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Journal of Veterinary Medicine and Animal Health

ARTICLES

Full Length Research Paper

A survey of ticks and East Coast fever among cattle in Fangak County, Jonglei State, South Sudan

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This study was carried out in Fangak County, Jonglei State, South Sudan, with aim to identify the main ticks species and follow north limit of the theileriosis in Jonglei. Three localities namely; Hai, Toggar and Bichoul kun village in Phoum payam were selected as suspected area for East Coast fever (ECF), (Group A) and Kuer kan in Manjang payams were selected as non suspected area for ECF (Group B). These groups A and B were based on animal movement, trade business and intermarriages (animals paid for dowry). A total of 120 sera were collected from cattle of different age groups. The serum samples were tested using indirect polymorphic immunodominant molecule (PIM) ELISA to detect *Theileria parva* antibodies. The results indicated that 5/44 (11.4%) samples from non suspected area (group B) and 48/76 (63.2%) samples from suspected area (Group A) revealed antibodies. The overall positivity was 53/120 (44.17%) which was highly significant (P < 0.001) according to the locations. Three tick genera were recorded, *Amblyomma*, *Hyalomma* and *Rhipicephalus*. The species were *A. variegatum*, *A. lepidum*, *H. rufipes*, *R. (B.) decoloratus*, *R. (B.) annulatus*, *R. e. evertsi* and *R. sanguineus*. The most abundant tick species was *A. variegatum*, constituting 62%, while the lowest tick recorded was *R. e. evertsi* with prevalence rate of 2%. No *R. appendiculatus* tick was seen in Fangak area; while *T. parva* antibodies were detected. Regarding the fact that *T. parva* antibodies were present in the area with 44.17% prevalence, more efforts are needed to determine the extension of ECF and its vector *R. appendiculatus* to the northern parts of Jonglei State and this result of ECF antibodies could be an alarm to migrate cattle owners from South Sudan.

Key words: Ticks, east coast fever (ECF), ELISA, South Sudan.

INTRODUCTION

South Sudan is known to be most populated with livestock in Africa, on other hand only equatorial area is known to be endemic while two region of Upper Nile and Bahr El Ghazal is known to be free from diseases except some pockets in Bor Jonglei State and Awerial in Lake State (Kavaria et al., 2012). Ticks and tick-borne diseases (TBDs) are the major problems according to the studies carried out in the area (FAO, 1983; Julla, 1994

and Marcellino et al., 2011b). The first assessment for ticks was carried out by Hoogstraal (1956) who found that there were 39 species of ticks in South Sudan in 150 localities. He found that *Rhipicephalus* (*B*) species, *Margaropus* spp and *Hyalomma* spp. were the main prevalent ticks. *R. appendiculatus*, the vector of East Coast Fever (ECF) caused by the *Theileria parva* is mainly distributed in areas of high rainfall and moderate temperature, such as the district of Kajo Kaji, Yei, Ngangala, Torit and Katire. In addition, these tick species was identified in Chukudum, Aswa River, Palotaka, Nimule and Juba (Morzaria et al., 1981 and Julla 1985, 1994). Korok (2005) reported the presence of eight species of ticks in Pibor area of Jonglei State while Salih et al., (2008) found ten species and three genera including the ECF vector in Central Equatoria State. Also, Marcellino et al., (2011b) reported the presence of seven species of ticks in Central Equatoria State among them was *R. appendiculatus*. On the other hand, Kivaria et al., (2012) conducted a survey in five states, and showed the presence of three genera and six species among which was the vector of ECF and presence of other main TBDs in those states.

In South Sudan, theileriosis was reported for the first time in 1950 by Hoogstraal (1956), the region then became endemic (Julla, 1994; Salih et al., 2007b and Marcellino, 2008). Then, ECF extended north direction and reached Bor area (Ochi et al., 2009; Kivaria et al., 2012). Recently the disease was reported by Malak et al., (2012) in Kajo kaji and Yei counties of central Equatoria. The impact of disease reported by Marcellino et al., (2011a) stated the losses (mortality) due to ECF was around 134325$ in two cattle camps where outbreaks for the diseases were reported. The study area is one of the active route for transporting animals from Equatoria and Bor areas, known endemic areas to ECF, to other states and north Sudan. The present study aimed at determining prevalence of ticks and ECF in Jonglei State with emphasis on ECF and determination of north limits with objectives, firstly to identify the main species of ticks prevalent in the area with special reference to the main vector of ECF, and secondly, to examine animals to follow the northward spread of ECF in Jonglei State.

MATERIALS AND METHODS

Study area

Fangak County in Jonglei State, located at 9°04′10 to 9°06 94°N, 30°53′03 to 30°88′41E is bordering Panyikang county of Upper Nile State in the North East, Ayod County to the South, Piji County to the east. Phoum al Zeraf town, the county headquarter, lies where the Bahr el Zeraf joins the White Nile from the east (Figure 1 and 2).

Vegetation cover

The area around the study region in Jonglei State is characterized by acacia tall trees forest and swampy grassland, seasonally inundated land or known locally as toich (swampy) alongside the Bhar jabel (White nile ,Kiir) and Bhar El Zaraf (Phow) with vast grazing area for cattle and wildlife reserve in the area. The annual rainfall ranges between 750 to 900 mm with the rain starting in mid April declining in October, while the mean ambient temperature is between 23 to 41°C in dry season (March, early April) and 19 to 34°C in wet season (June to August). On the other hand, elatives humidity average is between 47% in dry season and 89% in wet season.

Sampling

A convenience sampling method was applied (Smith, 2005). Thus, the target animals were the indigenous Nilotic type (*Bos indicus*). One hundred and twenty cattle was sampled. The animals were sampled from an area categorized into A, being suspected area and B, being non-suspected area for East Coast fever (ECF). Categorization into A and B areas was based on cattle movement and trading routes. Two payams (Administration Unit), Phoum in the mainland and Manjang on the island were selected as the study areas. The animals selected were 44 from Kure kan within Manjang Payam, Hai Toggar with 42 animals and Bichoul kun village with 34 animals, the males were 40 animals and females were 80 animals. Also, animals were categorized to three age groups; 1 year to less than 2 years, 2 years to less than 4 years and 4 years and above.

Serum sample

Animals were restrained and blood was collected from the jugular vein, in sterile vaccutainers without EDTA, then labeled and left overnight (12 h) to clot at room temperature (25°C). The serum was then separated and put in serum tubes, labeled and stored at -20°C until used for serological tests. The procedure of PIM ELISA was carried out as described by Katende et al. (1998).

Blood smears

One hundred and twenty blood smears were taken from the animals. The blood drop was taken from the ear vein of each animal on a clean slide, then spread with another clean slide and air dried, then fixed with absolute methanol labeled according to sample number and place of collection. The blood smears were stained using 10% Giemsa's stain before microscope examination under oil immersion (100x) was carried out.

Tick collection

Total body collection of ticks was carried out with care not to lose the mouth parts and preserved in vials containing 70% alcohol and labeled accordingly to correspond with the labelled serum samples.

Tick identification

Ticks were identified under a stereoscopic dissecting microscope, with key guidance for taxonomy as described by Hoogstraal (1956).

Statistical analysis

Ticks collected from animals were subjected to an appropriate general liner model (GLM) of statistical analysis system (SAS (Version 9) package. The SAS was used to perform analysis of variance (ANOVA) and mean separations were performed using
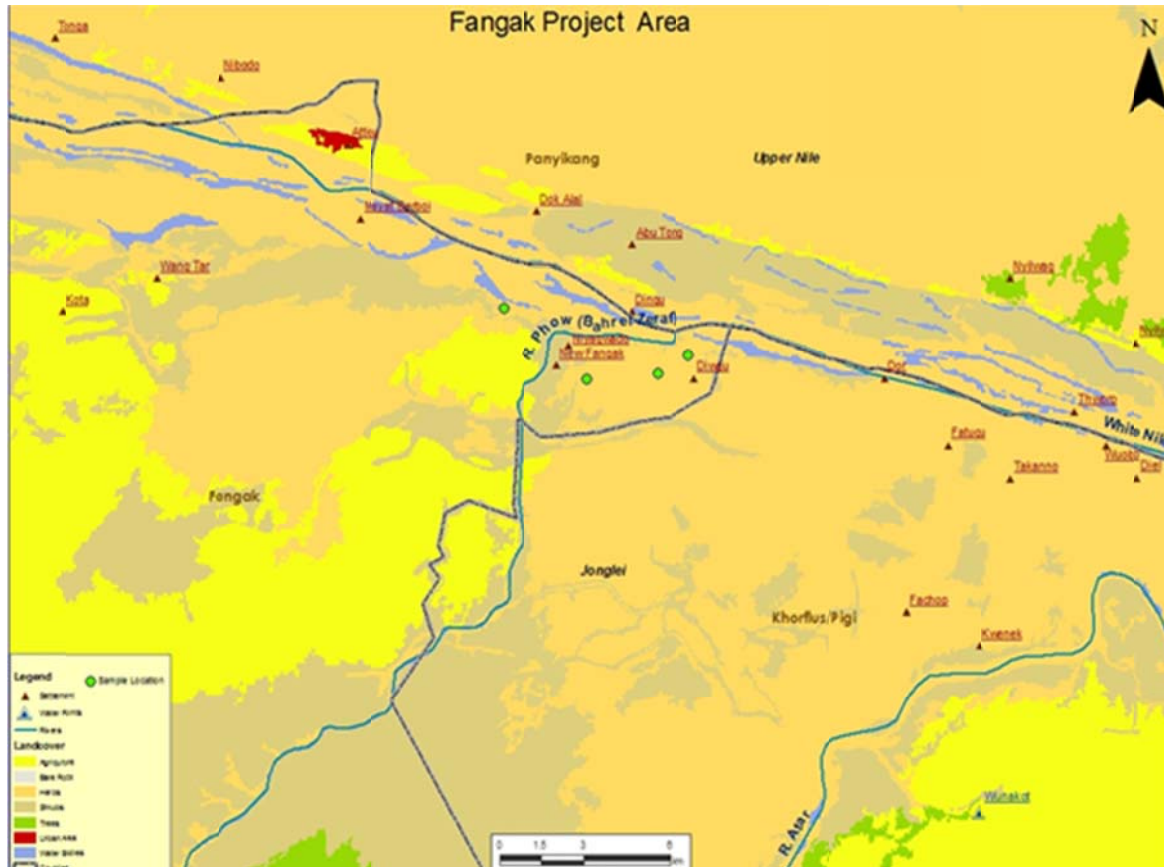
Figure 1. The map of Fangak county showing the sampling sites.

