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Case Report

Confirmatory diagnosis of contagious ecthyma (Orf) by polymerase chain reaction at Adet Sheep Research Sub-Center, Ethiopia: A case report

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An outbreak of contagious ecthyma (CE) was investigated in June, 2012 with morbidity rate of 22% in Adet Sheep Research Sub-Center, Ethiopia. The results of this investigation indicated that the outbreak was caused by infection with CE virus. A polymerase chain reaction (PCR) assay for rapid diagnosis was applied to five scab samples obtained from sheep suspected for CE. To confirm whether the causative agent was present in skin scrapings, PCR of the complete B2L gene to diagnose CE was used in this study. The expected PCR fragments, approximately 1206 bp in length were obtained from DNA which had been extracted from tissue scrapings. All five skin scab samples were confirmed positive to CE by PCR. In conclusion, detailed phylogenetic analysis of CE virus is suggested in order to know the genetic origin of the virus strain as well as for the future choice for immunoprophylaxis.

Key words: Adet Sheep Research Sub-Center, B2L gene, contagious ecthyma, polymerase chain reaction (PCR), sheep.

INTRODUCTION

Contagious ecthyma (CE) virus is the etiological agent of contagious pustular dermatitis and is the prototype of the genus *Parapox virus* (PPV), which is an oval, enveloped virus containing dsDNA genome within the genus *P. virus*, family Poxviridae (Damon, 2007). This epitheliotrophic virus causes a severe exanthematous dermatitis that afflicts domestic and wild small ruminants (Robinson and Balassu, 1981; Keshan et al., 2010). It is characterized by the formation of papules, nodules, or vesicles that progress into thick crusts or heavy scabs on the skin of the lips, on the oral mucosa, tongue, gingiva, and around the nostrils (Robinson and Balassu, 1981; Vikoren et al., 2008). Lesions can also be found occasionally on the teats of nursing animals and rarely on other organs (Vikoren et al., 2008). Characteristic of the disease are proliferative and often self-limiting lesions (Keshan et al., 2010).

The morbidity of the disease may reach 100%. The mortality rate related to CE is usually low, but it may be very high in small ruminants, especially when bacterial or fungal secondary infections occur (Robinson, 1983; Lughano and Dominic, 1996; Haig and Mercer, 1998).

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The disease does not only have an economic impact on farmers worldwide, but also have a considerable negative effect on animal welfare. Infected animals are sickly, fail to thrive, and are more susceptible to adventitious bacterial infections (Gallina et al., 2006). CE is an infection of public health significance and humans with immunodeficiency diseases, in particular can develop serious infections (Ara et al., 2008).

Although clinical diagnosis, histopathology and electron microscopy have been used for viral identification, only polymerase chain reaction (PCR) and genomic analyses can distinguish CE virus from other *P. virus* species. The PCR method based on the amplification of the B2L gene technique has become a powerful tool in molecular diagnosis of CE (Inoshima et al., 2000; Inoshima et al., 2001; Tikkanen et al., 2004). The diseases caused by *P. virus* ovis or Orf virus (ORFV) have worldwide distribution and have been reported from many countries (Hosamani et al., 2006). Although, outbreaks of CE have been reported in Ethiopia, confirmatory diagnosis using molecular technique has not been conducted. Thus, the objective of this study was to confirm CE virus from clinical samples by PCR assay by amplifying a part of B2L gene of CE virus.

MATERIALS AND METHODS

Study area description

This case study was conducted at Adet Sheep Research Sub-Center, found in Yilmanadensa district, Amhara Region, Ethiopia, which is located at about 40 km in South-East of Bahir Dar 11°10′ to 11°15′ N and 37°30′ to 37°40′ E at an altitude range between 1500 and 3000 m above sea level. It has a uni-modal type of rainfall receiving a mean annual rainfall of about 1270 mm (1051 to 1488 mm) which occurs from May to October. The research sub-center contained 112 Washera sheep in the time of sample collection.

Study animals and flock management

Washera sheep (known as Agew or Dangla sheep) of both sex and all age reared at the research sub-center were the study animals. This sheep breed is characterized by large body size, wide fat-tail usually curved upward tip, horizontally carried or semi-pendulous long ears, both sexes hornless, slightly concave facial profile with plain, patchy and spotted patterns of coat colour (Sisay, 2009).

Grazing constitutes the basal ration throughout the year. Sheep were allowed to graze the natural pasture for 8 to 10 h per day. During dry season where feed scarcity is common, sheep were supplemented with vetch hay, concentrated feed containing maize, noug cake and wheat bran. Pure breeding was performed in the research sub-center with sex ratio of 1 ram to 20 to 30 ewes. The flock was structured into five groups (each group containing 1 ram with 20 to 30 ewes).

Foot rot, CE, gastro-intestinal parasites, especially *Haemonchus contortus* and ticks were the common ovine diseases reported in the research sub-center. Tentative case reports from the research sub-center show that CE was the primarily sheep health constraint. An annual outbreak of CE repeatedly occurred during wet season Vaccination against CE has not been practiced in the research sub-center. Strategic vaccination was given for Ovine Pasteurellosis, Anthrax, and Sheep pox. Sheep were strategically dewormed with broad-spectrum anthelmintic against gastro intestinal parasites (GIP) according to the area rain fall pattern; at the beginning of rainy season (June), at mid dry season (March to April) and at the end of rainy season (October). Scheduled dipping and spraying against to external parasite infestation were also conducted.

Case presentation

At the beginning of wet season, June 2012, a high incidence of disease showing skin lesions in the form of papules, pustule, ulcers and brown thick scabs on the lips, gingival, knee and inter-digital region was observed among sheep in Adet Sheep Research Sub-Center.

For specimen collection, five (5) sheep were presented with skin lesions in the form of pustule, ulcers and brown thick scabs on the lips, gingival and wart-like lesions on the feet (knee and interdigital region), and ear (Figure 1A to F).

Specimen collection and laboratory technique

Skin scrapings were collected from five affected sheep and were stored frozen until testing. Then collected samples were dispatched to National Veterinary Institute (NVI), Debre Zeit for laboratory examination. PCR to amplify B2L gene of CE virus was applied.

DNA extraction and PCR assay

The collected scab samples were minced using sterile scissors and forceps and then triturated in a sterile pestle and mortar with phosphate buffered saline and made into 10% suspension. The mixture was clarified at 1800 rpm for 10 min and then transferred to a microcentrifuge tube and subjected to DNA extraction. The DNA was extracted from scab materials by using the phenol: chloroform:iso-amyl alcohol method as described by Klein (2004) with slight modifications. 200 µl of tissue samples were transferred into a 1.5 ml microcentrifuge tube; 200 µl PureLink™ genomic digestion buffer were added and mixed well; 20 µl proteinase K was added and gently mixed, and then incubated at 55°C water bath for 1 h; 20 µl RNase A was added and incubated at room temperature for 2 min, then the lysate was centrifuged at maximum speed for 5 min at room temperature to remove any particulate material; the supernatant was transferred to a fresh microcentrifuge tube; 200 µl PureLink™ genomic binding buffer was added and mixed well by vortexing; 200 µl 96 to 100% ethanol was added to the lysate and mixed well by vortexing for 5 s. Binding of DNA was made by removing the PureLink™ spin column in a collection tube, then lysate of 640 µl was added to the PureLink™ spin column. The column was centrifuged at 10,000 ×g for 1 min at room temperature. The collection tube was discarded and the spin column was placed into a clean PureLink™ collectin tube. Washing of DNA was also carried out by adding 500 µl of washed buffer 1 to the column; centrifuged the column at room temperature at 10,000 ×g for 1 min. The collection tube was discarded and the spin column was placed into a clean PureLink™ spin collection tube, then 500 µl washed buffer 2 was added. The columns were centrifuged at maximum speed for 3 min at room temperature and discard the collection tube. Eluting of DNA was carried out by placing the spin column in a sterile 1.5 ml microcentrifuge tube; 50 µl of PureLink™ genomic elution buffer was added to the column then incubated at room temperature for 5 min; the column was centrifuged at maximum speed for 1 min at room temperature. Finally, the purified DNA was stored at -20°C until analysis.

