropsied in the following 20 year period (1993-2012). Out of the 507 carcasses necropsied in the first 10 years only 11(2.2%) were found with plastic materials in the rumen, while 21 (8.0%) of the 263 carcasses necropsied between 1993 and 2012 has plastic materials in the rumen. Data were, however, not available for the years 1993, 2003 and 2004.

Overall prevalence of plastic materials in the rumen of sheep and goats carcasses presented for necropsy at the University of Nairobi from 1982 – 2012

From 770 carcasses of sheep and goats examined, 4.2% (32) were found to have plastic materials in their rumen. The prevalence of rumen plastic materials in goats (7.2%) was significantly higher than that of sheep (0.8%) ($\chi 2= 18.1$, $P = 0.0001$) (Table 1).

Prevalence of rumen plastic materials in the rumen of sheep and goat carcasses according to sex

Out of the 770 carcasses necropsied, 59.5% (458) were females and 40.5% (312) were males. Among carcasses found with plastic materials in the rumen, 40.6% (13) were males, while 59.4% (19) were females. There was no significant difference in the prevalence of rumen plastic materials between male and female carcasses ($\chi 2 = 0.05$, $P > 0.83$) (Table 2).

Prevalence of plastic materials in the rumen of sheep and goat carcasses according to the year of necropsy.

The highest number of sheep and goat carcasses presented for necropsy was recorded in 1986 (55 cases) while the lowest was in 2000 (1 case). However, the highest prevalence rate of plastic materials in the rumen was recorded in 2010 (13.6%) followed by 2011(10.3%). Out of the 30 years of data studied, 11 of the years did not record any positive case of rumen

Table 1: Overall prevalence of plastic materials in the rumen of sheep and goat carcasses presented for necropsy at the Faculty of Veterinary Medicine, University of Nairobi from 1982 - 2012.

Animal species	Number carcasses necropsied	Number with plastic materials	Prevalence %
Sheep	367	3	0.8
Goat	403	29	7.2
Total	**770**	**32**	**4.2**

Table 2: Prevalence of plastic materials in the rumen of sheep and goats carcasses according to sex

Animal species	Number carcasses necropsied	Number of carcasses with plastic materials	Prevalence %
Male	312	13	4.2
Female	458	19	4.1
Total	**770**	**32**	**4.2**

Table 3: Prevalence of rumen plastic materials in the rumen of sheep and goat carcasses at time periods between 1982 and 2012.

Time period	Number of carcasses necropsied	Number of carcasses with plastic materials	Prevalence %	Chi square (χ2)	*P - value*
1982-1987	268	6	2.2	3.63	0.06
1988-1992	236	5	2.1	3.39	0.07
1993-1997	128	9	7.0	2.79	0.10
1998-2002	33	2	6.1	0.27	0.60
2003-2007	20	3	15.0	5.07	0.02
2008-2012	85	7	8.2	3.47	0.06
TOTAL	**770**	**32**	**4.2**	**-**	**-**

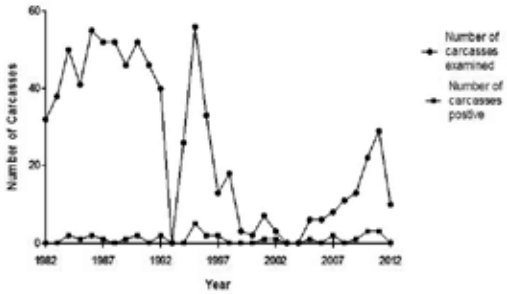


Figure 1: Cases of rumen plastic materials in sheep and goat carcasses necropsied at the Faculty of Veterinary Medicine, University of Nairobi between 1982 and 2012.

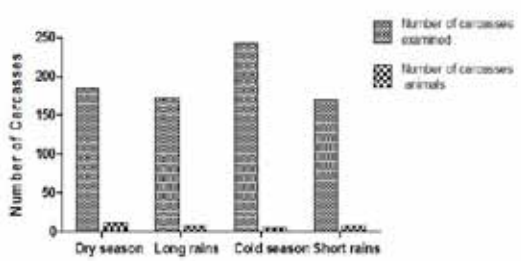


Figure 2: Seasonal distribution of cases rumen plastic materials in sheep and goat carcasses necropsied at the Faculty of Veterinary Medicine, University of Nairobi between 1982 and 2012.

plastic materials (Fig 1). The highest number (268 cases, 35.1%) of carcasses presented for necropsy was recorded in the period between 1982 and 1987, while the lowest number (20 cases, 2.6%) was recorded in the period between 2003 and 2007. However, this same period had the highest prevalence rate of plastic materials in the rumen with the period between 1982 and 1987 showing the lowest prevalence rate (Table 3).

Prevalence of plastic materials in the rumen of sheep and goat carcasses according to age groups

The carcasses presented for necropsy during the period were categorized into 2 age groups. Those less than one year old were considered as young and those more than one year old were considered as adults (Table 4). A majority of the carcasses necropsied were adults (56.8%, 437). The adults also showed a higher prevalence rate of rumen plastic materials of 5.5%.There was a significant difference in prevalence rates of rumen plastic material between adults and the young ($\chi 2$ = 4.19, P = 0.04) (Table 4).

Prevalence of rumen plastic materials in sheep and goat carcasses according to the season of necropsy.

Out of the 770 carcasses necropsied, 23.9% (184) were examined in the dry seasons, while 22.5% (173), 31.5% (243) and 22.1% (170) were examined during the long rains, cold season and short rains season respectively. Among the animals necropsied in the dry seasons, 11 had plastic bags in their rumen, while 8 were positive of plastic bags during the long rains season, 5 in the cold seasons and 8 in the short rain seasons respectively (Figure 2). The highest prevalence rate (6.0%) was observed during the dry season, followed by 4.7% in the short rain season and 4.6% in the long rain season. The lowest prevalence rate (2.1%) was observed in the cold seasons. However, the differences between the prevalence rates of rumen plastic bags in the various seasons of the year were not statistically significant (P > 0.05).

Discussion

The 4.2% overall prevalence of plastic bags in the rumen of sheep and goat carcasses gives an indication that sheep and goats reared in urban and peri-urban areas of Nairobi occasionally ingest plastic bags that may contribute to mortality of these animals. The prevalence rates of 7.2% in goat and 0.8% in sheep carcasses observed in this study, sharply contrasts those of Mohammed (2012) who reported a prevalence rate of 88.5% in goats and 5.5% in sheep in a retrospective study at the Central Veterinary Hospital in Khartoum, Sudan in the period between 2001 and 2011. The prevalence in goats is also lower than the 44.4% in goats at the Central Veterinary Hospital in Omdurman, Sudan between 1998 and 2002 (Mohammed *et al.,* 2006).

The comparatively low prevalence rates at necropsy in the current study can probably be attributed to the fact that not all animals with plastic foreign bodies will die and those that die may not end up at necropsy. Since sheep and goats are food animals, whenever their health conditions deteriorates or when the cost of treatment is not affordable, they are salvaged for domestic consumption. This

Table 4: Prevalence of plastic materials in in the rumen of sheep and goat carcasses according to age groups

Age animal necropsied	Number of carcasses necropsie	Number of carcasses with plastic materials	Prevalence %
< 1 year	333	8	2.4
> 1 year	437	24	5.5
Total	**770**	**32**	**4.2**

(P < 0.05)

may account for differences in prevalence rates between live animals presented for ante mortem examinations at veterinary clinics or at slaughter and carcasses presented for necropsy. This also supports suggestions that rumen impaction with foreign bodies can best be studied at the abattoir, since most of the cases are asymptomatic and most livestock will end up at the abattoir (Ramin *et al.,* 2008)

The higher prevalence of plastic materials in the rumen of goat carcasses than sheep carcasses agrees with previous findings of Mohammed *et al.* (2006), Ghurashi *et al.* (2009) and Mohammed (2012) who reported higher prevalence rate in goats than sheep in similar studies. This finding can be attributed to the dominance of goats as the preferred small stock kept in the urban and peri-urban areas in Kenya as they attract better prices than sheep due to consumer preferences (GoK, 2010; Kagira and Kanyari, 2010). Some households also prefer goats to sheep because goats could be milked for domestic use. Furthermore, goats are more difficult to confine and may wonder far away from home scavenging for food and hence more likely to ingest plastic materials. Conversely, other researchers have reported higher prevalence rates of rumen foreign bodies is sheep than goats in various abattoir studies (Halitat *et al.,* 1997; Igbokwe *et al.,* 2003; Remi-Adewunmi *et al.,* 2004; Okai *et al.,* 2007; Roman and Hiwot, 2010).

The absence of a significant difference in the prevalence of plastic materials in the rumen of male and female carcasses is in corroboration with similar findings in abattoir studies carried out in Ethiopia and Nigeria respectively (Abebe and Nuhu, 2011; Saulawa *et al.,* 2012). Other previous abattoir studies have reported a difference in the susceptibility of male and females to ingestion of plastic materials. This difference was attributed to the fact that, female animals are usually kept longer for periods for breeding and have higher nutritional demands during estrus, pregnancy and lactation (Igbokwe *et al.,* 2003; Remi-Adewunmi *et al.,* 2004; Roman and Hiwot, 2010; Omidi *et al.,* 2012).

The higher prevalence rate of plastics materials in the rumen of sheep and goat carcasses in the more recent years is consistent with previous reports in Sudan (Mohammed *et al.,* 2006; Ghurashi *et al.,* 2009). This observation can be attributed to increased urbanization resulting in increased environmental pollution with plastic materials (UNEP/MEMA, 2005; Ikara, 2006), resulting in places available for grazing becoming heavily polluted with plastics and other non-biodegradable waste materials. The finding of a significantly higher prevalence of plastic materials in rumen of older animals in the current study is similar to previous reports from abattoir studies indicating that occurrence of indigestible foreign bodies in the rumen was associated with older animals (Halitat *et al.,* 1997, 1998; Igbokwe *et al.,* 2003; Adewunmi *et al.,* 2004; Roman and Hiwot, 2010; Abebe and Nuhu, 2011; Saulawa *et al.,* 2012; Tesfaye *et al.,* 2012). This finding can be explained by the fact that ingestion and accumulation of foreign bodies in the rumen of livestock is a gradual process. Thus, these foreign bodies accumulate in the rumen over prolonged periods of time. It is therefore more likely to find waste plastic materials in the rumen of older animals that have spent longer periods in polluted environments than younger ones in the same environment.

Observations in the current study indicate that season may have an impact on ingestion of plastic materials in of sheep and goats. Though it may be difficult to associate occurrence of plastic materials in the rumen with season in a retrospective study, a higher prevalence rate of indigestible foreign bodies in the rumen of sheep and goats have been associated with hot dry seasons and summer (Mohammed *et al.,* 2006; Mohammed, 2012). This observation has been attributed to drought and animal feed scarcity during the dry seasons that compels livestock to ingest indigestible foreign bodies (Halitat *et al.,* 1997; Igbokwe *et al.,* 2003; Ramaswamy and Sharma, 2011). In the current study, more carcasses examined at necropsy during the dry season were found to have plastic materials in their rumen than the cold and raining seasons. Perhaps, scarcity of feed and mineral deficiencies of roaming and scavenging sheep and goats as suggested by the previous reports may be responsible for the observation in this study.

Conclusion

A finding of plastic materials in the rumen of carcasses of sheep and goat was a general indicator of the association between the system of production of small ruminants and the environment polluted with waste plastic materials. It suggests that the sheep and goats may have ingested these plastic materials through roaming and scavenging at polluted sites in urban and peri-urban areas of Nairobi. The overall prevalence rate of 4.2% of plastic materials in the rumen of carcasses of sheep and goats is quite significant. Though, presence of plastic materials in the rumen may not necessarily have been the cause of death, these plastic materials may have contributed to the general pathogenesis of the mortality of these animals. Good waste management and sound animal husbandry practices may be necessary to prevent mortalities associated with indigestible plastic materials in sheep and goats in Nairobi, Kenya.

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ASSESSMENT OF HELMINTH LOAD IN FAECAL SAMPLES OF FREE RANGE INDIGENOUS CHICKEN IN PORT HARCOURT METROPOLIS NIGERIA.

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Abstract

Helminths load in faecal sample of free range indigenous chicken in Port Harcourt Metropolis was examined. Faecal samples were collected from 224 birds in 15 homesteads and 4 major markets - Mile 3, Mile 1, Borokiri and Eneka Village market where poultry are gathered for sale. 0.2-0.5g of feacal sample was collected from birds between January and September, 2011. Feacal samples were collected from 224 birds in both dry and wet season. Feacal sample were analyzed for helminths load using wet preparation, sedimentation and floatation method for the isolation and identification of helminths and egg count per gram faecal sample. The result showed various species of helminths of the three common classes of poultry helminths parasites (nematodes, cestodes and trematodes). The most prevalent in the study area were Teanea spp 22.31%, Ascaris lumbricoides 11.16%, Enterobius vermicularies 12.3% and Trichuris trichura 9.96%. Season was found to have a significant effect ($P < 0.05$) on helminths load of indigenous chicken with higher value recorded in the wet season than the dry season (42.27±6.26 versus 25.00±4.86 respectively). Sex and strain was found to be insignificant, that is , it did not have effect on helminths parasites as there was no variation in the egg count. The result of this study reveals that all the indigenous chickens that were examined in the study area showed multiple infestations of the three classes of helminths It was concluded that the keepers of indigenous chickens should be educated on good management practices that will reduce the exposure of the birds to sources of infestation or rather confining the birds especially during the rainy season.

Key words: Indigenous chicken, Helminths, prevalence, free range

ÉVALUATION DE LA CHARGE HELMINTHIQUE DANS LES ECHANTILLONS FECAUX DE POULETS INDIGENES ELEVES EN LIBRE PARCOURS DANS LA METROPOLE DE PORT HARCOURT

Resume

La présente étude a examiné les charges helminthiques des échantillons fécaux de poulets indigènes élevés en libre parcours dans la métropole de Port Harcourt. Les échantillons fécaux ont été prélevés sur 224 oiseaux dans 15 exploitations familiales et 4 principaux marchés (Mile 3, Mile 1, Borokiri et le marché du village d'Eneka où les volailles sont rassemblées pour la vente). Des échantillons fécaux de 0,2-0,5g ont été prélevés entre janvier et septembre 2011 sur 224 oiseaux, pendant les saisons sèche et humide. Ces échantillons ont été analysés pour évaluer la charge helminthique en utilisant la méthode de préparation humide, la sédimentation et la flottaison pour l'isolement et l'identification des helminthes et la numération d'œufs par gramme d'échantillon fécal. Le résultat a révélé la présence de diverses espèces d'helminthes des trois classes courantes de vers parasites des volailles (nématodes, cestodes et trématodes). Les helminthes les plus répandus dans la zone d'étude étaient Teanea spp (22,31%), Ascaris lumbricoides (11,16%), Enterobius vermicularies (12,3%) et Trichuris trichura (9,96%). L'étude a constaté que la saison avait un effet significatif ($P <0,05$) sur la charge helminthique des poulets indigènes, les valeurs enregistrées étant plus élevées pendant la saison des pluies par rapport à la saison sèche (respectivement 42,27 ± 6,26 contre 25,00 ± 4,86). Les résultats ont révélé que le sexe et la souche n'avaient pas d'importance, c'est à dire qu'ils n'ont pas d'effet sur les helminthes parasites puisqu'il n'y a pas eu de variation au niveau du nombre d'œufs. Cette étude révèle que tous les poulets indigènes

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examinés dans la zone d'étude étaient infestés par plusieurs parasites des trois classes d'helminthes. Il a été conclu que les éleveurs de poulets indigènes devraient être formés aux bonnes pratiques d'élevage qui leur permettront de réduire l'exposition des volailles aux sources d'infestation ou de confiner les oiseaux en particulier pendant la saison des pluies.

Mots-clés : poulet indigène, helminthes, prévalence, libre parcours

Introduction

The contribution of poultry industry to national economy and even to human nutrition is quite appreciating (Matur, 2002). Poultry industry which is made up of the domesticated birds like geese, turkeys, pigeon and chicken (fowl) have indeed outgrown other sectors of livestock production such as cattle, sheep and goat. This is true especially in chicken production due to their potential of short-run return, simple managerial skills, quick income generation, and low land requirement per unit output. Indigenous chicken is reared basically by rural farmers and urban householder who use their egg and meat as a source of animal protein as well as means of income generation (Frantova, 2002). The indigenous chicken therefore plays a significant role in the livelihood of the rural farmers. This implies that proper attention has to be given to indigenous chicken production such that will boost its productivity in the rural areas.

Some of the factors contributing to the reduction of indigenous chicken production in most developing countries of the world include poor management skills, diseases prevalence such as coccidiosis, fowl pox, Newcastle and helminthiasis among others. It is important to state here that several studies have been conducted to examine effects of these diseases among the indigenous chicken from various perspective. For instance, it was reported by Sonaiya, (1990)). that the loses recorded in poultry especially the indigenous chickens have been as a result of disease causing agent such as virus, bacteria and parasites. He also stated that about 750 million chickens, guinea fowls and ducklings in Africa die every year as a result of infections. In most rural areas in Nigeria, indigenous chickens are kept under the free range system or extensive management system where the birds are fed with some grains in the morning and allowed to scavenge for other feed resources with little or no medication and provision of night shelter only. The birds are predisposed to predators and other parasitic organisms which they can pick up in the course of their scavenging. It has been reported that chickens reared under the cage system gave negative results for helminthes whereas those reared on free range had higher helminth infections (Abebe *et al*., 1997).

Indigenous chicken are therefore prone to a lot of environmental hazards that also pre-dispose them to other diseases. There is need to adopt strategies that will reduce parasitic infection especially helminths which will in turn increase productivity of indigenous chicken. Moreover, the findings of this study will also help to expose the indigenous chicken producers on the seasonal prevalence of helminths load among the indigenous chicken thereby proffering ways of reduction or controlling the exposure of these chicken during such seasons.

Helminths are parasitic infection of livestock species generally including the indigenous chicken. The disease caused by helminths is known as helminthiasis. Helminths parasites that affect poultry are classified into three main groups namely: nematodes cestodes and trematodes. Although these parasites can be present in the village chicken without apparent effects on the birds (Saidu *et al.,* 1994). their mode of infections among poultry especially indigenous chickens varies as they exhibits different life cycle. This study focused on examining the prevalence of these parasites among free range indigenous chickens in Port Harcourt metropolis. The study will serve as a database for identifying the most common helminth affecting indigenous chicken in the study area.

Materials and Methods

This study was carried out using birds from 15 homesteads where indigenous chickens are kept by families and four selected

major markets of Port Harcourt metropolis were poultry birds are gathered for sale. The market includes Mile One, Mile three, Borokiri and Oil Mill markets. Port Harcourt metropolis is in River State Nigeria located in Niger Dater Region. The city lies between latitude 4.472'21" N and longitude 6.39"55"E with escheated population of 1,382,592 following the 2006 Nigeria census. The materials used for this study includes; sample bottle, 10% formalin, physiological saline, slides, cover glass, applicator stick, rack, masking tape, centrifuge microscope. A total of 224 feacal samples was collected from three strains of indigenous chicken normal feather (NF), Frizzle feather (F) and naked neck (N) of both sexes. 0.2 - 3g of feacal sample was collected from each of the three strains of chicken of both sexes from the four major markets and homesteads of backyard poultry farmers in the study area. The chickens (birds) were monitored as they defecated and the freshly deposited feacal material was collected and put in a sample bottle containing about 4mls of 10% formalin to prevent contamination. The feacal sample was analyzed by shredding in 10% formalin using wet preparation method (Cheesbrough, 2000). A fresh physiological saline was dropped on one end of a slide and iodine on the other end. A small amount of the sample, 0.5g was mixed with saline and similar amount with iodine using a spatula. Each preparation was covered with a glass. The slides were examined systematically using X 10 objective lenses and X 40 objective to help magnify the sample for easy detection and identification of the parasites. Helminth eggs and protozoan cysts were isolated from the sample using one gram feacal sample and counted using a centrifugal floating technique (Anon, 1986). Eggs, cysts and other organisms present were identified by microscopic examination and the number of occurrence recorded. Data collected were analyzed using descriptive statistics and Generalized Linear Model of SAS (2003).

Results

Out of 224 specimens observed in the study, 162 were found to be positive whereas 62 birds were negative for helminthiasis, giving a prevalence rate of 72.3%. The prevalence of different categories of helminthes found in the feacal samples of birds used for this study is presented in Table 1. Ten different species of helminthes were identified. Teanea species of Cestodes family has the highest prevalence (22.3%) while two species of Nematodes *Ascaris lumbricoides* and *Enterobius vermicularies* recorded 11.16 and 12.3% respectively. Some other organisms which include yeast cells, protozoa and Ooyst of *Cryptosporidium parvum* were also found in the samples analyzed.

Average egg count per gram of feacal sample is shown in Table 2. No significant difference in mean egg count of helminths in the different strains of indigenous chicken.

Seasonal variation and effect of sex on helminths load are presented in Tables 3 and 4. There was significantly ($P<0.05$) higher infection during wet season than in the dry season. The sex of bird on the other hand showed no significant difference ($P>0.05$) on helminths load.

Discussion

Multiple infestations were common in the study with some of the 224 birds examined harbouring 1-4 species of helminths parasites including other organisms. Results showed that 10 species of the three common poultry helminths, nematodes, cestodes and trematodes was recovered in the faecal samples of free range indigenous chickens. The species include the following: *Ascaris lumbricoides, Enterobius vermicularies, Trichuris trichura, Trichoslonglus tenius, Teanea spp, Hymenolopsis nana, Dipybobothrum latum*, *Diphyllobothrium latum* Hook worm, *Hymenolopsis dominata, Fasciola gigantic*. Other organisms that were identified alongside the helminths were Yeast cell, *Entaemoba, spp* Intestinal flagellates cyst, *Isospora belli* and Oocyst of *Cryptosporidium parvum*.

From the different species of the three classes of helminths reported, *Enterobius vermicularies* a nematode was dominant both in the rainy (wet) and dry (hot) season (13.2 % versus 11.5 %), respectively. This result however, does not agree with earlier report (Jordan and Pattison, 1996) where it was concluded that nematodes constitute the most common

Table 1: Prevalence of Helminthes and other Organisms in Feacal Sample of Indigenous Chicken in Port Harcourt Metropolis

Helminths	Frequency	Percentage
Nematodes		
Ascaris lumbricoides	56	11.16
Enterobius vermicularies	62	12.3
Trichuris trichura	23	9.16
Trichoslongylus tenius	2	0.80
Cestodes		
Teanea Spp	112	22.21
Hymenolopsis nana	12	2.39
Diphyllobothrium latum	2	0.40
Hook Worm	24	4.78
Hymenolopsis dominata	18	3.59
Trematodes		
Fasciola gigantic	10	1.99
Other Organisms		
Yeast cells (fungi)	60	11.95
Entaeomoeba Spp (protozoa)	4	0.80
Intestinal flagellate cyst	11	4.38
Ooyst of Cryptosporidium parvum	2	0.80

Table 2: Average Total Egg Count of Helminths and other Organisms Prevalence in the Indigenous Chicken

Helminths	Mean ± SEM
Nematodes	
Ascaris lumbricoides	26.6±6.67
Enterobius vermicularies	31.96±7.19
Trichuris trichura	10.21±2.27
Cestodes	
Teanea spp	49.9±9.82
Hymenolopsis nana	21.66±6.00
Diphyllobothrium latum	10.00±-
Hook worm	9.58±2.25
Hymenolopsis dominate	64.37±27.94
Trematodes	
Fasciola gigantic	5.00 ± 0.0
Others organisms	
Yeast cell (fungi)	42.08 ± 22.96
Isospora belli	37.14 ± 27.98
Oocyst of Crytosporidum parvum	17.5 ± 12.5

Table 3: Effect of Season on Helminths Egg Count of Indigenous Chicken

Season	Mean ± Sem
Dry	25.00 ± 4.85[b]
Wet	42.22 ± 6.26[a]

[a, b] means that different column carrying different subscript differs significantly (P < 0.05)

Table 4: Effect of Sex on Helminths Egg Count of Indigenous Chicken

Sex	Mean ± Sem
Male	29.49 ± 4.379[a]
Female	37.95 ± 6.96[a]

groups of helminths parasite of poultry both in number of species and the number of losses they incur. This is because the percentage of Ascaris lumbricoides of the nematode class found is lower than the percentage of Teanea spp of cestodes class (22.21%) in this report. However, the Teanea spp was high in number in both dry and wet season 23.1% and 21.5% respectively; hence the result did not agree with the report of (Okech *et al.,* 2006) and (Pam *et al.,* 2006) who both concluded that Ascaris galli have been incriminated as the most common and important parasites of poultry.

The prevalence of 1.99% for *Fasciola gigantic* – a trematode reported in this study contradicts earlier reports of (Permin *et al.,* 1997) . who found no trematode in their study with rural scavenging poultry in Tanzania in relation to season and climate and in northern Jordan (Abdelqader *et al.,* 2008). The report however corroborates earlier assertion that *Fasciola hepatica* and *Fasciola gigantica* are common in the tropics (Fisher and Say, 1989). A possible cause for the difference may be attributed to the vegetation cover in the humid tropical environment which is characterized with high humidity and warm environmental conditions when compared to the other part of West African (Abebe *et al.,* 1997). One can suggest that both high humid zones and warm environment favours the development and prevalence of these parasites. Another factors could be the production system which is common among the indigenous chicken referring to them as the scavengers, following the report of Ondwassy *et al.,* 2000. who reported that helminths infestation in the indigenous chicken are very common because of the risk posed by the production system which relies on scavenging.

Considering the effect of sex on the prevalence of helminth parasites, result shows that sex (male and female) have no effects on the helminths load of the indigenous chicken in the study area. Hence one can suggest that helminths have no sex preference with reference to infestation. This result is contrary to earlier reports where sex differences in prevalence of Ascaris galli and Raillietina cesticillus was reported with higher percentages in the male than the female hosts (Permin *et al.,* 1997). Egg count per gram revealed that helminths parasites are more prevalence among the female birds. Although this study did not consider the production performance of the birds used for this research, there is an indication that the high prevalence of helminths in female birds could contribute to irregularities in laying hens under the free range system. It had earlier been reported that helminths infestation can lead to egg abandonment in laying hens depending on the degree of the load on the birds (Nnadozie, 2006).

Conclusion and Recommendation

Although helminths parasites of poultry birds are not of zoonotic importance since the pathogens cannot survive on contact with heat but its effect on indigenous chicken production calls for urgent attention especially in the rural areas where these birds are reared. Since the production system employed in rearing these birds is basically scavenging as practiced in most rural dwellings in the tropics, there is need to adjust the production system

of these birds especially in the rural area since it is of high economic value to the rural farmers.

Finally, it may be suggested through the result of this study, that wet season favours the infestation and survival and development of helminths prevalence among the indigenous chicken. This however could be attributed to the vegetation cover of the study area which is characterized by high rainfall pattern, relative humidity and temperature. Therefore, it becomes essential to consider the need to confine these chickens especially during the raining season in order to reduce the losses encounters by the rearers of these chickens during the period. The incorporation of semi-intensive system of production should be encouraged in indigenous chicken production where feed can be provided either as protein supplement from cheap, affordable and non-conventional but quality feed source to complement the kitchen residue and other things that these birds feed on especially during the raining season when these parasites thrives heavily so as to build up the immune system of the birds for more resistance to the diseases caused by worm infestation.

Good management system through vaccination and/or deworming progamme that is practiced in the exotic birds can as well be adopted in free-range system to reduce the rate of infection by helminthes thereby boosting the productivity of indigenous birds. There is also need for routine vaccination and/or deworming programme so as to reduce the losses encountered in the production system.

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PROSPECTIONS DES GLOSSINES ET AUTRES MOUCHES PIQUEUSES DANS LES CERCLES DE SIKASSO ET KADIOLO AU MALI EN PRÉLUDE À UNE CAMPAGNE DE SUPPRESSION.

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Résumé

Le but du projet TCP/MLI/3402 FAO est d'initier une campagne de protection efficace et durable des bovins contre la Trypanosomose Animale Africaine (TAA) et la résistance aux trypanocides par le contrôle de la mouche tsé-tsé combiné aux traitements stratégiques des animaux. Préalablement à la mise en œuvre des activités de lutte, une étude de base entomologique a été confiée au Laboratoire Central Vétérinaire (LCV).

35 villages ont été prospectés dans les cercles de Kadiolo (20 villages dans 9 communes) et Sikasso (15 villages dans 4 communes). Pour cette étude 350 pièges biconiques Challier – Laveissière ont été posés dans les galeries et au niveau des points de contact des différents cours d'eau et géo référencés à l'aide de GPS. La prospection entomologique dans la zone de l'étude a permis la capture de deux espèces de glossines : Glossina palpalis gambiensis et Glossina tachinoïdes. 786 glossines sont capturées dont 405 mâles et 381 femelles. Parmi les glossines capturées, il ya 691 Glossina palpalis gambiensis dont 349 mâles et 342 femelles, 95 Glossina tachinoïdes dont 56 mâles et 39 femelles. Dans cette étude le sex-ratio est de 1.12, donc légèrement en faveur des mâles. Le taux d'infection des glossines qui concerne le cercle de Kadiolo seulement est 0% (175/402 glossines).

Mots clés : Prospections, glossines, lutte, Mali.

PROSPECTING OF GLOSSINAS AND OTHER STINGING FLIES IN THE CIRCLES OF SIKASSO AND KADIOLO (MALI) IN PRELUDE TO A CAMPAIGN OF ABOLITION.

Boubacar Bass[1]*; Mama Bagayoko[1] ; Daman Traore[1]; Fatogoma Kone[1]

Summary

The objective of the project TCP / MLI / 3402 FAO is to introduce a campaign of effective and long-lasting protection of the cattle against Trypanosomose Animale Africaine (TAA) and resistance to trypanocides by the control of the tsetse fly combined in the strategic treatments of animals. Before the implementation of the activities of fight, a study of entomological base was confided to the Veterinary Central Laboratory (LCV).

35 villages were canvassed in the circles of Kadiolo (20 villages in 9 municipalities) and Sikasso (15 villages in 4 municipalities). For this study 350 traps biconiques Challier - Laveissière was put in galleries and at the level of the contact points of the various streams and the geography referenced by means of GPS (GLOBAL POSITIONING SYSTEM). The entomological prospecting in the zone of the study allowed the capture of two species of glossinas: Glossina palpalis gambiensis and Glossina tachinoïdes. 786 glossinas are captured among which 405 males and 381 females. Among the captured glossinas, him ya 691 Glossina palpalis gambiensis among whom 349 males and 342 females, 95 Glossina tachinoïdes among whom 56 males and 39 females. In this study sex-ratio (report male / female) is 1.12, thus slightly in favour of males. The rate of infection of the glossinas which concerns the circle of Kadiolo only is 0 % (175/402 glossinas).

Keywords: prospecting, glossinas, fight, Mali.

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Introduction

Les glossines sont des Diptères Brachycères Cyclorrhaphes de la famille des Glossinidae avec un seul genre : Glossina. Elles diffèrent des autres mouches par l'adaptation de leurs pièces buccales à la piqûre ; à la différence de quelques autres insectes hématophages (moustiques, taons), les deux sexes se nourrissent de sang. Les glossines sont des mouches allongées, robustes, de coloration brun noirâtre à brun testacé, jamais métallique ; leur longueur sans la trompe, est comprise entre 6 et 16 mg. Leur poids varie de 7 -14 mg chez Glossina tachinoïdes à 20 – 25 mg chez Glossina morsitans. Ces insectes transmettent à l'homme et aux animaux domestiques, plusieurs espèces d'un protozoaire flagellé du genre Trypanosoma qui vit dans les liquides biologiques et les tissus des hôtes provoquant une maladie aiguë ou par contre, bien que piqués et inoculés par les glossines, la plupart des mammifères sauvages (antilopes, girafes, buffles, lions, éléphants…) et quelques races d'animaux domestiques (taurins, N'dama, Baoulé, Lagune…) vivent, produisent et se reproduisent dans les zones infestées, bénéficiant d'une trypanotolérance. Ce serait peut être également le cas de certaines populations humaines des zones très humides.

La distribution de l'insecte est liée à son habitat : la végétation au bord des cours d'eaux, des lacs, des forêts - galeries et des vastes étendues de savane arbustive.

Au Mali les glossines occupent une superficie de 240.000 km2 à très grand potentiel agricole soit 16% du territoire national. La région de Sikasso est infestée à 100%, la région de Kayes à 76%, la région de Koulikoro à 60% et la région de Ségou à 44%.

Il est alors impératif de lutter contre cette maladie. L'un des moyens de contrôle de cette maladie est la lutte contre les vecteurs (Cuisance et al. 2003).

Dans la plupart des cas, les mouches tsé-tsé tendent à subsister, en raison de la survie de certaines d'entre elles après les interventions ou des mouches venant des régions non traitées, ou les deux (Politzar et Cuisance, 1984). Selon Bouyer (2006), les différents échecs enregistrés étaient liés à un manque de connaissances approfondies de l'écologie des glossines. La connaissance de l'écologie des glossines est donc primordiale pour comprendre l'épidémiologie de la maladie. La glossine, agent majeur de transmission des trypanosomoses animales et humaines est une contrainte pour le développement de l'agriculture et de l'élevage au Mali. Les activités de suppression des populations de glossines par l'installation de pièges et d'écrans imprégnés et le traitement épi cutané des animaux domestiques par des insecticides synthétiques importés et très onéreux, sont à la charge des communautés rurales.

La présence des glossines en Afrique explique la faible production agricole à cause de l'affaiblissement des animaux malades, et l'invalidité des personnes touchées.