Rayan- Einot- Gebrial–Welsch multiple range test (REGWQ) (Day and Quinn, 1989).

RESULTS

Prevalence of *T. parva* antibodies in the study area

Based on the indirect PIM ELISA ,the overall prevalence of *T. parva* antibodies was 44.17% (53/120) in overall cases prevalence ranging between 4.17% (5/120), 15.83% (19/120) and 24.17% (29/120) in Kuer kan, Bichoul Kun and Hai Toggar, respectively. The prevalence for relative occurrence range was between 9.49% (5/53) in Kuer Kan, 35.85% (19/53) in Bichoul Kun and 54.72% (29/53) in Hai Toggar. While the overall prevalence cases range was between locations, showing highly significant difference (P < 0.001). Sero-prevalence was found to be 55.88% (19/34) in Bichoul kun, 69.05% (29/42) in Hai Toggar Phoum payam and 11.34% (5/44) in Kuer kan Manjang payam (Table 1). Concerning sex, the overall prevalence was 28.3% (34/120), while within females the prevalence was 42.50% (34/80). Meanwhile overall prevalence rate was 15.83% (19/120) for male sex. Within males the prevalence was (19/40) 47.50%. The sero-prevalence rate by sex was not significant (P >

0.05) (Table 2). According to the age groups, the overall prevalence rate in animals of one year to less than two years was 3.33% (4/120) and the prevalence within this age group was 44.44% (4/9). The overall prevalence rate for those between two years and less than four years was 8.33% (10/120) while the prevalence with this group was 50% (10/20). While the overall prevalence for the age group four years and above was 32.5% (39/120) and the prevalence within this group was 42.86% (39/91). The relative occurrence prevalence revealed 73.58% in 4 years and above, 18.87 and 7.55% respectively for 2 years to less than 4 years and 1 year to less than 2 years old. The sero-prevalence by age was not significant (P > 0.05) (Table 3).

Blood smear

The results of 120 blood smears taken from animals in the three locations revealed no piroplasms.

Tick identification results

During the study, 328 ticks were collected from 120 animals in the three locations in Phoum and Manjang
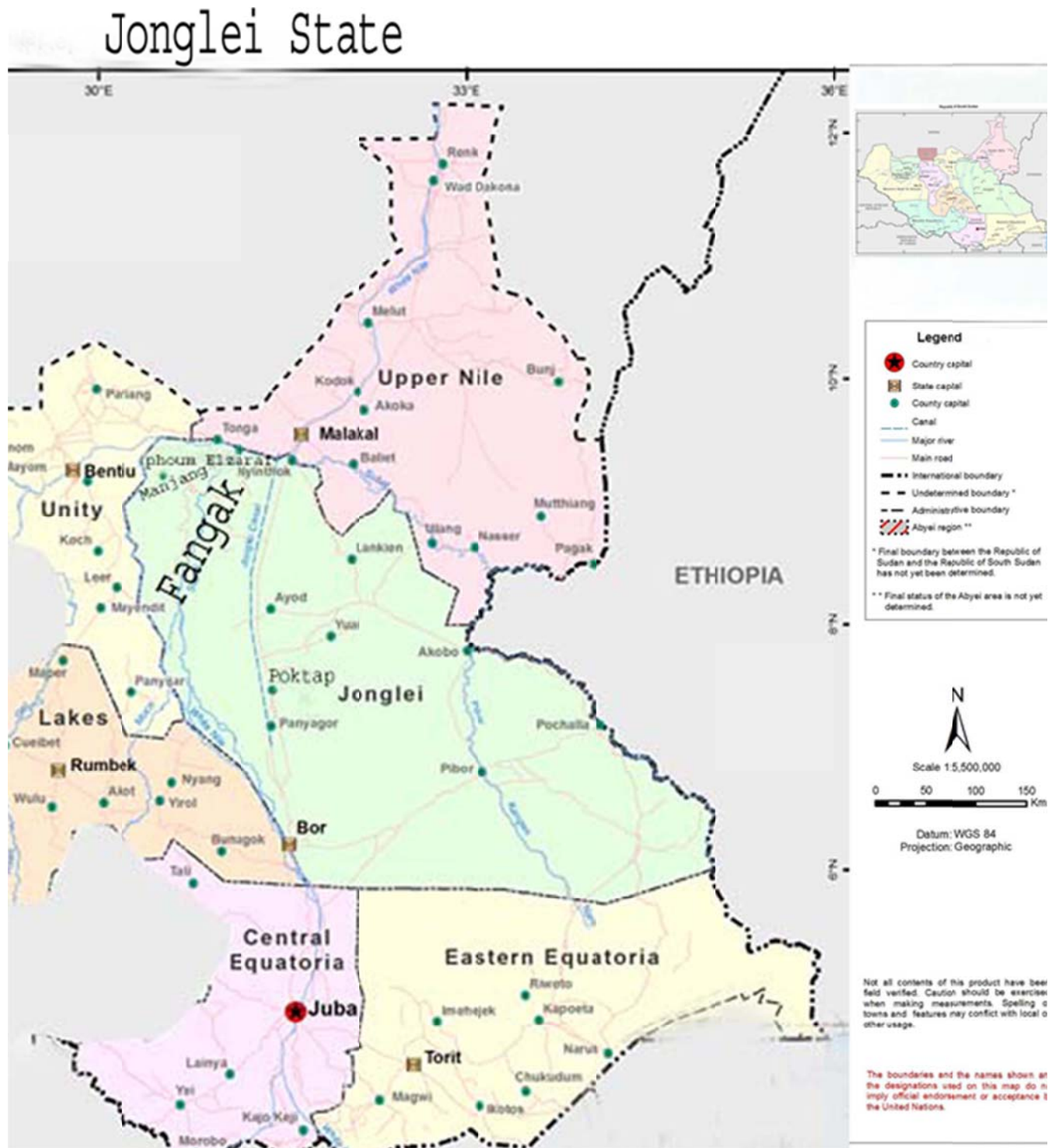
Figure 2. South Sudan Map location of Jonglei State.

Payams (administration units) (Table 4). Three tick genera and seven species were identified. Tick genera were *Amblyomma*, *Hyalomma* and *Rhipicephalus*. Tick species were *A. variegatum*, *A. lepidum*, *H. rufipes, R. (Boophilus) annulatus*, *R. (B.) decoloratus*, *R. e. evertsi*

and *R. sanguineus*. The two *Amblyomma* and two *Rhipicephalus* (B) species were found throughout the study area, while *R. e. evertsi* was found only in Kuer kan village Manjang payam. Mean while *R. sanguineus* was found only in Phoum payam. The highest tick count was

Table 1. Prevalence of *Theileria parva* antibodies tested by ELISA in different locations of Fangak, Jonglei State in September 2011.

Location	No. of Animals	Results positive (+Ve)	Percentage of positive reactors		
			Prevalence pre-cases (+Ve) %	Overall cases prevalence (+Ve) %	Relative occurrence prevalence (+Ve) %
Bichoul Kun village (Phoum payam) Suspected area	34	19	(19/34) 55.88	(19/120) 15.83	(19/53) 35.85
Kuer kan village (Manjang payam) Non suspected area	44	5	(5/44) 11.34	(5/120) 4.17	(5/53) 9.49
Hai Toggar (Phoum payam) Suspected area	42	29	(29/42) 69.05	(29/120) 24.17	(29/53) 54.72
Total	120	53	100	44.17	100

Table 2. Prevalence of *Theileria parva* antibodies tested by ELISA in different sex groups cattle, in Fangak, Jonglei state, September 2011.

Sex	No. of Animals	Results positive (+Ve)	Percentage of positive reactors		
			Prevalence pre-cases (+Ve) %	Overall cases prevalence (+Ve) %	Relative occurrence prevalence (+Ve) %
Female	80	34	(34/80) 42.50	(34/120) 28.3	(34/53) 64.15
Male	40	19	(19/40) 47.50	(19/120) 15.83	(19/53) 35.85
Total	120	53	100	44.17	100

recorded in Phoum payam Group A. (Table 4) summarizes the frequency of adult ticks in the study area. The most abundant tick species in decreasing order were *A.variegatum* (62%). *R.* (*B.*) *decoloratus* (14%), *H. rufipes* (8%), *R.* (*B.*) *annulatus* (6%), while the least frequent ticks were *R. sanguineus* (4%), *A. lepidum* (4%), *R. e. evertsi* (2%). The male ticks represented 63.4% (208/328) outnumbering the females 36.6% (120/328), in all locations.