The extracted DNA was subjected to PCR technique as per the procedure standardized in this laboratory. CE virus specific primers B2L gene, was used in the PCR assay. The primer sequences used

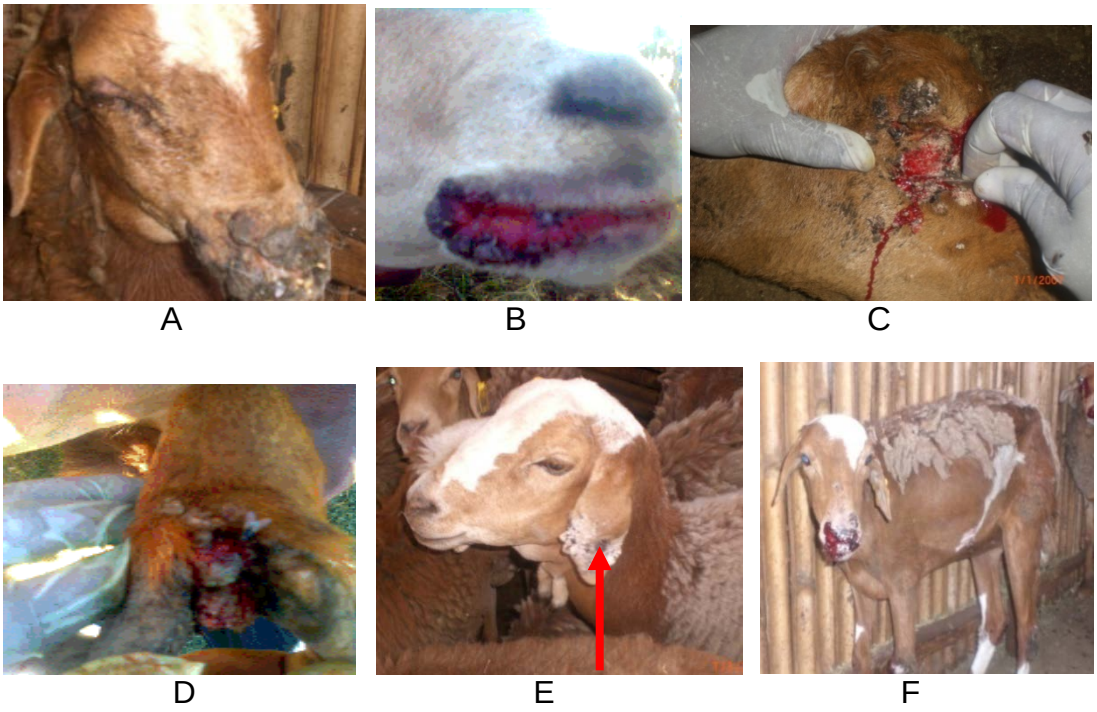

Figure 1. Observed clinical cases of CE virus infection in affected sheep flock at Adet Sheep Research Sub-Center. A, B and F: sheep with severe proliferative scaby and ulcerated lesions of ecthyma around the lips. C and E: wart-like multiple nodules around eyelids and ears. D: wart-like tissue projections in the interdigital space.

for amplification of B2L gene were: Forward primer: 5'-GCT CTA GGA AAG ATG GCG TG-3' Reverse primer: 5'-GTA CTC CTG GCT GAA GAG CG- 3'.

The PCR mixture was prepared in total volume of 20 µl containing DNA template 3 µl, forward primer 2 µl, reverse primer 2 µl, IQ supermix 10 µl, and distilled water 3 µl and then subjected to the following PCR cycling conditions in a thermal cycler (Applied Biosystem). Initial denaturation was at 94°C for 5 min followed by 30 cycles denaturation at 94°C for 60 s, annealing at 57°C for 1 min and extension at 72°C for 1 min and final extension at 72°C for 7 min. The amplified product was electrophoresis on 2% agarose gel, stained with ethidium bromide.

RESULTS AND DISCUSSION

During clinical examination and sample collection, the clinical picture was quite characteristics; the observable clinical signs were proliferative papular lesions in oral commissures, muzzle and lower jaw regions and tumor-like tissue projections in the interdigital space (Figure 1). The clinical diagnosis performed at Adet Sheep Research Sub-Center suggested CE with morbidity rate of 22% (25/112).

All five clinical samples collected from the outbreak were confirmed as positive with PCR, a product size around 1206 bp (Figure 2). The PCR result of five suspected samples was equal to the PCR reading of positive control CE virus which showed 1206 bp (Figure 2).

The traditional methods of diagnosis that depend on pathologic examinations and clinical signs are inaccurate, virus isolation is thought to be a gold standard, but it is time-consuming (Chan et al., 2007). But with the development of molecular biology, the PCR technique is being widely used to amplify the desired genomic fragments from tissue specimens of affected animals, and it has become a powerful tool in molecular diagnosis (Inoshima et al., 2000). Thus, to confirm whether the causative agent was present in skin scrapings, PCR of the complete B2L gene was used in this study. The expected PCR fragments, approximately 1206 bp in length, were obtained from DNA which had been extracted from tissue scrapings.

Apart from the present study, different authors have used PCR for the detection CE virus and they have reported that PCR assay is a rapid and specific detection system for the diagnosis of CE (Inoshima et al., 2000; Kanou et al., 2005; Klein and Tryland, 2005; Hosamani et al., 2007; Ramesh et al., 2008).

A conventional PCR method that is based on the amplification of complete sequence of the B2L gene has been used for the detection and phylogenetic analysis of ORFV (Inoshima et al., 2001; Guo et al., 2003; Tikkanen et al., 2004; Guo et al., 2004; Hosmani et al, 2006). Hence, the PCR assay using B2L gene primers proved to be a rapid detection system for the diagnosis of CE for field outbreaks without using other diagnostic techniques like cell culture system or electron microscopy.

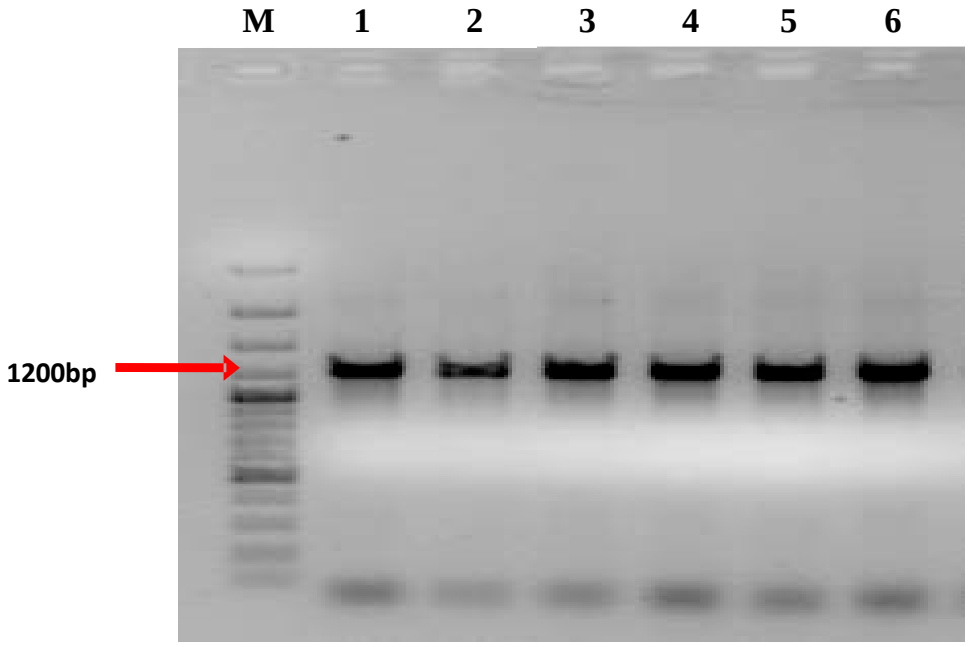

Figure 2. Agarose (2%) gel electrophoresis of major envelope (B2L) gene fragment obtained by PCR, stained with ethidium bromide. M: 100 bp Marker; Lane 1,2,3,4 and 5: PCR amplicon from contagious ecthyma suspected scab samples; Lane 6: PCR amplicon from known contagious ecthyma positive control (1206bp).