Plus de 70% de la population malienne a comme activité principale l'agriculture. Au mali, l'élevage occupe la troisième place des produits d'exportation après le coton et l'or. Lutter contre les glossines, c'est augmenter les surfaces cultivables et les productions agricoles, donc réduire la pauvreté rurale et améliorer la sécurité alimentaire au Mali.

Matériel et méthodes

Sites d'étude :

Le cercle de Kadiolo couvre une superficie de 5 375 Km2. Il est situé à l'extrême sud du Mali, limité à l'Est par le Burkina-Faso, à l'Ouest et au Sud par la RCI), au Nord par le cercle de Sikasso et au Nord-Ouest par le cercle de Kolondiéba.

Le cercle de Sikasso couvre une superficie de 19.100 km². Il est limité au Nord par les Cercles de Koutiala et Dioïla, au Sud par le Cercle de Kadiolo, à l'Ouest par les Cercles de Kolondiéba et Bougouni, à l'Est par le Burkina-Faso.

Matériel :

Loupe binoculaire, piège biconique Challier – Laveissière, GPS, microscope, boite de Pétri, lames et lamelles, trousse de dissection, solution physiologique

Méthodes :

La méthode de prospection préconisée pour cette étude fait appel au piège biconique

Challier – Laveissière très attractif pour les trois espèces rencontrées au Mali (Glossina palpalis gambiensis ; Glossina morsitans submorsitans ; Glossina tachinoides).

Les pièges biconiques sont géo référencés et placés tous les jours avant 8 heures à côté de l'eau, de la végétation et au soleil pour les espèces ripicoles et en savane pour l'espèce savanicole. La distance entre deux pièges consécutifs à quelques exceptions est de 200 m environ, celle entre le piège et le sol est environ 10 cm.

Le lendemain matin les pièges sont enlevés.

Nous avons placés dans chaque cage une étiquette qui porte : la date, la localité, le numéro (point de capture). Nous procédons au dénombrement des différentes espèces capturées, et par la méthode classique, nous séparons les glossines par sexe et les résultats sont portés sur l'étiquette.

Au cours de nos captures, d'autres mouches piqueuses qui transmettent la trypanosomose telles les tabanidés et les stomoxes sont identifiées. Les résultats sont aussi portés sur l'étiquette. La dissection des glossines concerne l'appareil piqueur, le tube digestif et les glandes salivaires, organes de développement des parasites pathogènes pour les bovins. Après avoir été individualisés, ces organes sont examinés au microscope pour rechercher la présence de trypanosomes.

Pour les enquêtes entomologiques, à chaque sortie l'équipe constituée comprend :

Un Chercheur spécialiste des T&T, chargé de la supervision de l'équipe sur le terrain.

Un technicien spécialisé en entomologie chargé de la pose et du relevé des pièges, de noter l'espèce et le sexe des glossines capturées, de disséquer quelques glossines capturées.

Résultats et discussion

Dans les villages où moins de 20 glossines ne sont pas en état de disséquer, pas de dissection pour la détermination du taux d'infection des glossines par les trypanosomes. Pour le cercle de Sikasso, on n'a pas pu procéder à la dissection des glossines parce que la fiche d'alimentation électrique de la loupe binoculaire a été endommagée suite à un court circuit électrique.

Distribution et DAP des glossines

200 pièges biconiques Challier – Laveissière ont été posés et géo référencés dans le cercle de Kadiolo, deux espèces de glossines capturées : Glossina palpalis gambiensis et Glossina tachinoïdes.402 glossines sont capturées dont 335 Glossina palpalis gambiensis et 67 *Glossina tachinoïdes*.

167 mâles et 168 femelles de Glossina palpalis gambiensis capturés.

Les captures les plus importantes pour Glossina palpalis gambiensis ont été observées à Gninasso (41 glossines), Zekoun et Galamakourou (39 glossines), Nimbougou (34 glossines). Par contre, à Katele et Dioumantene 0 glossine capturée.

Le plus grand nombre de mâles de Glossina palpalis gambiensis a été capturé à Gninasso (29 glossines) et le plus grand nombre de femelles à Zekoun (25 glossines).

32 mâles et 35 femelles de Glossina tachinoïdes sont capturés dans le cercle de Kadiolo. Le plus grand nombre a été capturé à Zekoun (13 glossines), la capture est nulle dans beaucoup de villages (Katele, Dioumantene, Niérouani, Sieou-Kourani, Nimbougou, Siranikoroba, Daoulasso).

150 pièges biconiques Challier – Laveissière, géo référencés ont été posés dans 15 villages du cercle de Sikasso, deux espèces de glossines sont capturées : Glossina palpalis gambiensis et Glossina tachinoïdes.

384 glossines sont capturés dont 356 Glossina palpalis gambiensis et 28 Glossina tachinoïdes.

182 mâles et 174 femelles de Glossina palpalis gambiensis sont capturés dans le cercle de Sikasso.

Des captures importantes sont observées à Diassadiè (59 glossines), Farako (56 glossines), Finibougou (51 glossines).

Des captures nulles sont enregistrées à Pitagalasso (0 glossine).

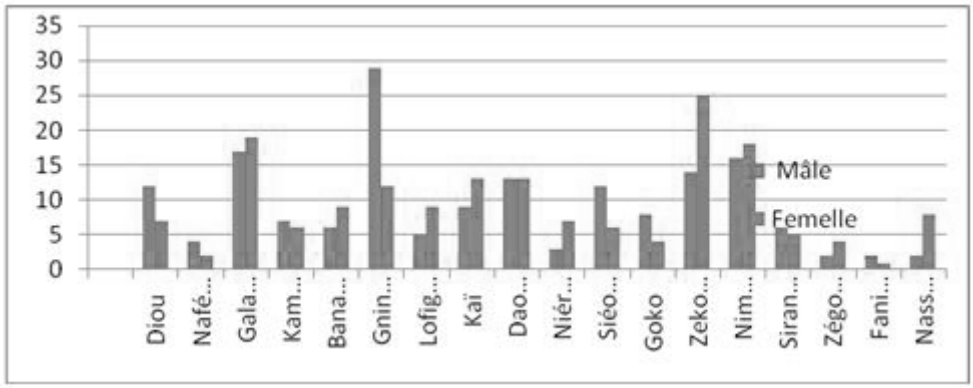


Figure 1 : Nombre de Glossina palpalis gambiensis capturé dans le cercle de Kadiolo.

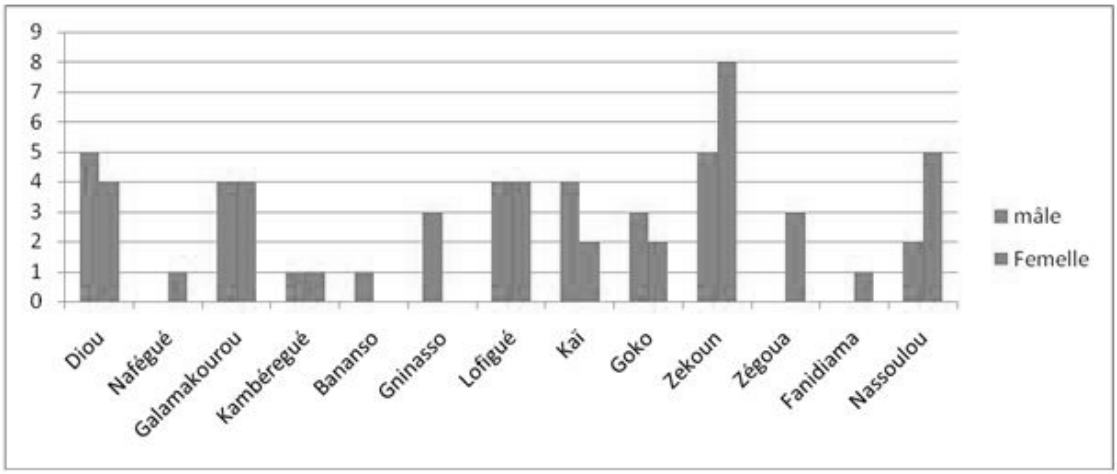


Figure 2 : Nombre de Glossina tachinoides capturé dans le cercle de Kadiolo.

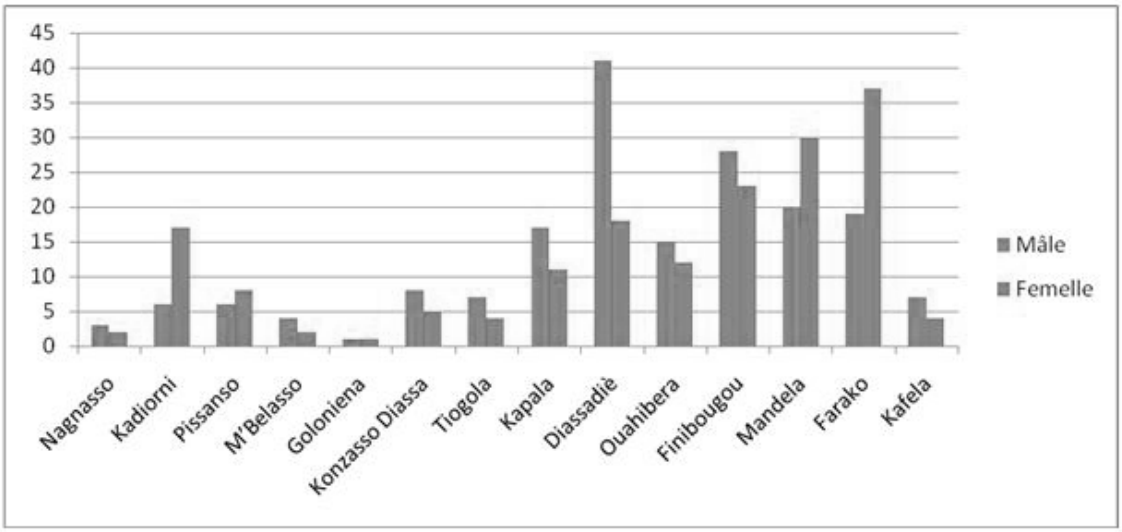


Figure 3 : Nombre de Glossina palpalis gambiensis capturé dans le cercle de Sikasso.

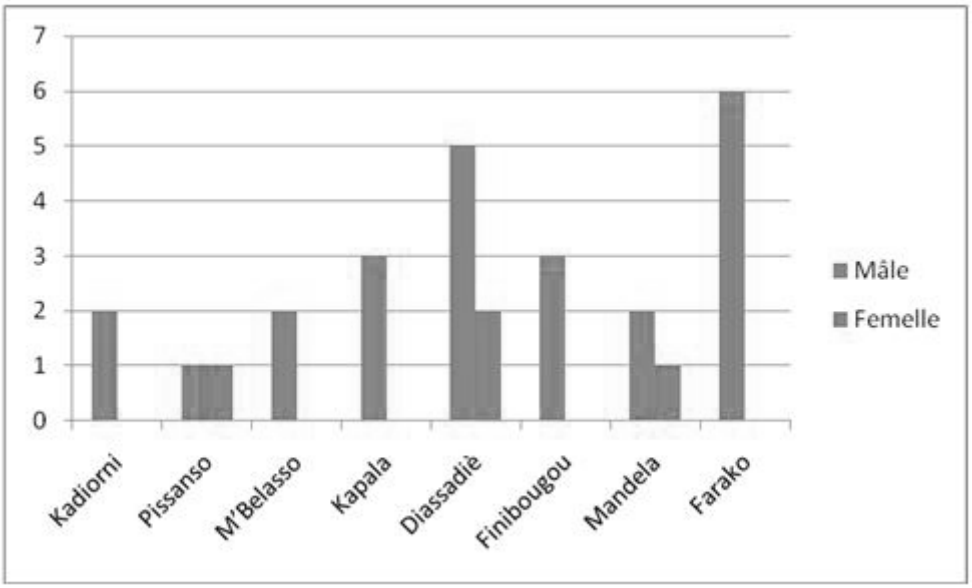


Figure 4 : Nombre de Glossina tachinoïdes capturé dans le cercle de Sikasso.

24 mâles et 4 femelles de Glossina tachinoïdes ont été capturés. Le nombre le plus élevé a été enregistré à Farako (6 glossines) et des captures nulles à Nagnasso, Pitagalasso, Konzasso Diassa, Tiogola, Ouahibera, Kafela.

Au total dans la zone du projet TCP/MLI/3402, 350 pièges biconiques Challier – Laveissière ont été posés dans les galeries et au niveau des points de contact des différents cours d'eau et géo référencés à l'aide de GPS.

La prospection entomologique dans la zone de l'étude a permis la capture de deux espèces de glossines : Glossina palpalis gambiensis et Glossina tachinoïdes. 786 glossines sont capturées dont 405 mâles et 381 femelles. Parmi les glossines capturées, il ya 691 Glossina palpalis gambiensis dont 349 mâles et 342 femelles, 95 Glossina tachinoïdes dont 56 mâles et 39 femelles.

Contrairement à notre étude où deux espèces de glossines (Glossina palpalis gambiensis et Glossina tachinoides) ont été capturé, Djitèye et al. 1997 ont capturé dans la même zone, trois espèces de glossines (Glossina palpalis gambiensis, Glossina tachinoides et Glossina morsitans submorsitans). Ceci peut s'expliquer par l'impact du changement global et surtout la rareté de la faune sauvage dont la présence de l'espèce Glossina morsitans submorsitans dépend largement.

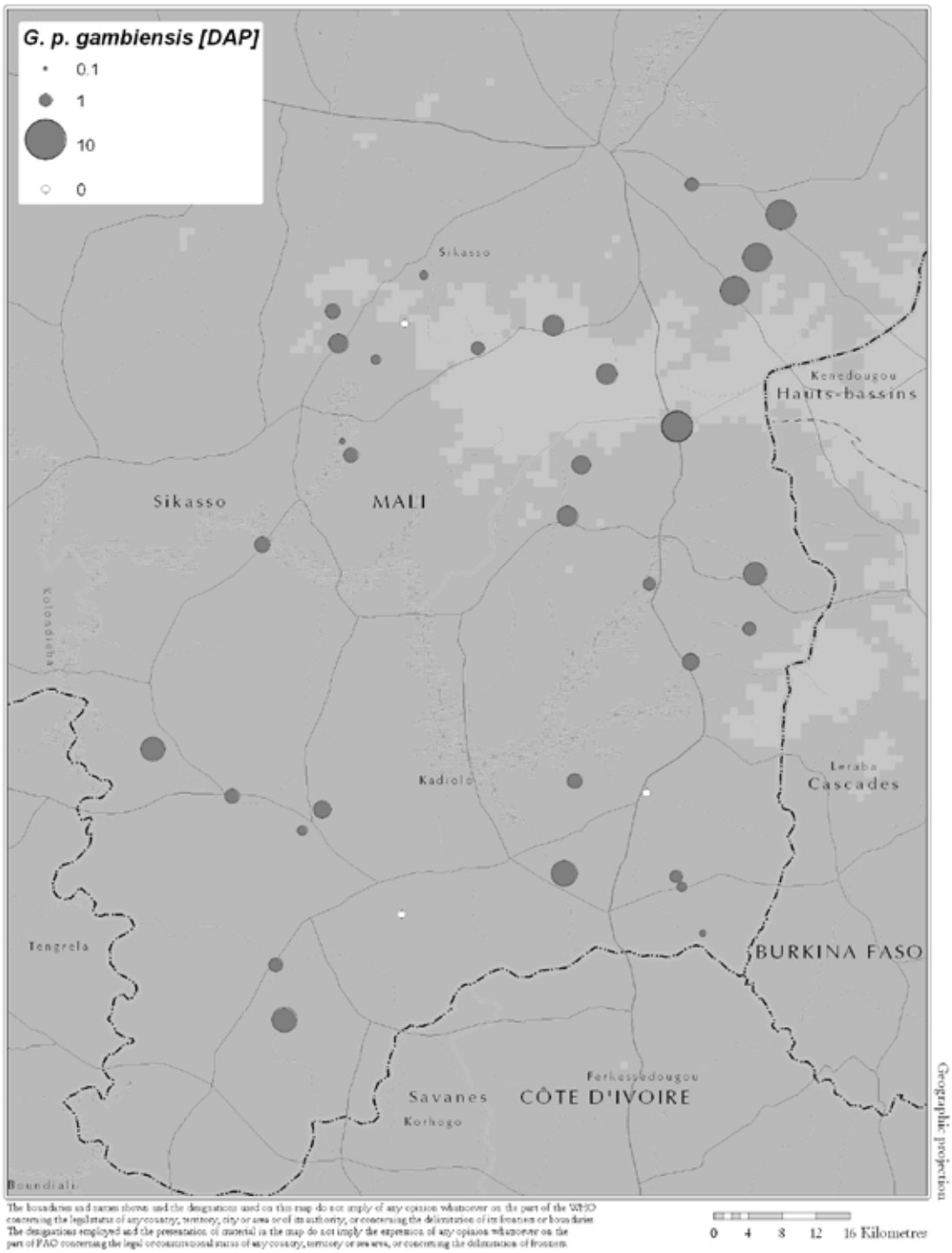
Bass et al. 2010, les résultats des prospections entomologiques dans le bassin du Bani ont permis d'identifier la présence de mouches tsé-tsé et d'autres insectes vecteurs de la trypanosomose animale africaine, comme les tabanidés et les stomoxes. La seule espèce de glossine rencontrée dans la zone d'étude est Glossina palpalis gambiensis.

Dans le cercle de Kadiolo, les plus fortes densités sont observées à Zekoun (5.2) et les plus faibles à Katele (0) et Dioumantene (0).

Dans le cercle de Sikasso, les plus fortes densités sont observées à Diassadiè (6.6) et les plus faibles à Pitagalasso (0).

Les DAP les plus élevées ont été obtenues à Zekoun (5.2), Mandela (5.3), Finibougou (5.4), Farako (6.2), Diassadiè (6.6) tandis que les plus faibles sont enregistrées à Katele (0), Dioumatene (0), Pitagalasso (0), Goloniena (0.2), Fanidiama (0.4), Nagnasso (0.5), Nafegué (0.7), M'Belasso (0.8) et Zégoua (0.9).

L'étude de Bass et al. 2014 confirme nos résultats où sur la base des résultats des prospections entomologiques, 3421 glossines sont capturées : parmi les glossines capturées, 80% (2740/3421) sont de l'espèce Glossina palpalis gambiensis et 20% (681/3421) de l'espèce Glossina tachinoïdes.



Carte 1 : DAP de Glossina palpalis gambiensis dans les cercles de Kadiolo et Sikasso

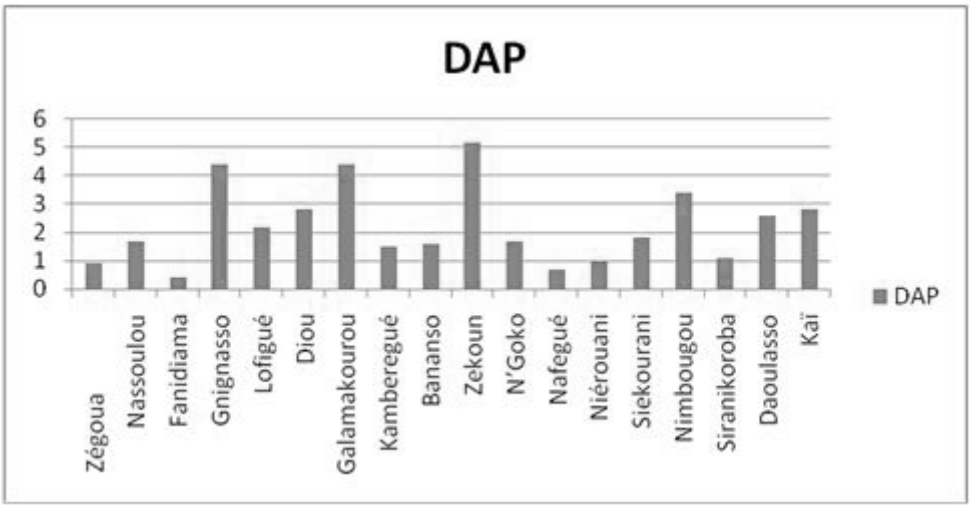


Figure 5 : DAP des glossines dans le cercle de Kadiolo.

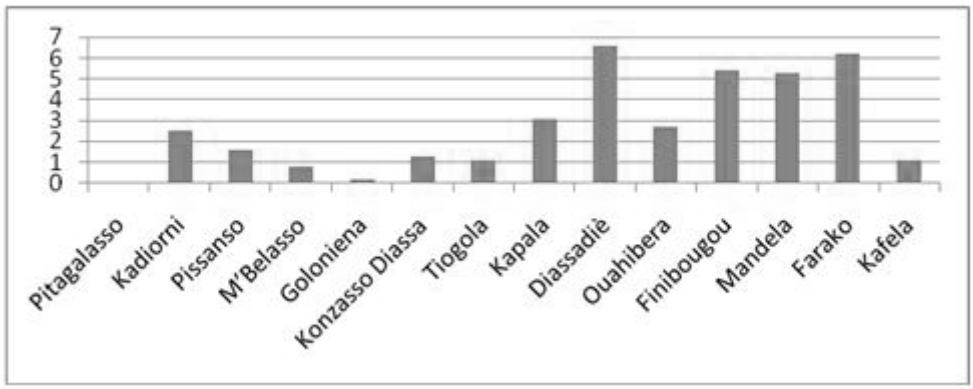


Figure 6 : DAP des glossines dans le cercle de Sikasso.

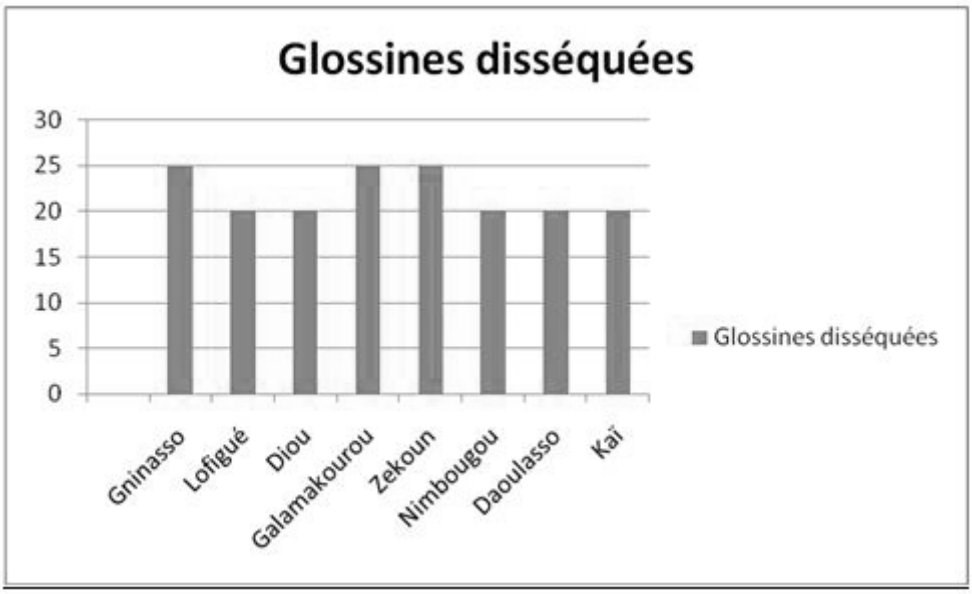
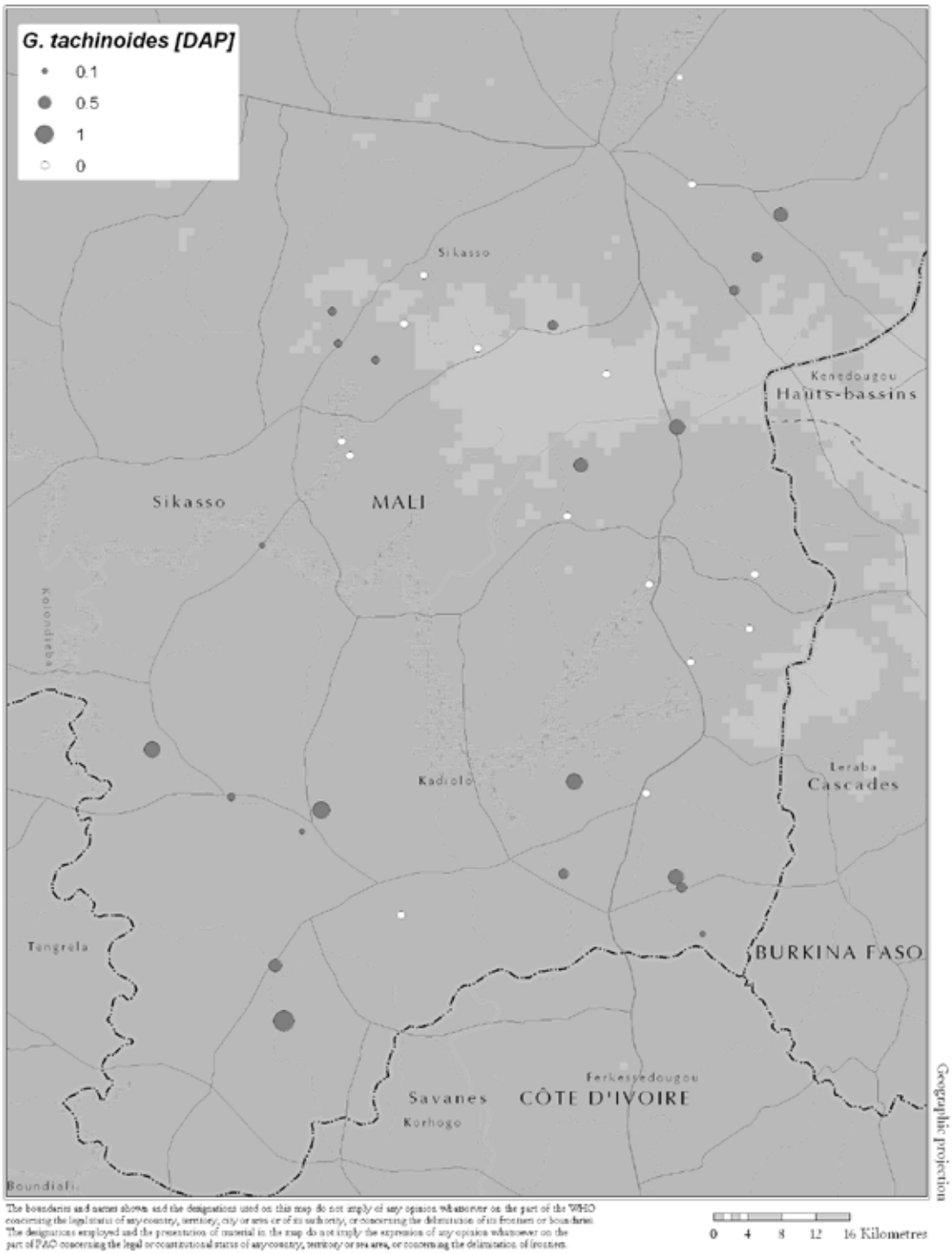


Figure 7 : Nombre de glossines disséquées dans le cercle de Kadiolo.



Carte 2 : DAP de Glossina tachinoïdes dans les cercles de Kadiolo et Sikasso

Taux d'infection des glossines

Dans toutes les localités où le nombre de glossines en état de disséquer est inferieur à 20, pas de dissection.

Dans le cercle de Sikasso, la fiche d'alimentation électrique de la loupe binoculaire a été endommagée suite à un court circuit, donc on n'a pu procéder à la dissection des glossines.

Comme indiqué dans le tableau 40, concernant seulement le cercle de Kadiolo, 175 glossines ont été disséquées et le taux d'infection est de 0%.

Ce faible taux de dissection parce que dans certains villages prospectés, le nombre de glossines capturées ne vaut pas 20 et beaucoup ne pouvaient être exploitées car les insectes sont en mauvais état ou détériorées par les fourmis et autres. La dissection a concerné le tube digestif et le proboscis des glossines. Dans l'étude de Bass et al. 2010, des dissections réalisées durant la prospection entomologique, il est apparu que le taux d'infection des glossines s'élève à 10% des glossines disséquées. Ces résultats contredisent les nôtres où le taux d'infection des glossines s'élève à 0%.

Sex-ratio des glossines capturées

A l'éclosion, le rapport mâles/femelles est voisin de 1 chez la plupart des espèces élevées au laboratoire, sauf pour Glossina morsitans submorsitans où il y a une distorsion génétique en faveur des femelles (68% de femelles dans les insectariums du CIRDES). Celle-ci serait contrôlée par un facteur lié au chromosome X. (CUISANCE. D)

Dans la nature, la sex-ratio est de 1 à l'éclosion, mais du fait de la plus grande longévité des femelles, il est en général en faveur des femelles lors de capture.

Mais il existe des variations en fonction du lieu, de l'heure du jour, de la saison, de l'espèce et du moyen de capture. Dans cette étude le sex-ratio (rapport mâles / femelles) est de 1.12, donc légèrement en faveur des mâles. Nos résultats confirment ceux de Bass et al. 2010 où le sex-ratio est 1.05. La sex-ratio est très variable au cours de l'année. Dans la forêt classée du KOU, le pourcentage des femelles capturées peut descendre au-dessous de 20 p. 100, mais peut aussi atteindre 50 p. 100. Le maximum se trouve durant les mois pluvieux ; un second maximum a lieu vers février et parfois mars. (Cuisance D) Le sex-ratio diffère entre deux points de capture mais en suivant les mêmes variations. Une analyse des

Tableau I : Sex-ratio des glossines capturées.

Villages	Sex-ratio
Zégoua	0.28
Nassoulou	0.30
Fanidiama	1
Katele	0
Gninasso	2.66
Lofigué	0.69
Diou	1.54
Dioumatene	0
Galamakourou	0.91
Kamberegue	1.14
Bananso	0.77
Zekoun	0.57
N'Goko	1.83
Nafegué	1.33
Niérouani	0.42
Sieou-Kourani	2
Nimbougou	0.88
Siranikoroba	1.2
Daoulasso	1
Kaï	0.86
Nagnasso	1.5
Pitagalasso	0
Kadiorni	0.47
Pissanso	0.77
M'Belasso	3
Goloniena	1
Konzasso Diassa	1.6
Tiogola	1.75
Kapala	1.81
Diassadiè	2.3
Ouahibera	1.25
Finibougou	1.34
Mandela	0.70
Farako	0.67
Kafela	1.75
Total	**1.12**

résultats a permis de mettre en évidence que la proportion des sexes varie en fonction d'un complexe de facteurs qui comprend le sexe de la mouche (comportement), le captureur (adresse, comportement), la végétation (visibilité variable au cours des saisons) et la saison. Les femelles sont moins actives que les mâles alors qu'elles sont en plus grand nombre en raison de leur plus grande longévité que celle des mâles.

Tableau 2 : Résultats de capture des autres mouches piqueuses du cercle de Kadiolo.

Localités	**Autres mouches piqueuses**	**Nombre**
Zégoua	*Tabanus gratus*	1
Fanidiama	Pas de capture	0
Katele	Pas de capture	0
Nassoulou	Pas de capture	0
Gninasso	*Atylotus agrestis*	1
Lofigué	Pas de capture	0
Diou	*Atylotus agrestis*	1
Dioumantene	Pas de capture	0
Galamakourou	Pas de capture	0
Kamberegue	*taenicola*	1
Zekoun	*Atylotus agrestis* *Tabanus gratus*	2 1
N'Goko	Pas de capture	0
Nafegué	Pas de capture	0
Niérouani	Pas de capture	0
Siekourani	Pas de capture	0
Nimbougou	Pas de capture	0
Siranikoroba	Pas de capture	0
Daoulasso	Pas de capture	0
Kaï	Pas de capture	0
Bananso	*Tabanus gratus*	17
TOTAL	//////////////////////	**24**

Vecteurs mécaniques capturés

Les tableaux 2 et 3 montrent la présence des vecteurs mécaniques (tabanidés et stomoxes) dans la zone du projet.

Le plus grand nombre a été capturé à Bananso, 17 Tabanus gratus. Dans beaucoup de localités (25 localités / 35), la présence des vecteurs mécanique n'a pas été signalée.

Ces vecteurs mécaniques permettant un relais de la transmission de Trypanosoma vivax, il serait judicieux qu'une étude sur ces vecteurs soit menée afin de vérifier cette hypothèse.

Dans l'étude Bass et al. 2010, le nombre de vecteurs mécaniques capturé est 8 fois supérieur que dans notre étude. Cet écart important peut s'expliquer par les meilleures conditions écologiques pour les vecteurs mécaniques dans le bassin du Bani.

Tableau 3 : Résultats de capture des autres mouches piqueuses du cercle de Sikasso.

Localités	Autres mouches piqueuses	Nombre
Nagnasso	*Pas de capture*	0
Pitagalasso	Pas de capture	0
Kadiorni	Pas de capture	0
Pissanso	*Tabanus gratus* *Tabanus taneola*	1 1
M'Belasso	Pas de capture	0
Goloniena	*Tabanus gratus* *Atylotus agrestis*	4 1
Konzasso Diassa	*Tabanus gratus*	4
Tiogola	Tabanus gratus	3
Kapala	Pas de capture	0
Diassadiè	*Pas de capture*	0
Ouahibera	*Pas de capture*	0
Finibougou	Pas de capture	0
Mandela	Pas de capture	0
Farako	Pas de capture	0
Kafela	Pas de capture	0
TOTAL	//////////////////////	**14**

Conclusion

Les prospections entomologiques dans la zone du projet TCP/MLI/3402 ont permis d'identifier deux espèces de glossines : Glossina palpalis gambiensis et Glossina tachinoïdes.

L'espèce Glossina morsitans submorsitans habituellement existante dans la zone du projet n'a pas été capturée à cause de l'impact du changement global.