The geographic distribution and population density of tick species in two proposed groups

The mean tick load per location was the highest in Kuer kan village with mean load (2.8 ± 0.15), while the lowest mean load was recorded in Bichoul kun and in Hai Toggar (2.3 ± 0.14). Both bulls and cows ((2.6 ± 0.14 and 2.6 ± 09 respectively), carried the same load. The animals between one and two years were found to carry more tick load mean (2.7 ± 0.33), while animals of four years and more showed a mean of 2.6 ± 0.09. The lowest tick load was realized in animals of two years and less than four years (2.4 ± 0.16). Animals with brown coat revealed the highest mean of tick load (2.7 ± 0.12) followed by white coat animals (2.6 ± 0.12) while the

lowest infested animals were of black coat with a mean of (2.3 ± 0.14) (Table 5) with no significant difference (P > 0.05).

Discussion

Ticks and tick-borne diseases (TBDs) are one of the most important causes of poor animal production and productivity around the world (FAO, 1983; Singla et al., 2007 and Salih et al., 2015). In the current study no parasites were seen in blood smears despite the detection of antibodies to ECF in serum sample with 44.17% positive cases. This is in agreement with finding of Malak et al., (2012) who reported prevalence of 35.60 and 70.60% in Yei and Kajo Kaji, respectively. Julla (1994) revealed 44.20% prevalence which is similar to this study and Morzaria et al., (1981) revealed 51.60 and 61.50% prevalence in Aswa river and 12.10% prevalence in Juba and 83.30% prevalence in Chukudum after outbreak. This study used indirect ELISA while the other three studies used IFA. These findings are in agreement with Marcellino (2008) with his finding in Terekaka County where he found *T. parva* antibodies without detection of *R. appendiculatus*. Although in Terekaka the prevalence was 71.80% while in this study it was 44.17%;

Table 3. Prevalence of *Theileria parva* antibodies tested by ELISA in different age groups of animals , in Fangak ,Jonglei State in September 2011.

Age group	No of animals	Results	Percentage of positive reactors		
		positive (+Ve)	Prevalence per-cases	Overall cases prevalence	Relative occurrence prevalence
			(+Ve) %	(+Ve) %	(+Ve) %
1 < 2 year	9	4	(4/9) 44.44	(4/120) 3.33	(4/53) 7.55
2 < 4 year	20	10	(10/20) 50.00	(10/120) 8.33	(10/53) 18.87
≤4 year	91	39	(39/91) 42.86	(39/120) 32.5	(39/53) 73.58
Total	(120)	(53) 44.17	100	44.17	100

Table 4. Tick species infesting cattle in two proposed groups (A and B) in Fangak, Jonglei State during September 2011.

Tick species	Group A(Phoum payam)		Group B (Manjang payam)		Percentage per group		Relative Occurrence (Total) 100%
	Male (%)	Female (%)	Male (%)	Female (%)	Group A (%)	Group B (%)	
Amblyomma variegatum	109 (33.23)	27 (8.23)	61 (18.59)	6 (1.82)	136 (41.46)	67 (20.42)	203 (62)
Amblyomma lepidum	5 (1.52)	3 (0.91)	5 (1.52)	0	8 (2.43)	5 (1.52)	13 (4)
Hyalomma rufipes	5 (1.52)	6 (1.82)	14 (4.26)	0	11 (3/35)	14 (4.26)	25 (8)
Rhipicephalus e. evertsi	0	0	2 (0.60)	3 (0.91)	0	5 (1.52)	5 (2)
Rhipicephalus sanguineus	7 (2.13)	7 (2.13)	0	0	14 (4.26)	0	14 (4)
Rhipicephalus(B)decoloratus	0	25 (7.62)	0	22 (6.7)	25 (7.62)	22 (6.7)	47 (14)
Rhipicephalus (B) annulatus	0	9 (2.72)	0	12 (3.65)	9 (2.72)	12 (3.65)	21 (6)
Total	126 (38.41)	77 (23.47)	82 (25.00)	43 (13.11)	203 (61.89)	125 (38.11)	328 (100)

he stated that due to seasonal migration of animals to endemic areas while here in Fangak County it is due to trade and intermarriage and rampant movement of animals between endemic and free zone with no proper control measures. Phoum payam act as a trade centre and main transporting station, despite the fact that Manjang Payam could be more suitable area for survival of vector of ECF due to vegetation cover in the area and similar environment of Bor county in south part of the state which known to be endemic area as reported by Kivaria et al., (2012). This is an indication that ECF can establish if the tick is introduced in this area. The sex of the animals does not have any epidemiological significance although female animals revealed in one occasion higher rates than males. This might be due to the fact that most of the herds comprises of females even most of animals paid for dowry were females. Age groups finding are similar to Salih et al., (2007a) who indicated that antibodies profile was found to increase significantly with advance in age. This study is also in agreement with Marcellino (2008) who found older animals from four years and above revealed higher prevalence than young animals of one year to less than four years. This study is in agreement with the study of Darghouth et al., (1996) who reported that older cattle had seropositive level to *T. annulata* antibodies more than younger ones.

This study agrees with Zessin and Baumann (1982) in Bahr Elghazal, South Sudan who detected *T. parva* using blood smears and lymph node smears. The current study detected the antibodies by ELISA with no presence of piroplasms in blood smears. No presence of the vector in the two areas might be due to the short time of study or the use of Oxyteracycline Long Acting by cattle owners that could have concealed the piroplasms. The trypanosomes were detected accidentally while carring the examination to find the piroplasms for TBDs. In addition area is endemic for trypanosomiasis with presence of mechanical vector. *Tabnus* spp observed do not have any impact for ECF unless on others TBDs. The results of ticks identified in this study are in agreement with those reported by Korok (2005). The finding that *A. variegatum* showed the highest prevalence rate which is in agreement with the finding of Ochi et al., (2009). Meanwhile, Korok (2005) reported *A. lepidum* was the highest, probably due to difference in climatic conditions. Fangak County experience frequent flooding and has more forest cover than Pibor which tends to be a semi-arid in nature. In contrast, while Korok (2005) found both sexes of *R* (*B*) *decoloratus* and *R* (*B*) *annulatus*, the current study showed the opposite, that is, females without males. Also, Korok (2005) found *R. praetextatus*, while this tick species was not reported in the current study.

Table 5. Mean (±SE) numbers of ticks encountered in Fangak county Jonglei State according to locations, sex, age group and animals colour September 2011.

Locations	No of animals	Mean (± SE)
Bichoul kun village	41	2.6 ± 0.13[a]
Kuer kan village	45	2.8 ± 0.15[a]
Hai Toggar	42	2.3 ± 0.14[a]
Hai Moaazker foqu	22	2.9 ± 0.21[a]
Sex		
Female	106	2.6 ± 0.09[a]
Male	44	2.6 ± 0.14[a]
Animals age (years)		
1 < 2	11	2.7 ± 0.33[a]
2 < 4	24	2.4 ± 0.16[a]
≤ 4	115	2.6 ± 0.09[a]
Animals colour		
Black	14	2.3 ± 0.14[a]
Brown	60	2.7 ± 0.12[a]
White	76	2.6 ± 0.12[a]

Means (± SE) followed by the same letter in each column are not significantly different at 5% level based on Rayan´s Qtest (REGWQ), (1989).

Generally, results of tick's collection were in agreement with the findings of Marcellino et al. (2011) and Julla (1994), but with the absence of *R. appendiculatus* in this study and absence of *A. lepidum* in Marcellino et al ., (2011b) but were present in Julla study (1994). This study agrees with Hoogstraal (1956) in Fangak where he encountered *H. rufipes* and *R* (*B*) *decoloralatus* and other species were reported in nearby areas like Malakal, Tonga and Atar. It could be concluded that ECF comprises a threat in the northern areas of Jonglei State if it is accidently introduced with its vector *R. appendiculatus*.

Conclusion

Regarding the fact that *T. parva* antibodies were present in the area with 44.17% prevalence, more efforts are needed to determine the extension of ECF and its vector *R. appendiculatus* to the northern parts of Jonglei State. Seasonal collection of ticks throughout the State and mapping of the distribution of *R. appendiculatus* in the State would help in formulating future plans for control of ECF in the State.

Impact

The finding of this study may address the cattle owners, stakeholders and policy maker to draw their attention to critical movement of ECF toward north part of the country and possibility of establishing itself in new areas and threaten most populated animal will impact of livelihood of those communities. Combined efforts by the Government, with different stakeholders to determine the presence of other T and TBDs in the State are required. The principal action should be developing and production of animal health policy to, among others, regulates animal movement within the State and to the adjacent States. Such a policy may also reduce incidence of cattle raiding amongst the tribes of Jonglei State. This result of ECF antibodies could be an alarm to migrate cattle owners from South Sudan.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGMENTS

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Full Length Research Paper

Serological evidence of foot-and-mouth disease virus (FMDV) antibodies in pigs from northeastern Nigeria

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Foot-and-mouth disease (FMD) is endemic in Nigeria with studies conducted in cattle, sheep and goats. A cross-sectional study for foot-and-mouth disease virus (FMDV) antibodies in field sera collected for African swine fever (ASF) surveillance was conducted in order to determine the status of FMDV in swine within the study area. Four hundred and fifty field sera collected as part of National ASF Surveillance Programme from two states of Taraba and Adamawa in Northestern Nigeria were selected and screened for FMDV non-structural proteins using 3ABC ELISA. Positive sera were serotyped using a Solid Phase Competitive ELISA (SPCE) for antibodies specific to FMDV serotype A and O. The results revealed an overall seroprevalence of 1.11% (5/450) with evidence of FMDV serotype A circulating in the swine population within the study area.