The morbidity rate associated with CE in this study was 22% which is lower than the report made by Lughano and Dominic (1996), in sub-Sahran Africa with morbidity rate of 70 to 90% in small ruminants and still high (80%) morbidity rate was also recorded in an unvaccinated flock (Animal Disease Fact Sheet, 2007). The discrepancy of the magnitude of morbidity among previous and current reports may be attributed due to differences in stage of outbreak investigation and patient animal management practices. From Adet Sheep Research sub-Center, CE outbreak was investigated early and diseased animals were isolated and well managed to prevent further spread. Softening ointments and provision of soft and palatable feeds may help in more severe cases and help to keep intake up (Animal Disease Fact Sheet, 2007). Accordingly, broad-spectrum antibiotic ointment along with soft and palatable feeds was given to CE affected sheep to control secondary complications and to improve feed intake.

As the disease is commonly introduced into a sheep flock by replacement ewes or breeding rams and by contact with bedding material, trucks, and vehicles contaminated by the CE virus, strict adherence of CE prevention and control strategies is crucial (Leite-Browning, 2008). Vaccination for CE is a relatively simple procedure and should be done routinely in all but completely isolated flocks. However, it is known that once CE disease is entered and circulated in the sheep farm/flock, the virus is very hardy in the environment. It can remain on the wool and hides for approximately one month after the lesions have healed and has been recovered from scabs after 12 years (CFSPH Technical Fact Sheets, 2006). Therefore, establishment of CE disease free flock with strict adherence to disease control and prevention strategies would help to effectively control CE in the study location.

CONCLUSION AND RECOMMENDATION

The PCR based assay proved that the outbreak that occurred at Adet Sheep Research Sub-Center was caused by CE virus with morbidity rate of 22%. As the present report is not exhaustive, detailed epidemiological study with phylogenetic analysis of CE virus should be conducted to know the genetic origin of the virus strain as well as for the future choice of CE immunoprophylaxis.

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Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Do dental abnormalities predispose horses to colic?

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Evaluation of dental abnormalities were carried out on a group of 74 polo horses with history of colic (colicky group) and another group of 70 randomly selected polo horses with no history of colic (non-colicky group) under similar environmental and management condition at Lagos Polo Club, Lagos, Nigeria in order to investigate probable correlation between dental abnormalities, routine dental care and predisposition to colic. Visual examination of the horses' oral/dental status was carried out after adequate physical and chemical restraint with intravenous administration of 2% xylazine hydrochloride at dose rate of 1.1 mg/kg body weight. Structured interview of handlers and review of dental health records where available were carried out to investigate routine dental care. One-way analysis of variance (ANOVA), Pearson correlation and linear multiple regression analysis were used to find out associations between dental abnormalities, routine dental care and colic. Thirty-eight (38) horses (51.4%) among the colicky group had dental abnormalities ranging from overjet (4.8%) to dental attrition (26.2%), while twenty-two (22) horses (29.7%) among the non-colicky group had dental abnormalities. Dental caries and sharp enamel point had significant difference ($p < 0.05$) on colic in horses and were positively correlated with colic. There were also positive significant correlation between fractured tooth and overjet ($r = 0.908$) and malposition and overjet ($r = 0.944$), respectively. Age and sex had no significant correlation with dental abnormalities and predisposition to colic, while local breeds were found to be more predisposed to colic due to dental abnormalities ($p < 0.05$). There was also a significant difference ($p < 0.05$) in horses that had no routine dental care and colic. In conclusion, this study indicates that dental caries and sharp enamel points are predisposing factors for colic in horses. Although not all forms of dental abnormalities predispose horses to colic, routine dental examination and care would be beneficial for early diagnosis and prevention of dental abnormalities that may predispose to colic.

Key words: Colic, horses, dental abnormalities, predisposed to colic.

INTRODUCTION

Colic is an important manifestation of gastro-intestinal problems in horses (Adeyefa, 1990) and it is the most prevalent cause of death and second only to lameness in terms of economic losses (Adeyefa, 1990; Cohen and Woods, 1999; NAHMS, 1998; Hillyer et al., 2001). The incidence of colic has been reported to range from 3.5 to 26% in different countries (Kaneene et al., 1997; Hillyer et al., 2001; Akinrinmade and Olusa, 2009) and some of the identified predisposing factors for colic are age, breed, diet and feeding practices, weather, exercises

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and previous history of colic among others (Uhlinger, 1992; Kaneene et al., 1997; Tinker et al., 1997; Cohen et al., 1999; Hillyer et al., 2001; Traub-Dargatz et al., 2001; Hudson et al., 2001).

Although, dental abnormalities have been perceived to be associated with occurrences of colic (Dabareiner, 1988; Easley, 1996; Ferraro et al., 2006; Olusa and Akinrinmade, 2009), no study has specifically investigated the role of dental abnormalities as a risk factor for colic in horses. Dental abnormalities are common occurrences in horses (Easley, 1996) and any dental problem that makes eating difficult could affect the general body condition of the animal (Henderson, 1990; Easley, 1996). The equine teeth are hypsodont teeth with long anatomic crown much of which are held reserved sub-gingivally in the alveolar bone (Dixon and Du Toit, 2011). Once fully formed, the tooth no longer grows in length but continue to erupt throughout life as occlusal wear takes place. Dental attrition and sharp enamel points are two of the most common dental abnormalities that occur especially in older horses due to irregular wears of the occlusal surfaces of the grinding teeth (Ferraro et al., 2006; Peters, 2006; Olusa and Akinrinmade, 2009). Although, it is generally believed that improperly masticated roughages and concentrates may lead to poor digestibility and subsequent impaction of the small colon or ceacum (Easley, 1996; Dabareiner, 1988; Ferraro et al., 2006), there are no sufficient findings to prove whether or not dental abnormalities could predispose horses to colic. Furthermore, although dental floating is a common routine dental care performed on horses, its beneficial role in apparently healthy horses has however not been ascertained (Carmalt et al., 2004; Carmalt and Allen, 2008).

Nigeria has about 200,000 horse population (Bourn, 1992; FAOSTAT, 2008) and in a previous study conducted among athletically fit polo horses converged during a polo tournament, high prevalence of dental abnormalities was found (Olusa and Akinrinmade, 2009). In another study, colic was found to have a high incidence rate of 14.5% and routine dental practices are often neglected (Akinrinmade and Olusa, 2009). The aim of this study therefore is to investigate if dental abnormalities and lack of routine dental care could predispose horses to colic.