En plus des glossines, d'autres insectes vecteurs de la Trypanosomose Animale Africaine aussi ont été identifiés : tabanidés et stomoxes. Les DAP plus élevées ont été obtenues à Diassadiè (6.6) et Farako (6.2) et les nulles à Katele (0) et Pitagalasso (0). La sex-ratio est légèrement en faveur des mâles. La dissection des glossines, qui a concerné le cercle de Kadiolo seulement a donné un taux d'infection de 0%. IL ressort clairement de l'analyse que la densité des glossines diminue davantage dans la zone du projet. Ce qui peut s'expliquer par les effets de sécheresse, du changement climatique et aussi l'anthropisation. En effet, ces résultats renforcent l'hypothèse que, dans certains cas, l'effet combiné d'un climat en général plus aride et d'une pression importante de l'agriculture sur le biotope naturel des glossines contribue à empêcher la réinstallation de ces populations après les opérations de contrôle. Dans d'autres cas cependant, les zones libérées ont été envahies à nouveau. Il serait souhaitable d'étudier ce phénomène avant la lutte, notamment en utilisant les outils génétiques qui permettent de mesurer les probabilités de ré invasion avec les conséquences importantes sur la méthode générale à adopter pour éradiquer progressivement ce fléau avec des risques de ré invasion minimaux. Les vecteurs mécaniques et principalement les tabanidés et les stomoxes ont été capturés aussi dans la zone du projet. Ces vecteurs mécaniques permettant un relais de la transmission de Trypanosoma vivax, il serait judicieux qu'une étude sur ces vecteurs soit menée afin de vérifier cette hypothèse.

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Remerciements particuliers aux secteurs vétérinaires de Kadiolo et Sikasso, aux mandataires sanitaires des localités concernées.

Sincères remerciements aux notabilités, aux guides.

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PREVALENCE DES TRYPANOSOMOSES BOVINES DANS LES CERCLES DE KADIOLO ET SIKASSO AU MALI AVANT LE DEMARRAGE DE LA CAMPAGNE DE LUTTE.

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Resume

L'objectif du projet TCP/MLI/3402 FAO est d'initier une campagne de protection efficace et durable des bovins contre la Trypanosomose Animale Africaine (TAA) et la résistance aux trypanocides par le contrôle de la mouche tsé-tsé combiné aux traitements stratégiques des animaux. Préalablement à la mise en œuvre des activités de lutte, une étude de base protozoologique a été confiée au Laboratoire Central Vétérinaire (LCV). 35 villages ont été enquêtés dans les cercles de Kadiolo (20 villages dans 9 communes) et Sikasso (15 villages dans 4 communes). Pour l'étude protozoologique 1208 prélèvements sanguins ont été effectués chez des bovins, 15 trypanosomes ont été identifiés dont 9 à Kadiolo et 6 à Sikasso. Tous les trypanosomes observés sont de l'espèce Trypanosoma vivax. La prévalence de la trypanosomose est de 1.12% dans le cercle de Kadiolo et 1.2% dans le cercle de Sikasso. Pour l'ensemble de la zone d'étude, la prévalence est de 1.16%. La moyenne du taux d'hématocrite est de 28.9 dans le cercle de Kadiolo et 33.4 dans le cercle de Sikasso. La moyenne du taux d'hématocrite pour l'ensemble de la zone est de 31.15

Mots clés : Prévalence, Trypanosomoses, Bovines, Lutte, Mali.

PREVALENCE OF THE BOVINE TRYPANOSOMOSES IN THE CIRCLES OF KADIOLO AND SIKASSO IN MALI BEFORE THE STARTING UP OF THE CAMPAIGN OF FIGHT.

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Summary

The objective of the project is to introduce a campaign of effective and long-lasting protection of the cattle against Trypanosomose Animale Africaine (TAA) and resistance to trypanocides by the control of the tsetse fly combined in the strategic treatments of animals. Before the implementation of the activities of fight, a protozoologique basic study was confided to the Veterinary Central Laboratory (LCV). 35 villages were investigated in the circles of Kadiolo (20 villages in 9 municipalities) and Sikasso (15 villages in 4 municipalities). For the protozoologique study 1208 sampling of blood were made at cattle, trypanosomes 15 were identified among which 9 to Kadiolo and 6 to Sikasso. All the observed trypanosomes is the sort Trypanosoma vivax. Prevalence of the trypanosomose is 1.12 % in the circle of Kadiolo and 1.2 % in the circle of Sikasso. For the whole zone of study, prevalence is 1.16 %. The average of the rate of hématocrite is 28.9 in the circle of Kadiolo and 33.4 in the circle of Sikasso. The average of the rate of hématocrite for the whole zone is 31.15

Keywords: Prevalence, Trypanosomoses, Bovine, Fights, Mali.

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Introduction

La trypanosomose animale est une maladie parasitaire à transmission vectorielle qui sévit à l'état endémique dans certaines régions d'Afrique noire où l'affection est strictement localisée (grossièrement entre le 12ème degré de latitude nord et le 15ème degré de latitude sud) (Itard, 1986). C'est une maladie infectieuse inoculable et non contagieuse, causée par des protozoaires flagellés qui se multiplient dans le plasma sanguin, la lymphe et divers organes des mammifères. 38 pays d'Afrique dont le Mali sont affectés par la maladie. Toure et Mortelmans (1991) estiment à 7 millions de km² l'aire de répartition des glossines dont 42% se situent en Afrique de l'Ouest. Au Mali comme dans beaucoup de pays au sud du Sahara, la trypanosomose animale reste un obstacle majeur au développement de l'élevage des régions rurales infestées (Hursey et Slingenbergh, 1995 ; Swallow, 1998 ; shaw, 2003). Elles sont à l'origine de pertes humaines, du bétail et de l'abandon par les paysans des terres souvent fertiles parce qu'ils fuient les zones où sévit la maladie (Hursey et Slingenbergh, 1995 ; Swallow, 1998 ; Shaw, 2003). L'Organisation mondiale de la santé (OMS, 1990) estime que 60 millions de personnes, 50 millions de bovins et 70 millions de petits ruminants y sont exposés au risque de la trypanosomose. Les pertes annuelles de viande sont estimées à près de 5 milliards de dollars américains (Mortelmans, 1986). La productivité du bétail et l'utilisation des animaux pour l'agriculture (engrais, traction) s'en trouvent donc affectées.

Au Mali, les espèces de trypanosomes pathogènes rencontrées chez le bétail dans les zones infestées par les glossines sont : T. congolense, T. vivax et T. brucei brucei. (Diall, 1993). Notons qu'à coté de la transmission cyclique, il existe la transmission mécanique assurée par les tabanidés et les stomoxes.

Malgré les progrès réalisés en épidémiologie, et les nouvelles méthodes de lutte contre les trypanosomoses transmises par les mouches tsé-tsé, ces parasites, continuent de représenter un problème sanitaire et économique majeur en Afrique au sud du Sahara (Hursey et Slingenbergh, 1995 ; Swallow, 1998 ; Shaw, 2003).

Il est alors impératif de lutter contre cette maladie.

Materiel et Methodes

Pour réaliser cette étude, des activités suivantes ont été menées conformément aux termes de références :
Etablissement d'un plan de travail qui va permettre de géo référencer les sites dans un système de grilles.

Détermination de la taille des échantillons.

Utilisation de la technique de Buffy Coat pour le diagnostic parasitologique et les frottis pour l'identification des espèces.

Détermination des PCV.

Pour la mise en œuvre des études parasitologiques initiales au profit du projet, la stratégie suivante sera adoptée :

Phase préparatoire :

Analyse de la documentation disponible

Préparation technique et logistique des enquêtes

Phase d'enquête :
Elaboration d'un plan d'enquête
Préparation du travail de terrain
Exécution du travail de terrain

Phase de traitement et analyse des données
Phase d'élaboration du rapport
Phase préparatoire

Etude documentaire :

Les données bibliographiques sont collectées sur les travaux déjà réalisés en matière de tsé-tsé et de trypanosomose au Mali et singulièrement dans la zone du projet.

Préparation technique et logistique des enquêtes :

Pour la préparation et l'organisation des travaux de terrain nous avons fait la mise au point de tous les moyens matériels et logistiques ainsi que des consommables nécessaires aux activités sur le terrain. Nous avons établis des calendriers de sortie et

présentés à la coordination du projet notre protocole d'enquête.

Des informations relatives aux effectifs animaux présents, au personnel sanitaire vétérinaire sont collectées.

Phase d'enquête

Pour effectuer les travaux de terrain, l'équipe constituée comprend :

Un Chercheur spécialiste des T&T, chef de mission chargé de la supervision de l'équipe et des examens parasitologiques sur le terrain.

Deux techniciens spécialisés en parasitologie chargés des examens parasitologiques sur le terrain, des prélèvements de sang.

Les équipes ont effectué des prélèvements dans 35 villages dans la zone couverte par le projet.

Les travaux de terrain portent sur :

Les différentes zones qui sont divisées en blocs correspondant à des regroupements de communes. Des villages sont choisis dans ces blocs en fonction de :

- la répartition géographique des localités à sonder
- la position par rapport aux cours d'eau ;
- la présence effective de bétail ;
- le type de conduite du troupeau (transhumant, sédentaire, laitier, animaux de trait).

Dans chaque village, au moins 40 têtes de bovins sont choisies au hasard dans 2 à 4 troupeaux géo référencés au GPS.

Les localités retenues sont distantes d'au moins 5 km les unes des autres. Dans chaque troupeau, 25 à 50% des animaux sont choisis au hasard et prélevés.

Un questionnaire est soumis aux producteurs. Il est destiné à recueillir les informations suivantes :

- La durée de séjour de l'animal au sein du troupeau
- La date du dernier traitement trypanocide et le produit utilisé.

La collecte d'informations sur les animaux sélectionnés : les paramètres tels que le sexe, la race et l'âge des animaux sélectionnés sont enregistrés suivant une fiche standard.

La recherche de trypanosomes : le sang prélevé sur anticoagulant est immédiatement analysé en utilisant la technique du Buffy Coat : centrifugation, lecture de l'hématocrite et examen en microscopie en fond noir. Ceci permet d'identifier les espèces de trypanosomes présentes, de déterminer leur fréquence relative et de calculer la prévalence parasitologique par site.

La préparation de confettis sur papier filtre et d'échantillons de Buffy coat pour les besoins ultérieurs de diagnose et de confirmation des cas suspects.

Phase de traitement et analyse des données :

Les données collectées sur le terrain et au laboratoire sont traitées et analysées par les méthodes appropriées pour la production d'un rapport détaillé.

Les données sont analysées à l'aide des logiciels EXCEL et GENSTAT.

Resultats

Au total 1208 prélèvements sanguins ont été effectués chez des bovins dans 35 villages dont 19 dans le cercle de Kadiolo et 15 dans le cercle de Sikasso.

15 trypanosomes ont été identifiés dont 9 à Kadiolo et 6 à Sikasso. Tous les trypanosomes observés sont de l'espèce Trypanosoma vivax.

La prévalence de la trypanosomose est de 1.12% dans le cercle de Kadiolo et 1.2% dans le cercle de Sikasso. Pour l'ensemble de la zone d'étude, la prévalence est de 1.16%.

La moyenne du taux d'hématocrite est de 28.9 dans le cercle de Kadiolo et 33.4 dans le cercle de Sikasso. La moyenne du taux d'hématocrite pour l'ensemble de la zone est de 31.15

Parmi les bovins concernés par les prélèvements, il y a 1143 N'dama (95%) et 65 zébus (5%).

Le diagnostic parasitologique a montré que Trypanosoma vivax est la seule espèce de trypanosome pathogène observée dans la zone du projet.

Aucun cas d'infection mixte ou par Trypanosoma brucei brucei n'a été diagnostiqué.

Tableau 1: Résultats des enquêtes protozoologiques dans le cercle de Kadiolo.

Localités	Latitude	Longitude	Nbre Prel.	Tbb	T.c	T.v	Total positifs	Moy. PVC	% Prév.
Zégoua	1052270	0558078	40	0	0	0	0	28	0
Nassoulou	1054232	0559783	40	0	0	0	0	30	0
Fanidiama	1049312	0556419	40	0	0	0	0	27.3	0
Katele	1063138	0561703	40	0	0	0	0	30.2	0
Gnignasso	1054052	0570469	40	0	0	0	0	29.1	0
Lofigué	1063985	0569880	40	0	0	0	0	26.7	0
Diou	1058770	0596945	40	0	0	0	0	33.9	0
Dioumatene	1053230	0590649	40	0	0	0	0	30.3	0
Galamakourou	1067339	0615000	40	0	0	0	0	31	0
Kamberegué	1062692	0606383	40	0	0	0	0	30.4	0
Zekoun	1039562	0599187	40	0	0	4	4	26.4	10
N'Goko	1041361	0603455	40	0	0	0	0	28.8	0
Nafegué	1058820	0598953	40	0	0	0	0	28.7	0
Niérouani	1083274	0563250	40	0	0	2	2	32	5
Siekourani	1073821	0560785	40	0	0	0	0	27.5	0
Nimbougou	1085612	0550526	40	0	0	3	3	34.2	7.5
Siranikoroba	1078178	0549377	40	0	0	0	0	34.9	0
Daoulasso	1091947	0570365	40	0	0	0	0	33.6	0
Kaï	1096972	0568750	40	0	0	0	0	27	0
Banansso	1087927	0603161	22	0	0	0	0	37.5	0

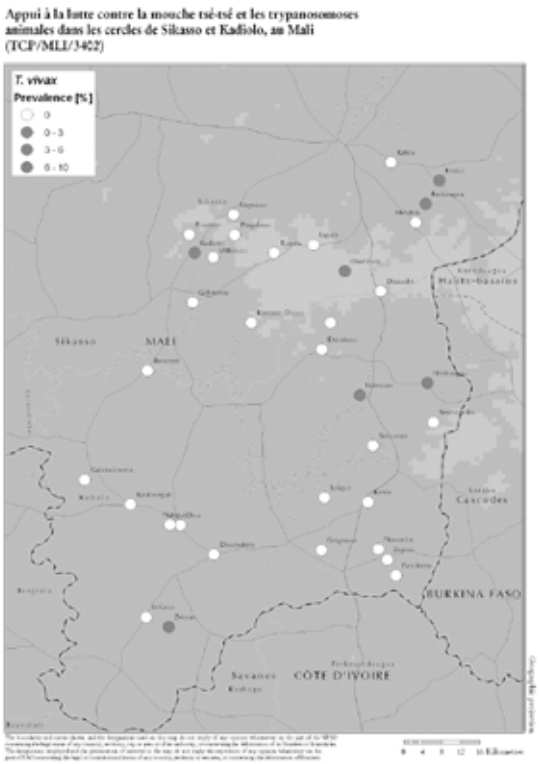
Tbb :Trypanosoma brucei brucei ; T.c :Trypanosoma congolense ; T.v :Trypanosoma vivax ; Nbre Prel. : Nombre de Prélèvements; Moy. PCV : Moyenne PCV ; % Prév. : % Prévalence

Tableau 2 : Résultats des enquêtes protozoologiques dans le cercle de Sikasso.

Localités	Latitude	Longitude	Nbre Prel.	Tbb	T.c	T.v	Total positifs	Moy. PVC	% Prév.
Nagnasso	1117371	0586982	30	0	0	0	0	33.9	0
Pitagalasso	1113587	0586806	30	0	0	0	0	34.1	0
Kadiorni	1110190	0594337	30	0	0	1	1	32.9	3
Pissanso	1113604	0595335	30	0	0	0	0	33.6	0
M'Belasso	1109374	0590827	30	0	0	0	0	32	0
Goloniena	1100827	0594688	30	0	0	0	0	36.8	0
Konzasso Diassa	1096967	0583631	30	0	0	0	0	33.9	0
Tiogola	1110204	0579341	30	0	0	0	0	32.7	0
kapala	1111692	0571955	30	0	0	0	0	32.9	0
Diassadiè	1102923	0559338	30	0	0	0	0	30.4	0
Ouahibera	1106717	0566069	30	0	0	2	2	34.9	6
Finibougou	1119382	0550851	30	0	0	2	2	33.1	6
Mandela	1115881	0552726	16	0	0	0	0	32.4	0
Farako	1023779	0548258	30	0	0	1	1	30.5	3

Localités	Latitude	Longitude	Nbre Prel.	Tbb	T.c	T.v	Total positifs	Moy. PVC	% Prév.
Kafela	1127258	0557358	20	0	0	0	0	37.8	0
Total	//////////	//////////	426	0	0	6	6	33.4	1.2
Siranikoroba	1078178	0549377	40	0	0	0	0	34.9	0
Daoulasso	1091947	0570365	40	0	0	0	0	33.6	0
Kaï	1096972	0568750	40	0	0	0	0	27	0
Banansso	1087927	0603161	22	0	0	0	0	37.5	0

Tbb :Trypanosoma brucei brucei; T.c :Trypanosoma congolense; T.v :Trypanosoma vivax; Nbre Prel. : Nombre de Prélèvements; Moy. PCV : Moyenne PCV; % Prév. : % Prévalence



Carte 1 : Prévalence de la trypanosomose animale dans les cercles de Kadiolo et Sikasso.

Les présents résultats ont révélé l'ascendance de Trypanosoma vivax sur Trypanosoma congolense, ce qui est dû à la raréfaction de Glossina morsitans submorsitans qui est un vecteur de Trypanosoma congolense.

Les vecteurs mécaniques permettent un relais de la transmission de Trypanosoma vivax. Il serait judicieux qu'une étude sur ces vecteurs mécaniques soit menée afin de vérifier cette hypothèse.

La faible prévalence obtenue pouvait aussi s'expliquer par la faible sensibilité de la méthode utilisée. En effet, la méthode du Buffy coat n'est pas très sensible pour mesurer la prévalence des infections dans les situations d'enzootie.

De plus la sélection extemporanée des animaux exposait au risque d'utiliser des animaux traités avec des chimio préventifs, réduisant les prévalences observées.

Le recul et la baisse de la densité des glossines ont réduit le contact entre ces dernières et les hôtes domestiques, en particulier les bovins, ce qui s'est traduit par les faibles prévalences obtenues.

En plus des trypanosomes, des microfilaires aussi ont été identifiés.

Indicateurs pour le Suivi

Pour le suivi-évaluation de l'impact de la campagne, des animaux sentinelles choisis seront prélevés pour la détection des trypanosomes par la méthode de Buffy Coat.

Les prélèvements se feront selon le rythme suivant :

- Une première visite avant le démarrage de la campagne.
- Une visite de saison sèche et une visite d'hivernage par an.
- Une visite à la fin de la campagne.

La comparaison des prévalences permet d'évaluer l'impact de la campagne.

L'impact de la campagne sera mesuré par une étude d'incidence bimensuelle des trypanosomoses animales chez le bétail de la zone d'intervention du projet. Pour cela, dans chaque village sélectionné, deux troupeaux seront choisis :

Le premier troupeau « sentinelle » appartenant à 3 familles d'éleveurs sédentaires possédant chacune environ 15 à 50 bovins en moyenne, qui pratiquent l'élevage traditionnel. Les animaux suivis seront échantillonnés par tirage aléatoire. Au total 35animaux seront retenus et marqués d'une boucle auriculaire.

Le second troupeau « sentinelle » sera composé d'animaux de trait appartenant à plusieurs agriculteurs. Ceux –ci possèdent entre 2 et 8 animaux, essentiellement destinés aux travaux champêtres. Les animaux de 2 à 3 agriculteurs seront retenus, ce qui correspond à 30 têtes par village.

Tous les animaux identifiés par une boucle auriculaire seront traités puis soumis à un suivi bimensuel du taux d'infestation trypanosomienne, pendant 12 mois. Les animaux dont les hématocrites sont inférieurs à 25% seront considérés comme douteux par conséquent, ils seront traités à la même dose que les animaux positifs.

Parallèlement, des sondages entomologiques (densité et taux d'infection des glossines) ciblés sur les points d'abreuvement de ces troupeaux seront réalisés lors du suivi bimensuel des troupeaux sentinelles pour évaluer l'évolution des populations des glossines. Pour ce faire, 10 pièges seront posés par site et placés à 100 m l'un de l'autre et relevés 24 heures après la pose.

Les mouches capturées seront comptabilisées. Par site, des glossines seront sélectionnées au hasard et disséquées pour la recherche d'infection de trypanosomes. Les organes (proboscis, intestin moyen et glandes salivaires) seront récoltés séparément et un échantillon aléatoire de 20% des organes sera analysé par la PCR.

Conclusion

Les enquêtes protozoologiques ont permis d'identifier une seule espèce de trypanosome : Trypanosoma vivax. Des microfilaires aussi ont été observées.

La moyenne du taux d'hématocrite la plus élevée a été enregistrée à Kafela et la plus faible à Zekoun. La prévalence de la trypanosomose la plus élevée a été enregistrée à Zekoun.

Le diagnostic parasitologique dans la zone du projet a montré que Trypanosoma vivax est la seule espèce de trypanosome pathogène observée avec 100% des infections trypanosomiennes.

La prévalence nulle de Trypanosoma congolense peut s'expliquer par la raréfaction dans la zone du projet de Glossina morsitans submorsitans qui est un vecteur de Trypanosoma congolense.

Aucun cas d'infection mixte ou par Trypanosoma brucei n'a été diagnostiqué.

Les hématocrites moyens calculés ont été supérieurs à 25% aussi bien chez les N'damas que chez les Zébus. 2% des individus (25/1208 bovins) présentent des valeurs inférieures à 25%, seuil considéré comme révélateur d'un état pathologique.

Remerciements

L'équipe de recherche remercie la direction du Laboratoire Central Vétérinaire.

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ENQUÊTE DE BASE PARASITOLOGIQUE DE LA TRYPANOSOMOSE ANIMALE DANS TROIS ZONES AGROPASTORALES DU BURKINA FASO

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Résumé

Les trypanosomoses animales constituent une contrainte majeure au développement de l'élevage dans les zones infestées de tsé-tsé qui en sont les hôtes intermédiaires. Dans une perspective de lutte contre la trypanosomose dans les zones agropastorales (ZAP) de Sidéradougou, Samorogouan et Barani, la "Panafrican Tse-tse and Trypanosomiasis Eradication Campaign" (PATTEC) au Burkina Faso, y a commandité une enquête parasitologique de base.

Pour les trois ZAP, un échantillon de 2396 animaux dont 1379 bovins, 474 ovins, 339 caprins et 191 asins, a été sélectionné au hasard dans 29 élevages. La détection des trypanosomes a été effectuée par la technique de buffy-coat à partir du sang collecté à la veine jugulaire.

Les infections étaient dues à Trypanosoma vivax ou T. congolense. Les prévalences globales étaient de 8,26%, 5,76%, 2,32% et 2,95% chez les bovins, les asins, les ovins et les caprins respectivement. Le mode d'élevage des bovins et l'utilisation des asins pour la force de traction constituent des facteurs de risque pour les infections trypanosomiennes avec des odds ratios de 2,99 IC 95% (1,98-4,55) et 16,94 IC 95% (2,06-139,1) respectivement. L'hématocrite moyen des animaux infectés était significativement inférieur à celui des animaux sains ($p<0,001$).

Cette étude a montré qu'il y a une variation entre les taux d'infection selon les ZAP et les espèces animales. La PATTEC doit adapter les mesures en prenant en compte la spécificité de chaque localité.

Mots-clés: Burkina Faso, PATTEC, Trypanosomoses, Zones Agropastorales,

BASELINE PARASILOGICAL SURVEY OF ANIMAL TRYPANOSOMOSIS IN THREE AGROPASTORAL ZONES IN BURKINA FASO

Abstract

Animal trypanosomosis constitute a major constraint to the development of livestock breeding in the zone infested by tsetse flies which are the intermediate hosts. In the perspective of trypanosomosis control in the agropastoral zones (APZ) of Sideradougou, Samorogouan and Barani, the Panafrican Tse-tse and Trypanosomiasis Eradication Campaign (PATTEC) Burkina Faso has financed a baseline parasitological survey in these areas.

For the three APZ, a sample of 2396 animals inclunding 1379 cattle, 474 sheep, 339 goats et 191 donkeys, was randomly selected in 29 herds. The detection of trypanosomes was carried out by the buffy-coat technique using blood sample collected by jugular vein puncture.

The infections were caused by Trypanosoma vivax or T. Congolense. The overall prevalences were 8.26%, 5.76%, 2.32% and 2.95% in cattle, donkeys, sheep and goats respectively. The system of cattle management and the utilisation of donkeys in draught power constitute risk factors for trypanosomosis infections with odds ratios of 2.99 IC 95% (1.98-4.55) and 16.94 IC 95% (2.06-139.1) respectively. The average PCV of infected animal were significantly less than those of healthy subjects ($p<0.001$).

This study showed that there was a variation between the rates of infection according to the APZ and the animal species. PATTEC must adapt the control measures by taking in account the specificity of each area.

Keywords: Burkina Faso, PATTEC, Trypanosomosis, Agropastoral Zones

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Introduction

L'économie du Burkina Faso repose essentiellement sur le secteur rural. La contribution de l'élevage au PIB est estimée entre 10 et 14% (MEDEV, 2004; MRA, 2000). Le cheptel se compose de près de 8 millions de bovins, 18 millions de petits ruminants, 1 million d'équidés et 2 millions de porcins (MRA, 2006). Cependant, l'élevage est majoritairement pratiqué selon un mode extensif basé sur l'exploitation du pâturage naturel avec des soins vétérinaires très limités. Ce mode d'élevage expose les animaux à de nombreuses maladies animales dont des foyers sont régulièrement reportés par les services vétérinaires (MRA, 2006). La trypanosomose animale reste la première entrave au développement de l'élevage au Burkina Faso à l'instar des autres pays subsahariens infectés par les tsé-tsé (Shaw, 2003 ; Swallow, 1998). Cette maladie est enzootique dans presque le tiers du pays. Selon Kamuanga et al. (2001) plus de 63% du cheptel bovin vivent en zones à risque trypanosomien.

Les pertes attribuables à la trypanosomose sont énormes mais souvent difficiles à évaluer avec exactitude. Toutefois, les pertes directes liées à l'achat de trypanocides préventifs et curatifs ont été évaluées à 3,9 millions US$ par an (Sow *et al.,* 2010). Le traitement trypanocide demeure pratiquement la seule voie de lutte contre la trypanosomose utilisée par les éleveurs pour le maintien des animaux sensibles à la maladie en zone infestée de glossines. Maints efforts ont été déployés par l'Etat Burkinabè, appuyé par des organismes internationaux pour réduire l'impact de la trypanosomose animale (Cuisance *et al.,* 1985a; Brandl, 1985).

A ce titre, plusieurs projets de lutte contre la mouche tsé-tsé, vecteur des trypanosomoses, ont été exécutés au Burkina. La lutte contre les glossines a été implémentée dans les zones agropastorales de Sidéradougou, de Samorogouan et Yalé (Cuisance *et al.,* 1985a ; Bauer *et al.,* 1999). Les campagnes avaient permis de réduire de façon significative les glossines et l'incidence des trypanosomoses animales dans les zones concernées. Les acquis de ces différentes campagnes n'ont pas pu être préservés parce que les communautés bénéficiaires et les services publics n'ont pas poursuivi la lutte antivectorielle. De plus, ces campagnes de lutte étaient localisées à des zones géographiques isolées, ce qui exigeait la continuation de l'effort de lutte et le maintien ad vitam aeternam des barrières pour prévenir la ré-infestation des zones assainies. A l'exception des campagnes de lutte précédentes, la Panafrican Tse-tse and Trypanosomiasis Eradication Campaign (PATTEC), a pour ambition l'éradication des tsé-tsé et de la trypanosomose (T&T) de l'aire d'infestation à l'échelle du continent. Le succès de la campagne d'éradication de PATTEC conférera une victoire définitive sur les T&T en Afrique.

La PATTEC est le plus récent programme de lutte qui vise à éradiquer les tsé-tsé et la trypanosomose, dans les 38 pays africains endémiques de trypanosomoses animale et humaine. Comme programme d'éradication, la PATTEC devrait être exécutée concomitamment par tous les pays membres afin d'aboutir à un bon résultat.

La stratégie d'implantation de la PATTEC au Burkina, consiste à créer des zones exemptes de mouches tsé-tsé et des trypanosomoses, en commençant par les zones identifiées prioritaires puis en étendant les zones d'intervention à travers une action soutenue dans les phases subséquentes. Les zones agropastorales sont considérées comme zones prioritaires de la PATTEC. C'est ainsi qu'une enquête de base parasitologique anté campagne de lutte a été commanditée. L'objectif de la présente enquête était de déterminer la prévalence des infections par les trypanosomes, l'état de santé des animaux, en se servant de la mesure de l'hématocrite et l'évaluation des facteurs associés aux infections et à la densité des tsé-tsé dans 3 zones agropastorales (ZAP) que sont Sidéradougou, Samorogouan et Barani.

Matériel et Méthodes

Sites de l'étude

Les zones agropastorales de Sidéradougou, Samorogouan et Barani sont situées à l'ouest et au sud du Burkina Faso

(Figure 1). Ces zones agropastorales ont été créées suites aux grandes sècheresses des années 1970 et 1980 en vue de réduire la pauvreté par l'augmentation des productions agricoles et animales. L'aménagement des zones pastorales avait aussi pour but la sédentarisation des éleveurs transhumants, l'intensification de l'élevage traditionnel et la gestion rationnelle des pâturages naturels.

La zone agropastorale de Sidéradougou est située au sud de Bobo-Dioulasso, au pied de la falaise de Banfora qui constitue sa limite Nord-ouest. Elle est localisée entre 10°40'-11°10' nord et 3°55'-4°30' ouest. La zone couvre une superficie de 3500 km2 et correspond au bassin de la rivière Koba. Elle est située dans la zone soudanienne avec une pluviométrie annuelle moyenne de 1 200 mm.

La zone agropastorale de Samorogouan, encore appelée Centre d'Encadrement des Zones d'Intensification de l'Elevage Traditionnel (CEZIET), couvre une superficie de 1400 km². Elle est située entre 11°13'-11°34' nord et 4°40'-5°11' ouest, dans la zone cotonnière. Les conditions climatiques sont analogues à celles de la ZAP de Sidéradougou. La pluviométrie annuelle est assez bonne et oscille entre 900 et 1100 mm.

La zone pastorale de Barani couvre une superficie de 490km2 et est située entre les parallèles 12°5' et 13°5' de latitude nord et les méridiens 3° et 4° de longitude Ouest. Elle est localisée au nord-ouest du pays et à l'Est du Département du même nom. Contrairement aux deux autres zones, la ZAP de Barani est indemne de tsé-tsé. Elle est plus aride que les deux premières avec une pluviométrie annuelle moyenne inférieure à 900mm.

Echantillonnage

Pour chaque zone agropastorale, une liste exhaustive des villages a été établie et 10 villages ont été tirés au hasard afin d'avoir une représentativité des résultats de cette enquête transversale. Par village, il était prévu de sélectionner au hasard 50 bovins, 30 petits ruminants (si possible 15 de chaque espèce) et 10 asins. Pour améliorer la représentativité de l'échantillon, au plus 5 animaux étaient tirés par élevage. Une fiche d'enquête individuelle a été utilisée pour consigner la note d'état corporel (NEC), le mode d'élevage, la race, l'utilisation de l'animal, le sexe, l'âge et les traitements trypanocides antérieurs. Pour chaque animal échantillonné, un prélèvement de sang a été effectué à la veine jugulaire sur tube



Figure 1: Les zones agropastorales de Sidéradougou, Samorogouan et Barani

hépariné pour la recherche parasitologique de trypanosomes vivants par la technique du buffy-coat (Murray *et al.,* 1977).

Examens parasitologiques

Le sang est prélevé dans des tubes capillaires puis soumis à une centrifugation différentielle (3000 tours/mn pendant 5mn). Après la lecture de l'hématocrite, les tubes capillaires sont sectionnés à environ 1mm en dessous du buffy-coat à l'aide d'un crayon diamant. Le buffy-coat est étalé entre lame et lamelle pour l'examen microscopique au grossissement x40. Le prélèvement est déclaré négatif après observation d'au moins 40 champs sans détecter de trypanosome.

Analyses statistiques

Les données ont été d'abord saisies sur le tableur Excel 2007® pour l'établissement de la base de données. Les analyses statistiques ont été effectuées à l'aide du logiciel STATA SE 9.2®. Les prévalences parasitologiques ont été calculées par ZAP et par groupes bien définis d'animaux. Des tests statistiques ont été utilisés pour comparer les moyennes (T-test et ANOVA) et les proportions (Test de $\chi 2$). Les facteurs de risque associés aux infections parasitologiques sont évalués par régression logistique (estimation de l'Odds Ratio). Les différences ont été considérées comme statistiquement significatives lorsque $p < 0,05$.

Résultats

Description de l'échantillon

L'échantillon recueilli était constitué de 2396 sujets composés comme suit : 1379 bovins, 474 ovins, 339 caprins et 191 asins (Tableau I). Ces animaux étaient issus de 29 villages des 3 ZAP qui ont fait l'objet de nos enquêtes. L'échantillon était composé de 43,02 et 22,2% de bovins transhumants à Sidéradougou et à Barani respectivement.

Pour les ZAP de Sidéradougou et de Samorogouan, les bovins étaient essentiellement des zébus à 93,4% et les petits ruminants étaient majoritairement des djalonké (91,77% pour ovins et 76,7% pour caprins). Pour la ZAP de Barani, il n'y avait que des zébus, des chèvres du sahel et des moutons bali-bali. Les animaux avaient en majorité un bon état corporel pour toutes les espèces et dans les 3 ZAP (Tableau II).

A l'exception des bovins, les sujets n'ayant pas reçu de traitement trypanocide dépassaient ceux l'ayant reçu (Tableaux III). Les sujets traités ont reçu pour la plupart du diminazène sauf les asins chez lesquels les traitements ont été surtout à base d'isométhamidium. La majorité des traitements trypanocides étaient à titre curatif. En effet, les traitements curatifs au diminazène représentaient 83,87%, 94,28% respectivement chez les bovins et les ovins. Tous les cas de traitements trypanocides chez les caprins étaient à base de diminazène.