Key words: Foot-and-mouth disease (FMD), antibodies, pig, Nigeria.

INTRODUCTION

Foot and mouth disease (FMD) is considered one of the most contagious diseases affecting economically important livestock species such as cattle, sheep and pigs in the 2007 Terrestrial Animal Health Code by the World Organisation for Animal Health (Office-International-des-Epizooties) (Orsel., et al., 2009). Seven immunologically distinct FMDV serotypes have been described, namely serotypes A, O, C (the so-called European types), Asia-1 and three South African Territories (SAT) types 1, 2 and 3. Serotypes A, O, C and Asia-1 constitute a distinct lineage separate from the SAT viruses (Vosloo, et al., 2009). However, it has been established that infection with one serotype of FMD virus does not confer immunity against another (Pereira, 1976). FMD cannot be distinguished clinically from other vesicular diseases, including swine vesicular disease, vesicular stomatitis and vesicular exanthema (OIE, 2012).

FMD is endemic in Nigeria with outbreaks occurring in cattle seasonally. Serotypes O, A, and SAT 2 have been reported as the cause of recent outbreaks in selected locations across the country (Fasina, et al., 2013). FMD affects all cloven-hoofed animals (Alexandersen and Mowat, 2005). However recent studies in Nigeria involving cattle, sheep, goats and pigs (Lazarus, et al., 2012) demonstrated evidence of antibodies in cattle, sheep and goats only with no evidence of antibodies from pig samples. In Nigeria, unlike other parts of Africa where abundant wildlife population exist, no information is available on FMD in wildlife species. However, we cannot

rule out wildlife-livestock interaction since the husbandry practice in most part of west and central Africa is majority pastoralism.

The role of pigs in the epidemiology of FMD has been recognized in outbreaks in FMD-free countries (Chen, et al., 2008; Gibben, 2011; Hayama, et al., 2012; Knowles, et al., 2001). However in endemic countries of Africa, surveys have demonstrated low to high prevalence of antibodies against FMD virus (Fernandez-Pacheco, et al., 2012; Wekesa, et al., 2014). In a study in Thailand, it has been demonstrated that pigs were not involved in outbreak cases (Chamnanpood, et al., 1995). However, in China and Uganda, FMDV have been isolated from pigs (Yang, et al., 2011; Kerfua, et al., 2013).
In Africa, FMD viruses are maintained in cattle and African buffaloes (*Syncerus caffer*) in domestic and wildlife ecology, respectively (Vosloo, et al., 2004). However, it has been reported that the pig-adapted Cathay strain of FMD virus apparently does not infect large ruminants in the field or experimentally and requires cells of porcine origin for primary isolation (OIE, 2012).

Typical cases of FMD are characterized by high fever, loss of appetite, salivation and vesicular condition of the feet, buccal mucosa and, in females, the mammary gland (Thomson, 1994). Clinical signs can vary from mild to severe, and fatalities may occur, especially in young animals (OIE, 2012). Even though FMDV outbreaks in pig have been reported in other countries, especially where pig production is intensive, no clinical case of FMD has been reported in swine in Nigeria. In previous related studies, no evidence of antibodies to FMDV was demonstrated in sera of pigs (Lazarus, et al., 2012). However, seroprevalence of FMD antibodies in cattle, sheep and goats were reported (Ehizibolo, et al., 2010; Fasina, et al., 2013; Ishola, et al., 2011; Lazarus, et al., 2012). Nigeria has a limited swine population of 9 million compared to the 17 million heads of cattle (WAHID, 2013). The pig production in most part of the north is backyard piggery system which is on a small scale production. In the country's southwest, more intensive commercialized piggery systems are in place with good market for pork. This study investigated the presence of antibodies against FMDV in pig sera collected as part of a National ASF Surveillance Programme in two states of Taraba and Adamawa north-eastern Nigeria.

MATERIALS AND METHODS

Study area and sample selection

Two States were conveniently selected from a list of states that submitted samples to the National Veterinary Research Institute, Vom for the National ASF Surveillance Programme in 2009.These are Taraba and Adamawa states; they are the second administrative unit in Nigeria administrative structure. These states share land borders with the Adamawa Province of Cameroun where most pastoralist cattle within the Northeast come from. These states also have suitable vegetation and climate that supports the local livestock industry in the country. Farmers in these areas practice the backyard piggery system in addition to keeping other livestock. FMD outbreak is a seasonal occurrence in this area and pigs reared on backyard piggery system normally come in contact with cattle reared within the community. More samples were selected from Taraba relative to Adamawa state, considering the distribution of piggery in Taraba to Adamawa. .

Serology

The ELISA serology was performed according to the manufacturer's instructions for PRIOCHECK FMD-3ABC NS protein ELISA (Sorensen et al., 1998; Brocchi et al., 2006). Briefly described, 80 µl of the ELISA buffer and 20 µl of the test sera were added to the 3ABC-antigen coated test plates. Negative, weak positive and strong positive control sera were added to designated wells on each test plate, gently shaken and incubated overnight (18 h) at 22°C. The plates were then emptied and washed six times with 200 µl of washing solution and 100 µl of diluted conjugate was added to all wells. The test plates were sealed and incubated for 60 min at 22°C. The plates were then washed six times with 200 µl of the washing solution and 100 µl of the chromogen (Tetra-Methyl Benzidine) substrate was dispensed to all wells of the plates and incubated for 20 minutes at 22°C following which 100 µl of stop solution was added to all the wells and mixed gently. Readings were taken on a spectrophotometer Multiskan® ELISA reader (Thermo Scientific, USA) at 450 nm and the OD450 values of all samples was expressed as Percentage Inhibition (PI) relative to the OD450 max using the following formula PI = 100 - (OD450 test sample/OD450 max) × 100. Samples with PI = ≥ 50% were considered positive, while those with PI < 50% were declared negative. Since the 3-ABC ELISA for FMD was = 100% specific and > 99% sensitive, the percentage prevalence was taken as true prevalence. All samples that tested positive for NSP using the 3ABC ELISA were further typed for structural proteins using a Solid Phase Competitive ELISA (SPCE) for antibodies to FMDV serotype A and O, (IZSLER Brescia, Italy),the test was performed according to the manufacturer's instructions.

RESULTS

The result showed an overall seroprevanlence of 1.11% (5/450) in the study area. 1.26% (5/389) were positive in Taraba State and 0% (0/56) in Adamawa state as indicated in Table 1. Furthermore, serotype specific ELISA test for FMDV serotype A and O revealed 1 out of the 5 positive samples, positive for FMDV serotype A.

DISCUSSION

In most communities within the study area, cattle, sheep, goats and pigs interact freely during grazing and their possible role in the maintenance and epidemiology of important animal pathogens are poorly understood. In this study, we attempted to screen sera of swine from areas that have reported FMD outbreaks for evidence of antibodies to FMDV. In Nigeria, pigs are not vaccinated for FMD and evidence of non-structural protein antibodies might suggest exposure to FMDV. The epidemiology of FMD in swine in Nigeria is poorly understood as a result

Table 1. Seroprevelence of FMDV in pigs and serotype detected.

States	Total number of sera tested	Number positive	Number negative	Seroprevalence % (95%CI)	Serotype O	Serotype A
Taraba	394	5	389	1.26 (0.5 - 3.0)	0	1
Adamawa	56	0	56	0 (0.5 - 21.0)	0	0
Total	450	5	445	1.11 (0.4 - 2.4)	0	1

of the lack of documented evidence from previous studies. This study however, revealed that 1.11% of the samples tested positive for FMD non-structural proteins which might be an indication of exposure to FMDV. In a previous study involving cattle, sheep, goats and pigs from some selected states in Northern Nigeria, samples from swine never tested positive for FMD (Lazarus, et al., 2012). However, in a related study 2% seroprevalence was reported in 869 pig sera collected in sub-Saharan Africa (Fernandez-Pacheco, et al., 2012). In a study in Kenya, serological evidence for SAT 1 FMDV infection in pigs was demonstrated without obvious clinical signs during an outbreak in cattle (Wekesa, et al., 2014). The low seroprevalence observed may be due to under reporting of FMD or as a result of a too small sample size to give a comprehensive picture of the presence of antibodies against FMDV. However, due to the cultural and farming practices in the study area the distribution of pigs is not compared to other livestock species as such few farmers engage in swine production and thus few pig populations. Though FMD has been reported to be severe in pigs (Yoon, et al., 2012), no clinical signs of the disease were observed in the sampled pigs. This may be one of the reasons why FMD is often considered not one of the important diseases of swine in Nigeria. This study has indicated that the pigs in the study area have been exposed to FMDV serotype A. This has been supported by the result of the SPCE serotypes A and O for samples that tested positive at NSP. The inability of the other samples tested positive at NSP to be able to be serotyped might be that they are positive for other serotypes than the A and O antigens in the SPCE. Currently we may not have evidence of how the transmission must have occurred in the swine population, but as a result of the farming system practiced, there may be a need to investigate if this is a result of some inter species transmission from cattle to pigs, since it did not give a clear clinical symptoms in pigs which may suggest an insufficient adaptation in the host. It has been demonstrated that host susceptibility to certain FMDV strains varies (Yoon, et al., 2012), and previous studies have observed pigs to play significant roles in the spread of FMDV serotypes O (Gibbens 2011; Hayama, et al.,

2012), Asia 1 (Yang, et al., 2011) and A (Mohamed, et al., 2011). It has been reported that subclinically infected pigs with low level antibody responses may have very limited ability to transmit infection (Kitching and Alexansersen 2002). Hence the findings in this study could indicate infections of pigs acquired from cattle, at low levels that could not develop clinical symptoms.