MATERIALS AND METHODS

Dental examination were carried out and compared within two groups of horses. Group A consists of 74 horses with history of colic (colicky group), while group B consists of 70 randomly selected horses with no history of colic (non-colicky group), both from a total horse population of about 340 horses. The horses were kept under similar environmental and management conditions at the Lagos Polo Club, Lagos, Nigeria. Owners consent was taken and the protocol was approved by the Research and Ethics Committee of the College of Veterinary Medicine, University of Agriculture, Abeokuta, Nigeria. The horses were physically restrained in wooden crush and sedated with intravenous administration of 2% xylazine hydrochloride at dose rate of 1.1 mg/kg body weight. The mouth of the horses was flushed with clean water using drenching gun. The lips were parted and dental examinations were carried out on each horse by two veterinarians as previously described (Olusa and Akinrinmade, 2009). Abnormalities were regarded as any malformation or a state of being unlike the normal condition. A list and definition/description of dental abnormalities investigated and found were as follows: (1) Dental attrition: an occlusal wear of tooth surface; (2) Dental caries: presence of cavities in the infundibulum of tooth which usually contains food debris that promotes bacteria activities; (3) Fracture tooth: any tooth split into two or more parts. The split part(s) could be missing or still present; (4) Gingivitis: an acute or chronic inflammation of the gums generally characterized by congestion and swelling; (5) Hook tooth: portion of dominant lower or upper caudal or cranial cheek teeth overhanging the opposite teeth; (6) Sharp enamel points: these are sharp projections that generally form on the buccal side of the upper cheek teeth and the lingual side of the lower cheek teeth, causing lacerations or ulcers on the cheeks or tongue; (7) Malposition: an improper setting of a tooth in its root socket. The condition may affect more than one tooth and such malpositioned teeth may be prone to secondary dental disorders; (8) Overjet: is a condition in which upper incisors protrude in front of lower incisors, but the upper and lower incisors still make some level of contact with each other.

The age of the horses were estimated according to Parker (2003) and grouped into one of three groups, namely, those below 5 years old as group I (< 5 years); those between 5 and 10 years old as group II (5-10 years) and those above 10 years old as group III (> 10 years). Breeds and sex were determined and geldings were regarded as males in this study. Structured interview of handlers and review of dental health records where available, were used to investigate routine dental care. Data were analyzed by SPSS (16.0 Version). Types of dental abnormalities observed were presented in percentages, while inferential statistics of one-way analysis of variance (ANOVA), linear multiple regression and pearson correlation were carried out in order to analyze association between dental abnormalities, routine dental care and colic within and between the two groups. A 95% confidence interval was used and values were taken as statistically significant at ($P < 0.05$).

RESULTS

The sex, breeds and age distribution of the two groups of horses are as shown in Table 1. A total of forty-two (42) dental abnormalities were found in thirty-eight (38) horses (51.4%) out of the seventy-four (74) colicky horses examined for oral/dental soundness, while in the non-colicky group, a total of twenty-six (26) dental abnormalities were found in twenty-two (22) horses (29.7%). Table 2 compares the types of dental abnormalities found in the 2 groups of horses, while Table 3 presented the prevalence of dental care. Dental caries [$F (2, 3) = 13.000$] and sharp enamel point [$F (1, 4) = 21.000$] had significant difference ($p < 0.05$) on colic in horses and are positively correlated with colic (Table 3). There were also positive significant correlation between fractured tooth and overjet ($r = 0.908$) and malposition and overjet ($r = 0.944$), respectively (Table 4). Age and sex had no significant correlation ($p > 0.05$) with dental abnormalities and predisposition to colic, while local breeds were found to be more predispose to colic due to dental abnormalities ($p < 0.05$) (Table 5). Only 6% of respondents to the self-conducted structured interview provided routine

Table 1. Sex, breed and age distribution of colicky and non-colicky horses.

Group of horses	Sex		Breed			Age (Years)		
	Male	Female	Argy	Local	ND	<5	5-10	>10
Colicky horses	25	49	25	38	11	14	36	24
Non-colicky horses	33	37	28	33	9	26	29	15
Total	58	86	53	71	20	40	65	39

Argy: Argentine thoroughbred; Local: Arab, Chad, Sudanese, Dangola and their crosses; N.D: Not determined.

Table 2. Comparison of prevalence of some dental abnormalities in colicky and non-colicky horses.

S/N	Dental abnormalities	Colicky horses {No/Prevalence (%)}	Non-colicky horses {No/Prevalence (%)}
1	Dental attrition	11 (26.2)	9 (34.6)
2	Dental caries	3 (7.1)	2 (7.7)
3	Fracture tooth	4 (9.5)	1 (3.8)
4	Gingivitis	5 (11.9)	2 (7.7)
5	Hook tooth	6 (14.3)	4 (15.4)
6	Malposition	3 (7.1)	1 (3.8)
7	Overjet	2 (4.8)	0 (0)
8	Sharp enamel point	8 (19.0)	7 (26.9)
	Total	42 (100)	26 (100)

Table 3. Prevalence of routine dental care in colicky and non-colicky horses.

Dental care	Colicky horses {No/Prevalence (%)}	Non-colicky horses {No/Prevalence (%)}	Total
Routine dental care (at least once in 12 months)	5 (6.8)	8 (11.4)	13
No dental care	69 (93.2)	62 (88.6)	131
Total	74	70	144

dental care to their horses. There was a significant difference ($p < 0.05$) in horses that had no routine dental care and colic.

DISCUSSION

The result of this study showed that only dental caries and sharp enamel points are significantly associated with colic and are capable of predisposing affected horses to colic. Not all forms of dental abnormalities had any significant association with colic. Dental attrition is an occlusal wear of tooth surface which begins when opposing teeth come into occlusion and their occlusal surfaces grinds off each other (Baker, 1991; Kene and Agbo, 1998; Kene and Uwagie-Ero, 2001; Dixon et al., 2011). The degree of wear usually depends on the type of tooth, the species of animal and the texture of food material being chewed (Jubb et al., 1993) and any asymmetry in the position of the jaw or of the teeth could result unto uneven dental wear (Dixon et al., 2011). Although dental attrition has the highest prevalence in both groups of horses studied, it was not significantly associated with colic ($p>0.05$). It could thus have been more of physiological rather than pathological origin (Kene and Uwagie-Ero, 2001). Dental attrition is thus not a predisposing factor for colic.

Dental caries had significant difference in predisposition to colic in horses. Caries is characterized by destruction of the calcified dental tissue with bacterial fermentation action of the food debris hidden in cavities in the infundibulum of the affected check tooth (Dixon et al., 2011). Although the most common type of dental carries identified in equine teeth is maxillary cheek teeth (CT) infundibularcemental caries with prevalence ranging from 13 to 100% in horses over 12 years of age (Colyer, 1906; Honma et al., 1962; Dixon et al., 2000; Brigham and Duncanson, 2000), high level of severe peripheral dental caries involving all classes of teeth (incisors, canines and CT) have also been found in horses (Dixon et al., 2011).

Table 4. Effects of dental abnormalities on colic in horses.

Dental abnormality	ANOVA	Sum of squares	df	Mean square	F	Sig.
	Between groups	1.333	2	0.667		
Dental attrition	Within groups	6.000	3	2.000	0.333	0.740
	Total	7.333	5	-		
	Between groups	4.333	2	2.167		
Dental caries	Within groups	0.500	3	0.167	13.000	0.033
	Total	4.833	5	-		
	Between groups	2.333	2	1.167		
Fractured tooth	Within groups	4.500	3	1.500	0.778	0.534
	Total	6.833	5	-		
	Between groups	1.333	2	0.667		
Gingiritis	Within groups	1.500	3	0.500	1.333	0.385
	Total	2.833	5	-		
	Between groups	1.333	2	0.667		
Hook tooth	Within groups	2.000	3	0.667	1.000	0.465
	Total	3.333	5	-		
	Between groups	5.333	2	2.667		
Malposition	Within groups	2.000	3	0.667	4.000	0.142
	Total	7.333	5	-		
	Between groups	1.333	2	0.667		
Overject	Within groups	2.000	3	0.667	1.000	0.465
	Total	3.333	5	-		
	Between groups	7.000	2	3.500		
Sharp enamel	Within groups	0.500	3	0.167	21.000	0.017
	Total	7.500	5	-		

Dental caries: dental caries had significant difference on colic in horses, since $F(2, 3) = 13.000$, $p < 0.05$. Sharp enamel: sharp enamel had significant difference on colic in horses, since $F(1, 4) = 21.000$, $p < 0.05$.