Prévalences parasitologiques de la trypanosomose

Les examens parasitologiques ont révélé des prévalences variables d'une zone à une autre. Les prévalences les plus élevées ont été rencontrées dans la ZAP de Sidéradougou (Tableau IV). Dans la ZAP de Barani, des cas de trypanosomose ont été détectés seulement chez les bovins. Les bovins et les asins ont été les espèces les plus infectées par les trypanosomes et la ZAP de Sidéradougou est la zone la plus infectée. Les prévalences parasitologiques de la trypanosomose ont atteint 18,12% et 17,4% dans la ZAP de Sidéradougou respectivement pour les bovins et les asins. Les animaux étaient infectés par Trypanosoma vivax ou T. congolense et quelque fois par les deux à la fois. De manière générale, les infections étaient dues à T. vivax. Toutefois, cette tendance est inversée dans la ZAP de Sidéradougou chez les bovins. Les infections mixtes ont été uniquement reportées dans cette dernière ZAP. Dans toutes les ZAP, les petits ruminants ont été moins infectés par rapport aux bovins et asins.

Etat sanitaire des animaux par l'hématocrite

Le Tableau V donne les valeurs de l'hématocrite chez les différentes espèces ainsi que sa variation en fonction du statut d'infection à la trypanosomose. L'hématocrite moyen a été bon chez toutes les espèces sauf chez les bovins où il a été en deçà de 30% (28,9%). Les animaux infectés par la trypanosomose ont eu un hématocrite très significativement inférieur

à celui des sujets sains (p = 0,000). Parmi, les animaux atteints de trypanosomose, toutes espèces confondues, les sujets infectés par T. congolense seul ou en infection mixte avaient un hématocrite beaucoup plus bas que ceux infectés par T. vivax seul (p = 0,000).

Facteurs de risque associés à la trypanosomose dans les ZAP du Burkina Faso

Le Tableau VI donne l'impact des facteurs de risque associés à l'infection de la trypanosomose dans les 3 ZAP du Burkina Faso. Ainsi, l'âge, le sexe, la NEC, les traitements

Tableau I: Répartition de l'échantillon par espèce et par zone agropastorale

ZAP	Bovins	Ovins	Caprins	Asins
Sidéradougou	502	172	144	69
Samorogouan	474	133	97	33
Barani	403	169	98	89
Total	**1379**	**474**	**339**	**191**

Tableau II: Répartition des animaux par catégories de note d'état corporel et par ZAP

ZAP	Bovins			Ovins		Caprins		Asins		
	N1	N2	N3	N2	N3	N2	N3	N1	N2	N3
Sidéradougou (%)	3,2	34,7	62,1	18,6	81,4	0,7	99,3	1,5	21,7	76,8
Samorogouan (%)	1,3	42,4	56,3	13,5	86,5	9,3	90,7	-	3	97
Barani (%)	12,7	76,4	10,9	37,9	62,1	19,4	80,6	4,5	42,7	52,8
Totaux (%)	5,3	49,5	45,2	24	76	8,6	91,4	2,6	28,3	69,1

N1: NEC<2; N2: 2≤ NEC <3; N3: NEC ≥ 3, ZAP: zone agropastorale

Tableau III: Proportion des animaux traités selon l'espèce et la zone agropastorale

ZAP	Bovins (%)				Asins (%)			
	T. v	T. c	Mixte	Total	T. v	T. c	Mixte	Total
Sidéradougou	5,17	12,35	0,60	18,12	8,71	5,80	2,89	17,4,
Samorogouan	2,95	0	0	2,95	3,03	0	0	3,03
Barani	1,75	0,24	0,24	2,23	0	0	0	0

ZAP	Ovins (%)				Caprins (%)			
	T. v	T. c	Mixte	Total	T. v	T. c	Mixte	Total
Sidéradougou	4,65	1,16	0	5,81	4,86	2,77	1,38	9,01
Samorogouan	0,75	0	0	0,75	1,03	0	0	1,03
Barani	0	0	0	0	0	0	0	0

T. v = T. vivax ;T.c = T. congolense ; Mixte = T. vivax + T. congolense

Tableau V: Variation de l'hématocrite (%) selon le statut parasitologique par espèce

Espèces animales	Moyenne	Négatifs	Positifs	T	*p-value*
Bovins	28,90±5,7	29,3±5,5	24,45±5,7	9,01	0,000**
Ovins	30,12±5,2	30,25±5,1	25,18±4,7	3,25	0,000**
Caprins	30,34±6,2	30,55±6,1	23,2±4,6	3,76	0,000**
Asins	32,31±6,2	32,84±5,8	23±5,5	5,47	0,000**

** *Différence très significative à p< 0,001.*

Tableau VI: Résultats de la régression logistique multiple pour les facteurs associés au statut parasitologique, par espèce animale,

Variables descriptive	Bovins (n=1379)		Ovins (n= 474)		Caprins (n=339)		Asins(n=191)	
	OR (CI 95%)	*p-value*	OR (CI 95%)	*p-value*	OR (CI 95%)	*p-value*	OR (CI 95%)	*p-value*
Age	1,04 (0,97-1,12)	0,220	0,67 (0,38-1,17)	0,103	0,72 (0,43-1,21)	0,437	0,92 (0,71-1,18)	0,435
Traction (oui vs non)	1,67 (0,77-3,62)	0,147	-	-	-	-	16,94 (2,06-139,1)	0,008*
Sexe (Femelle vs Mâle)	1,09 (0,55-2,16)	0,676	2,75 (0,32-23,7)	0,329			0,75 (0,20-2,79)	0,671
Mode d'élevage (Sédentaire vs Transhumant)	2,99 (1,98-4,55)	0,000*	-	-	-	-	-	-
NEC (bon vs mauvais)	1,34 (0,89-1,99)	0,068	0,78 (0,19-3,12)	0,697			3,30 (0,38-28,78)	0,257
Traitement (oui vs non)	0,87 (0,69-1,09)	0,281	0,84 (0,38-1,85)	0,682			1,83 (0,19-17,57)	0,562
Race	0,84 (0,45-1,56)	0,594	0,77 (0,19-3,02)	0,668	0,17 (0,01-3,38)	0,227	-	-
Hématocrite (bon vs mauvais)	0,21 (0,14-0,31)	0,000*	0,25 (0,06-0,99)	0,048*	0,15 (0,04-0,56)	0,004*	0,05 (0,014-0,21)	0,000*

*OR = Odds Ratio, bon NEC> 2, mauvais NEC ≤ 2, bon hématocrite≥25%, mauvais hématocrite < 25%. * significatif à p<0,05*

trypanocides et la race n'ont pas eu une influence significative sur les prévalences parasitologiques chez les différentes espèces. Toutefois, chez les bovins, la prévalence était plus élevée chez les sujets âgés de plus de 2 ans (8,46%) que chez ceux ayant au plus 1 an (5%) ; bien que, la différence n'ait pas été significative (p>0,05).

Cependant les bovins transhumants ont été beaucoup plus infectés par la trypanosomose que les animaux sédentaires (OR = 2,99 (1,98-4,55) ; p = 0,000). Chez les asins, la prévalence de la trypanosomose chez les animaux de traction a été plus élevée que chez les ânes qui n'étaient pas

Discussion

La prédominance de zébus s'explique d'une part, par le fait que tous les bovins de Barani sont des zébus et d'autre part, par les meilleures performances de production des zébus par rapport aux taurins baoulé (Maichomo *et al.,* 2009). Dans la ZAP de Barani, les petits ruminants sont des moutons bali-bali et des chèvres du sahel. Dans les ZAP de Sidéradougou et de Samorogouan, la majorité des petits ruminants étaient des Djalonké. Cette composition des petits ruminants est fonction des zones climatiques. La zone de Barani est dans la zone sahélienne, indemne de glossine (PATTEC, 2010). Les races sahéliennes s'adaptent très bien à ce milieu. Les ZAP de Samorogouan et de Sidéradougou, situées plus au sud, sont dans la zone infestée tsé-tsé et de trypanosomose, la majorité des petits ruminants étaient des Djalonké qui ont une certaine résistance à la trypanosomose (Geerts *et al.,* 2009).

Presque tous les animaux avaient un bon état corporel (NEC> 2). Ceci pourrait s'expliquer par l'abondance du fourrage vert pendant la période de l'enquête qui a coïncidé avec la saison pluvieuse (juillet 2010). Cependant, les parasitoses gastro-intestinales et autres parasitoses sanguines telles que les babésioses et l'anaplasmose, qui n'ont pas été prises en compte dans notre étude, pourraient justifier les états corporels moyens des bovins.

La médication trypanocide a été plus importante chez les bovins que chez les autres

espèces. Cela se justifierait par le fait que les bovins, en majoré étaient composés de zébus, race plus sensible à la trypanosomose que les taurins. De plus en raison de leur importante valeur économique, les bovins reçoivent régulièrement des traitements préventifs ou curatifs contre les trypanosomoses. Le diminazène a été le plus utilisé chez les ruminants, ce qui témoigne d'une grande proportion de traitements curatifs. Ce fait explique également un manque de programme de protection des animaux contre la trypanosomose. Chez les petits ruminants, majoritairement des Djalonké en zone infestée de tsé-tsé, le faible niveau de médication serait dû à leur trypanotolérance (Geerts *et al.,* 2009). Cependant le faible taux de traitement chez les ânes pourrait être expliqué d'une part par la négligence des soins vétérinaires chez les ânes et d'autre part à cause de leur résistance naturelle (Sow *et al.,* 2014 ; MRA, 2008). De plus, l'utilisation de l'isométamidium qui provoque des réactions locales au niveau des points d'injections et qui, par cnséquent, occasionnent des jours de repos des ânes traités, pourrait aussi expliquer la rétissence des propriétaires (Eisler *et al.,*1996 ; Sow, 2013). Les taux de traitements trypanocides chez les différentes espèces sont semblables à ceux trouvés par Sow et al. (2013) dans la région de la Boucle du Mouhoun, une des zones d'intervention du programme PATTEC.

La prévalence de la trypanosomose légèrement plus élevée chez les caprins que chez les ovins s'expliquerait par la meilleure prise en charge des ovins par rapport aux caprins. En effet, 21,95% des ovins recevaient des traitements trypanocides contre 5,02% des caprins. De plus, les traitements des ovins étaient à la fois préventifs (isométhamidium) et curatifs (diminazène) alors que ceux des caprins étaient seulement curatifs (diminazène). Ce résultat est contraire à celui de Sow et al. (2013) dans la boucle du Mouhoun. D'autres études ont justifié la faible infection des caprins grâce à leur habilité à se défendre physiquement contre les glossines par des mouvements du corps et des tremblements de la peau (Simukoko *et al.,* 2007).

Pour les ZAP de Samorogouan et de Barani, les prévalences parasitologiques sont dans l'ordre de grandeur des valeurs trouvées dans la région de la Boucle du Mouhoun (Sow *et al.,* 2013). Mais ces prévalences restent inférieures à celles trouvées chez des bovins au Nord du Ghana, du Togo et de la Côte d'Ivoire. Cependant, la prévalence enregistrée chez les bovins dans la ZAP de Sidéradougou était comparable aux valeurs rapportées dans ces zones (Napala *et al.,* 1999 ; Mahama *et al.,* 2004 ; Soffo, 2010).

De toutes les 3 zones, les prévalences parasitologiques les plus faibles ont été enregistrées dans la ZAP de Barani. Cette ZAP est située hors de la zone de distribution des tsé-tsé au Burkina Faso, et ceci a été confirmé par de récentes enquêtes entomologiques dans la zone (PATTEC, 2010). La ZAP de Sidéradougou située plus au Sud que les 2 autres, a un climat qui offre des conditions écologiques beaucoup plus favorables aux tsé-tsé donc un risque trypanosomien plus élevé (de la Rocque *et al.,* 2001). C'est ce qui expliquerait les prévalences plus élevées dans cette ZAP. Cette éventuelle explication est soutenue par la prédominance de T. congolense transmise presque exclusivement par les glossines (Moloo et Kutuza, 1988) dans la ZAP de Sidéradougou.

Les prévalences parasitologiques ont été plus élevées chez les bovins et les asins (Tableau IV). Le cas d'infection chez les bovins se justifierait d'une part, par le fait qu'ils sont majoritairement des zébus (trypanosensibles) et d'autre part, par le fait qu'ils sont le plus souvent envoyés au pâturage et vers les points d'eau pour leur abreuvement. C'est au niveau, surtout des points d'eau et le long des forêts galeries qui sont les habitats de glossines riveraines. En effet, les enquêtes entomologiques antérieures dans les ZAP de Sidéradougou et de Samorogouan ont permis de capturer Glossina tachinoides et G. palpalis gambiensis (de la Rocque *et al.,* 2001 ; Clausen *et al.,* 1992). Dans cette ZAP de Barani, les bovins positifs à la trypanosomose sont des animaux transhumants et les infections seraient survenues lors de la transhumance. Toutefois, une transmission mécanique locale n'est pas totalement exclue. En effet, sur les 9 cas, il y avait une seule infection due à T. congolense. La transmission mécanique de T. vivax par

des insectes piqueurs a été démontrée (Desquesnes et Dia, 2004, 2003).

Trypanosoma vivax fut l'espèce la plus rencontrée dans notre étude, elle a été identifiée dans toutes les 3 ZAP. Excepté chez les bovins dans la ZAP de Sidéradougou, T. vivax était prédominant chez toutes les espèces animales. Cette prédominance de T. vivax s'expliquerait par le fait que les glossines riveraines le transmettent efficacement. De plus, T. vivax est transmis avec succès par les vecteurs mécaniques (Itard, 2000 ; Desquesnes et Dia, 2003; 2004). Cette tendance a été documentée par des études antérieures dans la région de la Boucle du Mouhoun et bien d'autres régions infestées par les tsé-tsé au Burkina (Sow *et al.,* 2013 ; Bengaly *et al.,* 2001). Cependant Desquesnes et al. (1999) ont rapporté une décennie plus tôt un taux d'infection plus élevé de T. congolense que T. vivax chez les bovins dans la ZAP de Sidéradougou.

L'hématocrite moyen a été très significativement plus faible chez les sujets positifs toute espèce confondue ($p<0,001$). La présence de l'anémie est l'un des signes les plus importants de la présence d'infection des trypanosomes surtout *T. congolense* (Murray et Dexter, 1988). Toutefois, l'hématocrite moyen chez les bovins apparemment en bonne santé était en deçà de la valeur usuelle de l'hématocrite qui est d'environ 30% (Hoste *et al.,* 1982). En cas d'infection de trypanosomose l'hématocrite peut baisser jusqu'en dessous de 20% en seulement 3 ou 8 semaines d'infection par *T. congolense* ou *T. vivax* respectivement (Dayo, 2004). Les valeurs de l'hématocrite et leur variation selon le statut d'infection à la trypanosomose rejoignent les résultats de Sow et al. (2013) dans la région de la Boucle du Mouhoun.

L'utilisation des animaux et le mode d'élevage constituent des facteurs de risque trypanosomien pour les asins et les bovins. La transhumance expose les animaux aux piqûres des glossines d'où un risque trypanosomien plus élevé. La transhumance se fait toujours vers le sud du pays où les densités de glossines sont plus élevées. En effet, vers la frontière de la Côte d'Ivoire, zone de transhumance, les densités apparentes sont très élevées pour les deux espèces riveraines (jusqu'à 60 glosines/piège/jour) (Rayaissé *et al.,* 2009). Les bovins transhumants descendent régulièrement vers le sud, où le fourrage est plus abondant pendant la saison sèche, et reviennent en début d'hivernage dans leur région d'origine. Le rôle de la transhumance dans l'augmentation du risque trypanosomien a été prouvé dans la Région de la Boucle de Mouhoun au Burkina Faso (Sow *et al.,* 2013).

Les asins utilisés pour traction animale étaient plus infectés par les trypanosomes que les animaux non soumis à cette tâche. En effet, les ânes attelés aux charrettes assurent la corvée d'eau et de bois. Ceci nécessite la fréquentation des rivières et les forêts galeries le long des cours d'eau qui sont les gîtes des glossines d'espèces riveraines (Cuisance *et al.,* 1985b). Ce fait a également été rapporté par Sow et al. (2013) chez les ânes de la région de la Boucle du Mouhoun.

Conclusion

L'étude a permis d'établir une situation de base parasitologique des trypanosomoses animales dans les trois ZAP enquêtées. Il ressort que la trypanosomose n'est pas la contrainte dans la ZAP de Barani, où seul des cas d'infections ont été trouvés chez les bovins transhumants. Les ZAP de Sidéradougou et Samorogouan subissent la maladie, bien que les prévalences soient assez faibles dans la dernière. Les traitements trypanocides sont les seuls moyens de lutte pour le maintien des animaux sensibles. Les résultats de cette étude pourraient aider la PATTEC dans l'élaboration de sa stratégie de lutte contre les tsé-tsé et les trypanosomoses animales dans les trois ZAP.

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

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Abstract

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

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

DIAGNOSTIC PRÉSOMPTIF DE L'ENCÉPHALOMYÉLITE AVIAIRE CHEZ LA CAILLE JAPONAISE À IBADAN (NIGERIA)

Résumé

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

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

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Introduction

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

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

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

Case Report

History

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

Clinical findings

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

Pathology

Post mortem examination of the carcasses revealed no visible lesion. A tentative diagnosis of *Avian encephalomyelitis* was made with a differential diagnosis of Vitamin E deficiency. Tissue samples were harvested from the brain, fixed in 10% neutral buffered formalin and routinely processed for paraffin embedding. Sections of 4µm thickness were stained with hematoxylin and eosin for microscopic examination.

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

Discussion

Avian encephalomyelitis (AE) is known to be a disease of young chickens, pheasants, pigeons, Japanese quail, and turkeys, ducklings, patridges (Bodin *et al.,* 1981; Toplu and Alcigir, 2004; Welchman *et al.,* 2009). Clinical disease has not been reported in adult birds but they could be infected showing reduction in egg production. The history, clinical findings, absence of gross pathology and histopathological

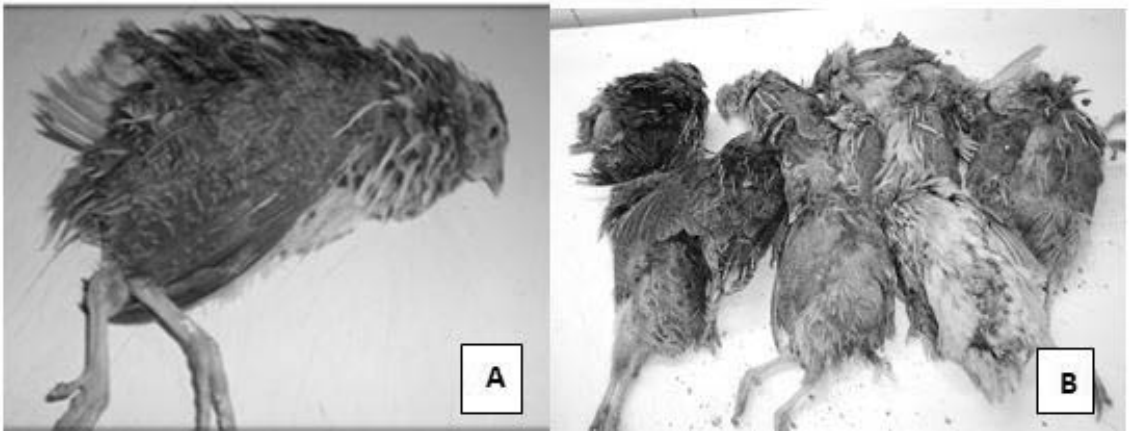


Plate 1: A, moribund Japanese quail presented by farmer showing lateral recumbency and paralysis; B, some mortalities experienced on farm.

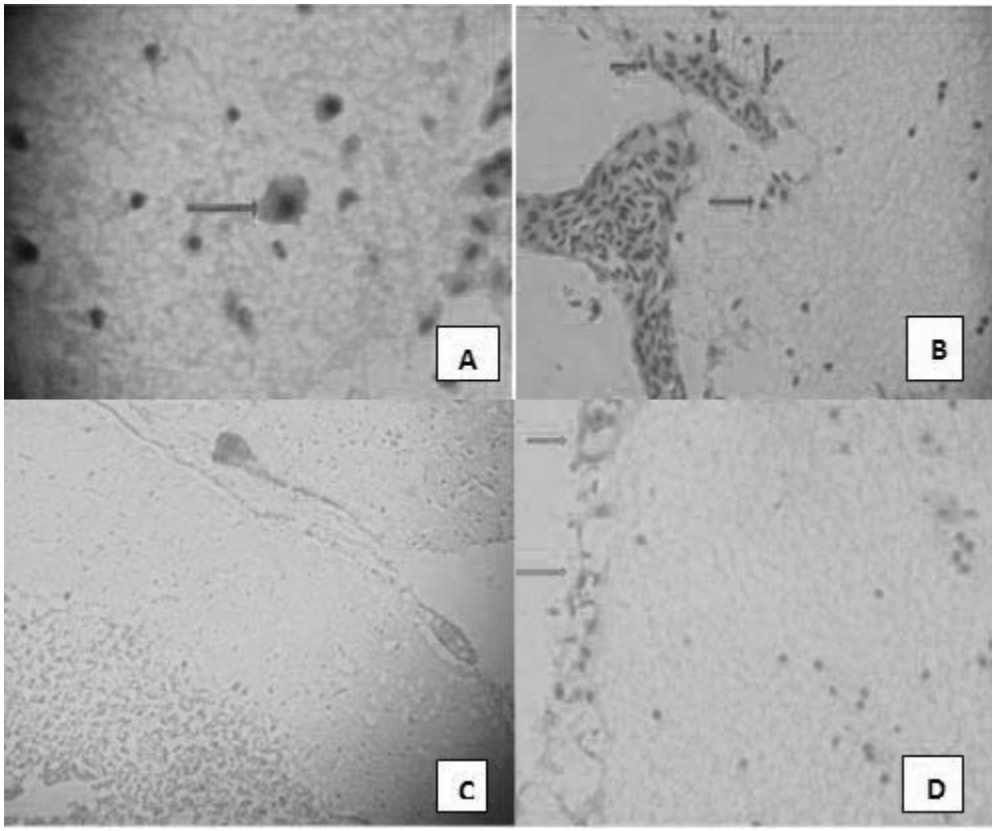


Plate 2: Photomicrographs of cerebrum and cerebellum. H&E stain.
A, neuronal chromatolysis (arrow) with diffuse gliosis in cerebrum (x400); B, congested blood vessels(arrows) in the meninges of cerebellum(x100); C, congested meninges and diffuse gliosis in cerebellum (x100); D, lymphocytic infiltrations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

lesions observed in this outbreak are characteristic of horizontally transmitted Avian encephalomyelitis (Calnek, 2008) except for the age of outbeak . Findings from this outbreak are similar to those reported by Hill and Raymond (1962) in quail chicks. It is worthy of note that this is the first report of the disease in Japanese quail in Nigeria.

Recently, there has been an upsurge in the domestication of Japanese quail in Nigeria after the initial introduction by the Nigeria Veterinary Research Institute, Vom, in 1992. Intensification in rearing has been accompanied by health challenges with little or no disease control measures instituted by either Veterinary authorities or individual farmers in the country. However, the age of the flocks at the time of this outbreak is worthy of consideration. It is believed that the dose of infection relative to the size of the birds played a major role in this outbreak. A recent study in Southwest Nigeria by Oladele and Onwuka (2013) revealed inefficiency of AE virus maternal antibody transfer to progeny among breeder flocks which could be responsible for the apparent poor control of Avian encephalomyelitis in chicken flocks in this region, evident by incessant outbreaks. The Japanese quail is a small-sized bird even as adults. The adult male weighs between 100 and 140g while the female weighs between 120 and 160g (Randall and Bolla, 2008). This genetic trait coupled with apparent endemicity of the disease in southwest Nigeria is believed to be responsible for this outbreak.

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

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TIME SERIES ANALYSIS AND PUBLIC HEALTH IMPLICATIONS OF SUSPECTED BOVINE TUBERCULOSIS IN CATTLE SLAUGHTERED IN LAFENWA MUNICIPAL ABATTOIR, ABEOKUTA, OGUN STATE, NIGERIA – 2004-2012

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Abstract

Bovine tuberculosis is a major zoonotic disease with worldwide distribution, especially in developing countries like Nigeria, where the disease is endemic with no concrete eradication programme. The study was conducted to investigate the magnitude, seasonality and trend of meat rejection resulting from suspected cases of bovine tuberculosis in slaughtered cattle in Lafenwa municipal abattoir ,Abeokuta, South-western Nigeria over a period of nine years using time series analysis and also, to discuss the public health implications. This is with a view that, decision making and policies can be made to control and/or eradicate this disease. Seasonal pattern with more cases during the dry season than wet season was observed while an increasing long term secular trend of cases of rejection is also predicted in the long run if there is no proven control program in place. This study further reinforced the fact that bovine tuberculosis is endemic in Nigeria and is seasonally connected.

Key words: Suspected cases, bovine tuberculosis, Abattoir, time series analysis, Retrospective study, public health implications

ANALYSE DE SÉRIES CHRONOLOGIQUES ET IMPLICATIONS POUR LA SANTÉ PUBLIQUE DE LA TUBERCULOSE BOVINE SUSPECTÉE CHEZ LES BOVINS ABATTUS DANS L'ABATTOIR MUNICIPAL DE LAFENWA À ABEOKUTA DANS L'ÉTAT D'OGUN AU NIGERIA – 2004-2012

Résumé

La tuberculose bovine est une zoonose majeure présente dans le monde entier, en particulier dans les pays en développement comme le Nigeria, où la maladie est endémique mais ne fait l'objet d'aucun programme concret d'éradication. L'étude a été menée dans le but d'évaluer l'ampleur, le caractère saisonnier et la tendance du refus de viande à cause de suspicions de cas de tuberculose bovine chez les bovins abattus dans l'abattoir municipal de Lafenwa à Abeokuta, dans le sud-ouest du Nigeria, sur une période de neuf ans, en utilisant l'analyse de séries chronologiques. L'autre objectif était de discuter des implications de cette maladie sur la santé publique. L'impact recherchée était d'amener les autorités responsables à prendre des décisions et à élaborer des politiques pour le contrôle et / ou l'éradication de cette maladie. Une tendance saisonnière, comportant un nombre plus élevé de cas pendant la saison sèche par rapport à la saison humide, a été observée, tandis qu'une tendance d'augmentation croissante de cas de refus est également prédite sur le long terme si aucun programme de contrôle efficace n'est mis en place. Cette étude a davantage corroboré le fait que la tuberculose bovine est endémique au Nigeria et a un caractère saisonnier.

Mots-clés : cas suspects, tuberculose bovine, abattoir, analyse de séries chronologiques, étude rétrospective, implications pour la santé publique

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Introduction

The global and national burden of Tuberculosis (TB) cannot be over-emphasized. Globally, an estimated 8.6 million new cases of TB and 1.3 million death from the disease (including 320 000 deaths among HIV-positive people) was recorded in 2012 (WHO, 2013). The number of TB deaths is currently increasing unacceptably with ninety-five percent (95%) of cases occurring in people living in developing countries, despite the 2015 target set by the W.H.O for the total control and eradication of the disease, set within the context of the Millennium Development Goals (MDGs), nearly 20 years after declaring it as a global public health emergency (WHO, 2006). Nigeria is currently ranked as the 9th most tuberculosis burdened country in the world. In Nigeria with a population of over 168 million people, a national survey carried out in 2012 estimated the prevalence of 161 tuberculosis (TB) cases per 100,000 inhabitants (WHO, 2013).

Bovine tuberculosis (BTB) is caused by *Mycobacterium bovis*, a slow growing acid-fast bacillus and member of the *Mycobacterium tuberculosis* complex, which includes *M. tuberculosis*, *M. africanum* and *M. microti*. It is a chronic disease that is very infectious and contagious to both domestic and wild animals, including humans (Radostits *et al.,* 2003). The disease is characterized by the formation of typical granulomatous lesions with varying degrees of necrosis, calcification and encapsulation in tissues and organs, such as lungs, lymph nodes, intestines, liver, kidneys and thoracic wall (Shitaye *et al.,* 2007).

Though, cattle serve as the principal reservoir species for *M. bovis*, hence the name 'Bovine Tuberculosis'. The term is likewise used to describe *M. bovis* infection in other species of animals to demonstrate the bovine source of the infection. Bovine tuberculosis have also been reported in most domestic animals such as pigs, goats, sheep, cats, camels, horses, and in wild-animals such as lions, badgers, deer, cheetahs, leopards, african buffalos and elephants (Ayele *et al.,* 2004).

Bovine tuberculosis has been classified as a 'neglected zoonosis' by the WHO in conjunction with FAO and OIE, with special reference to developing countries, while the International des Epizooties (OIE) classified it as a 'List B' disease, due to its socioeconomic or public health significance in international trade of animals and animal products. The growing awareness of neglected zoonoses including bovine tuberculosis has led to initiatives supported by the WHO/FAO/OIE to investigate, calculate and mitigate the unknown risk from these animal diseases on livestock productivity, human health and livelihoods (WHO, 2009).

One of the contributing factors to the increased epidemicity of human tuberculosis is 'Anthropozoonosis', a phenomenon characterized by transfer of infection from animals to humans. Over the years, global awareness has been raised on the Anthropozoonotic nature of tuberculosis at the human-livestock-wildlife interface (Pavlas, 1999). There have been many reports of interaction between humans and livestock and /or wildlife that have resulted in outbreaks. Other results have shown increased prevalence of the disease in cattle herdsmen, dairy farmers, butchers, meat inspectors and other abattoir workers, with alarming rate in HIV-infected patients, thereby emphasizing the increased public health significance and zoonotic pathway of disease transfer between humans, cattle and other animals. Eradication of BTB has also been found to have beneficial effect in other animal species and man (Pavlas, 1999).

Transboundary movements of cattle within and between countries and continents have also in many ways facilitated the worldwide distribution of bovine tuberculosis. Transmission between animals is thought to occur mainly by inhalation of contaminated aerosol and therefore affects the lungs primarily (Radostits *et al.,* 2003). However, infection can also occur via the gastro-intestinal tract or become systemic and affect other organs, such as the urinary tract or the mammary lymph nodes (Ashford *et al.,* 2006).

Transmission of tuberculosis from cattle to humans mostly occurs through the consumption of infected meat and unpasteurized milk, and also close contact

with infected animals as seen on dairy farms and in abattoirs. Tuberculosis in humans due to *M. bovis* has been found to be both clinically and pathologically indistinguishable from cases caused by *M. tuberculosis* (Evans *et al.,* 2007) and the primary location of lesions is depended on the route of infection and dissemination pathway to nearby organs, but principally found to be extra-pulmonary in most cases.

The WHO reported in 1998 that 3.1% of tuberculosis cases in humans worldwide are attributable to *M. bovis* and that 0.4–10% of sputum isolates from patients in African countries could be *M. bovis*, despite the fact that *M. bovis* is more often associated with extra-pulmonary disease in humans and hence might be difficult to diagnose in humans (Cosivi *et al.,* 1998). In Nigeria, the prevalence of bovine tuberculosis in cattle ranging from 2.5% in 1976 to 14% in 2007 has been reported from different parts of the country (Cadmus *et al.,* 2004; Abubakar, 2007).

This nine (9) year longitudinal survey of BTB cases in cattle in this abattoir was instituted, because of the public health risk of Bovine tuberculosis in Nigeria as previously reported, coupled with the recent insurgence and menace of multi-drug resistance TB in the population, which we hypothesized, might be in some way linked to BTB (Daniel and Osman, 2011; Olusoji *et al.,* 2013). This highly drug resistance TB have also been reported in cattle and might not be unrelated to the indiscriminate and unsupervised use of antibiotic drugs in cattle farms or herds. The previous complaints by state veterinarians and meat inspectors reporting farmers' attitude of turning in diseased cattle for slaughter at the abattoir coupled with a potential zoonotic high risk behavior identified among meat handlers and butchers in this abattoir, through a customary practice of hiding diseased meat from meat inspectors and swallowing or eating the visibly infected parts of the lungs (locally called Fuku-Elegusi) in order to reassure customers that the meat on display is safe to eat and convince customers or meat sellers to buy meat from them, despite constant warnings and sanctions from meat inspectors to stop this practice among butchers in this abattoir.

The aim of this study was to investigate the seasonality and trend of suspected bovine tuberculosis cases in slaughtered cattle in a municipal abattoir in a period of nine years and to discuss the public health implications. This is with a view that, decision making and policies can be made to control and eradicate this disease.

Materials and Methods

Study Location:

This study was conducted at Lafenwa abattoir in Abeokuta North Local Government of Ogun State. Abeokuta is the Capital city of Ogun state, Southwest of Nigeria. The Lafenwa abattoir is the largest in the State, situated at 709'39" N, 3020'54"W, in the rainforest vegetation zone of Nigeria close to the Ogun River, which is the largest river and main source of drinking water in Ogun state share boundaries with the republic of Benin on the West, Lagos state on the South, Oyo state on the North, and Osun and Ondo state on the East (Google Earth, 2006).

The climatic condition of the state is characterized by two distinct seasons – the wet and dry seasons. The wet season occurs between April and October with a break in August while the dry season lasts from November to April with a cold harmattan between December and January. The state has an estimated population of about 3,751,140 persons, comprising 1,864,907 males and 1,886,233 females (National Population Commission, 2006). The main source of meat in the state is from cattle slaughtered at the abattoir. Though most of these cattle come from neighboring countries like Republic of Benin, Togo and Mali and also some others from northern states of Nigeria, small scale livestock farming of indigenous breeds (mainly Muturu) do exist, but this accounts for less than 10% of the state's cattle population.