Conclusion

This is the first report of serological evidence for FMDV serotype A in pigs in Nigeria without obvious clinical signs. Although it has not been reported that pigs may play an obvious role in the epidemiology of FMDV in Nigeria, this study demonstrates that they can be infected and could become important in the epidemiology of the disease when exposed to a virulent strain of FMDV. Therefore, we recommend that contacts between cattle and pigs in communal grazing areas should be limited to avoid interspecies transmission of FMDV and other important animal pathogens. This study is limited by small sample size and incomplete epidemiological data generated. Another major limitation to the study is the SPCE Kit used which was able to detect serotypes A and O. It may be necessary to serotype all NSP positive samples in future studies for all the FMD endemic serotypes within Nigeria.

RECOMMENDATION

We therefore recommend more in depth research into the possible role of pigs in the epidemiology of FMDV in Nigeria and possibly attempt to isolate and characterize FMDV in pigs.

Conflict of Interest

The authors have not declared any conflict of interest.

System (NADIS), Federal Department of Livestock Nigeria, Staff and management of the Foot and Mouth Disease Research Laboratory, National Veterinary Research Institute, Vom.

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Full Length Research Paper

Serological evidence of foot-and-mouth disease virus (FMDV) in camels (*Camelus dromedaries*) in Nigeria

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Foot-and-mouth-disease (FMD) is one of the most important trans-boundary animal diseases in Africa with outbreaks occurring mostly in cattle. However, there is scarcity of information on the potential role of camels in the epidemiology of FMD virus in West Africa. A total of 360 camel sera collected from abattoir in Nigeria from different geo-political zones (North, West and East) were screened for the presence of antibodies produced against 3ABC non-structural proteins (NSP) for foot-mouth-disease virus (FMDV) using a commercially available kit prioCHECK® FMDV NS. Thirty nine, (10.83%) out of the 360 sera samples were tested positive for 3ABC NSP ELISA. The 39 positive samples were further subjected for sero-typing using solid-phase competitive ELISA (SPCE) for antibodies to FMDV serotype A and O (Solid-Phase Competitive ELISA, IZSLER Brescia-Italy). Two out of the 39 sera samples were positive for serotype A and the remaining were negative for both serotype A and O. This appears to be the first report of evidence of FMD antibodies in dromedaries in West Africa and that dromedaries may be susceptible to FMDV infection.

Key words: 3ABC; camel, ELISA, foot-and-mouth-disease (FMD), non-structural proteins (NSP), sera.

INTRODUCTION

Foot-and-mouth disease (FMD) is the most economically important disease of cloven hoofed animals. The virus is highly contagious, affecting almost exclusively among animals such as cattle, sheep, goats, Bactrian camels and pigs (Wernery and Kinne, 2012). The disease affects both domestic and wild animals. The disease is characterized by lesions in hairless area and myocardial degeneration in calves has been observed (Wernery and Kinne, 2012). Many countries around the globe have been certified free for FMD by Office International des

Epizooties (OIE) (Wernery and Kaaden, 2004). FMD virus has continued to circulate in other continents of the World like Asia, South America, Middle East and Africa. This has affected the economy of such continents significantly due to the effects on international trade of susceptible animals and their products.

The Camelidae are found in countries like North and East Africa, Middle and East Asia as well as South America where FMD is endemic (Du et al., 2009). The dromedary camels are also found in West Africa, Nigeria

in particular because of its significance in trade and meat products. There are divergent opinions as to whether Camelidae family are susceptible to FMD or not, or they may serve as a reservoir host of the virus (Yousef et al., 2012). The two closely related camel species of Bactrian and dromedary camels possess noticeably different susceptibility to FMD virus (Larska et al., 2009). Bactrian camels under experimental studies can easily develop obvious clinical sign of FMD (Larska et al., 2009), while several investigation appear to suggest that dromedaries are less susceptible to inoculation with FMD virus serotype O but that they do not present a risk in transmitting the disease to susceptible animals (Wernery and Kaaden, 2004; Alexandersen et al., 2008). However, Kumar et al. (1983) have described the isolation of FMDV serotype O from one of two randomly selected dromedaries in India, and Moussa et al. (1987) in Egypt described a strain of type O FMD virus isolated in Giza from a camel with vesicular, ulcerative stomatitis and they suggested that dromedaries are susceptible to natural FMD infection (Yousef et al., 2012).

FMD is caused by an RNA aphthovirus of the family picornaviridae. There are seven immunological serotypes (A, O, C, SAT 1, 2, 3 and Asia1) that exist and over 60 subtypes of the virus are circulated Worldwide (Wernery and Kinne, 2012). Four (A, O, SAT 1 and 2) out of this seven serotype exist in Nigeria (unpublished data). Foot-mouth-disease virus (FMDV) is a small non-enveloped virus and has a genome of 8.5 kb which encodes for structural as well as non-structural proteins (NSPs) (Yousef et al., 2012). The viral capsid composed of four structural proteins, VP1, VP2, VP3 and VP4 (Fry et al., 2005).

A structural protein produces antibodies to FMDV in vaccinated animals, whereas infected animals produce antibodies to both the structural and non-structural proteins (Yousef et al., 2012). Meanwhile, assays to demonstrate antibodies against non-structural proteins have potential to differentiate infected animals from vaccinated (Berger et al., 1990; Rodriguez et al., 1994; De Diego et al., 1997; Clavijo et al., 2004). The disease is endemic in some parts of Europe, Africa, Middle East and Asia, but places like North America, Australia, New Zealand and many countries in Western Europe are free from the disease and have stringent regulations preventing the introduction of the virus (Wernery and Kinne, 2012).

The first reported case of FMD outbreak in Nigeria was in 1924, which was attributed to type O virus (Libeau, 1960). Subsequently, other serotypes (A, SAT 1 and SAT 2) were reported, and each of these introductions was associated with trade cattle entering Nigeria from neighboring countries, (Nawathe and Goni, 1976; Owolodun, 1971; Durojaiye, 1981; Abegunde et al., 1988). It should be understood that trans-humance production system is the predominating system of animal management in the Sub-Saharan Africa and many of

these individuals traverses national borders (especially those of Central African Republic, Chad, Niger, Cameroun, Benin and Nigeria) in search of feed and water resources for their livestock without any recourse to quarantine and control measures. Furthermore, camels are frequently moved across the desert of Niger, Chad, Benin and Central African Republic to Nigeria across areas that FMD outbreak is endemic in cattle and other small ruminants. Therefore, it is possible that camels may play a possible role in the maintenance and transmission of FMDV and may carry FMDV over a very long distance and across the borders (Yousef et al., 2012). However, because of the limited information concerning FMDV in camels in West Africa, Nigeria in particular, this study was aimed to investigate the serological evidence of natural exposure of camels *(Camelus dromedaries)* to FMD virus. The investigation was to detect the presence of antibodies against non-structural proteins (NSP) using competitive ELISA and solid-phase competitive ELISA (SPCE) for serotype specific FMDV antibodies, to evaluate the role of camels in the epidemiology of FMD in Nigeria and by extension West Africa.

MATERIALS AND METHODS

A total of 360 abattoir camel sera samples were collected from North Western Nigeria (Kano and Sokoto States) and North Eastern Nigeria (Borno State) over a period of one year (November, 2010 to October, 2011). The sera were collected from all the slaughtered camels in the said abattoir without evidence of any clinical signs of FMD, even though the camels had unrestricted contact with susceptible ruminants (cattle, sheep and goats) that had history of infection with FMD. Whole blood was collected in a wide mouth sample collection bottles which was allowed to clot at room temperature for about 3 to 4 h. The serum was harvested and transferred into a cryovials for storage at -20°C until testing. All positive sera were serotype for antibodies against FMDV serotypes A and O. The prioCHECK® FMDV NS commercial ELISA kit (Prionics Lelystad B.V, The Netherlands) for detection of antibodies against non-structural proteins (NSP) of FMDV was used for testing serum samples of cattle, sheep, goats camels and pigs. The assay was performed according to the manufacturer's protocol and the optical density (OD) value was read using Multiskan® spectrophotometer (Thermo Scientific, USA) at a 450 nm wavelength and results expressed as a percentage inhibition (PI) of the controls and the test sera which was calculated using the formula in the protocol (Sorensen et al., 1998).