Certain fed diet such as diet low in pH and consisting largely of simple carbohydrates like processed maize and low roughages have been reported to predispose to caries (Dixon et al., 2011). Infundibular and peripheral caries occurring in equine teeth can predispose affected teeth to an increased rate of occlusal wear, tooth fracture, and apical infection (Dixon et al., 2011). These anomalies will invariably make mastication of feed materials incomplete and subsequent feed indigestion which could then result into colic. These pathologies may therefore further substantiate the association found between horses with dental caries and the increased tendency to develop colic found in this study. In view of the high incidence of dental caries which has been reported in ruminants in Nigeria (Kene and Agbo, 1988; Kene and Uwagie-Ero, 2001) and recent studies which put the

Table 5. The relationship between dental abnormalities in colicky and non colicky horses.

Correlation	Dental attrition	Dental caries	Fractured tooth	Gingivitis	Hook tooth	Malposition	Overjet	Sharp enamel
Dental attrition	1							
Dental caries	0.224	1						
Fractured tooth	0.612	0.493	1					
Gingiritis	0.366	0.585	0.720	1				
Hook tooth	-0.270	0.664	0.349	0.759	1			
Malposition	0.636	0.784	0.800	0.512	0.270	1		
Overject	0.674	0.581	0.908*	0.542	0.200	0.944**	1	
Sharp enamel	0.000	0.581	0.489	0.759	0.800	0.270	0.200	1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). There was a positive significant correlation between fractured tooth and overjet with r = 0.908, at p < 0.05. There was a positive significant correlation between malposition and overjet with r = 0.944, at p < 0.01

prevalence of dental caries in horses in Nigeria at 3.5 to 7.7% (Akinrinmade and Olusa, 2009; Olusa and Akinrinmade, 2009), more emphasis should be placed on routine dental care in all species and in particular in horses to forestall colic and economic losses.

Sharp enamel points may lead to soft tissue ulceration of the buccal mucosal and in severe cases biting problems and quidding (Dixon et al., 2011). The significant association found between sharp enamel points and colic in this study could substantiates the perceptions that pain caused by injured sharp enamel points on the buccal mucosal may results in reduced feed intake and hence poor body condition and poor athletic performance.

Dental abnormalities are of major importance in the UK and the US where survey had shown that 10% of equine practice time is spent on dental related work in UK and it is the third most common equine medical problem encountered by large animal practitioners in the US (Traub-Dargatz et al., 1991; Dixon et al., 2011). Equine dentistry is an important but often neglected area of equine veterinary practice in Nigeria and elsewhere (Peters et al., 2006; Olusa and Akinrinmade, 2009) as only 6% of respondents to the structured interview practice some form of routine dental care on their horses. The non-significant difference for other forms of dental abnormalities found in both the colicky and non-colicky horses in this study may suggest that not all dental irregularities are major determinant factor in horses' abilities to prehend, masticate and digest their feeds. Since other factors such as fermentation are known to take part in the complex processes of digestion, the presence of some dental abnormalities alone may not sufficiently result into compromise in the gastrointestinal tract functions as to result into colic.

A positive significant correlation found between fractured tooth and overjet and malposition and overjetmay suggest that the presence of one dental abnormality often encourage or predispose to development of more abnormality. Overjet is a condition in which upper incisors protrude in front of lower incisors, while malposition is an improper setting of a tooth in its root socket. Malposition could affect more than one tooth and such malpositioned teeth may be prone to secondary dental disorders. The non-symmetry of the dental arcade in overjet and malposition might be a factor responsible for it tendency to fracture.

Age and sex had no significant correlation with dental abnormalities and predisposition to colic while local breeds were found to be more predisposed to colic due to dental abnormalities ($p < 0.05$). This finding might be accidental as local breeds were more represented (49.3%) in the sampled population and they are usually not given the preferential treatment (like dental floating) accorded to the more expensive imported Argentine thoroughbred horses. Dental abnormality can be found in all age categories and both sexes of horses.

Routine dental care such as periodical mouth was with warm saline and more important and common dental floating or rasping has been performed on horses for hundreds of years (Scrutchfield, 1999). Dental floating are generally performed to: (1) relieve discomfort associated with oral soft tissue injuries caused by sharp enamel points; (2) reduce dental elongations, which place stress on affected teeth and jaws; (3) improve mastication and digestion of feedstuffs; (4) alleviates stresses on abnormally worn teeth; and (5) prevent discomfort and improve performances in the horse wearing a bit and bridle (Knottenbelt, 1999; Gatta et al., 1995; Carmalt et al., 2004; Carmalt and Allen, 2008; Easley, 2011). Although dental floating is being performed on a regular basis, controversy exist regarding its clinical usefulness in apparently healthy horses as there is very little scientific evidence to support this practice (Scrutchfield, 1999; Carmalt et al., 2004; Carmalt and Allen, 2008; Easley, 2011). In this study, there was a significant difference ($p < 0.05$) in horses that had no routine dental care and colic. Sharp enamel points are common dental abnormalities in horses and are easily recognizable during routine dental examination. Reduced feed intake

and improperly chewed roughages due to pain elicited on the buccal cavity secondary to lacerations or ulcers on the cheeks or tongue caused by sharp enamel points could result into quidding, choke, chronic colic and general unthriftiness (Easley, 1996; Ferraro et al., 2006). Since dental rasp or floating could be used to effectively correct sharp enamel points (Scrutchfield, 1999), routine dental examination and care are therefore essential for sound dental health.

In conclusion, this study indicates that dental caries and sharp enamel points are predisposing factors for colic. Although not all forms of dental abnormalities predispose horses to colic, routine dental examination and care would be beneficial for early diagnosis and prevention of dental abnormalities that may predispose to colic. There is minimal published knowledge on the normal bacteriology of the equine mouth and even less on bacteria that incite dental caries formation. More studies to investigate the aetieo-pathogenesis of identified dental abnormalities in colic are therefore recommended.

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Conflict of Interest

The author(s) have not declared any conflict of interests.

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Slaughter surveillance for tuberculosis among cattle in three metropolitan abattoirs in Ghana

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Despite its existence in Ghana, there is very little information on the extent or nature of bovine tuberculosis. This state of affairs may pose a serious public health threat through risks associated with the consumption of beef from infected cattle, dairy milk and other bovine products. A study to screen bovine carcasses with lesions suggestive of mycobacterial infection at necropsy in three selected abattoirs in Accra was conducted. A total of 2,886 cattle slaughtered in 3 abattoirs in the Greater Accra Region of Ghana between June and October, 2009 were examined at necropsy for lesions suggestive of bovine tuberculosis. Specimens taken from suspicious lesions were first subjected to Ziehl-Neelsen microscopy and then cultured on Löwenstein-Jensen media containing both pyruvate and glycerol. One hundred and fifty five (155) tissue samples were elicited from only lesions presenting with classical patho-morphological features consistent with bovine tuberculosis in organs found in 145 cattle. These results indicate that 5% (or 145/2886) of the cattle carcasses inspected at slaughter in the Accra region exhibited lesions suggestive of bovine tuberculosis and this poses a serious public health threat. Visual inspection at necropsy, provided done proficiently, could serve as the primary screening measure for beef contaminated with mycobacterial species in abattoirs in resource-poor settings. Microscopic examination, because of its revealed high specificity in this work may be employed, only as a supplementary test, in difficult cases.