Data collection:

Records of carcasses rejected due to suspected BTB cases by gross TB visceral lesions detected during routine post-mortem meat inspection by meat inspectors at Lafenwa municipal abattoir in Abeokuta from 2004 - 2012 were obtained from the Veterinary Public

Health Department of the State Ministry of Agriculture and Natural Resources. This was done following approval from Director of Veterinary Services in the State. There were no complete records of daily, monthly or annual number of cattle slaughtered during the period under consideration, hence it was not considered in the analysis. Rejections or condemnations were made based on visual post mortem observation of tuberculous or granulomatous lesions in various parts of the carcass including lung, intestine, liver and other viscera organs. Records of rejected carcasses were made based on the number of partial or total rejections made per month and in turn per year. Data were compiled and tabulated on monthly basis as shown recorded in table 1.

Statistical Analysis

Data gathered from the records of rejected carcasses during post mortem inspection were collated and tabulated over a period of nine years. These were further analyzed using the Microsoft Excel 2010 and Epi InfoR 3.5.3 version

Graphically representation of data collected and analyzed described seasonal pattern and trend in monthly and yearly pattern over a period of nine years. A further analysis was carried out by smoothing the graph using two period moving averages to assess for seasonality while logarithmic, polynomial, linear forecast trend-lines were used to forecast the long term trends of the disease in the nearest future if not subject to any control program hereafter.

Results

The graphs of the time-series of suspected cases of BTB associated rejections/ condemnations in slaughtered cattle in Lafenwa municipal abattoir for the period of 2004-2012 are presented in the Figures 2 to Figure 5.

A total of 5,566 rejections cases of gross TB lesions were observed during routine post-mortem meat inspection for the period of 9 years. The rate of BTB condemnations were also found to increase steadily during the dry seasons (November to December and January to March), while declining gradually in April, just before the start of the rainy season, there was a sudden drop in the cases, with the lowest drops recorded in the month of August for the entire period of the survey. This later rose and increased from September into the dry season or ending of each year (Figure 2). This trend was noted for entire period of the

Table 1: Number of rejection of suspected cases of bovine Tuberculosis during post-mortem at Lafenwa abattoir, Abeokuta, Ogun State, Nigeria

Years of Study	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**
Jan.	2	8	4	2	3	35	129	158	80
Feb.	3	9	2	5	9	33	171	156	87
Mar.	1	5	2	5	5	46	172	153	72
Apr.	7	4	0	1	4	44	152	140	39
May	4	0	4	1	11	91	173	63	41
June	2	0	3	0	0	7	107	193	61
July	6	3	0	0	7	107	193	61	85
Aug.	8	4	0	0	3	42	90	79	106
Sept.	8	4	0	0	25	162	134	54	138
Oct.	11	3	6	0	25	115	177	114	111
Nov.	22	4	0	2	45	147	131	106	133
Dec.	17	3	5	0	28	149	115	134	113
Total	**91**	**47**	**26**	**16**	**170**	**1027**	**1796**	**1327**	**1066**



Figure 1: Map showing the study location in Abeokuta, Ogun State, South-western Nigeria.
Source: Google Earth, 2006

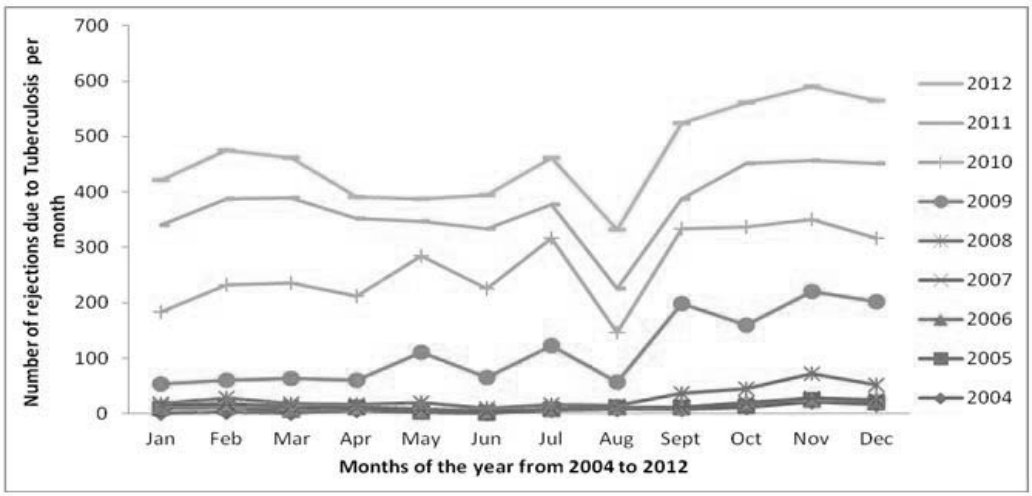


Figure 2: Seasonal patterns of BTB associated rejections made in Lafenwa abattoir over a period of nine years (2004 – 2012)

survey with slight exceptions in some years. The series also exhibited a number of peaks, aside from the small scale fluctuations, the significant peak appeared to be separated by more than a few months. With the highest peaks observed in the months of dry season (Figure 2). Figure 3 shows similar monthly trends for the number of BTB associated condemnation made within the period of study with year 2010 showing the highest monthly peaks (Figure 3, 4). Cyclical seasonal pattern was observed between the year 2010 and 2012 following smoothing while an increasing long term secular trend of cases of condemnations or rejections were observed (Figure 5). The annual cases of BTB condemnation increased from 2004 to 2010,

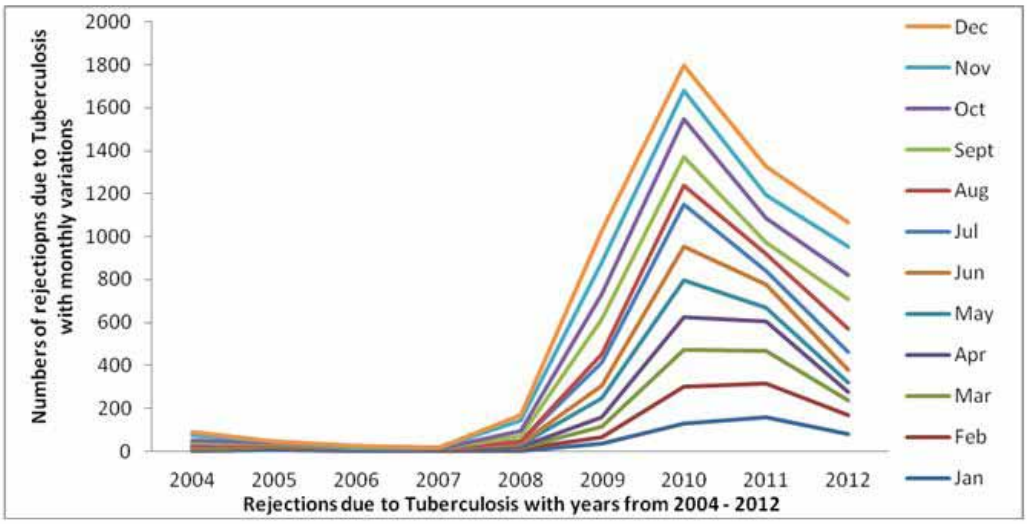


Figure 3: Monthly trends in Bovine Tuberculosis associated rejections made in Lafenwa abattoir over period of nine years [2004 – 2012].

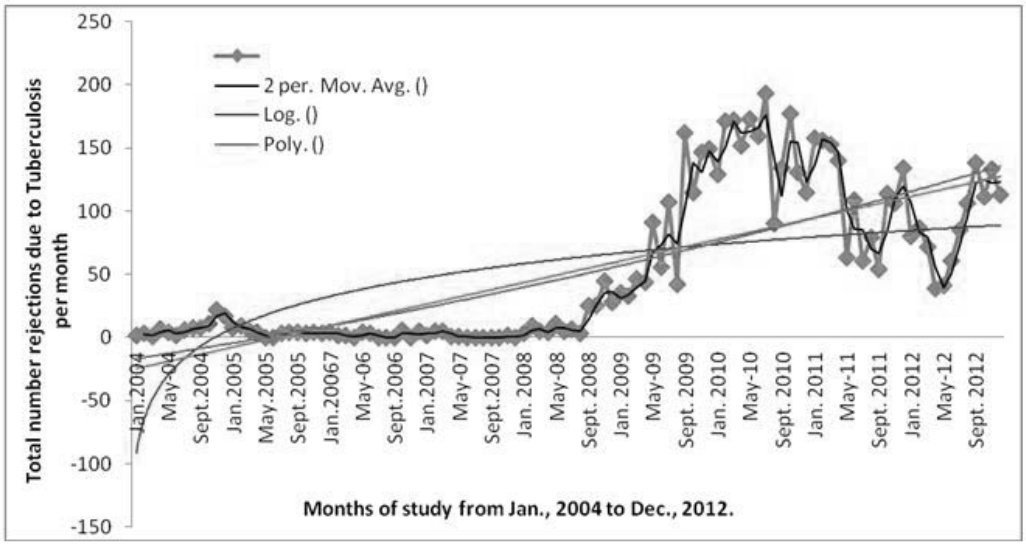


Figure 4: Trend and Seasonality of BTB associated rejections made in Lafenwa abattoir over a period of nine years [2004 – 2012] with logarithmic, polynomial trends and two period moving average smoothing.

where it peaked in 2010 and was followed by a steady decline until 2012. Monthly condemnation rate ranges from 0 to 193 cases over this period while annual condemnation rate ranges from 16 cases (lowest) in the year 2007 to 1,796 cases (highest) in the year 2010 (Figure 5).

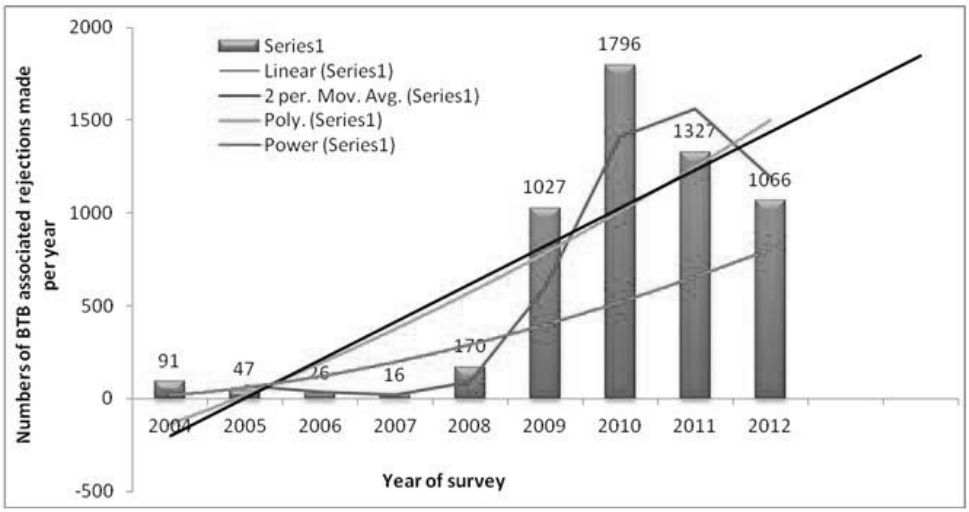


Figure 3: Monthly trends in Bovine Tuberculosis associated rejections made in Lafenwa abattoir over period of nine years [2004 – 2012].

Discussion

Bovine tuberculosis (BTB) is a major zoonotic disease with worldwide distribution, especially in developing countries where the disease is endemic. This study has further demonstrated the fact that the disease is still endemic in Nigeria. Although, successful control leading to the eradication of the disease has been accomplished in some countries by the application of the 'test-and-slaughter' strategy, all, efforts to control the disease in both animals and humans have not been successful in developing countries like Nigeria. However, this may be due to the frequent and concurrent presence of multiple risk factors (Müller *et al.,* 2013).

In Nigeria, routine tuberculin diagnosis is not done on most farms or prior to slaughter due to cost, therefore, thorough post-mortem meat inspection in relation to bovine TB lesions in the abattoir or slaughter houses serves as an ancillary or improvised method of surveillance system. Other factors such as proper abattoir hygiene and clean handling of meat are also necessary to reduce zoonotic tuberculosis.

The reason for the annual fluctuation in cases of BTB condemnation during the study period is not clear. The condemnation rate (less than 200 cases per year) of BTB between 2004 and 2007 might be due to lower occurrence of the disease on the field. This lower rate of condemnation has also been reported by Nwanta et al. (2011) in abattoirs in Enugu, Nigeria, between year 2004 and 2008 with the exception of year 2006. However, inadequacies in capacity, especially in Nigeria where an average of two meat inspectors is assigned to 200 slaughtered cattle for inspection, lack of thoroughness of the veterinary staff carrying out the meat inspection, could have also played major roles in lower condemnation rate during this period. This agrees with Corner et al. (1990) and Shitaye et al. (2006) who reported that post-mortem surveillances for detection of bovine TB lesions in particular, depending on the work load, time and diligence of the inspector conducting the examination.

In addition, poor cooperation or blatant refusal of some butchers to present their carcasses for meat inspection coupled with the fact that there is no compensation programme from the government for rejected carcasses thereby forcing most butchers to hid apparently diseased carcasses from veterinary inspection and rejection in an attempt to forestall losses to rejection leading to the distribution of diseased carcasses in the market and ultimately, the food chain

Another contributory factor to the high cases of BTB associated With rejections especially between 2008 and 2012 may be accounted for by the porous nature of Nigerian borders and lack of or defective quarantine

services in Nigeria . Ogun state, which is being located at the south western border of Nigeria is usually subjected to unsupervised influx of BTB unscreened cattle from neighboring countries of Benin Republic, Togo and Mali and also the northern part of Nigeria during transhumance or nomadism and cattle trade. This has been previously reported by Cadmus *et al.,* (2004) and may have been one of the major contributors to the increase in BTB cases in this abattoir. Also, the increase in trend of BTB associated with carcass rejection within the period of 2008 and 2010 may also be attributed to failure of test and slaughter policy, disease monitoring and surveillance by the constituted authorities and other stakeholders to control and eradicate bovine tuberculosis in Nigeria.

This study documents the time series of occurrence of suspected BTB rejection in slaughtered cattle in Lafenwa Abattoir for the first time. The findings are of public health concern especially among the high risk beef consuming population with a rising immune-compromised individuals in most urban areas in Nigeria.

In assessing seasonal pattern and trend, the rate of condemnation of BTB in the abattoir which was consistently higher in the dry season especially in month of November compared to rainy season with lower cases in the month of August, year 2008 to 2012 following smoothing revealed that, occurrence of BTB is seasonally connected. Similar finding have also been observed and reported by Bikom and Oboegbulem (2007) in Cross river State Abattoir in Southern Nigeria. This however may be due to the role of livestock movement especially by nomadic transhumance can play in the occurrence of BTB during the search for water and grazing pasture due to seasonal changes.

The higher cases in the BTB associated rejection occurred during the dry season and this may be due to the fact that BTB is is predisposed by overcrowding and increased occurrence may have been precipitated by the harsh weather condition during this season. Several herds are often seen migrating together from different regions while at the same time sharing grazing areas and watering sources together along the way, which can facilitate spread and transmission of BTB (Oloya *et al.,* 2007). According to Humblet et al. (2009), the proportion of human cases of tuberculosis due to *M. bovis* will also depend on the importance of infection in animals within the herds, meaning that BTB are easily transmitted in an overcrowded herd of cattle, which is a regular practice by herdsmen in Nigeria.

The rejection rate of BTB (as seen by the logarithmic, polynomial scale and linear forecast trend) in this study points to the increasing trend of the disease in the long run; this invariably means that the health of the public is increasingly at risk, especially the butchers and meat handlers who are constantly being exposed on a daily basis to the infected carcasses owing to the fact that they are involved in the meat processing and could be a source of infection to their families. The direct economic repercussion of BTB on livestock and its indirect consequences for human health calls for increased surveillance and awareness campaign. The knowledge of the prevalence/incidence rate with time is very essential in defining the course of the disease and an effective control strategy for the disease.

The study was based on a nine year retrospective temporal study (time-series) of BTB suspected rejections in slaughtered cattle and has further showed the increasing endemicity of BTB in Nigeria. Since a seasonal pattern of BTB suspected cases have been observed in this time-series study, with only slightly variable amplitude of fluctuation. It will be good to suggest that a seasonal disease-modifying factor might have contributed to the incidence of the disease, excluding factors related to practices in the abattoir, which must have limited the true prevalence of the disease in the slaughtered cattle. The regularity of peak seasonality in BTB suspected cases in this survey may be vital in initiating an excellent program that will allow for a better implementation of the control measures. This will also help to alert abattoir hygiene workers and meat-inspectors to be more thorough and also provide them with better facilities during those peak seasons.

Nevertheless, the study enables us to conclude that the risk of BTB transmission

from slaughtered animals to humans appear potentially high in view of the relatively high numbers of carcasses rejected in the abattoir due to suspected cases of BTB during period of study and also poor hygiene habits demonstrated by the workers in the abattoir in addition to the fact that the abattoir is located a stone-throw away from the largest river (River Ogun) in the State that serves as source of recreation, fishing and drinking water for the population close to it and that most of the effluents and wastes from the abattoir are drained into the river.

This study underscores the need to test for BTB in all animals before they are brought to the abattoir for slaughter and also the institution of a very efficient and thorough ante-mortem and post-mortem examinations by the veterinarians. Diseased animals should not be allowed anywhere near the abattoir, and herdsmen or farmers that are in the practice of transporting diseased animals should be heavily sanctioned and penalized. Though the eradication of tuberculosis from livestock is expensive, for the cases to bearable or negligible rate Government and Corporate bodies must play their part in the total eradication and control of the disease by funding intensive surveillance and monitoring of livestocks and their owners, slaughtering of infected livestocks and payment of compensation to butchers with rejected carcasses to cushion the economic losses from such rejection compensation for those who owns each slaughtered BTB laden/ infected animal.

In order to control BTB in Nigeria, it is therefore recommended that the 'test and slaughter strategy' that has been proven to be efficient in industrialized world should be employed in Nigeria too. Animals and herdsmen coming from neighboring countries and northern Nigeria should also be properly tested and quarantined before being allowed free entrance into the State for transhumance pastoralism or cattle trade.

To better control the disease, good collaboration between public health officials, veterinarians, medical doctors, scientists, animal managers/ herdsmen, public stakeholders, epidemiologists and policy-makers 'One Health Approach' will go a long way in managing and protecting the health of humans, livestocks, wildlife and the environment from future outbreak of the disease. This multi-transdisciplinary collaboration will further address the wildlife–human–livestock boundary zoonosis and broaden the scope of options for total eradication. Government should also engineer the establishment of special research Laboratories at strategic locations within the six geopolitical zones and adequately equip them with equipped with diagnostic and research facilities devoted to the surveillance and control of disease with zoonotic importance such as Tuberculosis.

Further studies will be necessary to highlight the impact of the wild-life domestic interface and transhumance on the transmission of the disease in the State. Protective kits should be provided for abattoir workers and butchers at affordable prices and they should be simultaneously trained and educated on the public health implications of the disease and why proper hygiene is essential, while handling animals within the abattoir and need for regular medical check-up. Further studies will be needed to investigate the prevalence of the disease and also assess the public health risk and debilitating extent of the diseases on the populace that gets their meat from the abattoir, including workers at the abattoir. . It is also important to investigate the epidemiological impact of BTB in humans within the Abeokuta metropolis through determination of the true prevalence of M. bovis in humans, especially those that work in the abattoir and in close proximity to slaughtered animals.

Impact

To the best of our knowledge, this research paper will be the first to demonstrate the trend of condemnation of suspected bovine tuberculosis in slaughtered cattle using syndromic data in this part of Nigeria. In addition, the research work elucidated the seasonality nature of condemnation of bovine tuberculosis in this abattoir which could be very helpful in understanding and correlating the epidemiological transmission of tuberculosis in humans and animal product with respect to season.

This research work also revealed the possible trend of condemnation as well as risk of bovine tuberculosis in this abattoir and public health within this region of Nigeria. This research work has contributed in understanding the seasonality nature of bovine tuberculosis especially on the field and it has also revealed the failure of control of this disease vis-a-vis test and slaughter policy in Nigeria. In addition, this research has revealed the indirect role abattoir system could play in epidemiology of bovine tuberculosis in humans.

Acknowledgement

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Author Contributions

All authors participated equally in carrying out this research and also preparing this manuscript for publication.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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THE ISOLATION AND ANTIBIOGRAM OF AEROBIC NASAL BACTERIAL FLORA OF APPARENTLY HEALTHY GRASSCUTTERS (THRYONOMYS SWINDERIANUS TEMMINCK, 1827)

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Abstract

Grasscutter meat is an important source of animal protein in many West African countries. Despite the obvious potential of the grasscutter towards protein availability, attempts towards domestication have been hampered by diseases of which pneumonic conditions are of extreme importance. This study evaluates the normal nasal microflora of apparently healthy grasscutter with a view of understanding the nasal bacterial flora dynamics in health. Sixteen healthy adult grasscutters were examined for nasal normal microflora using the culture method. The disc diffusion technique was used for the antibiotic sensitivity test. The following microorganisms were identified from the normal flora of the grasscutters, they are; *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterococcus sp*, *Micrococcus sp*, *Bacillus cerus*, *E. coli*, *Serratia sp*, *Streptococcus sp*, *Pasteurella multocida*, *Streptacoccus, sp*., *Mannheimia heamolytica*, *Klebsiella sp*., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus sp*. and *Proteus sp*. There was no significant sex variation in the populations of the nasal normal microflora. Most of the bacteria were found to be susceptible to cloxacillin, ceftrazone, cefunoxime, ciprofloxacin and gentamycin, while they were resistant to ceflazidime, ampicillin, erythromycin, co-trimazole augmentin and nitrofurentoin. The findings provided the basis for the understanding of normal nasal bacterial flora and the possibility of their involvement in pneumopathies associated with this animal model and it will provided basis for the use of appropriate antibiotics to combat respiratory infections in grasscutter in captivity.

Keywords: nasal, microflora, grasscutter, domesticate, pneumopathies

ISOLEMENT ET ANTIBIOGRAMME DE LA FLORE BACTERIENNE AÉROBIE DES CAVITES NASALES DES AULACODES APPAREMMENT EN BONNE SANTE (THRYONOMYS SWINDERIANUS TEMMINCK, 1827)

Résumé

La viande d'aulacode est une importante source de protéines animales dans de nombreux pays d'Afrique de l'Ouest. En dépit du potentiel évident de l'aulacode pour la disponibilité de protéines, les tentatives de sa domestication ont été entravées par les maladies, parmi lesquelles les pneumopathies sont d'une extrême importance. La présente étude évalue la microflore nasale normale d'aulacodes apparemment en bonne santé, en vue de comprendre la dynamique de la flore bactérienne nasale en matière de santé. Seize aulacodes adultes en bonne santé ont été soumis à un examen de la microflore normale nasale en utilisant la méthode de culture. La technique de diffusion en gélose a été utilisée pour le test de sensibilité aux antibiotiques. Les microorganismes suivants ont été identifiés dans la flore normale des aulacodes : Staphylococcus epidermidis, Staphylococcus aureus, Enterococcus sp, Micrococcus sp, Bacillus cerus, E. coli, Serratia sp, Streptococcus sp, Pasteurella multocida, Streptacoccus, sp., Mannheimia heamolytica, Klebsiella sp., Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus sp. et Proteus sp. On n'a pas relevé de variation significative entre les sexes dans les populations de la microflore normale nasale. Les résultats de l'étude ont révélé que la plupart des bactéries étaient sensibles à la cloxacilline, au ceftrazone, au cefunoxime, à la ciprofloxacine et à la gentamicine, alors qu'elles étaient résistantes

à la ceflazidime, à l'ampicilline, à l'érythromycine, au co-trimazole, à l'augmentin et à la nitrofurentoine. Les résultats ont permis de comprendre la flore bactérienne nasale normale et la possibilité de son implication dans les pneumopathies associées à ce modèle animal, et ils serviront de base pour l'utilisation d'antibiotiques appropriés dans la lutte contre les infections respiratoires des aulacodes en captivité.

Mots-clés : nasal, microflore, aulacode, domestiquer, pneumopathies

Introduction

Grasscutters (*Thryonomys swinderianus Temminck*, 1827) have assumed important role in the Nigerian livestock economy by being a good source of protein and income for the rural poor. It also serves a good source of foreign exchange earnings needed to maintain food security and increased earnings. Being a wild hystricomorphic rodent and widely distributed in the African sub-region, it had been exploited as source of animal protein (Opara and Fagbemi, 2010). In Nigeria, Ghana and other regions of West Africa where grasses provide their natural habitat and food, they are called "grasscutters" while in South Africa where they are associated with cane plantations, they are called cane rat (Baptist and Mensah, 1986).

This animal is the most preferred (Martin, 1985) and most expensive bushmeat in West Africa including Nigeria, Togo, Benin, Ghana and Cote d voire (Baptist and Mensah, 1986; Asibey and Addo, 2000) where they are hunted aggressively by most rural dwellers to complement protein supply by the conventional livestock like sheep, cattle and goat (Byan et et al. 2009).

For the search of alternative animal protein sources which are preferred by most populace, there had been popularized attempts to domesticate and breed wild animals, especially African grasscutters (Yeboah and Adamu, 1995; Opara et. al., 2000). Meat derived from this animal form over 80% of the animal protein supply of some communities in Africa (Ntiamoah – Baidu, 1987; FAO, 1989) hence a large market is available for the meat in the West African sub region as it is regarded as a delicacy (Asibey and Child, 1990, Adamu, 1993). To alleviate this problem, attempts are being made in the sub-region to domesticate the grasscutter (National Research Council, 1991; Addo 2002) and make it more readily available, gain economic benefit and also reduce the environmental destruction that accompanies its collection from the wild.

Hence cane rat farming is proposed as a model of wildlife production for sustainable development in West and Central Africa (Auzei and Wilkie, 2000). Although attempts to domesticate this rodent have continued (Eben, 2004), such efforts had produced less desired results (Opara et. al., 2006) possibly because of poor understanding of the biology, lack of veterinary care and other management factors. Although studies have been carried out on the ethology of this species (Codjia, 1985; Holzer et. al., 1985), feeding (Akomedi, 1988), selection systems (Senou et. al., 1992), reproduction (Adjanohoun, 1988, 1992a, b) and the technical feasibility of grasscutter farming (Adoun, 1992a), focus on diseases and the control of diseases in this animal has not been accorded due attention. Some of the few investigations into the disease pattern of cane rats revealed that trauma, septicaemia, respiratory lesions were responsible for deaths (Jori *et al.,* 2001). Since pneumonia is a major condition in cane rats, emphasis of research should be on arriving at an aetiological diagnosis in most the infectious pneumopathy. Most of the infectious agents that cause respiratory disease in nature are usually normal inhabitants of the system hence with the increasing need to domesticate grasscutter; this requires proper knowledge and understanding of nasal bacterial dynamics in health and diseases of the animal. To this end, it's expedient to understand the bacteria flora of the respiratory tract of cane rats as studied in sheep, goats, cattle, and camel (Salihu and Kazeem 2001, Emikpe *et al.,* 2009). This study was therefore designed to isolate, characterize and reveal the antibiogram of bacteria from the nasal passageway of apparently healthy grasscutters (Thryonomys swinderianus Temminck, 1827).

Materials and Methods

Study Location

The study was carried out at the domestication unit of the department of wildlife and ecotourism management within the University of Ibadan, Ibadan, Nigeria. The University of Ibadan is situated some 6 kilometers to the north of the city of Ibadan at Latitude 7°26" North and Longitude 3° 54" East at a mean altitude of 277 meters above sea level. The annual rainfall is approximately 1,220mm (48") most of which falls between the months of April and October inclusive, given a predominantly dry season from November to March.

Experimental Animals

The study was carried out from November 2011 to July 2012. Sixteen (16) apparently healthy 12 month old grasscutters obtained from Tropical farm domestication unit in Imo State, Nigeria were used for the study. The farm is a well established farm that majors in the breeding and domestication of the animal. All animals for the study belonged to a selected strain of animals adapted to captivity. The grasscutters were housed in cages in the department of wildlife and ecotourism management domestication unit. The grasscutters were initially quarantined for two weeks where they were provided with compounded feed supplemented with elephant grass (Pennisetum purpureum) and water was provided ad libitum.

Methods

The nasal samples were collected a day after arrival by inserting sterile cotton tipped applicator sticks or swab into the nasal passage after proper cleaning and disinfection of the external wares. Each nasal swab on swab stick was carefully cut into a well labeled bottle containing 2mls brain heart infusion broth. The swabs were transported in a cool box to the laboratory for bacterial culture.

Each nasal swab was removed from the bottle and streaked over the plates containing blood agar – base supplemented with 7% sheep blood and MacConkey agar. The streaking was further spread with inoculating loop to aid colony isolation. The plates were labeled and incubated aerobically at 37^0C for 24 - 48 hours (Carter 1984). The cultural growth characteristics, Gram's staining properties and cellular morphology under 100X objective of light microscope were noted. Mixed colonies and Gram negative bacteria were sub cultured on both blood and McConkey agars and incubated aerobically for further 24 hours. Pure culture of single colony type, from both blood and McConkey agars were transferred onto nutrient agar- slants for a series of biochemical tests including catalase, oxidase and fermentative/oxidative tests for final identification following standard procedures (Quinn et al 1994). For antibiotic sensitivity test, the disc diffusion technique was employed and inhibition observed as clear zones around the antibiotics. Inhibition zones were measured using meter rule and measurement greater than 0.5cm was regarded as susceptibility.

Results

Descriptive statistics was used to summarize the data generated from the study. The relative abundance of each species / genera was expressed as a percentage in comparison to the total number of isolates. Two hundred and thirty-two (232) isolates were obtained. The following microorganisms were isolated: Staphylococcus epidermidis, Staphylococcus aureus, Enterococcus sp., Micrococcus sp., Bacillus cerus, E. coli, Serratia sp, Streptococcus sp., Pasteurela sp., Mannheimia haemolytica, Klebsiella sp., Pseudomonas aureus, Bacillus sp., Proteus sp. The bacteria isolates and their absolute / relative abundance are presented in Table 1 while the antibiotics sensitivity test was presented on Table 2. Most of the bacteria were found to be susceptible to cloxacillin, ceftrazone, cefunoxime, ciprofloxacin and gentamycin, while they were resistant to ceflazidime, Ampicillin, erythromycin, co-trimazole Augmentin and Nitrofurentoin.

Discussion

This study showed that a variety of bacterial flora inhabit and colonise the nasal passageway of apparently healthy grasscutter (Thryonomys swinderianus Temminck, 1827). Various investigations on the bacteria flora of the respiratory tract of diseased and apparently

Table 1: Nasal Bacteria isolates from of apparently healthy grasscutters

Bacteria	freq of occurrence	%
Staphylococcus epidermis	28	12.1
Staphylococcus aureus	45	19.4
Enterococcus sp	22	9.5
Micrococcus sp	11	4.7
Bacillus cerus	32	13.7
E. coli	12	5.2
Serratia sp	14	6
Streptococcus sp	9	3.8
Pasteurella sp	14	6.0
Mannheimia haemolytica	5	2.2
Klebsiella sp	7	3.0
Pseudomonas aureus	13	5.6
Bacillus sp	14	6.0
Proteus sp	6	2.8
Total	**232**	**100**

Table 2: The antibiotic sensitivity test of Nasal Bacteria isolate of apparently healthy Grasscutters

Bacteria	CAZ	CXC	GEN	CTR	ERY	CLX	COT	AUG	AMP	CRX	CPR	OFL	AUG	NIT	Total
Staphylococcus epidermis	+	+	+	+	R	+	+	+	R	+	+	+	+	R	11
Staphylococcus aureus	R	+	+	+	+	+	+	R	R	+	R	+	R	R	8
Enterococcus sp	+	+	+	R	+	+	+	R	R	+	+	+	R	R	9
Micrococcus sp	R	+	+	R	+	+	R	+	+	R	+	R	+	R	8
Bacillus cerus	R	+	R	+	R	R	+	R	R	R	+	+	+	+	7
E. coli	R	+	R	R	R	+	R	R	+	+	+	+	R	R	6
Serratia sp	R	+	+	R	R	+	R	+	+	R	+	R	+	R	7
Streptococcus sp	+	R	R	+	+	R	R	R	+	+	R	+	+	R	7
Pasteurella sp	+	R	R	+	+	R	R	+	R	+	+	+	+	R	8
Mannheimia haemolytica	R	+	+	+	R	R	+	R	R	R	+	+	+	R	7
Klebsiella sp	R	R	R	+	+	R	+	R	R	+	R	R	+	R	5
Pseudomonas aureus	+	R	+	+	R	R	+	+	+	R	R	+	R	R	7
Bacillus sp	+	+	+	+	R	+	R	+	R	+	+	R	+	R	9
Proteus sp	+	R	+	+	+	+	R	+	+	+	+	R	+	R	10
	7	5	5	4	7	6	7	7	8	5	4	5	4	13	

Key: R (Resistance) *+ (Susceptible)*

CAZ: Ceflazidime , CXC: Cloxacillin, GEN: Gentamicin, CTR: Ceftriaxone ERY: Erythromycin, CRX: Cefunoxime, CPR: Ciprofloxacin, OFL: Ofloxacin AUG: Augmentin, NIT: Nitrofurentoin, COT: Cotrimoxazole, AMP: Ampicillin

healthy domestic animals in various parts of the world have focused on cattle (Pandey and Sharma, 1987), camel (Al-Tarazi 2001) and goats (Emikpe *et al.,* 2009) with fewer reports on bacterial flora changes in normal and diseased nasal passage of domesticated grasscutter.