Solid-phase competitive ELISA (SPCE) for antibodies to FMDV serotype A and O

Commercial SPCE kit (IZSLER Brescia, Italy) was used for detection of serotype-specific antibodies (A and O) to foot-and-mouth disease virus according to the manufacturer's instructions. The criteria for the validity of the test are that the spectrophotometric readings must be ≥1 OD in the wells of the negative control while the positive control serum is expected to give ≥90% inhibition at 1/10 dilution and >50% inhibition at the second dilution (I/30). For screening purposes, the test sera is considered positive when it produces an inhibition ≥70% at the 1/10 dilution and negative when

Table 1. Abattoirs from where camel samples were collected and results of samples tested.

Abattoir location	No. of animals tested	No. of samples tested positive	NSP prevalence (%)	95% CI
Maiduguri	68	1	1.47	0.07 - 7.04
Kano	260	37	14.23	10.37 - 18.88
Sokoto	32	1	3.13	0.15 - 14.46
Total	360	39	10.83	7.93 - 14.37

Table 2. Solid-Phase Competitive ELISA (SPCE) for antibodies specific to FMDV serotypes O and A.

Abattoir location	NSP positive	FMD serotype O	FMD serotype O % (95%CI)	FMD serotype A	FMD serotype A% (95%CI)
Maiduguri	1	0	0 (0 – 95)	0	0 (0 – 95)
Kano	37	0	0 (0 – 7.78)	2	5.4 (0.92 – 16.73)
Sokoto	1	0	0 (0 – 95)	0	0 (0 – 95%)
Total	39	0	0 (0 – 7.39)	2	5.1 (0.86 – 15.93)

producing an inhibition of <70% at the dilution of1/10.

RESULTS

The overall result indicated 10.83% (95% CI: 7.93 to 14.37) of the total serum samples collected from Maiduguri, Kano and Sokoto abattoirs to be positive for antibodies against FMD NSP (Table 1). All sera that tested positive for FMD NSP were further analyzed for antibodies against FMD structural proteins for serotypes A and O using a SCPE and the results is presented in Table 2. The optical density of camel sera was read on a MultiSkan spectrophotometer reader at 450 nm wavelength.

DISCUSSION

FMD is endemic in most of Sub-Saharan Africa, in particular with unrestricted movement of susceptible animals across the border with no or less control measure instituted to the susceptible animals. Because of the poor veterinary services in most African countries and limited information on the role of camels (dromedary camels) in the epidemiology, few documented evidence exists on its epidemiology of FMD. Camels however, move frequently across the Sahara desert for grazing and trade purposes thereby mixing freely with susceptible animals in endemic countries like Niger, Nigeria, Chad and Central African Republic. These results indicate serological evidence of FMD non-structural proteins in dromedaries camel which may be as a result of exposure to FMDV since camels are not vaccinated against FMDV. It could be that during FMD outbreak, camels come in contact with infected susceptible animals like cattle, sheep and goats which are often herded together by most pastoralists.

These results contradict several reports by researchers that tested camel sera in Africa and the United Arab Emirates for evidence of FMD with negative results (Wernery and Kaaden, 2004). Also, the finding of Moussa and Yousef (1998) that the antibodies identified by Richard (1979) were non-specific inhibitory substances frequently observed in camel sera may be correct but with the use of ELISA test, it is confirmed that dromedaries develops antibodies against FMDV and this results is in agreement with studies by Moussa et al. (1987) in Egypt which indicated the susceptibility of dromedaries to natural FMD infection. Similarly, Metwally et al. (1986) did experimental studies in dromedaries in Egypt with FMD serotype O1/2/72 Egypt with no clinical signs observed, but the virus was re-isolated from one camel between one to three weeks post-inoculation (PI), both dromedaries sero-converted but with low titers, which lasted for six weeks and this contradicted the studies by Moussa et al. (1987) where the dromedary did not develop any antibodies to the artificial infection which suggest the lack of antibodies development is as a results of route of inoculation.

From these results, it is clear that dromedaries can contract the FMDV by contacts with FMD infected animals, but may not pose risk of transmitting the disease to susceptible animals. According to Wernery and Kaaden (2004) it seems that dromedaries do not become FMD carriers because FMDV has not been isolated from oesophageal-pharygeal fluid (OPF) 14 days after viral exposure and the statement agrees with the study of Farag et al. (1998) where they were unable to isolate FMDV from 30 probang samples harvested from

dromedaries on different farms in Saudi Arabia where FMD was said to be endemic. This study was limited because of the availability of the ELISA kit that could test only for two FMD structural protein serotypes A and O. Therefore, other serotypes might have been remained undetected as a result of the non-availability of a kit that could test for other FMDV serotypes that circulates within this region.

However, from the results obtained dromedaries appear to be susceptible to infection with FMDV but may likely not play a significant role in the epidemiology of FMD. However, further research on the epidemiology of FMD in dromedaries in West Africa is necessary where the disease is endemic.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Prevalence of bovine tuberculosis in cattle slaughtered at Gombe township abattoir, Gombe State, Nigeria

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A study to determine the prevalence of bovine tuberculosis in cattle slaughtered in the Gombe township abattoir was conducted from March to July, 2009. Three hundred and twenty (320) cattle comprising of four breeds, slaughtered at the abattoir were examined post mortemly for bovine tuberculosis. The four breeds and number of each examined were White Fulani (155), Red Bororo (92), Sokoto Gudali (53) and Muturu (20). Eighty five (26.6%) cattle were found to be infected with bovine tuberculosis. On the basis of animal characteristics, white Fulani breed 60 (38.7%), females (cows) 58 (62.4%) and adults 45 (14.1%) had the highest prevalence of bovine tuberculosis than other breeds, males (bulls), young adults and young, respectively. There was significant association between sex, age and breed of cattle with infection (P < 0.05). The study suggests that bovine tuberculosis is a disease common to cattle in Gombe, though with moderate prevalence and could be of economic and public health importance.

Key words: Bovine, tuberculosis, cattle, Gombe township abattoir, Nigeria.

INTRODUCTION

Bovine tuberculosis is a chronic contagious respiratory disease of cattle, usually caused by *Mycobacterium bovis* (Edward, 1998). Cattle are considered to be the main hosts of *Mycobacterium bovis*, although isolations have been made from many other livestock and wildlife species and transmission to humans poses a public health problem (OIE, 2010; Gupta et al., 2009; Hiko and Agga, 2011 and Mamo et al., 2011). Bovine tuberculosis along with other diseases is thus a serious problem in cattle rearing and seriously affects the productivity of the livestock industry in developing countries like Nigeria (Radostitis et al., 2007; Cosivi et al., 1998; Fikre et al.

2014; Berg et al., 2009; Demelash et al., 2010; Regassa et al., 2010 and Firdessa et al. 2012). In Ethiopia as well as other African countries like Nigeria, bovine tuberculosis is endemic in cattle and prevalence varies depending on the geographical location, breed and the husbandry practices. In such developing countries, the occurrence of bovine tuberculosis is widely distributed in areas where pasteurization of milk is rarely practiced and control measures are applied sporadically (Cosivi et al., 1998). In Gombe, milk pasteurization has not been largely practiced and no proper control measures have been put in place, thus people residing in the area
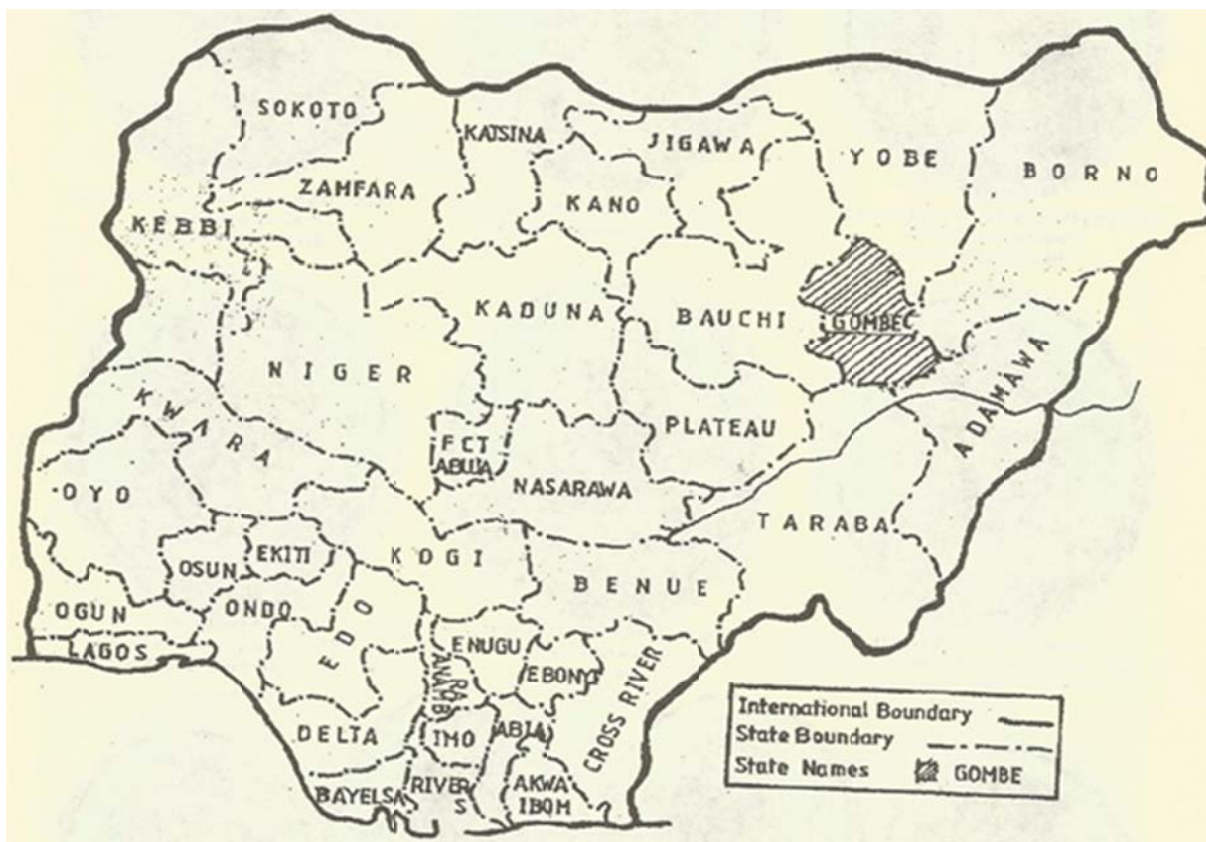
Figure 1. Map of Nigeria showing Gombe State, Nigeria.