Key words: Beef, lesions, Ziehl-Neelsen microscopy, *Mycobacterium bovis*, bovine tuberculosis, TB, necropsy, slaughter, surveillance.

INTRODUCTION

Bovine tuberculosis (BTB) is a chronic infectious zoonotic disease primarily infecting cattle and it is caused by *Mycobacterium bovis* (*M. bovis*), a member of the *Mycobacterium tuberculosis*-complex (MTBC). As with

other members of the MTBC, *M. bovis* can be classified as an acid-fast Gram-positive bacterium. The MTBC is responsible for tuberculosis (TB) in humans. It is estimated that *M. bovis*, the aetiologic organism of TB in bovines is also responsible for about 5% of all TB infections in humans (Cosivi et al., 1998; Michel et al., 2010). Cattle and Buffalo, both belonging to the family Bovidae are considered the facilitative natural hosts of *M. bovis,* though infections have been found in other members of Bovidae (goats, sheep, Greater kudu and the Common duiker). Mammalian families such as Cervidae (various deer and antelopes), Equidae (horses), Suidae (pigs), Sciuiridae (squirrels) and Mustelidae (badgers) are also important as reservoirs in the epidemiology of *M. bovis* (Gutpa et al., 2009). The predatory family of the big cats, Felidae (lions, tigers, leopards and lynxes), is also an important reservoir of BTB. In Africa, the Greater Kudu (*Tragelaphus strepsiceros*), common duiker (*Sylvicapra grimmia*), African buffalo (*Syncerus caffer*), warthogs (*Phacochoerus africanus*) and Kafue lechwe (*Kobus leche*) are considered the wild-life reservoirs of *M. bovis*. The rather broad host range of *M. bovis* makes it an important factor in the control and management of human TB as a disease (Ayele et al., 2004; Denis et al., 2007). Commonly in cattle, the disease is spread either through the respiratory route (by the inhalation of contaminated aerosol drops) or in humans, through the ingestion of contaminated bovine products such as beef and unpasteurised milk (Neill et al., 1994; Roxo, 1998).

At necropsy, BTB in bovines presents as granulomatous lesions or tubercles in such organs such as lungs, spleen and liver. These tubercles can also be found in the lymphatic system (mediastinal, retropharyngeal, mandibular, pre-scapular and portal lymph nodes among others). In disseminated cases these tubercles can be calcified or caseous in pathology, and also multiple small granulomas may form in numerous organs and in the surfaces of cavities, giving rise to the miliary form of the disease.

It is not uncommon to find only a few lesions presented at necropsy in infected carcasses (The Centre for Food Security and Public Health, 2005; http://www.cfsph.iastate.edu]). One important consequence resulting from infected bovines is the expression of *M. bovis*, the aetiolgic agent of bovine tuberculosis, in the milk of lactating cows (Saad et al., 2013; Baquir et al., 2013; Thakur et al., 2010; Pardo et al., 2001). Apparently healthy lactating cows have been found to shed viable *M. bovis* bacilli in their milk (Danbirni et al., 2010). The threat to public health stemming from the risk in the consumption of beef from infected cattle cannot be overemphasised. Though Ghanaian culinary culture involves, in the main, intensive cooking of beef and other protein products, the risk of infection is real since cooking may not always be an effective bulwark against *M. bovis* infection (van der Merwe et al., 2009). In the case of dairy milk however, the risk of infection can be eliminated by pasteurisation.

In the past, a study to assess beef quality at necropsy using Ziehl-Neelsen (ZN) microscopy of suspicious beef samples obtained from the main abattoir in Kumasi, the second largest city in Ghana, indicated a significant level of contamination by acid-fast species. In that study, 73.1% of carcasses harbouring lesions suggestive of BTB were found to be acid-fast on pre-culture microscopic examination (Adu-Bobi et al., 2009). There is also a paucity of information regarding the prevalence of BTB amongst cattle herds in Ghana. A survey using the Standard Single Intradermal Comparative Tuberculin Test (SCITT) and carried out in the Dangme-West District of the Eastern Region of Ghana revealed that the prevalence of bovine tuberculosis disease in some kraals investigated was 50% even though the total average prevalence was 13.8% (Bonsu et al., 2000). Elsewhere in Africa, varying prevalence rates based on lesion detection or gross pathology at meat inspections have been reported. For instance, prevalence of BTB infections in meat of 5.2, 4.5 and 3.5% have been reported in various abattoirs in Ethiopia, a country with the largest cattle population in Africa (Ameni and Wudei, 2003; Teklu at al., 2004; Shitaye et al., 2006).

In Ghana, the Ministry of Food and Agriculture (MOFA), through its Veterinary Services Division (VSD), has a policy to subject all livestock slaughtered in government-certified abattoirs to necropsy before the meat is passed for human consumption. This is done in an effort to control the spread of BTB and other zoonoses from cattle and other livestock to humans. Unsuitable carcasses, particularly those with generalised infections are removed from the food chain and destroyed.

Due to the zoonotic potential of BTB, coupled with the lax regulation governing cattle herd movement and the permit regime across the country, it is important to have reliable information on the disease at the point of slaughter. Metropolitan abattoirs provide an ideal and controlled environment as a monitoring point for the screening of carcasses at necropsy. Here, we report on a base-line study to screen bovine carcasses at necropsy from the two main abattoirs and a certified emergency abattoir located in the only livestock market in the Greater

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Accra Region of Ghana. The main objective was to screen bovine carcasses with lesions suggestive of mycobacterial infection in three selected slaughter points and thereby estimate the prevalence of TB-like suspicious infections at necropsy. In addition, three other objectives of this study were: (1) Using culture to estimate the percentage TB-like (suspect) lesions that subsequently are confirmed as acid-fast and (2) finally to describe the pre-culture and post-culture distributions of TB-like lesions in different organ sites in the carcasses. Carcasses harbouring lesions suggestive of BTB in the opinion of the Veterinarian were submitted to the laboratory where they were processed for an initial microscopy before culture, cultured and then finally, isolates obtained from lesions yielding a positive culture were then subjected to confirmatory microscopy for the presence of acid-fast bacilli (AFB).

MATERIALS AND METHODS

Study sites

The current study was carried out in two (2) government-approved metropolitan abattoirs located in the Greater Accra Region, namely the Tema and the Accra Abattoirs. However, a certified emergency abattoir, constructed purposely for the slaughter of livestock at the Turako livestock market was also included as the third sampling point in the study. Livestock merchants buy mostly cattle and other ruminants (sheep and goats) from different parts of the country, particularly from Northern Ghana and the Accra Coastal plains. Ghana is estimated to have a cattle population of only about 1.25 million (Addo et al., 2011) and therefore cattle are also procured from the neighbouring countries of Burkina Faso, Ivory Coast and Togo and from farther afield as Mali and Niger by these merchants to augment and supply the domestic cattle trade in Ghana. The Turaku livestock market, in a suburb of the port city of Tema, near Accra, is the first point of call in the Accra Region by the livestock merchants before their cattle are purchased for slaughter.

Cattle breeds

In Ghana, the predominant breed is the West African Short-Horn or WASH (*Bos taurus brachyceros*) constituting approximately 60% of the cattle population and therefore this breed dominates the trade in domestic stocks. Breeds like the *Sanga* (*Bos taurus africanus*), a cross between WASH and Zebu (*Bos primigenius indicus*), also feature significantly in the domestic cattle trade. The imported cattle stocks are dominated by *Sanga* and Zebu breeds like the White Fulani and Sokoto Gudali. Due to the lack of a permit system in operation regarding cattle movements in Ghana and the practice of multiple sales and purchases by several dealers, the precise geographical location or origin of batches of animals cannot usually be determined with any degree of certainty. In this generally lax system, the public health implications of trading in cattle infected with mycobacterial diseases cannot be further emphasised. At the abattoirs and in specified kraals, Ministry of Food and Agriculture-certified Veterinary Officers perform ante-mortem examinations on cattle by checking on their stress level, fur texture and colour, sex and other relevant body conditions. Inspections are then carried out at necropsy, where the carcasses are examined for lesions indicative of tuberculosis and other pathological disease states. For this study, Veterinarians were also available to perform necropsy at the Turaku abattoir whenever sampling was required.