The isolation of S. aureus and E. coli in nasal samples from the nasal passage in this study is similar to that obtained in rabbits (Ajuwape and Aregbesola, 2002). E. coli which is known to be usually harmless in their normal habitat but when found in other places could cause pulmonary and urogenital tract infection (Pelczar *et al.,* 1986). This may also be associated with possible faecal contamination. The consistent isolation of Mannheimia haemolytica and P. multocida from the lungs of cane rats and that of goats in health and in different respiratory syndrome (Emikpe and Akpavie 2010, Emikpe *et al.,* 2013) may indicate their possible role in infectious pneumopathy observed in this species.

Although stress factors with or without viral infection has been reported to suppress the muco-ciliary clearance mechanism which allows the proliferation of bacterial commensals in the respiratory tract (Brogden *et al.,* 1998, Emikpe *et al.,* 2013), considering the stress of weather, domestication and poor managemental conditions which the animals are constantly subjected to, the possible role of these entities in the pneumopathies observed in cane rats need further clarifications. Apart from the possible pathogenic role of the normal nasal bacterial flora, drug resistance of some of the pathogenic bacteria has become a rampant, proven, serious problem to both animal and human health care providers. The organisms isolated from grasscutters in this study were found to be susceptible to to cloxacillin, ceftrazone, cefunoxime, ciprofloxacin and gentamycin, while they were resistant to ceflazidime, Ampicillin, erythromycin, co-trimazole Augmentin and Nitrofurentoin. This observation further showed that the organisms are more susceptible to newer generation of antibiotics which further strengthens a possible relationship between misuse or unrestricted use of an antibiotics and drug resistance (Diker *et al.,* 1994).

This investigation provides a guide for the improvement of treatment plan in the management of grasscutters using appropriate antibiotics and stress reduction strategies.

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EFFECT OF SUBCLINICAL MASTITIS CAUSED BY SS-HAEMOLYTIC STREPTOCOCCI ON MILK YIELDS IN KENYAN CAMELS (CAMELUS DROMEDARIUS)

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Abstract

Mastitis is a major constraint to milk production in camels. A survey was in Marsabit and Isiolo counties of Kenya to quantify losses in milk yield associated with subclinical mastitis caused by ß-haemolytic Streptococci in the one-humped camel (*Camelus dromedarius*). Four hundred and twenty (420) pair wise quarter milk yield comparisons were obtained from randomly selected camels. Mastitis prevalence was determined by clinical examination, physical milk examination, on-the-spot screening by CMT and isolation of ß-*haemolytic Streptococci* from hygienically collected quarter milk samples using standard culture methods. Data were submitted to the analysis of variance for comparing 2 means. Differences in milk production within fore and hind quarters were attributed to mastitis. Milk loss was 0.114L and 0.172L per mastitic fore and hind quarter per camel per day respectively. Regardless of quarter, loss in milk production was significant ($F = 30.51, p<0.001$). In the studied camel herds, the prevalence of subclinical mastitis due to Lancefield Group B *Streptococcus* (GBS) was 64 %, 5.4% for Lancefield Group C *Streptococcus* (GCS), 2.2 % for Lancefield Group G *Streptococci* (GGS) and 23 % for untypable ß-*haemolytic Streptococci*. To the authors' best knowledge this is the first report on the involvement of Lancefield Group G *Streptococci* in mastitis of camels. Investigation of the impact of high level of milk contamination with GBS to elucidate camel-human transmission dynamics is necessary. A more sustainable approach to control of camel GBS infection using vaccine is recommended.

Key words: camels, subclinical mastitis, *Streptococcus agalactiae*

EFFET DE LA MAMMITE SUBCLINIQUE CAUSÉE PAR LES STREPTOCOQUES BÊTA HÉMOLYTIQUES SUR LES RENDEMENTS LAITIERS DES DROMADAIRES KENYANS (CAMELUS DROMEDARIUS)

Résumé

La mammite est un obstacle majeur à la production laitière des dromadaires. Nous avons mené une enquête dans les comtés de Marsabit et d'Isiolo au Kenya dans le but de quantifier les pertes de rendement laitier associées à la mammite subclinique causée par les streptocoques bêta hémolytiques chez les dromadaires (Camelus dromedarius). Quatre cent vingt (420) comparaisons de paires de rendement laitier par quartiers de pis ont été effectuées chez des dromadaires choisis de manière aléatoire. La prévalence de la mammite a été déterminée par l'examen clinique, l'examen physique du lait, le dépistage sur le terrain par le CMT et l'isolement de streptocoques ß-hémolytiques à partir d'échantillons de lait par quartiers de pis, recueillis dans des conditions hygiéniques en utilisant des méthodes de culture classiques. Les données ont été soumises à l'analyse de variance pour comparer deux moyens. Les différences de production laitière des quartiers antérieurs et postérieurs du pis ont été attribuées à la mammite. La perte de lait était de 0,114L et 0,172L respectivement pour les quartiers antérieurs et postérieurs atteints de mammite par chameau par jour. Indépendamment du quartier du pis, la perte en production de lait

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était significative (F = 30,51, p <0,001). Chez les troupeaux de dromadaires étudiés, la prévalence de la mammite subclinique était de 64% pour les streptocoques du groupe B (SGB) de Lancefield, 5,4% pour les streptocoques du groupe C (GCS) de Lancefield, 2,2% pour les streptocoques du groupe G (GGS) de Lancefield et 23% pour les streptocoques ß-hémolytiques non typables. D'après les auteurs, il s'agit du premier constat de l'implication des streptocoques du groupe G de Lancefield dans la mammite de dromadaires. Il est nécessaire de mener une étude de l'impact du niveau élevé de contamination du lait par les SGB pour élucider la dynamique de transmission chameau - homme. Une approche plus durable de contrôle de l'infection des dromadaires par les SGB à l'aide d'un vaccin est recommandée.

Mots-clés : chameaux, mastite subclinique, Streptococcus agalactiae

Introduction

The one humped camel (*Camelus dromedarius*) is the most important dairy animal in the vast arid and semi-arid lands of the Greater Horn of Africa (Kadim *et al.,* 2008) with an estimated population of 15 million in North East Africa, primarily in Somalia, Sudan, Kenya and Ethiopia from official FAO statistics, available on website www.fao.org. Kenya has an estimated camel population of 2.97 million with an annual milk production of approximately 340 million litres (KNBS, 2010). For pastoral communities the camel is potentially the most valuable species of livestock kept in the arid and semi-arid lands, which constitute over 75 per cent of Kenya's landmass. The camel maintains milk production when all other livestock stop lactating and due to its' unique ability to forage far from water sources is best suited to maintain the ecological balance of the arid rangelands. Camel milk contributes 40% of calories to the diet of pastoralist communities. In dairy cattle subclinical mastitis is the single most important infectious disease negatively affecting milk production. *S. agalactiae* mastitis reduces milk yields by as much as 20% to 40% (Tuasikal et.al., 2012). For camels the effect of subclinical mastitis on milk production is not well documented. In a limited study on one ranch herd in Kenya (Matofari *et al.,* 2005) it was found that milk yield from quarters infected with infectious mastitis pathogens was lower than that from quarters infected with environmental mastitis pathogens.

To alleviate poverty and enhance food security for economic growth (Millennium Development Goal 1) and to protect livelihoods of particularly food insecure pastoralist communities it is imperative that camel productivity in the arid regions be improved. Natural resources in the dry lands are the one constraint that seriously limits any production increases based on increased herd size. However, if mastitis prevalence in camels can be reduced improved milk productivity is achievable without putting any additional stress on the very fragile arid rangeland environment (Abdurahman, 2006). Husbandry practices are an important factor in level of mastitis prevalence. Camel mastitis is economically important due to its' negative effect on milk production (Akweya, 2012) and hence on the food security of pastoralist households. Information on the mastitis prevalence, its' aetiology and the relative importance of the different mastitis pathogens in reducing milk yields in camels are a prerequisite for development of effective mastitis control strategies to improve camel milk production. The aim of this study was to determine the prevalence of sub-clinical mastitis in camels (*Camelus dromedarius*), caused by various ß-*haemolytic Streptococci* and to quantify milk losses associated with mastitis.

Materials and Methods

Study area and sample collection

In November 2010, a survey was conducted in 17 camel herds in Isiolo and Marasabit counties employing stratified random sampling procedure, first of herds within a county followed by random sampling within the herds. Milk was collected from camels in their normal habitat with the help of herders. Camel teats were swabbed with 70% alcohol, first milk streak discarded, approximately 10ml of milk were collected into sterile universal bottles and the CMT was

carried out for all milk samples. Milk samples were then transported in a continuous cold chain to an ISO17025 certified veterinary laboratory (Analabs Ltd.) for bacteriological examination.

Estimation of mastitis prevalence

Every quarter of the camel udder was tested for clinical and sub-clinical mastitis. The udder was gently massaged to test for pain or inflammation. In addition, consistency of milk extracted was checked for presence of flakes, clots or traces of blood, all of which denote clinical mastitis and such cases were recorded. There is a positive correlation between California mastitis test (CMT) and presence of mastitis pathogens (Younan *et al.,* 2001; Salehu and Fayer, 2011). Thus milk from every quarter was subjected to CMT as an indicator of sub-clinical mastitis. The results were entered into animal data cards and later transferred to statistical software for analysis. A camel was considered mastitis positive if at least one of the quarters reacted positive in the CMT test.

Milk yield data collection

Animal data cards were used to collect information relating to selected camels. This information included the animal's age and breed, the owner and the geographic location. Extracted milk was quantified for each quarter using a graduated measuring cylinder. Milk yields were compared separately between the front right and the front left quarter and between the hind right and the hind left quarter respectively. Data were entered in individual animal data cards and later entered and managed in excel program where differences in milk volumes between front and hind quarters were calculated. For analysis differences in milk yield were attributed to CMT reaction of the examined quarters, all other factors held constant. Data were submitted to analysis of variance with volume of milk as the dependent variable, while animal level (age, breed, CMT reaction) and other (geographic location, farmer) variables were considered as the independent variables /determinant factors.

Bacterial culture

One loopful (10µl) of milk sample was aseptically streaked on blood agar (BA, Oxoid, No CM0055) and Edward's Agar (EA, Oxoid, No CM0027) and spread on the plates employing standard inoculation procedure. A further 200 µL of the milk sample was aseptically inoculated in Todd Hewitt Broth (THB) which is an enrichment media for Streptococci. These were aerobically incubated at 37 ± 1 ^{0}C and examined after 24 ± 4 hrs. If no significant growth was observed on any of the BA and EA plates, THB enrichment cultures were sub-cultured on BA and EA, and incubated at 37 ± 1 ^{0}C for 18-24 hrs.

Preliminary identification of mastitis bacteria

The level of growth, colony morphology and colour, type of haemolysis, Esculin hydrolysis and gram stain reaction, were recorded. To differentiate Staphylococcus and Streptococcus spp, catalase reaction was performed on all Gram-positive cocci samples employing the rapid slide technique described by Cheesbrough (2000). A drop of hydrogen peroxide was placed on a slide, a test colony introduced on the drop and observed for bubbling. Catalase negative reaction indicated presence of Streptococcus spp and the colony was purified on BA and EA for further analysis.

Lancefield grouping of ß-haemolytic Streptococci

ß-haemolytic catalase negative gram-positive Esculin hydrolysis negative cocci on EA were tested for presence of Lancefield groups polysaccharide antigen B, C, F, G in the cell wall using the Oxoid Strep Plus Kit (OXOID latex reagent types B(No DR0587G), C (No DR0588G), F (No DR0590G), G (No DR0591G), strep plus polyvalent control (No 0579M) according to the manufacturers protocol. Briefly, an instant room temperature nitrous acid extraction procedure was employed to extract group specific antigens from the colonies, the extracts neutralised and antigens identified visually by agglutination.

Data analysis

Streptococcus strains isolated

Using the results from the laboratory examinations, summary (descriptive) statistics on the prevalence of mastitis and ß-haemolytic Streptococcus groups in milk samples were

generated. Mastitis prevalence was calculated as number of CMT positive camels divided by total number of camels sampled. All descriptive analyses were done in Excel® software (Microsoft Corporation, USA).

Quantification of milk loss to subclinical mastitis

Quantification of milk loss was analysed using analysis of variance (Fischer, 1925) whereby presence or absence of mastitis as defined by a positive CMT score of 1-3 in at least one of the quarters was used as a grouping variable and the quantity of milk collected as the dependent variable. Significant differences in milk production between mastitic and non-mastitic quarters were identified based on $p<0.05$.

Results

Prevalence of camel mastitis

Two hundred and five lactating camels drawn from seventeen herds were examined in this study. Although the quarter was the sampling unit, a camel was considered mastitis positive if at least one of the quarters reacted positive in the CMT. Eighty seven per cent (87.3 %) of camels examined had sub-clinical mastitis and per herd prevalence ranged from 65-100 % (Table 1).

Prevalence of streptococcal organisms in milk

A total of 383 CMT positive milk samples were transported to the laboratory but only 281 samples (73.4%) were analysed for presence of ß-haemolytic Streptococci. Of these 281 milk samples, 270 produced growth on Edwards Agar (EA). Of the 270 EA cultures 243 yielded catalase negative gram positive cocci. But only 186 of these 243 cultures could be typed according to Lancefield. Table 2 shows the Lancefield grouping results of the 186 streptococcal isolates: GBS - 119/186 (64%), GCS - 10/186 (5.4%), GFS - 1/186 (0.5%), GGS - 4/186 (2.2%) and untypable ß-haemolytic Streptococci (57/243; 23%).

Milk reduction and economic loss due to camel mastitis

Four hundred and twenty (420) pair wise comparisons were obtained from randomly selected camels that were subjected to a CMT test. Milk loss in mastitic quarters was 0.114L and 0.172L equivalent to Ksh6.84 and Ksh10.32 for fore and hind quarters respectively per camel per day. Regardless of quarter, loss in milk production was significant ($F = 30.51$, $p<0.001$).

Discussion

The high prevalence of GBS in camel population in Isiolo and Marsabit counties of Kenya could probably pose high risk of transmission to consumers and handlers of raw camel milk. *Streptococcus agalactiae* is primarily a commensal bacterium in gastrointestinal and genitourinary tracts of healthy adults with a worldwide genotypic diversity of human isolates corresponding to a number of clonal complexes (CCs) which colonize humans (Schuchat, 1998; Brochet *et al.,* 2009; Huber *et al.,* 2011). It is a major cause of neonatal morbidity and mortality (Gibbs *et al.,* 2004), vaginitis (Maniatis *et al.,* 1996; Romanik *et al.,* 2007; Abdul-Rahman *et al.,* 2008) and purulent vaginal discharge in humans (Clark and Atendido, 2005). Indeed, genitourinary tract GBS infection is an emerging medical problem among people with underlying medical conditions (Schuchat, 1998; Scanziani *et al.,* 1999; de Steenwinkel *et al.,* 2008), whose carriage, distribution and impact varies strongly with geographical region (de Steenwinkel *et al.,* 2008; Zadoks and Fitzpatrick, 2009).

Since *S. agalactiae* has a broad host tropism and is pathogenic to humans (Elliott *et al.,* 1990), our results are of great medical concern. GBS isolated from several animal hosts show common ancestry (Elliott *et al.,* 1990) and in particular camel GBS strains from Kenya are closely related to human strains (Younan, *et al.,* 2000). However, a molecular study on the zoonotic potential of GBS from camels in Kenya and Somalia found a clear genetic distinction between human and camelid *S. agalactiae* populations (Liljander *et al.,* 2013). This notwithstanding, investigation of the impact of high level of milk contamination with GBS infection in human consumers to elucidate camel-human transmission dynamics of *S. agalactiae* infections in Kenya is necessary.

Table 1: Prevalence rates of mastitis in camel herds in Isiolo and Marsabit counties

County	Herd	Sample size	Prevalence	Percentage
Isiolo	1	10	8/10	80
	2	10	9/10	90
	3	7	6/7	86
	4	5	5/5	100
	5	21	19/21	90.5
	6	25	24/25	96
	7	13	12/13	92
	8	12	11/12	91.7
	9	12	10/12	83
	10	19	18/19	94.7
	11	16	14/16	87.5
	13	6	5/6	83
	14	8	7/8	87.5
Marsabit	15	3	3/3	100
	16	5	5/5	100
	17	10	8/10	80
Total		**205**	**179**	**87.3**

Table 2: Prevalence rates of mastitis and ß-haemolytic Streptococcus groups in milk samples

		Prevalence (%)		
CMT positive (n=281)	*GBS (n=186)*	*GCS (n=186)*	*GFS (n=186)*	*GGS (n=186)*
87.3%	**64%**	**5.4%**	**0.5%**	**2.2%**

GBS = B-antigen; GCS= C-antigen; GFS= F-antigen; GGS = G-antigen positive samples respectively. CMT = California Mastitis Test.

Data implied an endemic status of camel mastitis. Elsewhere, high prevalence of camel mastitis has been reported in Saudi Arabia (Salehu and Fayer, 2011), Jordan (Hawari and Hassawi, 2008), traditionally managed camels in Northern Kenya (Matofari *et al.,* 2003) and eastern and North Eastern Ethiopia (Mengistu *et al.,* 2010; Seifu and Tafesse, 2010). The mastitis-associated reduction in camel milk production we observed corroborates earlier findings (Younan *et al.,* 2001; Matofari *et al.,* 2005). Pathogenic and environmental streptococci are known to induce remarkable milk yield reduction in lactating camel (Matofari *et al.,* 2005). Indeed sub-clinical mastitis due to *S. agalactiae* impacts greatly on milk production in cattle and camels and loss of intact teats due to destruction of the gland tissue (Younan *et al.,* 2001).

The wide tissue tropism of *S. agalactiae* infections in camels suggest that its epidemiology may be different from that in cattle thus requiring specific control schemes (Younan *et al.,* 2000; Younan and Bornstein, 2007). Unlike in cattle where post-milking teat dip and dry cow therapy have significantly lowered somatic cell count associated with *S. agalactiae* (Keefe, 1997), the anatomy of camel udder limits effectiveness of common antibiotics to treat camel mastitis. Indeed, *S. agalactiae* intra-mammary infections in camels have been traced over prolonged periods with no spontaneous cure (Younan *et al.,* 2001). This scenario provides a sustained focus of infection complicating the epidemiology and control of the disease in camels. Short term mitigation against physical factors, hygienic handling of camel milk, provision of transport

or infrastructural facilities and veterinary personnel, use of CMT screening kits to segregate milk from animals with sub-clinical mastitis and provision of inputs like aluminium cans and cooling points can reduce mastitis prevalence (Akweya, 2012). Considering the importance of the camel as a source of livelihood, the poor hygiene in arid and semi-arid lands (ASALs) and ineffectiveness of common antibiotics, a more sustainable approach to control using vaccine is recommended and should be explored (Maione *et al.,* 2005; Rosini *et al.,* 2006; Margarit *et al.,* 2009).

Impact

This work has highlighted the high level of GBS infection in camels that sustains a focus of infection. The potential for camel-human transmission in the camel keeping community is unknown yet Streptococcus agalactiae causes serious health problems in humans. This leads to a public health concern for proper treatment of camel milk intended for human consumption. Understanding of the inherent difficulties of GBS mastitis control in camels is the reason for recommending search for a vaccine. Adoption and exploration of recommendation given in this manuscript can deliver safe milk and hopefully transform the economies of camel keeping counties in Kenya.

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CO-ADMINISTRATION OF ALBENDAZOLE AND LEVAMISOLE TO CONTROL MULTIPLE ANTHELMINTIC RESISTANT NEMATODES IN A SHEEP FARM IN KABETE KENYA

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Abstract

Albendazole (ABZ) and levamisole (LEV) were co-administered to evaluate their ability to control natural helminth infections in a sheep farm where resistance to the individual anthelmintic had previously been reported. Thirty two sheep of mixed ages and sex were randomly allocated to four equal groups. Group 1 and 2 were treated with ABZ and LEV respectively. ABZ and LEV were co-administered to group 3 while group 4 was the untreated control. Rectal faecal samples were collected from all the animals on the day of treatment (0 DPT) and fourteen days post-treatment (14 DPT) and the eggs per gram of faeces (EPG) determined. On both sampling occasions, pooled feacal samples from the respective groups were separately cultured for strongyle larval stage three (L3) identifications. Anthelmintic efficacies were evaluated based on faecal egg count reduction percentage (FECR%). Resistance to both drugs was still evident at FECR% of 71% and 75% for LEV and ABZ respectively. Co-administration of the two drugs resulted in a higher efficacy at 95.4% FECR %. L3 recovered from faecal culture 14DPT indicated that Haemonchus spp. survived treatments with ABZ and LEV given separately and when co-administered. *Trichostrongylus spp*. survived the LEV treatment but was highly susceptible to ABZ given alone or when co-administered with LEV, while Oesophagostomum species survived ABZ given separately. Combining these two drugs may therefore offer a temporary solution in helminth control on the farm as other control measures are sort.

Key words: Efficacy, resistance, albendazole, levamisole, co-administration

ADMINISTRATION CONCOMITANTE DE L'ALBENDAZOLE ET DU LÉVAMISOLE POUR LE CONTRÔLE DE PLUSIEURS NÉMATODES RÉSISTANTS AUX ANTHELMINTHIQUES DANS UNE FERME OVINE À KABETE (KENYA)

Résumé

L'albendazole (ABZ) et le lévamisole (LEV) ont été administrés concomitamment pour évaluer leur capacité à contrôler les infections par les helminthes naturelles dans une ferme ovine où la résistance aux anthelminthiques individuels avait déjà été signalée. Trente-deux ovins d'âges mixtes et des deux sexes ont été affectés de manière aléatoire à quatre groupes de même taille. Les Groupes 1 et 2 ont été traités respectivement avec l'ABZ et le LEV. L'ABZ et le LEV ont été administrés simultanément au Groupe 3, tandis que le Groupe 4 a servi de témoin non traité. Des prélèvements rectaux de matières fécales ont été faits sur tous les animaux le jour du traitement (0 DPT) et quatorze jours après le traitement (14 DTC), et les numérations d'œufs par gramme de fèces (EPG) ont été déterminées. Aux deux occasions d'échantillonnage, des prélèvements composites des divers groupes ont été mis en culture séparément pour les identifications de strongles au troisième stade larvaire (L3). Les efficacités des anthelminthiques ont été évaluées sur la base du pourcentage de réduction du nombre d'œufs fécaux (% FECR). La résistance aux deux médicaments était toujours évidente au FECR de 71% et de 75%, respectivement pour le LEV et l'ABZ. L'administration concomitante des deux médicaments s'est révélée très efficace, avec un FER de 95,4%. La L3 récupérée à partir de la culture fécale à 14DPT a indiqué que la Haemonchus spp a survécu aux traitements à l'ABZ et LEV administrés séparément et même à l'administration concomitante des deux produits. L'espèce *Trichostrongylus spp* a survécu au traitement LEV mais était très sensible à l'ABZ

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administré seul ou en cas d'administration concomitante avec le LEV, tandis que l'espèce Oesophagostomum a survécu à l'ABZ administré séparément. L'association de ces deux médicaments peut donc être une solution temporaire de lutte contre les helminthes à la ferme en attendant que d'autres mesures de contrôle soient déterminées.

Mots-clés : efficacité, résistance, albendazole, levamisole, administration concomitante

Introduction

Gastrointestinal parasitism is one of the most important disease complexes of sheep. Over the past several decades, the parasites have been controlled through the use of anthelmintics, but the emergence of anthelmintic resistance (AR) has threatened this chemotherapeutic approach. In some countries anthelmintic resistance has reached alarming proportions in small ruminant industry, where numerous reports indicate widespread resistance to one or more anthelmintics1,2,3,4. In the farm under investigation, multiple anthelmintic resistances to LEV, ivermectin (IVM), levamisole – rafoxanide combination and albendazole had been earlier reported5. Treatment of animals with a combination of drugs from different classes where resistance to the individual drug exists has resulted in improved efficacy. In some countries, the combined drugs are available as commercial formulations4. Where this is not available, the different classes of drugs may be co-administered at dose rates recommended by the individual manufacturers. On farms with multiple resistance, the specific nature of resistance will determine whether the combination drenches are effective or not and only drench testing can determine this. The main goal of the current trial was therefore to compare the efficacy of ABZ and LEV given either separately or co-administered to sheep naturally infected with GI nematodes resistant to both drugs.

Materials and Methods

Study site and worm control history

The study was carried out on a farm in Kabete, 20 Km west of Nairobi. The sheep enterprise on the farm consisted of 120 dorpers kept permanently on the farm and grazed in the same paddocks for 2 years. The animals had been moved to these paddocks after the previous ones were found to be highly contaminated and the helminths resistant to a number of anthelmintics previously used for their control[5].

Experimental animals and sampling

Initially, rectal faecal samples were collected from the entire flock examined for the presence of helminth eggs, and a modified McMaster technique as described in the MAFF6 manual used to determine the eggs per gram (EPG) of faeces. Thirty two animals with at least 100 epgs were identified and randomly assigned to four groups with equal numbers. Group 1 animals were given a drench of albendazole (Valbazen® Ultravetis East Africa Ltd, Nairobi, Kenya), group 2 were injected subcutaneously with levamisole (Levacide® Norbrook Laboratories Ltd, Karuri, Kenya) and in group 3 the two drugs were co-administered at the manufactures recommended dose rates. The fourth group remained as untreated control. Rectal faecal samples were again collected from the selected animals on the day of treatment (0DPT) and 14 day post treatment (14DPT) then processed as earlier described. FECR% and the 95% confidence limit for the reduction were calculated according to the method described in the World Association for the Advancement of Veterinary Parasitology (WAAVP7. AR was declared when the FECR% was less than 95 % and the lower 95 % confidence limit was less than 90 %. Analysis of variance was used to compare the FECR% and the 95% CI for the different drug treatments. Pooled faecal samples from the respective groups were cultured on both sampling occasions for strongyle L3 identification.

Table 1: The Faecal Egg Count Reduction Percentage (FERC%) and its Confidence Interval (CI) calculated according to the method of the World Association for the Advancement of Veterinary Parasitology (WAAVP) developed by Coles *et al.,* (1992).

Treatment	Arithmetic mean eggs per gram (EPG) of faeces (Range)						
	Pre-treatment (day 0)		Post-treatment (day 14)				
	Control	Treated	Control	Treated	FECR%	95% CI	Remarks
ABZ	4086 (300-10000)	2750 (100-7900)	1350 (100- 3100)	338 (0 - 1300)	75	61.2 – 83.9	Resistant
LEV	4086 (300-10000)	5800 (1200-12000)	1350 (100 - 3100)	386 (0-2400)	71	40.9 – 86.2	Resistant
ABZ + LEV	4086 (300-10000)	2975 (100-9100)	1350 (100 - 3100)	63 (0-300)	95.4	92 – 97.3	Susceptible

Key: ABZ = Albendazole, LEV = Levamisole

Table 2: The percentage composition of third stage strongyle larvae recovered from pooled pre-treatment and post – treatment faecal cultures

Day Post-treatment (DPT)	Strongyle genera	Percentage composition of third stage strongyle larvae			
		Control	ABZ	LEV	ABZ + LEV
DPT = 0	*Haemonchus*	60	61	62	65
	Trichostrongylus	30	27	25	21
	Cooperia	7	3	5	4
	Oesophagostomum	3	7	8	7
	Nematodirus	-	2	-	3
DPT = 14	*Haemonchus*	63	90	56	97
	Trichostrongylus	25	5	40	3
	Cooperia	8	2	3	-
	Oesophagostomum	4	3	1	-

Key: ABZ = Albendazole, LEV = Levamisole

Results

The results of the FECR % and their corresponding 95 % CI are shown in Table 1. The FECR % indicated resistance to ABZ (75%) and LEV (71%) when administered separately and susceptibility when the two drugs were co-administered (95.4%). Co-administration of the two drugs resulted in a significant increase ($p < 0.05$) in the FECR% and the 95% CI (92 – 97.3%) compared to that of the individual drugs. The percentage composition of L3 recovered from faecal culture of pooled samples at 0DPT and 14DPT are shown in Table 2. Haemonchus spp. survived treatments with ABZ and LEV given separately and when co-administered. *Trichostrongylus spp*. survived the LEV treatment but was highly susceptible to ABZ given alone or when co-administered with LEV, while Oesophagostomum species survived ABZ given separately.

Discussion

Modern anthelmintics are used at an efficacy of about 99% against susceptible strains3. The World Association for the Advancement of Veterinary Parasitology

(WAAVP) recommends that, AR is declared when the FECR% is less than 95 % and the lower 95 % confidence limit is less than 90 % 7. In the current study, the results of the FECR% for ABZ and LEV when administered separately were less than 95 % and the lower 95 % confidence limit were less than 90% an indication that resistance to these drugs as earlier reported5 still persisted in the farm. Once AR has established, there is no evidence of reversing to susceptibility even after prolonged withdrawal of the drug8. This was evident in this farm where ABZ had been withdrawn for the previous two years. It is important therefore that management practices that prolong the effectiveness of all classes of anthelmintic be applied at all times.

Resistance to multiple classes of anthelmintic commonly used in control of sheep endoparasites is common in many parts of the world3,4,9. Resistance to benzimidazoles (BZ) is common to Haemonchus contortus, Trichostrongylus colubriformis and Telardosagia circumcincta. Resistance to LEV is relatively rare in Haemonchus contortus, but common in *T. colubriformis* and T. circumcincta9. The results of this study are in agreement with these observations as the faecal cultures showed Haemonchus as the main parasites resistant to ABZ and LEV given separately and when co-administered. *Trichostrongylus spp*. showed resistance to LEV treatment but showed susceptibility to ABZ given separately and when the two drugs where co-administered.

Treating simultaneously with 2 drugs from different anthelmintic classes is one of the methods of preventing the development of AR and can prolong their use for over 20 years10. However, once resistance alleles accumulate in worm populations, this strategy will probably not be successful. Nevertheless, treatment with 2 drugs of different anthelmintic classes where resistance to the individual drug exists can still be of great benefit. Compared with individual drug effects, anthelmintics of different chemical classes administered together induce a synergistic effect, resulting in clinically relevant increases in the efficacy of treatment. This synergistic effect is most pronounced when the level of resistance is low. Once high-level resistance to both drugs is present, the synergistic effect is unlikely to produce acceptable levels of efficacy. In the present study, the FECR% for ABZ (75%) and LEV (71%) when administered separately suggest a fairly low level of resistance. The synergistic effects when the two drugs were co-administered greatly raised the FECR% (95.4%) and 95% CI (92 – 97.3) and thus produce acceptable levels of efficacy. Co-administrations of the 2 drugs may therefore offer a temporary solution in helminth control on the farm as other control measures are sort.

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SURVEY OF DISEASE STATUS OF CHICKENS IN SOME POULTRY FARMS IN SOUTH-WEST NIGERIA

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Abstract

A survey of health status of chickens in poultry farms in some local government areas (LGA) of Ogun State was carried out to determine the common chicken disease. Structured interview guides were administered through stratified and random sampling in six LGAs of Ogun state, viz. Odeda, Ewekoro, Ifo, Obafemi-Owode, Abeokuta South and Abeokuta North LGA, respectively. A total of 80 respondents who were the owners of the Poultry Farms were successfully interviewed and data collected were subjected to descriptive statistics to establish prevalent chicken diseases, and Chi-square analysis to determine associations between socio-economic characteristics of respondents and disease status of their flock. Coccidiosis was the major cause of ill-health in flocks. Significant relationships were established as follows: access to extension services and flock size (p=0.001), extension services and feeding of birds (p=0.001), extension services and shelter (p=0.003), extension services and healthcare (p=0.006), extension services and total sick (p=0.001), extension services and action on sick (p=0.044), access to veterinary services and flock size (p=0.003), veterinary services and feeding (p=<0.001), veterinary services and sheltering (p<0.001), veterinary services and healthcare (p<0.001), veterinary services and action on sick birds (p<0.001). It was concluded that socio-economic characteristics of farmers in the study area could influence health status of chickens.

Key words: Disease surveillance, poultry management, socio-economic characteristics.

ENQUÊTE SUR LA SITUATION SANITAIRE DES POULETS DANS CERTAINES FERMES AVICOLES DU SUD-OUEST DU NIGERIA

Résumé

Une enquête sur la situation sanitaire des poulets de fermes avicoles de certaines collectivités locales de l'Etat d'Ogun a été effectuée dans le but de déterminer les maladies courantes du poulet. Des guides d'entrevue structurés ont été administrés par échantillonnage stratifié et aléatoire dans six collectivités locales de l'Etat d'Ogun, à savoir : Odeda, Ewekoro, Ifo, Obafemi-Owode, Abeokuta Sud et Abeokuta Nord. Au total, 80 répondants, propriétaires de fermes avicoles, ont été interviewés avec succès, et les données recueillies ont été soumises à des statistiques descriptives dans le but de déterminer les maladies de volailles répandues, et à l'analyse du chi-carré pour déterminer les relations entre les caractéristiques socio-économiques des répondants et la situation sanitaire de leurs troupeaux. La coccidiose est la principale cause du mauvais état de santé des troupeaux. Des relations significatives ont été établies de la manière suivante : l'accès aux services de vulgarisation et la taille des troupeaux (p = 0,001), les services de vulgarisation et l'alimentation des oiseaux (p = 0,001), les services de vulgarisation et le logement (p = 0,003), les services de vulgarisation et les soins de santé (p = 0,006), les services de vulgarisation et l'ensemble des oiseaux malades (p = 0,001), les services de vulgarisation et l'action sur les malades (p = 0,044), l'accès aux services vétérinaires et la taille des troupeaux (p = 0,003), les services vétérinaires et l'alimentation (p = <0,001), les services vétérinaire et le logement (p <0,001), les services vétérinaires et les soins de santé (p <0,001), les services vétérinaires et l'action sur les oiseaux malades (p <0,001). Il a été conclu que les caractéristiques socio-économiques des aviculteurs dans la zone d'étude étaient susceptibles d'influencer l'état de santé des poulets.