consume a lot of raw milk predisposing them to human tuberculosis. Residents of the area could also be infected through drinking water that is contaminated with *M. bovis,* consumption of under cooked meat and drinking of milk directly from the udder of cows. Despite the existence of potential risk factors in the area, the occurrence of bovine tuberculosis has not been investigated, as previous studies estimating prevalence of bovine tuberculosis in the area are unavailable. There is therefore lack of adequate data on the epidemiology and public health implication of this disease in the area. The present study was thus, designed to determine the prevalence of bovine tuberculosis in Gombe using post mortem examination, in order to provide baseline information on the prevalence of the disease in the area (O'Reilly and Daborn, 1996). This type of information is of importance to public health officials.

MATERIALS AND METHODS

Study area

The study was conducted in Gombe township abattoir. Gombe town is the capital of Gombe State in North eastern Nigeria (Figure 1). Gombe town lies between latitude 10°08[1]N and 11°24[1]E and longitude 11°02[1]N and 11°18[1]E while Gombe State lies between

Latitude 9°30[1] and 12°30[1]N and Longitude 8°45[1]and11°45[1]E. The State covers a land area of 20,265 sq. km, with a topography that is mainly mountainous, undulating and hilly, with flat open plains. The climate is warm, with temperatures not exceeding 30°C from March to May, in which March to May are the hottest months. Average daily temperatures are 34°C in April and 27°C in August. The area experiences two seasons; the rainy season, from April to October and dry season, from November to March. Annual average rainfall ranges between 850 to 1000 mm. The relative humidity ranges from 70 to 80% in August and decreases to about 15 to 20% in December. The natural vegetation is typically that of the Guinea Savanna grassland with some concentration of woodlands. This provides enough grazing land and pasture for cattle rearing. Gombe State is predominantly an agrarian state with more than 80% of the population engaged in agricultural production. Cereals such as groundnut, maize, guinea corn, millet and cowpea are predominantly grown in the area and provide enough fodder for the animals. The projected population is about 2,755,387, a majority of which are the Fulani people whose major occupation is cattle rearing (Diary 2012, Gombe State Government, Federal Republic of Nigeria). Cattle slaughtered in Gombe township abattoir from March to July 2009, were the study animals.

Sampling procedure

Three hundred and twenty (320) cattle were examined, which comprised of 155 White Fulani, 53 Sokoto Gudali, 92 Red Bororo and 20 Muturu breeds. Visits were made to the abattoir once a week for a period of three months (April to June). A total of 12 visits

were made during which a total of 320 cattle were sampled. Twenty five cattle were sampled in four visits, 26 in two visits, 27 in two visits, 28 in two visits and 29 in two visits. About 35 to 40 cattle were slaughtered each day in the abattoir, more than 50% of which were sampled during each visit. On each visit, the number of cattle slaughtered, the breed, sex and age were noted. The animals slaughtered in the abattoir were bought from the Tashan Dukku and Pantami cattle markets in Gombe. The animals were brought from other parts of the State and from neighbouring States like Borno, Yobe, Adamawa, Bauchi, Taraba and Kano States. The number of cattle slaughtered at the abattoir, the breed, sex and age, were confirmed by veterinary officers on duty at the abattoir. The current food security regulations in Nigeria, is that cattle suspected for tuberculosis should not be slaughtered for human consumption. Such cattle should be treated and certified tuberculosis free before being slaughtered for human consumption. Any cattle to be slaughtered for human consumption should be certified as being tuberculosis free. This regulation is to be enforced by veterinary officers at the abattoir.

Breed determination

White Fulani

The White Fulani breed has a commonly white coat colour on a black skin with black ears, eyes, muzzles, hooves, horn and tail tips. The breed is characterized by medium to long, high lyre shaped horns. That is, the horns are slender, medium to long size, measuring 81 to 107 cm and are lyre shaped, curved outwards and upwards, with an outward turn at the tip. It either has well developed thoracic humps or humps intermediate with the cervico-thoracic humps. The head is long, wide across the fore head and with a straight or concave appearance. The neck is strong, providing an upward carriage for the head. The average adult wither height is 130 cm. The udder is well developed, of a good shape and strong attachment. The teats are well positioned and are of medium to reasonably large size (Tawah and Rege, 1996 and Gates, 2007). The general shallowness of the body and lack of width, gives the breed a leggy appearance.

Red Bororo

The Red Bororo has red coat colour, with long and lyre shaped horns measuring 80 to 105 cm. This breed is adapted to long distance trekking in the pastoral management system. The Red Bororo is more tolerant to heat and trypanosomiasis than the Sokoto Gudali and more resistant to dermatophilosis and intestinal helminthes than the Muturu thus, has low mortality rate (Tawah and Rege, 1996 and Gates, 2007). It is the most numerous and wide spread breed in Nigeria, representing about 37.2% of the national cattle population. It has excellent potentials for milk and beef and its conformation and body size makes it suitable for draught. Its average lactation period is about 220 days. The average birth weight ranges from 18.2 to 24.2 kg. The mature weight of bulls and cows in the improved system of management is 350 to 665 and 250 to 380 kg, respectively. The slaughter and carcass weights are 325 and 166 kg.

Sokoto Gudali

The Sokoto Gudali has multiple coat colours and the most common one is the black and white coating with light underside, with very short horns. The Sokoto Gudali has deeper body than the White Fulani breed. The head is long and wide between the eyes and across the forehead, with a straight or slightly convex facial profile.

The ears are long, large and convex, sometimes pendulous. The hump is thoracic in position. The average ranges from 130 to 138 cm for males and 116 to 132 cm for females. This breed is owned and managed by Fulani and Hausa pastoralists and transhumant herders (Gates, 2007) who feed them on communally owned grazing lands and browse especially in the dry season. This breed is known for its hardiness to the arid northerly environments and its beef quality and milk. The mature weight ranges from 495 to 660 kg for males and 240 to 355 kg for females.

Muturu breed

The Muturu breed has black and white patches of coat colour, with large udder for milk production (Getty et al., 1975 and Gates, 2007). It is the smallest cattle breed known. The height for withers is 95 cm for males and 88 cm for females. The weight is 147.2 kg for males and 110.0 kg for females. The management level where these cattle are kept is low. The breed maintains good body by grazing and browsing throughout the year. The breed is trypanotolerant and also tolerates ticks and tick-borne diseases. It has significant cultural values and is used for socio-cultural purposes, sacred and dedicated to shrines. It is sometimes used for work and is seldom milked.

Age determination

The age was determined using dentition according to (Getty et al., 1975). Cattle less than one year old (young) had temporary incisors that erupted at birth but could also have deciduous molars or premolars and never both. For cattle between one to three years of age, which were considered as young adults, those between 13 to 14 months had their full set of deciduous incisors but were temporary short, broad and bright. Those between 15 to 18 months of age had their deciduous incisors evident. The incisors were larger and narrower when compared with those of 13 to 14 months of age. The eruptions of the first central permanent incisors were indicative of cattle between 18 to 24 months of age. Cattle aged between 25 to 36 months had their middle incisors well erupted and developed permanently. The eruption of the second and third intermediate incisors indicated that the animal was 36 months old. Cattle above three years of age, which were considered as adults, had their incisors erupted and a few of them had their teeth worn down.

Post mortem examination of the lungs and abdominal cavity tuberculosis

The lungs and abdominal cavity of slaughtered cattle were examined for the presence of nodular tubercles. The lungs were serially sectioned for tuberculosis lesions and palpated to get infected lungs. Infected lungs identified to have tubercles, were incised in order to feel the gritty nature of the tubercles, abscesses were also incised to observe the pus formed inside them, as a result of the *M. bovis* infection. Method for identifying lesions was the same as the typical post mortem examination of cattle in the abattoir. This guaranteed the sensitivity and specificity of the method used for detecting tuberculosis. The lungs were also examined for inflammations. Examination of animals for bovine tuberculosis was carried out with the technical assistance of the veterinary officers at the abattoir, in order to guarantee the reliability and validity of the results. However, no physical examination or radiography was conducted. Only postmortem and laboratory examinations were carried out. After detailed postmortem examination, gross pathological tuberculous lesions were detected from the lungs and abdominal cavity. Both normal and infected

lungs were collected in specimen bottles containing 70% ethanol and taken to the histopathology laboratory of the Department of Veterinary Pathology and Microbiology, Ahmadu Bello University Zaria, Nigeria for laboratory analysis.