Sample collection

A total of 2,886 cattle were slaughtered and examined at necropsy between June and October, 2009, with an approximate total average of 20 cattle being slaughtered and examined daily. Out of the 2,886 cattle examined, 2,420 (83.9%), 425 (14.7%) and 41 (1.4%) were examined in the Accra, Tema and the Turaku Abattoirs, respectively. The slaughter protocol at the Accra and Tema abattoirs involved the ante mortem examination and selection for slaughter of only apparently healthy animals. For the present study, 155 tissue samples with gross visible lesions, suggestive of tuberculosis, were detected at necropsy and collected from 145 cattle (comprising 73 bulls and 72 cows out of total of 2,886 screened between June and October, 2009). In all, 108, 36 and 11 suspicious tissue samples were taken from the Accra, Tema and the Turaku Abattoirs, respectively. At sampling, approximately 4 g of suspicious beef carcasses with lesions suggestive of mycobacterial infection were excised with sterile surgical blades into a small stoppered sterile plastic container (4.7 cm long and 4.1 cm in diameter). They were labelled according to the abattoir address, tissue type, sex and date of collection. The samples were then immediately placed on ice and transported to the laboratory where they were stored at -20°C prior to processing and analysis.

Data management and analysis

Specimen data like tissue type, sex, abattoir address, date of collection, results of microscopy and culture were entered into Microsoft Office Excel Version 2007 Software and, where appropriate, descriptive parameters such as sums, percentages and fractions were then computed.

Sample decontamination, acid fast microscopy and culture

All 155 tissue samples were processed for smear microscopy and culture as follows: Stored tissue samples were thawed and sterile surgical blades used to rid them of as much non-lesioned lipoid and connective tissue as possible. Approximately 1 g of tissue with exhibiting gross visible lesions was sliced into a sterile petri-dish. The lesions were then scraped off into appropriately labelled tubes containing 5 ml sterile double distilled pyrogen-free water and homogenised by maceration. This was then decontaminated according to standard protocol (Kubica et al., 1963). Described briefly: 5 ml of freshly-prepared decontamination solution (consisting of equal volumes of 1 M sodium hydroxide and 0.1 M sodium citrate dihydrate ($Na_3C_6H_5O_7.2H_2O$), containing 0.01% (w/v) N-acetyl-L-cysteine (NALC) and 0.5% w/v phenol red solution) was added to an equal volume of the homogenate (suspended in sterile double distilled pyrogen-free water) and incubated for 15 min. A neutralization buffer (0.15 M $NaHPO_4$, pH 5.0) was added to neutralize the decontamination mixture before centrifugation at 3000 g for 30 min (neutralisation being effected when the colour changed from orange to pink). The supernatant was discarded and the pellet formed re-suspended (by vortexing) in 300 µl of phosphate-buffered saline (140 mM NaCl, 2.6 mM KCl, 10.0 mM Na_2HPO_4 and 1.7 mM KH_2PO_4). Using sterile pasteur pipettes, re-suspended pellets (2 to 3 drops) were then inoculated in duplicates onto Löwenstein-Jensen (LJ) slants (one incorporating glycerol and

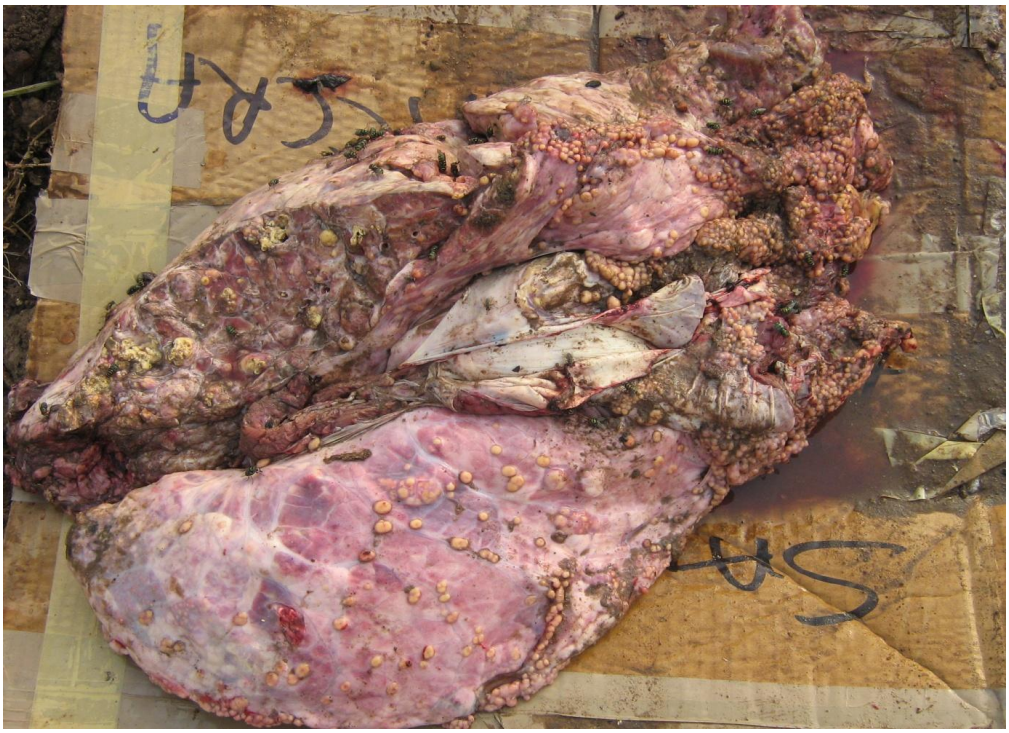

Figure 1. Disseminated Bovine Tuberculosis lesions seen, calcified and invasive, in lungs of a bull carcass at necropsy in the Accra abattoir. Observe the calcified and necrotic granulomas invading the periphery of the lobe in the foreground, and the interior of the sectioned lobe in the background.

the other pyruvate), incubated at 37°C and then observed weekly for eight weeks. Using a sterile 0.1 µl plastic loop, the re-suspended pellets were appropriately spread and heat-fixed (80°C for 10 min) onto labelled slides. Standard ZN microscopy was then performed. Also, briefly described, the heat-fixed smears were first stained with 3% Carbol-fuchsin for 5 min, decolourised with 20% sulphuric acid for 5 min and counter-stained with 0.3% methylene blue for 30 to 60 s. The slides were carefully examined under a microscope (10× ocular and 100× oil immersion) for the presence or absence of acid-fast bacilli. Presence of acid-fast bacilli was indicated by pink rods on a blue background. Slants were passed positive for culture based on the morphology of successful growths. Tubes showing no growths after 8 weeks of observation were concluded negative and appropriately discarded. All harvested growths were further subjected to confirmatory ZN microscopy.

RESULTS

Necropsy: Tissue samples with visible lesions at the Accra and Tema abattoirs

Morphologically, lesions indicative or suggestive of tuberculosis infection ranged from caseous necrosis with central mineralisation to disseminated and grossly-calcified granulomas. A picture of lungs presented in the carcass of a bull (slaughtered at the Accra abattoir) depicting classical features of calcified and invasive granulomas is shown in Figure 1.