Mots-clés : surveillance des maladies, gestion avicole, caractéristiques socioéconomiques

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Introduction

Animal production in general and chickens in particular play important socio-economic roles in developing countries. Food securities, generation of income and religious/ cultural considerations are amongst the major reasons for keeping chickens. Chickens are the most widely distributed of all livestock species in Nigeria with a population of 166 million birds (FAOSTAT, 2007). The poultry sub-sector is the most commercialized of all the sub-sectors of Nigerian agriculture with those that are commonly reared being chickens, ducks, guinea fowls, turkeys, pigeons and more recently ostriches (Adene and Oguntade, 2006). Those that are of commercial or economic importance given the trade in poultry, however, are chicken, guinea fowls and turkeys, amongst which the chickens predominate (Adene and Oguntade, 2006), so much so that the term poultry is often taken by most people to refer to chickens.

Nigeria is a country with heavy human population and this population is continuously on the rise. This increase has led to the high demand for the available animal and poultry products in all parts of the country. Among the cheapest and highly affordable protein sources for this teeming population are poultry products (meat and egg). There is a really good rationale why chicken is so popular just about everywhere. Poultry meat is regarded as the most diet-friendly of all of the other meats. It is an abundant source of easily digestible proteins, vitamins and minerals. Chicken white meat is the most useful meat type – with minimal amount of fats, it is second only to fish. The protein amount of chicken white meat is equal to that of beef or pork, but it cooks considerably faster. Chicken is filling but in addition the meat is easily digested (Devriendt, 2012).

As lucrative as the business of chicken farming is, it is not without its drawbacks. Like any venture, it is faced with a number of constraints, chief of which is the susceptibility of chickens to a wide array of diseases (Damerow, 1994). Disease cause severe economic losses in poultry production. The loss is not only due to the death of birds but also loss in production. A farmer should always therefore remain on the alert to notice any symptom evinced by the flock so that control or treatment measures can be initiated early and the loss minimized (Prabakaran, 2003). Infrastructural capacity to diagnose the main causes of disease losses accurately will therefore prove necessary for countries seeking to develop a sustainable poultry industry (Bagust, 2008).

A health survey is an important tool that can be used by the government and agricultural bodies to investigate into the disease status of livestock in a given area which would in turn enable them sensitize and make farmers aware of these diseases. Merriam Webster Online Dictionary (2011) defines a survey as an act of querying (someone) in order to collect data for the analysis of some aspect of a group or area. The minimization of outbreaks of chicken diseases is very important for further improvement and development of the chicken farming industry in Nigeria, which would invariably bring about increased availability of chicken products and enhance the diet of Nigerians. Conducting a survey of chickens on poultry farms in some local government areas of Ogun State, to determine the disease status of the domestic fowls in this geographical location, which was the aim of this study, will be the first step towards solving the problems facing poultry health in the state and in the nation as a whole.

Materials and Methods

The study area of the survey comprised six local government areas of Ogun State viz.: Abeokuta North, Abeokuta South, Ewekoro, Ifo, Obafemi-Owode and Odeda Local Government Areas, all in the old Egba division of Ogun State in southwest Nigeria. It lies below the Olumo Rock, home to several caves and shrines (Wikipedia, 2011).

Poultry farming is a well-embraced business venture in Ogun State, with a sizeable number of farms established and a high percentage of these farms specialized in production of chicken (Gallus gallus domesticus). Average number of birds for small farm size is 301, for medium farm size is 740, while that of large size is 2,288.

Data Collection: The data for the survey was collected with the use of well structured questionnaires from extension agents and chicken farmers of selected poultry farms in the study area. Due to unequal distribution of poultry farms in the six local government areas under study, 14 farms were surveyed in Odeda, 10 in Ewekoro, 17 in Ifo, 19 in Obafemi-Owode, 10 in Abeokuta North, and 10 in Abeokuta South, bringing the total number of respondents to eighty (80). The method of sampling was both stratified and random, that is, the study area was stratified into local governments and farms selected randomly within each local government. The structured interview guides was divided into three sections to acquire information from farm personnel on the following: Socio-economic status of respondents; Management information on the birds; Disease status of the birds.

Data Analysis: Data analysis was done with the use of analytical tools (the Chi-Square) Test Statistics of the SPSS 17 Package (2008). Descriptive Statistics Tools of the same package was used to determine the most common of the diseases identified in all of the farms visited, and to establish the relationship between the socio-economic status of respondents and disease status/management of the birds.

Results and Discussion

Common Clinical Signs in the Study Area

Figure 1: shows the frequency of observed clinical signs in the farms visited. Weight loss has the highest incidence – 34, followed by anorexia and bloody diarrhoea (29 and 26 respondents, respectively). Respiratory problems were identified by 21 respondents, discoloured faeces by 18, stunted growth by 17, immobility and inactivity by 10, and limb paralysis by 7 respondents. Limb paralysis is the least observed with just (7). Two listed other clinical signs and 16 said they saw no disease symptoms in their flock.

As shown in Figure 2, Coccidiosis was the disease most identified by the farmers and therefore has the highest probable prevalence in the study area (35%) followed by Chronic Respiratory Disease at 13.8%, Newcastle Disease at 6.3%, Gumboro and Salmonellosis at 3.8%, respectively. The least prevalence was shown by Fowl Pox at 1.3%; 35% of the respondents claimed to have identified no diseases in their flock. This result is almost similar to a retrospective study carried out in Sokoto State, Nigeria between 2004 and 2008 in which it was reported that out of a total of 1,034 cases recorded, 337 (32.9%) was Infectious Bursal disease (IBD), 147 (14.0%) was coccidiosis, while 128 (12.4%) and 102 (9.9%) were Newcastle disease (NCD) and Fowl typhoid (FT), respectively (Adamu *et al.,* 2009).

Disease Status of Chickens in the Study Area

Abeokuta-North Local Government Area: Total numbers of birds on farms visited in Abeokuta-North LGA were 40,689 and number of sick birds was 140 (Figure 3). Weight loss was the common clinical sign identified while the most common probable chicken disease was Coccidiosis.

Abeokuta-South Local Government Area: Total numbers of birds surveyed in Abeokuta South were 18,627 and number of sick birds, 182 with Ibara being the location with the highest number of sick birds (Figure 3). Anorexia, inactivity, diarrhoea and discoloured faeces showed the same degree of prevalence among the clinical signs. Coccidiosis was also the most common probable disease.

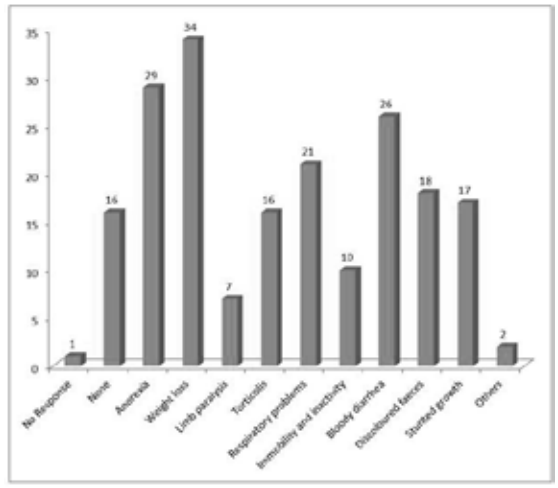


Figure 1: Frequency of clinical signs in the study area

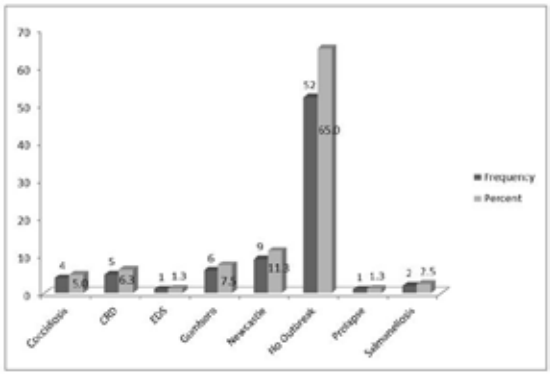


Figure 2: Probable disease outbreaks in the study area

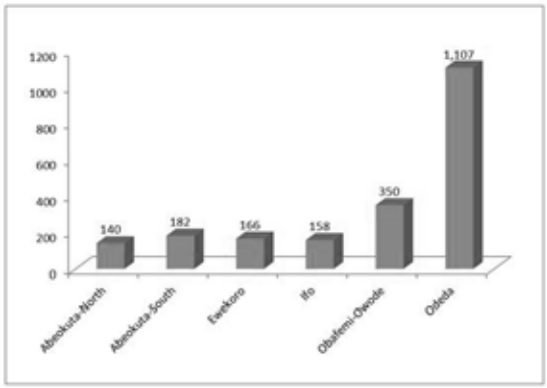


Figure 3: Number of Sick Birds in the Study Area

Ewekoro Local Government Area: Total number of birds on farms surveyed in Ewekoro Local Government Area was 10,086 and the total number of sick birds was 166 as seen in Figure 5. Diarrhoea was the most prevalent clinical sign and coccidiosis was the probable disease of highest prevalence.

Ifo Local Government Area: Total number of birds on farms surveyed in this local government area was 52,795 with the number of sick birds totalling 158. The two farms visited in Atan had the highest number of incidence of sick birds. Respiratory problem was the common disease symptom followed by stunted growth. Coccidiosis was the most common probable disease.

Obafemi-Owode Local Government Area: Total numbers of birds surveyed in the LGA were 90,640 and number of sick birds was 350. Weight loss was the common clinical sign while the most common probable disease was also coccidiosis.

Odeda Local Government Area: The total number of birds on all farms visited in Odeda local Government Area was 82,435 while the total number of sick birds was 1,107 (Figure 5). The high incidence of disease was Chronic Respiratory Disease. Weight loss was the most common clinical sign and the most common probable disease was coccidiosis.

Table 1: Chi-Square Analysis of Relationships between some Socio-economic Characteristics of Respondents and Disease Status/Management of their Flock

The chi-square analysis of the relationship between the socio-economic characteristics of the respondents and disease status/management of flock (Table 1) revealed that there was no significant relationship between the following: age of respondent and total birds, shelter, health services, total sick, action on sick, common diseases; sex of respondents and total birds, total sick, disease outbreak, common disease; marital status and total sick, action on sick, disease outbreak, common disease; level of education and total sick, disease outbreak; family size and all factors except disease outbreak; years in business and all factors; extension services and disease outbreak, common disease; veterinary services and total sick, disease outbreak, common disease; reason for business and total sick, common disease, respectively ($P > 0.05$).

However, there were significant relationships between age of respondent and feeding of birds ($P=0.044$), age of respondent and disease outbreak ($P=0.022$), sex of respondent and feeding of birds ($P=0.002$), sex of respondent and sheltering of birds ($P=0.003$), sex of respondent and healthcare provision ($P=0.023$), sex of respondent and action on sick birds ($P=0.047$), marital status of respondent and flock size ($P=0.008$), marital status of respondent and feeding of birds ($P<0.001$), marital status of respondents and sheltering of birds ($P<0.001$), marital status of respondents and healthcare provision ($P=0.001$), educational level of respondents and flock size ($P=0.003$), educational level of respondent and feeding, sheltering, healthcare provision ($P<0.001$), educational level of respondent and action on sick birds ($P=0.003$), family size and disease outbreak ($P=0.018$), access to extension services and flock size

Table 1: Chi-Square Analysis of Relationship between Socio-economic Characteristics of Respondents and Disease Status/Management

Socio-Economic Characteristics	Total Birds	Feeding	Shelter	Health Services	Total Sick
Age	9.464 (P=0.149)	9.812 (P=0.044)*	5.166 (P=0.271)	2.205 (P=0.698)	6.197 (P=0.401)
Sex	3.519 (P=0.318)	12.555 (P=0.002)*	11.510 (P=0.003)*	7.525 (P=0.023)*	0.983 (P=0.805)
Marital Status	17.527 (P=0.008)*	21.497 (P<0.001)*	20.245 (P<0.001)*	18.382 (P=0.001)*	9.976 (P=0.126)
Level of Education	30.169 (P=0.003)*	47.765 (P<0.001)*	52.357 (P<0.001)*	46.731 (P<0.001)*	10.585 (P=0.565)
Family Size	37.996 (P=0.150)	31.014 (P=0.055)	15.985 (P=0.718)	23.957 (P=0.244)	18.393 (P=0.952)
Years in Business	14.987 (P=0.242)	5.845 (P=0.483)	6.422 (P=0.600)	3.394 (P=0.907)	12.589 (P=0.400)
Ext. Services	27.487 (P=0.001)*	23.277 (P=0.001)*	19.884 (P=0.003)*	18.167 (P=0.006)*	29.394 (P=0.001)*
Vet. Services	28.844 (P=0.003)*	56.233 (P<0.001)*	43.093 (P<0.001)*	57.576 (P<0.001)*	13.962 (P=0.124)

**Significant (P<0.05)*

(P=0.001), extension services and feeding of birds (P=0.001), extension services and shelter (P=0.003), extension services and healthcare (P=0.006), extension services and total sick (P=0.001), extension services and action on sick (P=0.044), access to veterinary services and flock size (P=0.003), veterinary services and feeding (P=<0.001), veterinary services and sheltering (P<0.001), veterinary services and healthcare (P<0.001), veterinary services and action on sick birds (P<0.001).

Conclusion

From the study, it was concluded that Coccidiosis is the most prevalent of the probable disease, irrespective of scale of production, age, sex or level of education of farmers as shown by the lack of significant relationship between the socio-economic characteristics of farmers and common diseases. High prevalence of coccidiosis may be due to the lack of vaccination programmes against the disease. The weight loss, anorexia and bloody diarrhoea high frequency could be associated with Coccidiosis infection. The survey also revealed that the socio-economic status of farmers had an impact on flock management in areas of healthcare provision, sheltering and feeding.

Impact

The occurrence of poultry disease in this study area is highly correlated with the socio-economic status of the farmer. The access of the farmers to extension services and provision of veterinary care was a great advantage in the curbing of prevalent diseases and thus a better health status of the birds. This will mean a better performance of the birds, food security and thus a higher profitability, and poverty alleviation amongst farmers.

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LEUKOCYTE PROFILE OF DIFFERENT BREEDS OF THE NIGERIAN CATTLE IN HAEMOPARASITIC INFECTION

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Abstract

An examination of the leukocyte profile of different breeds of cattle was carried out to determine the effects of haemoparasites, sex and breed on the leukocytes. The effects of haemoparasite, sex and breed on the leukocytes were determined using parasitological methods. A total of 452 cattle comprising of 174 cows and 278 bulls were examined. The breeds included 78 'Red Bororo', 14 'Sokoto Gudali' and 360 'White Fulani'. The total white blood cell (WBC) count and differential counts of WBC were determined using Neubauer Chamber and Thin blood smear techniques. Data generated were analysed using Genstat statistical software, with sex, breed and haemoparasite species detected as factors. Results showed that 22% of the cattle were infected with haemoparasites while 78% were parasite-free. The parasites found were *Trypanosoma congolence* (4%), *Anaplasma centrale* (2%), *Babesia bovis* (14%), *A. centrale* + *B. bovis* (1%) and *Babesia divergens* (1%). Parasite species identified significantly did not influence ($P<0.001$) the WBC and the differential counts (neutrophils, basophils, eosinophils, lymphocytes and monocytes). Since there is fluctuation of the various fractions of the leukocytes in the course of haemoparasite infection, this may explain for the insignificant influence of the factor (haemoparasite) on the mean values of the leukocytes.

Key words: Differential counts, Nigerian cattle breeds, Parasitology.

PROFIL LEUKOCYTAIRE DES DIFFÉRENTES RACES DE BOVINS NIGÉRIANS DANS LES INFECTIONS HÉMOPARASITAIRES

Résumé

Un examen du profil leucocytaire de différentes races de bovins a été réalisé pour déterminer les effets des hémoparasites, du sexe et de la race sur les leucocytes. Les effets des hémoparasites, du sexe et de la race sur les leucocytes ont été déterminés en utilisant des techniques parasitologiques. Au total, 452 bovins comprenant 174 vaches et 278 taureaux ont été examinés. Les races comprenaient 78 « Bororo rouges », 14 « Sokoto Gudali » et 360 « Fulani blancs ». La numération totale des globules blancs (WBC : white blood cell) et les numérations différentielles de WBC ont été déterminées à l'aide des techniques Neubauer et du frottis sanguin mince. Les données générées ont été analysées à l'aide du logiciel statistique Genstat ; et le sexe, la race et les espèces d'hémoparasites ont été détectés comme facteurs. Les résultats ont montré que 22% des bovins étaient infectés par des hémoparasites tandis que 78% n'avaient pas de parasite. Les parasites étaient *Trypanosoma congolence* (4%), *Anaplasma centrale* (2%), *Babesia bovis* (14%), *A. centrale* + *B. bovis* (1%) et Babesia divergens (1%). Les espèces de parasites identifiés n'avaient pas d'influence significative ($P<0,001$) sur la numération de WBC et les numérations différentielles (neutrophiles, basophiles, éosinophiles, lymphocytes et monocytes). L'absence de fluctuation des différentes fractions de leucocytes au cours de l'infection hémoparasitaire peut expliquer l'influence négligeable du facteur (hémoparasite) sur les valeurs moyennes des leucocytes.

Mots-clés : numérations différentielles, races bovines nigérianes, parasitologie.

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Introduction

According to Bourdin (1980) and International Livestock Research Institute (ILRI), attack of farm animals by pest and diseases militate against the expansion of livestock production and could be costly in term of death, reduced performance and curative treatment. For example, diseases such as tick-borne diseases, anaplasmosis and babesiosis cost a total loss of about US$170 million per annum while trypanosomosis cost about US$500 million per annum in Africa (ILRI, 1995). The social losses in product efficiency caused by animal diseases in Nigeria were US$141.5 million dollars between 1984 and 1986 and social losses in consumption of about US$ 660 million dollars in Nigeria.

The diseases of cattle include haemoparasitic infections, ectoparasites, bacterial and viral diseases and others. Amongst these, the haemoparasites have caused a great health problem in cattle. The haemoparasites of importance in cattle are; *Trypanosoma, Babesia, Anaplasma, Theileria, Cowdria* and *Eperithrozoon*. Haemoparasitic diseases transmitted by arthropods caused by various species of *Babesia, Trypanosoma* and *Anaplasma* occur through-out the world but is frequently of greatest importance in the tropics where the maintenance of the vector populations have favourable conditions. In Nigeria, haemoparasitic diseases are of great importance in the cattle industry.

Babesia species are intracellular parasites invading the erythrocytes. These parasites cause the haemoparasitic disease called babesiosis. The disease is transmitted by tick vectors of the species of *Hyalloma* and *Boophilis*. The disease is characterised by anorexia, pyrexia, dehydration, jaundice, high parasitemia, cathexia, pulmonary edema with death sometimes from pulmonary edema (Goffi et *al.*, 1985).

Trypanosoma species are also intracellular parasites of the erythrocytic series. These parasites are transmitted mechanically by haematophagous tabanids flies (*Glossina* species). These parasites cause Animal African Trypanosomiasis (AAT) in animals and Human African Trypanosomiasis (HAT) in humans. Human trypanosomosis is known as sleepin sickness while animal trypanosomosis is known as Nagana (John, 2004).

The AAT and HAT are debilitating diseases in both man and livestock. The clinica signs of the diseases caused by *Trypanosom* organisms are pyrexia, anorexia, dehydration icterus, cachexia, depression, hepatomegaly splenomegaly, generalised lymphadenopath Death results from non-response to treatmen at terminal ailment or lack of therapy (Hussai et *al.*, 1987). This disease causes 55000 human and 3 million livestock deaths annually an hinders mixed farming through reduced worl efficiency of draft animals (Abenga et *al.*, 2002) Trypanosomiasis is responsible for about 80% of Nigerian landscape being unsuitable fo livestock production (Okwelum et *al.*, 2013).

Anaplasma species are equally intra erythrocytic, transmitted by *Hyalomma* an *Boophilus* species (adult and nymph) tick and are also transmitted mechanically by haematophagus flies as reported by Ewin (1981) causing anaplasmosis in ruminants. Th disease is characterised by anaemia, pyrexia i high parasitemia and icterus. This disease cause a very significant loss in cattle production i Nigeria.

In Nigeria, haemoparasitic diseases ar especially important due to its adverse effect in livestock production. In Nigeria, more tha one tick-borne disease occurs and is commo there could be mixed infections of differen haemoparasitic diseases which make it difficul to quantify losses due to haemoparasiti diseases.

Haemoparasites don't have only direc effect on host but also develop man complications in haematology especiall the leukocytes and physiology of cattle. Th destruction done by *Babesia, Trypanosom Anaplasma* species is not only limited t fever and uncontrolled death but ma also be the cause of icterus, haemolysis dehydration, haemoglobinuria, anorexia hyperplasia, pulmonary edema, asphyxia lymphadenopathy, splenomegaly, hepatomegaly immunosuppression (Ferris, 1984; Uilenberg 1998).

A large amount of work has been o haemoparasites and studies on cattle

haematology in the phase of infection by many research works through- out the world but in south-west Nigeria, this subject has not been attended to in the past. In view of this situation, this study is intended to take-up the haematologic studies focusing on the leukocytes of haemoparasite infected Nigerian cattle and establish species of haemoparasites and their effects on the leukocytes (lymphocytes, monocytes, basophils, eosinophils and neutrophils).

Materials and Methods

The research was approval by the Research Committee of the Federal University of Agriculture Abeokuta, Nigeria.

Study Area

The study was carried out in Abeokuta, capital city of Ogun State in South-west Nigeria, Most of the animals slaughtered in the abattoir were originally raised in various parts of northern Nigeria, and have been bought from nomadic/migrant Fulanis as trade cattle in various markets, mostly located in Imeko and Aiyetoro areas of Yewa region of the state. Some cattle traders got their animals from migrant Fulanis who have settled with their animals in the southwest. The cattle were predominantly crosses of White Fulani, Sokoto Gudali and Red Bororo breeds. However, sampled animals were classified based on physical observation of predominant phenotypic traits of pure breeds in the mixed-bred cattle population.

Sampling

A total of four hundred and fifty two (452) blood samples were collected at random from cattle, a sample from each cattle in the abattoir. Before the collection of blood samples, sex and breed of the cattle were recorded. Five millilitres (5ml) of blood was collected at slaughter into labelled specimen bottles that contained anticoagulant, Ethylene Diamine Tetraacetic Acid (EDTA; 1mg/ml of blood). Samples were immediately conveyed in a cold box to the Parasitology laboratory for processing and examination in the College of Veterinary Medicine, FUNAAB, Nigeria.

Examination of the Samples for Blood Parasites

The blood samples were processed and examined using parasitological methods as described by Woo (1970) for diagnosing the presence of blood parasites. Isolates were identified based on morphological and biometrical data, according to Hoare (1972).

Thin Blood Smear

Thin blood smear was made by spreading a drop of blood on the slide forming single cell layer. This was air-dried, labelled and fixed in methanol for 5 minutes. This was followed by staining in 10% Giemsa for 30 minutes, rinsing in physiological buffered solution and air-drying before examining for blood parasites under x100 (oil immersion) microscope.

Blood Cell Count

Total white blood cell counts were obtained using Neubauer Chamber. The differential blood counts were determined as proportion of different types of leucocytes (WBC) on the blood smear and were expressed in percentages.

Statistical Analyses

Animals were categorised in respect of the haemo-parasite species found in their blood and expressed as percentages of the total number of animals in each category to show the prevalence rate for each parasite species. Categories were then referred to as groups. The groups were then subjected to Analysis of Variance (ANOVA) as unbalanced design using the Genstat statistical package (GenStat Release 7.2 DE, Copyright 2007, Lawes Agricultural Trust, Rothamsted Experimental Station). Sex and 'breed' of animals were considered in a two-way ANOVA while parasite species found was considered in a one-way ANOVA. Parameters in percentage (the differential counts of WBC) were subjected to angular transformation before ANOVA to correct for non-normality; however, tabulated means (±standard error of means) were derived from raw values, as measured. Means were separated using Duncan Multiple Range Test to test for significance.

Results

Results showed that 22% of the cattle were infected with haemoparasites while 78% were parasite-free. The parasites were *Trypanosoma congolence* (4%), *Anaplasma centrale* (2%), *Babesia bovis* (14%), *A. centrale* + *B. bovis* (1%) and *Babesia divergens* (1%).

Across the sexes, it was observed that there was no significant ($p>0.05$) difference in the total WBC and the differential counts as shown in Table 1. In the females 174 animals were sampled; WBC was 6±0.1; Neutrophils 29.4±0.09; Lymphocytes 68.9±0.60; Eosinophils 0.9±0.10; Basophils 0.3±0.10; Monocytes 0.5±0.10.

In the males 278 animals were sampled, there was no significant difference ($p>0.05$) in the WBC and all the differential counts. WBC was 6±0.2; Neutrophils 29.5±0.47; Lymphocytes 68.8±0.50; Eosinophils 0.8±0.10; Basophils 0.3±0.04; Monocytes 0.6±0.10.

The breeds sampled were Red Bororo (RB), Sokoto Gudali (SG) and White Fulani (WF). Among the RB, 78 animals were sampled, WBC was 6.0±0.10; Neutophils 29.3±0.87; Lymphocytes 69.2±0.88; Eosinophils 0.6±0.12; Basophils 0.4±0.10; Monocytes 0.5±0.15.

Amongst the SG, 14 animals were sampled; WBC was 6.02; Neutrophils 29.3±0.87; Lymphocytes 69.2±0.88; Eosinophils 0.6±0.12; Basophils 0.4±31; Monocytes 0.1±0.14.

Amongst the WF, 360 animals were sampled; WBC was 6.0±0.2; Neutrophils 29.5±0.41; Lymphocytes 68.7±0.44; Eosinophils 0.8±0.07; Basophils 0.3±0.04; Monocytes 0.6±0.10.

In Table 2 the effect of haemoparasites on the leukocytes is shown.

WBC (X 109/L): *Anaplasma centrali* 6±0.3; *Babesia bovis* 7±0.8; *A. central* + *B. bovis* 5±0.7; *Babesia divergens* 6.0±0.3; *Trypanosoma congolense* 5.0±0.3; Parasite-free 6.0±0.1.

Neutrophils (%): *Anaplasma centrali* 29.9±3.00; *Babesia bovis* 29.1±0.99; *A. central* + *B. bovis* 27.3±4.67; *Babesia divergens* 31.4±6.71; *Trypanosoma congolense* 29.1±0.72; Parasite- free 29.5±0.41.

Lymphocytes (%): *Anaplasma centrale* 68.1±2.95; *Babesia bovis* 69.2±1.01; *A. central* + *B. bovis* 69.3±2.91; *B. divergens* 67.8±7.41; *Trypanosoma congolense* 69.9±1.83; Parasite-free 68.8±0.44.

Eosinophils (%): *Anaplasma centrale* 1.3±0.68; *Babesia bovis* 1.02±0.2; *A. centrale* + *B. bovis* 1.67±1.67; *Babesia divergens* 0.4±0.40; *Trypanosoma congolense* 0.7±0.28; Parasite-free 0.8±0.27.

Basoplils (%): *Anaplasma centrale* 0.2±0.20; *Babesia bovis* 0.2±0.08; *A. central* + *B. bovis* 0.0±0.00; *Babesia divergens* 0.4±0.40; *Trypanosoma congolense* 0.05±0.05; Parasite- free 0.3±0.04.

Monocytes (%): *Anaplasma centrale* 0.4±0.34; *Babesia bovis* 0.5±0.16; *A. central* + *B bovis* 1.7±11.20; *Babesia divergens* 0.0±0.00; *Trypanosoma congolense* 0.3±0.15; Parasite-free 0.5±0.07.

Discussion

The results of this study revealed a 4% prevalence rate for Trypanosome infection, exclusively caused by *Trypanosoma congolense*. The prevalence rate was similar to a 5.9% rate reported for N'Dama in lower Benue river area by Kalu (1995) but lower than 8.5% in Nsukka abattoir (Agu and Akuakonam, 2005). However, there were no significant effects of sex and breed of animals, or parasite species found, on all the blood indices measured. These could be due to the fluctuation in the numbers of the different leukocyte fractions i.e. lymphocytes, monocytes, eosinophils, basophils and neutrophils whose population usually vary with the stage of infection. Total leukocytes may drop initially due to absolute decrease in granulocytes followed by leukocyte response that is characterised by increased number of B cells (two to three folds). The T cells may recover but the monocyte cells often depressed. Sometimes granulocytopenia persists as a result of continued phagocytosis of matured cells and their precursors in the bone marrow accompanied by phagocytosis of matured cells in the spleen, liver and

Table 1: Differential counts of white blood cells (±SEM; ranges in parenthesis) of cattle according to sex and breed.

		WBC (X 109/L)	Neutrophils%	Lymphocytes%	Eosinophils%	Basophils%	Monocytes%
†Sex	N						
Female	174	6±0.1 (3-9)	29.4±0.59 (14-52)	68.9±0.60 (46-85)	0.9±0.10 (0-6)	0.3±0.10 (0-5)	0.5±0.10 (0-8)
Male	278	6±0.2 (3-9)	29.5±0.47 (12-50)	68.8±0.50 (48-88)	0.8±0.10 (0-6)	0.3±0.04 (0-5)	0.6±0.10 (0-6
*†Breed							
Red Bororo	78	6±0.1 (4-9)	29.3±0.87 (12-45)	69.2±0.88 (50-88)	0.6±0.12 (0-4)	0.3±0.10 (0-5)	0.5±0.15 (0-6)
Sokoto Gudali	14	6±0.2 (4-7)	29.5±2.52 (16-48)	69.4±2.64 (49-84)	0.6±0.34 (0-4)	0.4±0.31 (0-4)	0.1±0.14 (0-2)
White Fulani	360	6±0.2 (3-9)	29.5±0.41 (14-52)	68.7±0.44 (46-85)	0.8±0.07 (0-6)	0.3±0.04 (0-5)	0.6±0.10 (0-8)

** Breed classification was based on physical observation of predominant phenotypic traits of pure breeds in the mixed-bred cattle population*

† No significant main or interaction effect of sex or breed of cattle on all parameters (P > 0.05)

Table 2: Differential counts of white blood cells (±SEM; ranges in parenthesis) of cattle according to haemoparasites

*Parasite Species	N	WBC (X 109/L)	*Neutrophils%*	*Lymphocytes%*	*Eosino-phils%*	*Baso-phils%*	*Mono-cytes%*
Anaplasma central	9	6±0.3 (4-7)	29.9±3.00 (14-46)	68.1±2.95 (46-65)	1.3±0.69 (0-5)	0.2±0.20 (0-2)	0.4±0.34 (0-3)
Babesia bovis	63	7±0.8 (4-9)	29.1±0.99 (16-52)	69.2±1.01 (46-84)	1.0±0.20 (0-6)	0.2±0.08 (0-4)	0.5±0.16 (0-5)
A. centrale + B. Bovis	3	5±0.7 (4-6)	27.3±4.67 (20-36)	69.3±2.91 (64-74)	1.67±1.67 (0-5)	0.0±0.00 (0-0)	1.7±1.20 (0-4)
Babesia divergens	5	6±0.3 (6-7)	31.4±6.71 (15-52)	67.8±7.14 (46-85)	0.4±0.40 (0-2)	0.4±0.40 (0-2)	0.0±0.00 (0-0)
Trypanosoma congolense	20	5±0.3 (3-8)	29.1±1.72 (16-41)	69.9±1.82 (57-84)	0.7±0.28 (0-4)	0.05±0.05 (0-1)	0.3±0.15 (0-2)
Parasite-free	352	6±0.1 (3-9)	29.5±0.41 (12-52)	68.8±0.44 (48-88)	8±0 0..07	0.3±0.04 (0-5)	0.5±0.07 (0-8)

**No significant effect of parasite species on all the leukocytes (P > 0.05).*

haemolymphnodes. Variations in the depression and proliferations of the different fractions of the white blood cells during the course of the disease or infection may be responsible for the non-significant difference between the haemoparasite-infected and parasite-free animals.

Impact

This study has revealed that the total white blood cells and the differential counts which are the lymphocytes, monocytes, neutrophils, basophils and oesinophils increase in number during haemoparasite infection but there is fluctuation of these cell groups during the course of infections and not a steady increase in numbers. Therefore, the number of these leukocyte differentials may not exactly tell if there is a current haemoparasitic infection or not.

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PREVALENCE OF BRUCELLA ANTIBODIES IN SHEEP AND SPRINGBOK (ANTIDORCAS MARSUPIALIS) REARED TOGETHER IN THE KARAS REGION, NAMIBIA

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Abstract

An outbreak of brucellosis in sheep in 2009 on a farm in the adjacent Hardap Region of Namibia and the lack of information on the brucellosis status of springbok prompted a serological investigation of brucellosis in sheep and springbok in the Karas Region, Namibia as these two species are utilized for meat. The main aim of the study was to find out if springbok reared with sheep are infected with brucellosis. Sera collected from sexually mature naïve sheep (n=332) and from springbok (n=345) on 11 randomly selected commercial farms and from adult sheep (n=472) and springbok (n=9) on eight commercial farms identified as positive for Brucella melitensis between 2008 and 2010 tested negative for Brucella antibodies. However, 10% (95% CI: 2.78-26.0) of the tested sheep on one farm tested positive for B. ovis antibodies confirming the presence of this agent in the region. On the eight exposed farms, both sheep and springbok tested negative for Brucella antibodies, providing evidence that control measures that were implemented following the detection of the disease had been effective. It was concluded that sheep and springbok on the eleven farms had not been exposed to Brucella melitensis and B. abortus infections and that on previously positive farms the infection had been eliminated in sheep and had not spread to springbok.