Laboratory analysis of infected and normal lungs

A specimen of an infected lung was analyzed histopathologically to identify the presence of granulomatous tubercles, which contained gram positive bacilli. A sample was considered positive for *M. bovis* if there were gram positive bacilli. Tissue sections of the lungs were taken and fixed in 10% buffered neutral formalin for 72 h. Sections were mounted on clean glass slides, dried at room temperature and stained with haematozylin and eosin. These were cover slipped and dried in an oven. The slides were examined under X10 and X40 objectives of the light microscope. This analysis gave the clear difference between the normal and infected lung. It also predicted the presence of *Mycobacterium bovis* that causes the disease.

Data analysis

The prevalence was expressed as a percentage by dividing the number of animals infected by the number of animals examined and multiplying by 100 (Margolis et al., 1982). The chi square test (X^2) was used to determine any possible association between prevalence of the disease and sex, age and breed of cattle. A statistically significant association between the result and the variable (sex, age and breed) was said to exist if the calculated P < 0.05. The null and alternative hypotheses were stated. The degree of freedom (df) was determined as (n - 1). The P - value (α) was set at 0.05 (95% CI). The decision was to reject Ho if $X^2_{cal} \geq X^2_{tab}$ and to accept Ho if $X^2_{cal} \leq X^2_{tab}$. Chi square was calculated using the formula;

$$X^2 = \frac{\sum (O - E)^2}{E}$$

RESULTS

Three hundred and twenty (320) cattle were examined, which comprised of 155 White Fulani, 53 Sokoto Gudali, 92 Red Bororo and 20 Muturu breeds. Postmortem examination showed that 85 (26.6%) of the 320 cattle examined were infected with tuberculosis. These comprised of 60 (38.8%) White Fulani, 9 (17.0%) Sokoto Gudali, 14 (15.3%) Red Bororo and 2 (10.1%) Muturu breeds. Prevalence of tuberculosis was higher in females 58 (62.4%) than in males 27 (11.9%) and Fourteen (29.8%) young, 26 (19.4%) young adults and 45 (32.4%) adults were infected. Chi square test showed association between sex, age, breed and infection (P < 0.05). (Table 1).

DISCUSSION

The prevalence of 26.6% obtained in this study is higher than that of Fikre et al. (2014) who reported prevalence of

11.3 and 20.% at animal and herd level respectively in and around Mekelle in Northern Ethiopia, Ameni and Erkihun (2007) who reported prevalence of 11 and 15% at animal and herd level respectively in Adama town in Ethiopia, Regassa et al. (2010) reported 11.6% at animal level in Hawassa town and its environs in Southern Ethiopia and Anon (2007) which was 2.1% in Maiduguri North eastern Nigeria but lower than 34.1 and 53.6% prevalence at herd and individual animal level respectively reported by Tsegaye et al. (2010), 46.0% prevalence reported in Ethiopia by Demelash (2008) and prevalence of 23.7 and 43.4% at animal and herd level respectively reported by Elias et al. (2008). The observed differences in prevalence could be due to the breeds and number of cattle examined and the production system. In the present study, four breeds of cattle totaling 320 animals were examined, with more males examined than females, more adults examined than young adults and young and the animals were reared under the extensive production system. It is likely that as the number of animals examined increases and the age and sex varies, the number of animals infected may also increase (O'Reilly and Daborn, 1996 and Asseged et al., 2000). Animal reared under the extensive production system are less prone to infection than animals reared under the intensive production system (Fikre et al., 2014). The intensive production system promotes close contact between animals, thereby favouring the spread of the disease. According to Griffin et al. (1993), Ameni et al. (2003) and Elias et al. (2008), poor managerial inputs increase the risk of bovine tuberculosis. Thus, the status of bovine tuberculosis could be improved by adopting sanitary measures that improve hygiene conditions of the animals. In the present study, female animals (cows) had a higher prevalence compared to males (bulls). This is in agreement with the findings of Inangolet et al. (2008) and Fikre et al. (2014). This could be as a result of the fact that cows are confined to a barn and kept long for production purpose which may facilitate infection and acquisition of the disease (Bikon and Oboegbulem, 2007). Dairy cows experience greater production stress and gathering of cattle during milking increases the risk of transmission as shown by bovine tuberculosis modeling in New Zealand (Barlow, 1997). Another explanation is that slaughtered females tend to be older than slaughtered males. The older the animal, the more the opportunity for exposure to tuberculosis, and the greater the likelihood that lesions will be larger and therefore easier to detect on postmortem examination. The higher rate of infection in adults and young adults compared to calves (young), suggests that, as the animal ages, the rate of infection increases and the longer the animal lives, the more the disease manifests itself. The finding of lower prevalence in Muturu breed compared to the other breeds could be that, genetically the Muturu breed is less prone to infection than the other breeds. The high prevalence of the disease in the White Fulani breed

Table 1. Prevalence of tuberculosis in cattle slaughtered at Gombe township abattoir, Gombe State, Nigeria.

Variable	Number of animals						
	Examined	Positive (%)	df	p-value (α)	X^2_{cal}	X^2_{tab}	Decision
Sex	320	85 (26.6)	1	0.05	86.20000	3.84146	Reject Ho
Male	227	27 (11.9)					
Female	93	58 (62.4)					
Age (years)	320	85 (26.6)	2	0.05	6.19000	5.99147	Reject Ho
< 1	47	14 (29.8)					
$1 \le x \le 3$	134	26 (19.4)					
> 3	139	45 (32.4)					
Breed	320	85 (26.6)	3	0.05	23.09000	7.81473	Reject Ho
White Fulani	155	60 (38.7)					
Sokoto Gudali	53	9 (17.0)					
Red Bororo	92	14 (15.2)					
Muturu	20	2 (10.0)					

df = degree of freedom, N = 320 (total number examined).

probably indicates its high susceptibility and low immunity to infection than the other breeds. Thus, susceptibility to infection may be breed-dependent and could be due to some intrinsic and extrinsic factors. It is also likely that certain breed of cattle may be more susceptible to tuberculosis due to housing, management and feeding differences. Human tuberculosis cases exist in the area, and this has necessitated the establishment of tuberculosis treatment centre within the state. The zoonotic risk of bovine tuberculosis is often associated with the consumption of unpasteurized milk infected with *M. bovis*. Consumer of milk in Gombe, generally prefer raw milk because of its taste, availability and lower price. The consumption of unpasteurized milk is a regular practice in Gombe, especially among the Fulani speaking race, where milk forms a major part of their daily meal. The cultural habit of most Fulani cattle rearing families drinking milk directly from the udders of their cattle, could be a predisposing or major risk factor of humans to *M. bovis* infection (Cosiviet al., 1998; Ayele et al., 2004). The culture of sharing human habitation with cattle is a common practice in Gombe, thus, aerosol transmission from cattle-to-man could also be considered. Humans may also be exposed to *M. bovis* infection through food and unhygienic practices. *M. bovis* according to Dankner et al. (1993) and Dela'rua (2006) is genetically similar to *M. tuberculosis* and could cause identical clinical signs in humans. The disease transmission could be cyclical from cow-to-man-to-cow (Cosivi et al., 1998), underlying the existence of higher risk of dissemination of mycobacteria among the cattle and human populations. Radostits et al. (2007) reported that infected cattle could be the main source of infection to other cattle and may act as maintenance and reservoir hosts. Bikon and Oboegbulem (2007), Cadmus et al. (1999; 2004), and Dusai and Abdullahi (1994) earlier reported bovine tuberculosis as a

common disease of cattle in Nigeria. Bovine tuberculosis is thus an endemic problem in Gombe and Nigeria at large, with no well-defined national eradication control programme and could be of zoonotic and public health importance (Alhaji, 1976; Cosivi et al., 1998; Cadmus et al., 2004). Like in North America, carcasses with suspected lesions are condemned and often not allowed for human consumption. Thus, it is recommended that proper meat inspection at the abattoir should be intensified and properly conducted, to avoid the selling of infected lungs to the public. This entails detailed ante-mortem and postmortem inspection of animals brought for slaughter at the abattoir. Meat inspectors should be trained to check for miliary tubercles in the head, fore and hind limbs, lungs, heart, liver, spleen, kidneys and mammary glands and associated lymph nodes. Adequate palpation, with resultant production of gritty sound on incision of some of these organs and lymph nodes would form the basis of tentative diagnosis of tuberculosis. This form of diagnosis will help to a great extent in reducing the spread of zoonotic tuberculosis. When meat inspection procedures are properly carried out, cattle with visible lesions of tuberculosis could be identified. Only cattle that have been certified as disease free should be slaughtered at the abattoir for public human consumption.

CONCLUSION AND RECOMMENDATIONS

The public should be educated on the need to avoid the consumption of infected lungs and the impending dangers of their consumption. Infected lungs should be discarded, burnt or buried and not consumed by the public and owners of such should be adequately compensated. Proper surveillance/ambulatory services should be carried out within the state and its environs, for

diagnosis and treatment of infected animals with the appropriate drugs. The use of novel diagnostic techniques is recommended to monitor and control the spread of the disease in the area and in other areas at risk in Nigeria (Cui et al., 2013; Li et al., 2014).

Conflict of Interest

The authors have not declared any conflict of interest.

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