Key words: springbok, sheep, Brucella melitensis, Brucella abortus, Brucella ovis, Namibia

PREVALENCE DES ANTICORPS DE BRUCELLA CHEZ LES MOUTONS ET LES SPRINGBOKS (ANTIDORCAS MARSUPIALIS) ELEVES ENSEMBLE DANS LA REGION DE KARAS EN NAMIBIE

Résumé

Un foyer de brucellose ovine apparu en 2009 sur une ferme de la Région Hardap de la Namibie et le manque d'informations sur l'état de la brucellose des springboks ont entraîné une enquête sérologique de cette maladie chez les ovins et les springboks de la région de Karas en Namibie, car ces deux espèces sont utilisées pour la viande. Le principal objectif de l'étude était de déterminer si les springboks élevés avec des ovins peuvent attraper la brucellose. Les sérums prélevés sur des ovins naïfs sexuellement matures (n = 332) et sur des springboks (n = 345) dans 11 exploitations commerciales choisies de manière aléatoire et sur des ovins (n = 472) et springboks (n = 9) adultes dans huit fermes commerciales identifiées comme étant positives pour Brucella melitensis entre 2008 et 2010 ont donné des résultats négatifs pour les anticorps de Brucella. Cependant, 10% (IC 95% : 2,78 - 26,0) des ovins examinés sur une (1) ferme ont donné des résultats positifs pour les anticorps de B. ovis, confirmant ainsi la présence de cet agent pathogène dans la région. Sur les huit fermes exposées, les ovins et les springboks ont été déclarés négatifs pour les anticorps de Brucella, une preuve de l'efficacité des mesures de contrôle mises en œuvre suite à la détection de la maladie. Il a été conclu que les ovins et les springboks dans onze fermes n'avaient pas été exposés aux infections de Brucella melitensis et B. abortus, et que dans les fermes auparavant positives l'infection avait été éliminée chez les ovins et ne s'étaient pas étendues aux springboks.

Mots-clés : springbok, ovin, Brucella melitensis, Brucella abortus, Brucella ovis, Namibie

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Introduction

The contribution of game species to Namibia's economy through trophy hunting, game meat exports, live game sales and as a tourist attraction is well documented (Weidlich, 2007). Although game species are confined to national parks, game reserves, conservancies, relatively large numbers exist on commercial farms, where they are reared together with domestic livestock. In the Karas Region of Namibia, sheep and springbok (Antidorcas marsupialis) are the major animal species reared on commercial farms. About 500 000 sheep are slaughtered annually through the regional local and export abattoirs. During the hunting season (April-August), approximately 15 000 springbok are harvested by professional hunters for local meat consumption and for meat exports.

Brucellosis is an economically important zoonotic disease that is endemic in many African countries (Mangen *et al.,* 2002) and affects humans, domestic and wild animals. It is caused by Gram-negative bacteria of the genus Brucella.

In humans, B. melitensis, B. abortus, B. canis and B. suis are the species associated with the disease known as Mediterranean or undulant fever. Infection in humans is commonly acquired through occupational exposure to infected animals or through indirect contact with infected material such as aborted fetuses, the consumption of unpasteurised milk and dairy products (Godfroid *et al.,* 2010).

The main species of importance in domestic and wild ruminants are B. melitensis, B. abortus and B. ovis. B. melitensis was first isolated in Karakul sheep in Namibia in 1953 (Godfroid *et al.,* 2004) and is a common cause of brucellosis in sheep and goats (SANCO, 2001; Robinson, 2003). Sporadic cases of ovine and caprine brucellosis caused by B. abortus infections have been reported, but clinical disease is rare (McDermott *et al.,* 2002; FAO, 2003). Brucellosis has been reported in a wide variety of wild herbivores reared with domestic herbivores on ranches (McDermott *et al.,* 2002) and Brucella antibodies have been detected in a number of game species such as bushbuck (Tragelaphus scriptus), common eland (Taurotragus oryx), impala (Aepycros melampus), greater kudu (Tragelaphus strepsiceros), common duiker (Sylvicapra grimmia),Thomson's gazelle (gazelle thomsonii), Kafue lechwe (Kobus leche kafuensis), Oryx (Oryx beisa) and wildebeest (Connochaetes taurinus) (Paling *et al.,* 1988; Thorne, 2001; Godfroid, 2002; Muma *et al.,* 2007). Game species may acquire Brucella infections from livestock species (SANCO, 2001) and act as reservoir hosts of such infections (Godfroid *et al.,* 2010). In Namibia, Brucella antibodies have been reported in eleven unspecified antelope species (Depner, 1993).

The rearing of sheep and springbok together on commercial farms may result in the cross-infection of brucellosis between the species enabling the disease to enter the human population through the handling of livestock and game or their products (Böhm *et al.,* 2007). Cross-infection of brucellosis between springbok and sheep may complicate control measures for brucellosis, as game species may serve as reservoirs of infection (Muma *et al.,* 2007).

To meet the sanitary requirements of importing countries, the Directorate of Veterinary Services, Namibia embarked on an annual brucellosis testing of sheep farms to certify sheep flocks as free of the disease. An outbreak of brucellosis in sheep on a farm in a region adjacent to the Karas Region in 2009 (Magwedere *et al.,* 2011) and the lack of information on the brucellosis status of springbok despite the use of their carcasses for meat necessitated this study. It was decided to carry out a serological study of Brucella (B. melitensis, B. abortus B. ovis) antibodies in sheep and springbok because brucellosis due to B. melitensis and B. abortus is zoonotic and B. ovis is endemic in sheep in the region. The aim of the study was to find out if Brucella infections occur in springbok reared with sheep.

Materials and Methods

Study Area

The Karas Region of Namibia is located at the southern end of Namibia and shares borders with South Africa to the south

and east, the Hardap Region to the north and the Atlantic Ocean to the west. The region is divided into four magisterial districts. This study was conducted in the Keetmanshoop, Karasburg and Bethanie districts which have farms that rear sheep and springbok together. The region has a hot and dry climate, with unpredictable average summer rainfalls (October to March) of between 142-152mm. In the hottest months, temperature can go above 40oC, whilst in winter temperatures frequently drop to below the freezing point at night (NMS, 2011).

Farm Selection and Sample Size Determination

A two stage sampling approach was used to select farms for the serological study. Seventeen farms approved for springbok harvesting by the Directorate of Veterinary Services in the Karas Region in 2009 for the purposes of exporting meat through the regional export abattoir were grouped into three magisterial districts. A total of 11 farms with no brucellosis vaccination history were selected by simple random sampling from each district.

The formula for determining disease prevalence as described by Martin et al. (1987) was used to calculate the number of samples to be taken in sheep and springbok on each farm.

Sheep brucellosis testing results from 2008 to 2010 were used to identify eight previously positive or exposed farms for sampling in sheep and springbok to determine the effectiveness of control measures that were implemented following positive results - by checking for the absence or presence of the disease in both sheep and springbok. The sample size for this study was determined using the formula for detecting the absence or presence of disease in a population as described by Martin et al. (1987), as the diseased sheep on positive farms had already been culled, but no action had been taken in regard to springbok sharing pastures with sheep.

Collection of Sera from Sheep and Springbok

The number of sera collected from sheep and springbok on the eleven farms are shown in Table 1. A total of 332 sera were collected from sheep. Each sheep sampled had 10ml of blood taken from the jugular vein using individual sterile 20G needles and sterile plain vacuum tubes (BD Vacutainer Systems, Pre-Analytical Solutions, United Kingdom). A total of 345 blood samples were collected from the jugular vein of springbok immediately after shooting and killing using individual sterile 20G needles and sterile plain vacuum tubes (BD Vacutainer Systems, Pre-Analytical Solutions, United Kingdom). Sampling was done from springbok populations that were known to share pastures with sheep as confirmed by the farm owner/ manager. In addition, the number and species of game animals on each farms was collected at each sampling visit.

The number of sera collected from sheep and springbok on the eight previously positive farms is shown in Table 2. A total of 472 sera were collected from sheep and nine sera from springbok.

Identification, Packing and Dispatch of Sera

All collected blood tubes were identified with respect to the farm and animal species, date of sampling and given an individual identification number to prevent the mixing of samples from different animals and farms. The tubes were then placed in identified metal containers and packed in such as way as to prevent damage and leakage during transport

Table 1: Number of sheep and springbok sampled on the eleven commercial farms

Farm	Sheep sampled	Springbok sampled
1	30	28
2	31	31
3	30	35
4	30	32
5	31	30
6	30	31
7	30	30
8	30	29
9	30	34
10	30	32
11	30	33
Total	**332**	**345**

Table 2: Number of sheep and springbok samples collected from the exposed farms

District	Farms tested	Sheep sera sampled	Springbok sera sampled
Keetmanshoop	5	292	5
Karasburg	2	119	2
Bethanie	1	61	2
Total	**8**	**472**	**9**

to the regional laboratory. Ice packs were added to transport containers to preserve the samples during transportation from the place of collection to the regional laboratory. At the regional laboratory, the blood was allowed to clot, serum separated and stored in a refrigerator. Cooled sera were dispatched in refrigerated containers (4oC) to the Central Veterinary Laboratory in Windhoek.

Serological Testing of Sheep and Springbok Sera

Testing for Brucella antibodies (B. melitensis or B. abortus) was done using the Rose Bengal Test (RBT) as a screening test and samples testing positive on the RBT were confirmed using the Complement Fixation Test (CFT) as described in the OIE Manual (2004). The antigenic suspensions used in the detection of Brucella antibodies (B. abortus and B, melitensis) in the RBT and CFT tests were obtained from B. abortus strain 99 as described in the OIE Manual (2004). Any visible agglutination was considered as test positive in the RBT test. Brucella ovis serology was done using the CFT as described in the OIE Manual (2004). In the CFT test, titres of 1:8 and above were recorded as positive based on the presence or absence of haemolysis.

Data Analyses

Data from the study was stored and processed using Microsoft Excel®, Microsoft Corporation 2007. To account for the clustering effect of sampling on farms, 95% confidence intervals around the mean prevalence were adjusted according to Reiczigel et al. (2010).

Results

Animal Numbers and Species

The number of animal species reared on the eleven commercial farms is shown in Table 3. Sheep and springbok were the main species on the farms representing 69% and 21% of the total animal population respectively, but goats, cattle, blesbok and Oryx were recorded on some farms in variable numbers. The dorper breed was the most common breed of sheep on all the farms, but other breeds such as the karakul and merino were observed. The average size of the springbok herd per farm was 370 ± 269 (range: 40-1000). The sheep population on the farms was more than double the number of springbok on 10 farms (91%). The overall stocking density for all animal species was between 0.03 animals/ha and 0.34 animals/ha.

Sheep and Springbok Serological Results

Serological results for sheep sera are shown in Table 4. Brucella antibodies (B. melitensis and B. abortus) were not detected in sheep on the 11 farms. However, on one farm, 10% (95% CI: 2.78%-26%) of the sampled sheep tested positive for Brucella ovis antibodies. Brucella antibodies (B. melitensis, B. abortus and Brucella ovis) were not detected in 345 springbok sera collected from a total population of 4070 springbok reared with sheep on eleven commercial farms.

Results for Previously Exposed Farms

All 472 sheep sera and 9 sera collected from the eight exposed farms (that tested positive for Brucella antibodies between 2008 and 2010) tested negative for Brucella (B. melitensis, B. abortus, B. ovis) antibodies.

Discussion

Results of this study confirm that sheep and springbok were the main species of animals on commercial farms and that the sheep population on the farms was more than

double the size of the springbok population. Other species of livestock and game such as cattle and kudu were also present in low numbers on the farms.

Antibodies against Brucella were not detected in both sheep and springbok sera, although the sera were taken from sexually mature animals which are expected to harbor permanent Brucella infections (FAO, 2006; CFSPH, 2009). These results are consistent with the fact that the farms had no serological and clinical history of brucellosis as confirmed by the records at the regional state veterinary office. The extensive management of sheep and springbok in hot and dry conditions prevailing on the farms may have played a part in reducing the survival and transmission of Brucella bacteria on pasture (SANCO, 2001). As confirmed by this study, the stocking density of both species was very low and not favorable for the transmission of brucellosis. According to Godfroid (2002), the establishment and sustainability of brucellosis in a species depends on infectious dose, host susceptibility,

Table 3: Number of domestic and game species on the 11 commercial farms

Farm No.	Farm size (ha)	Sheep		Goats		Cattle		Springbok	Other
		ewes	rams	does	bucks	cows	bulls		oryx
1	9000	900	70	0	0	0	0	300	0
2	10324	2604	30	128	12	104	2	650	0
3	4812	890	15	25	2	25	1	435	0
4	6543	754	10	0	0	0	0	345	0
5	20600	2300	70	0	0	70	2	1000	60
6	7493	500	6	300	7	0	0	40	9
7	13347	643	20	0	0	76	3	300	70
8	12831	240	8	0	0	0	0	150	0
9	10365	1880	40	80	10	101	2	400	60
10	9561	1700	70	250	4	61	2	100	23
11	5237	740	48	281	32	52	22	350	0
Totals	**110113**	**13151**	**387**	**1064**	**67**	**489**	**34**	**4070**	**222**

Table 4: Serology results for sheep on the 11 commercial farms

Farm no.	Sheep population	No. tested	Number positive for:		% positive for B. ovis
			B. melitensis/B. abortus antibodies	*B. ovis antibodies*	
1	970	30	0	0	0
2	2634	31	0	0	0
3	905	30	0	0	0
4	764	30	0	0	0
5	2370	31	0	0	0
6	506	30	0	0	0
7	663	30	0	0	0
8	248	30	0	0	0
9	1920	30	0	0	0
10	1770	30	0	0	0
11	788	30	0	3	10% (2.78-26.0)*
Totals	**12386**	**332**	**0**	**3**	

contact with infected animals, management and environmental factors. In this study, sheep and springbok were naïve and susceptible, but environmental and management factors were not ideal for the survival and spread of the agent even if the agent had been introduced from outside. Although Brucella antibody titers can fluctuate in infected adult sheep (FAO, 2010), it is unlikely that this could have been the reason for the failure to detect antibodies in sheep, because sampling was carried out over a period of nine months. The other species of livestock and game present on the farms were not considered for the serological study because they occurred in low numbers.

Sera collected from sheep and springbok on the eight previously exposed farms tested negative for Brucella antibodies, confirming the absence of brucellosis on these farms. Only nine sera were collected from springbok reared with sheep on exposed farms as a result of a lack of cooperation from the farmers. Although the nine sera tested negative for Brucella antibodies, the sample size was deemed too small to make inferences about the brucellosis status of springbok herds reared with brucellosis exposed sheep. These results confirm the effectiveness of the test-and–slaughter interventions implemented by the Directorate of Veterinary Services to control B. melitensis infections in sheep and goat populations. According to the protocol, when a flock tests positive for brucellosis, the farm is placed under quarantine and all sheep above six months of age are serologically tested for Brucella antibodies. All sheep that test positive on the CFT test are eliminated. Quarantine restrictions on the remaining sheep are removed after two consecutive negative CFT serological results at least three months apart. In addition, the protocol encourages farmers to keep a closed flock and purchase replacement stock from brucellosis-free flocks (DVS, 2009).

The results of this study are in agreement with the results of a study by Karesh et al. (1997) in impala in Namibia in which no positive reactors were found. Although other wild ruminant species have tested positive for Brucella antibodies in Namibia (Depner, 1993; Karesh *et al.,* 1997) and in Southern Africa (McDermott *et al.,* 2002; Paling *et al.,* 1988; Thorne, 2001; Godfroid, 2002; Muma *et al.,* 2007), there is no record of springbok testing positive for Brucella antibodies in the literature. In the current study, the absence of reactors in springbok is a reflection of the status of the farms rather than the difficulty of transmission of the disease between springbok and sheep (Ferroglio *et al.,* 1998), as the later also tested negative. The role of springbok in the epidemiology of sheep brucellosis could not therefore be inferred due to the negative results recorded. The serological tests for Brucella antibodies in sheep were directly transposed onto springbok sera, but it is unlikely that these tests could have influenced the results as all sheep sera also tested negative. Positive reactors in sheep have been recorded on some commercial farms in Namibia. Therefore, the absence of reactors in sheep in this study may be attributed to lack of exposure to the agent on the tested farms.

B. ovis, a common cause of ram epidydimitis, orchitis and infertility in Southern Africa (Blasco *et al.,* 2004), was detected in sheep, but not in springbok reared with serologically positive sheep. These results are consistent with the fact that B. ovis has not been reported in wild ruminants in Southern Africa (Blasco *et al.,* 2004). Close contact is necessary for the transmission of B. ovis (OIE Manual, 2004). It was unlikely that the degree of close contact necessary for the transmission of B. ovis between sheep and springbok could be achieved on the study farms because of the shy nature of game species. The detection of B. ovis antibodies in sera from three rams confirms that this agent is present in the region. The prevalence of B. ovis antibodies (10%) detected was high considering that the rams were not housed but extensively managed on pasture. Group penning of rams is known to facilitate the transmission of B. ovis infections (OIE Manual, 2004). The serological reactions observed in this study were due to active infections or past exposure to the agent because the rams were not vaccinated. The detection of B. ovis in sheep is of economic significance because of the associated reproductive losses in the affected flocks.

In conclusion, B. ovis was confirmed in rams, but no evidence of brucellosis (B. melitensis or B. abortus) was found in sheep and springbok reared together. These results indicate that the slaughter of sheep and the harvesting of springbok meat for local and export meat markets in the Karas Region may not present an occupational health risk. The measures implemented by the Directorate of Veterinary Services to control brucellosis on previously positive farms had been effective in eliminating the disease from the farms.

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THE HISTOMORPHOLOGY OF THE AFRICAN PALM SQUIRREL (EPIXERUS EBII) LACRIMAL GLAND.

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Abstract

The histomophology of the dorso-lateral lacrimal gland of the African Palm Squirrel –Epixerus ebii, was investigated to establish its normal histology. The grossly almond shaped organ appeared as a pink coloured U-shaped structure with lobules on low magnification. The organ was coated by thin connective tissue fibres. The lobules averaging seven in number contained tubuloalveoalar glands. The cells of the glands were typically serous cells with low columnar shape containing basally located ovoid to spherical nucleus. While some gland acini lumen contained foamy eosinophilic secretions, other acini contained foamy eosinophilic secretions with basophilic materials. These acini were designated types I and II respectively. Periodic acid Schiff reaction revealed that the basement membrane were PAS positive while the gland secretions were poorly PAS positive. This data will fill the knowledge gap and aid biologists', comparative anatomists' in further investigative research, while it will be of interest to wildlife clinicians in this species disease management.

HISTOMORPHOLOGIE DE LA GLANDE LACRIMALE DE L'ÉCUREUIL DES PALMIERS (EPIXERUS EBII)

Résumé

L'histomorphologie de la glande lacrymale dorso-latérale de l'écureuil des palmiers africain – Epixerus Ebii, a été étudiée dans le but d'établir son histologie normale. L'organe qui avait une grossière forme d'amande, paraissait comme une structure de couleur rose en forme de U avec des lobules à faible grossissement. Il était recouvert de minces fibres de tissu conjonctif. Les lobules au nombre de sept en moyenne contenaient des glandes tubulo-alvéolaires. Les cellules des glandes étaient typiquement des cellules séreuses en forme cylindrique réduite contenant un noyau ovoïde ou sphérique situé à la base. Si les cavités de certains acini des glandes contenaient des sécrétions éosinophiles mousseuses, d'autres acini contenaient des sécrétions éosinophiles mousseuses avec des matériaux basophiles. Ces acini sont appelés respectivement Types I et II. La réaction PAS (Periodic Acid Schiff) a révélé que la membrane basale était positive pour PAS, tandis que les sécrétions des glandes étaient faiblement positives pour PAS. Ces données permettront de combler le déficit de connaissances et aideront les biologistes et les anatomistes comparatifs dans les recherches ultérieures, et seront également intéressantes pour les cliniciens de la faune sauvage dans la prise en charge des maladies de cette espèce.

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Introduction

The mammalian lacrimal glands produce and secrete aqueous solution containing mucus and proteineous substances (Janssen and van Bijsterveld, 1983; Mohammadpour, 2009). The product of these glands – tear fluid aids to preserve normal corneal wellbeing (Fullard and Snyder, 1990). The tear film consists of a superficial oily layer produced by the tarsal glands; a central aqueous layer which is a main constituent of tear film is produced by the lacrimal gland; and thin glycoprotein layer secreted by the conjuctival epithelium goblet cells (Junquira and Carneiro, 2003; Klećkowska-Nawrot and Dziegiel, 2008). This tear film is involved in provision of lubrication, prevention of spill over of tears from the lid margin, nourishment of the cornea and helps in adherence of the precorneal film to the corneal surface (Fullard and Snyder, 1990; Michele *et al.,* 1994). In most mammals, the bulk of tears are produced and secreted from the dorsal lacrimal gland. In general histology, the lacrimal glands are tubuloalveolar structures that produce serous or mucoserous secretions.

From available literature, the morphology of dorsal lacrimal glands of humans (Draper *et al.,* 1999; Obata, 2006); cattle (Pinard *et al.,* 2003), Camel (Mohammadpour, 2008; Al-Ramadan and Abdelhadi, 2012), dogs (martin *et al.,* 1988), pigs (Klećkowska-Nawrot and Dziegiel, 2008; Henker *et al.,* 2013) , rabbits (Michele *et al.,* 1994; Rehorek, 2011), sheep (Garguilo *et al.,* 2000; ElHafez *et al.,* 2014), and rats (Draper *et al.,* 1998) has been described. There is, however, dearth of information about the histomorphology dorsal lacrimal gland of the African palm squirrel in available literature. The major objective of the present investigation is to describe and establish its normal histomorphology as it will of interest to Zoologists', Physiologists', and wild life clinicians.

Materials and Methods

Five adult African palm squirrels of both sexes captured in the wild from Olokoro Umuahia in Abia state, Nigeria from March to November 2012 using metal cage traps were used for the study. Olokoro Umuahia is in the rainforest vegetation of southern Nigeria characterized by heavy rains and thick well grown mangrove forest trees. They were immediately transferred to the Veterinary anatomy laboratory of Michael Okpara University of Agriculture, Umudike, for acclimatization. During this period, the animals were fed with grasses, oil palm fruit and water ad libitum.

The squirrel on the day of sacrifice was sedated with chloroform and later decapitated. The weight of the animal was taken with Mettler balance (Model Ohaus scout PRO-200) with a sensitivity of 0.1gm. The eye was extirpated and the dorso-lateral lacrimal gland was dissected out. The lacrimal gland was dissected out and slices fixed in 10% neutral buffered formalin. The slices were passed through graded ethanol, cleared in xylene, impregnated and embedded in paraffin wax. Sections 5µm thick were obtained with Leitz microtome model 1512. They were stained with haematoxylin and eosin for light microscopy examination (Bancroft and Stevens, 1990). Glycogen and glycol-conjugates were demonstrated using periodic acid Schiff (PAS) procedure with and without prior digestion with diastase (Lillie and Greco, 1947; Ikpegbu *et al.,* 2011). The slides were examined and photomicrographs taken with – Motican 2001 camera (Motican UK) attached to Olympus

Results

The grossly almond shaped lacrimal gland was seen lobated with low magnification (fig.1). These secretory lobules that average seven in number, were fused by thin connective tissue fibres containing blood vessels (fig. 1). The fused secretory lobules formed a pink coloured U-shaped structure with an empty central cavity (fig. 1). The gland was covered by thin connective tissue fibres (fig. 2).

Higher magnification revealed that this tubuloalveolar gland is typically serous cells with low columnar shape, containing basally located ovoid to spherical shaped nuclei (fig.2). The cytoplasm was predominantly eosinophillic. Well developed myoepithelial cells were at the base of the secretory cells (fig. 2). The central

cavity of some acini was secretion filled, while others had a slightly clear lumen (fig. 2). Most of the tubuloalveolar lumen secretions contained foamy granules. This will be designated type I gland cells.

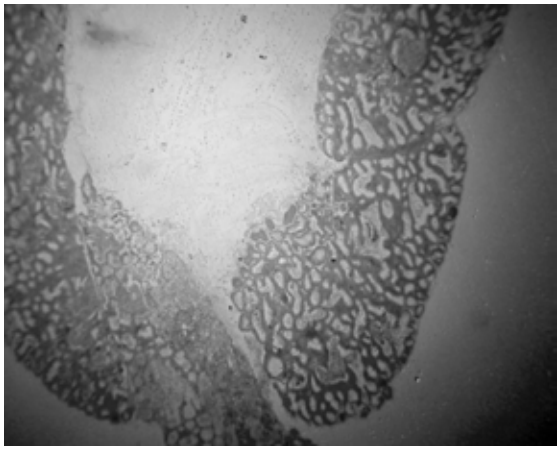


Figure1: Section of the U-shaped lacrimal gland showing the lobules L. Note the interlobular connective tissue (white arrow). H&E. (Scale bar = 4µm).

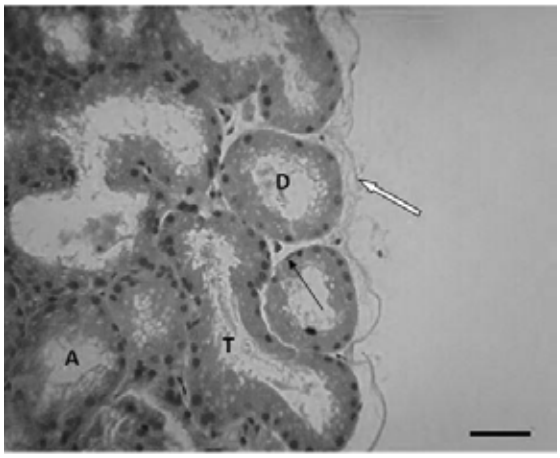


Figure 2: Section of lacrimal gland showing tubular T, and alveolar A, parts of the type I tubuloalveolar gland. Note the intercalated duct D, myoepithelial cell (black arrow), gland capsule (white arrow), . H&E. (Scale bar = 40µm).

In some acini, the central lumen contained light eosinophilic secretions with foamy granules and dark to basophilic materials (fig. 3, 4). These basophilic materials looked like desquamated nuclear materials. The secretory cells in these types of acini were mostly cuboidal cells (fig. 3,4). This type of tubuloacina cells predominated in an average of two lobes per lacrimal gland. This is the type II gland cells. The intralobular ducts were lined by simple columnar cells containing myoepithelial cells at the base.

Mucin histochemistry reaction revealed that the basement membrane `of the tubuloalveolar glands were all PAS positive (fig.5). While little secretions of type I glands were poor PAS positive, a lager content of type II gland was fairly PAS positive.

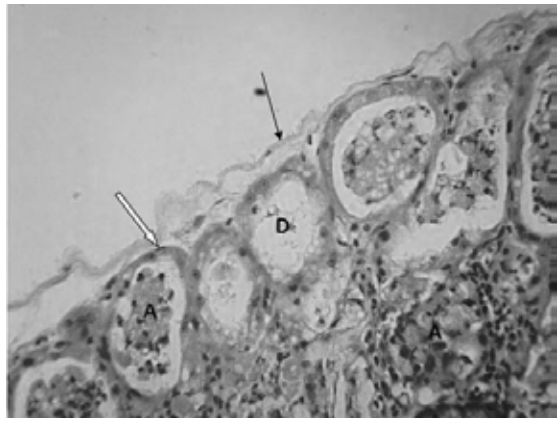


Figure 3: Section of lacrimal gland showing the type II acini gland. Note the intercalated duct D, myoepithelial cell (white arrow), and gland capsule (black arrow). H&E. (Scale bar = 40µm).

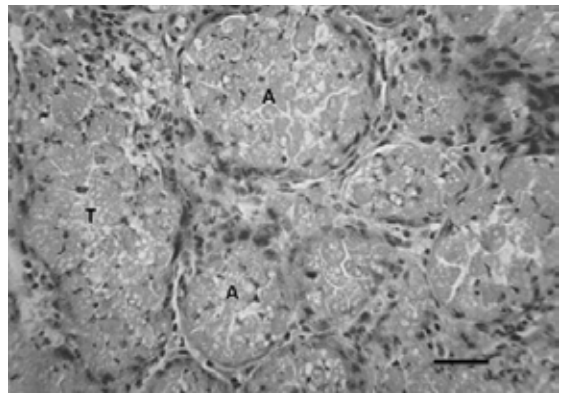


Figure 4: Section of lacrimal gland showing a lobule containing only tubular T, and alveolar A, parts of the type II tubuloalveolar gland. Note the presence of basophilic materials in majority eosinophilic secretion. H&E. (Scale bar = 40µm).

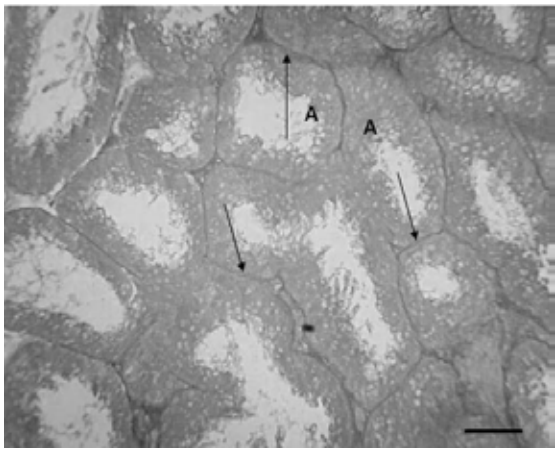


Figure 5: Section of lacrimal gland showing PAS positive basement membrane (black arow), of the secretory acini A. PAS (Scale bar = 40µm).

Discussion

This paper for the first time in available literature investigates the normal histomorphology of the lacrimal gland of the African Palm Squirrel Epixerus ebii. The lobulated lacrimal gland as seen in this study has been reported in the pigs, cattle and sheep (Pinard *et al.,* 2003; Klećkowska-Nawrot and Dziegiel, 2008; Abbasi, 2014). This lobulation has been related to the investment of the protective capsular connective tissue fibres into the gland parenchyma. The pink coloured organ reflects a typical serous gland.The general organ secretory micro-architecture of serous tubuloalveolar is typical of mammalian lacrimal gland (Gargiulo *et al.,* 1999), but a parenchyma of mixed seromucous cells has been reported in pig, horse and Lori sheep (Abbasi, 2014); in the dogs, mucous cells have been reported. The presence of foamy secretions may indicate presence of lipids which will help in protection of the Squirrel cornea in the wild. Also the abundant serous fluid apart from cleansing the cornea may contain some lysozymes that will aid in maintaining corneal health through its preoteolytic action on bacteria or other pathogenic agent (Kijlstra and Kuizenga, 1994). The myoepithelial cells will aid in expulsion of the secretory granules from the cell into the lumen and through the intralobular duct by the mechanism of its contractile ability (Redman, 1994; Moore *et al.,* 1987; Amano *et al.,* 2012). The presence myoepithelial cells hve been reported in the rabbit lacrimal gland (Michele, 1994). The presence of two types of gland acini may indicate varying types of exocrine secretion. Type I acini must be through merocrine exocytotic vesicle while type II will be of holocrine secretion. The evidence of holocrine secretion in type II can be related to the presence of basophilic materials which can be of nuclear origin, while the low cuboidal epithelium may suggest replacement of acini cells by surrounding cells. The presence of type II acini may suggest that the lacrimal gland in this species undergoes regeneration through holocrine secretion. It is also possible that holocrine secretion may be induced to increase lipid content of the tear fluid. This increased lipid will aid corneal lubrication, reduction in rate desiccation of aqueous fluid, and also being an arboreal animal, it will help protect the squirrel cornea from small mechanical injury caused by the constant challenge of objects like tree branches and leaves. Type I acini has been reported in the cattle while type II acini has been reported in Bison bison , but the difference in acini types was related to species variation (Pinard *et al.,* 2003). In the musk shrew (Suncus murinus) lacrimal gland, two acini types have been described based on mucin histochemical reaction. While some acini have both acid and neutral secrotory cells, others had only neural mucin secretory cells (Sakai, 1989). An apocrine mucous secretion has been described in geese harderian gland (Boydak and Aydin, 2009)

The presence of PAS positive basement membrane is typical of mammalial epithelial basement membranes (). The poorly PAS positive secretions contain carbohydrate moieties that will contribute to the nourishment of the corneal epithelium (). This poor PAS reaction may be due to the conjugated form of carbohydrates as glycoproteins (Shackelford and Kapper, 1962). The presence strong PAS positive acini has been reported in the American Bison and Cattle (Pinard *et al.,* 2003), Lori sheep (Abbasi, 2014).

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AFRICAN UNION - INTERAFRICAN BUREAU FOR ANIMAL RESOURCES (AU-IBAR)

Bulletin of Animal Health and Production in Africa
Guide for Preparation of Papers
Notes to Authors

The Editor in Chief
January 2014

Aims and scope

The Bulletin of Animal Health and Production in Africa (BAHPA) of the African Union Interafrican Bureau for Animal Resources (AU-IBAR) is a scientific journal which publishes articles on research relevant to animal health and production including wildlife and fisheries contributing to the human wellbeing, food security, poverty alleviation and sustainable development in Africa. The bulletin disseminates technical recommendations on animal health and production to stakeholders, including policy makers, researchers and scientists in member states. The Bulletin is the African voice on animal resources issues specific to Africa.

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

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

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- Marketing, economics
- Infectious and non infectious disease
- Parasitology
- Genetic improvement and biotechnology
- Animal production, nutrition and welfare
- Science and policy in animal health and production
- Beekeeping and honey bees
- Ecology and climate change impacts on animal resources in Africa
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- Fisheries and aquaculture development
- Food safety and food hygiene
- One health
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- Animal resources trade and value chain
- Socio economics and economics of animal resources development

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- *Reports:* Makarewicz JC, Lewis T, Bertram P, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. US EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.
- *Conference Proceedings:* Stock A, 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.
- *Thesis:* Strunk JL, 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, Unpublished PhD thesis, Michigan State University, East Lansing, MI.
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