At necropsy, from a total of 2,886 carcasses inspected, 145 (5%; 145/2,886) disclosed lesions suggestive of BTB. However, because a few of the cattle each had more than one organ presenting lesions, 155 suspicious tissue samples were obtained instead of 145 suspicious samples. Out of these 155 suspicious tissue samples, 69.7% (n = 108), 23.2% (n = 36) and 7.1% (n = 11) were collected, respectively from the Accra, Tema and Turaku abattoirs. In terms of organ involvement, the majority of lesions were found in lymph nodes that is, 74.2% (n = 115). Extensive invasions by lesions were also found in the lungs (15.5%, n = 24) and liver (8.4%, n = 13). The head, cardiac and mammary (udder) tissues contributed one tissue sample each (0.6%, n = 1) (Figure 2). Of the 115 infected lymph nodes, 68.4% (n = 106) were pre-scapular lymph nodes, 2.6% (n = 3) were each supra-mammary lymph and retro-pharyngeal lymph nodes, 1.7% (n = 2) were bronchial lymph nodes and 0.8% (n =1) was a mesenteric lymph node (Figure 3).

Pre-culture acid fast microscopy

Pre-culture microscopy revealed that, out of the 145

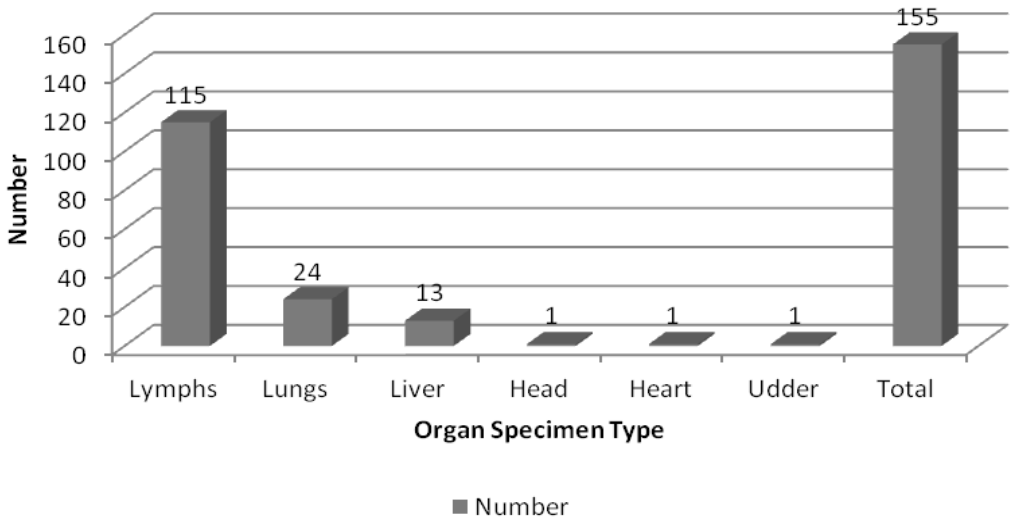

Figure 2. Distribution of organ specimen exhibiting tuberculous lesions at necropsy.

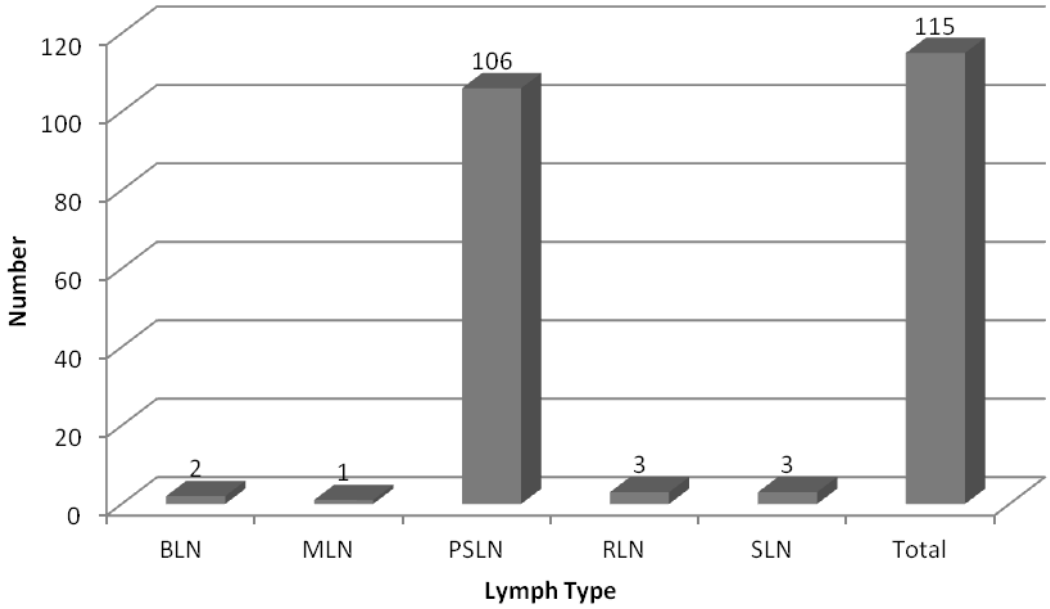

Figure 3. BLN=Bronchial lymph node; MLN=Mesenteric lymph node; PSLN=Pre-scapular lymph node; RLN=Retro-pharyngeal lymph node; SLN=Supra-mammary lymph node**.**

infected cattle disclosing at least one suspicious specimen at necropsy, 30.3% (n = 44; 44/145) of the cattle furnished lesioned samples tested positive for acid-fast bacilli whilst 69.7% (n = 101; 101/145) furnished lesioned samples which were negative for AFB. Overall, and in terms of the 155 individual sample analysed, lesions from lymph nodes represented the highest number of tissue samples that were positive by pre-culture microscopy that is, 16.8% (n = 26; 26/155), followed by lung tissues that is, 9.0% (n = 14; 9/155) and then liver that is, 5.2% (n = 8; 8/155). Two samples, one each taken from head and cardiac tissue, were found to be positive whilst a sample taken from the udder was negative (Table 1). For the 106 pre-scapular lymph nodes screened with ZN microscopy pre-culture, 17.9% (n = 19; 19/106) were positive for acid-fast bacilli and 82.1% (n =

Table 1. Pre-culture Ziehl-Neelsen microscopy results of all tissue samples.

Tissue type	ZN+ve (%)	ZN-ve (%)	Total
LN	26 (22.6)	89 (77.4)	115
LT	14 (58.3)	10 (41.7)	24
LIV	8 (61.5)	5 (38.5)	13
HD	1 (100)	0 (0)	1
CRD	1 (100)	0 (0)	1
UD	0 (0)	1 (100)	1
Total	50	105	155

ZN+ve = Ziehl-Neelsen positive; ZN-ve = Ziehl-Neelsen negative; LN=Lymph nodes; LT=Lung tissue; LIV=Liver tissue; HD=Head tissue; CRD=Cardiac tissue; UD=udder tissue.

Table 2. Tissue distribution of all culture-positive isolates.

Tissue type	Number of culture isolate
Cardiac tissue	1
Head	1
Udder/Mammary tissue	1
Mesenteric lymph node	1
Bronchial lymph node	2
Retro-pharyngeal lymph node	3
Supra-mammary lymph node	3
Liver	9
Lung tissue	17
Pre-scapular lymph node	95
Total	133

(CRD=Cardiac tissue; HD=Head tissue; UD=Udder tissue; MLN=Mesenteric lymph node; BLN=Bronchial lymph node; RTPLN=Retropharyngeal lymph node; SMLN=Supra-mammary lymph node; PSLN=Pre-scapular lymph node; LIV=Liver tissue; LT=lung tissue).

87; 87/106) were negative (Table 1). One out of the 3, supra-mammary lymph nodes screened tested positive and 2 tested negative (Table 1). All 3 of the retropharyngeal lymph nodes, all 2 bronchial lymph nodes and the only mesenteric lymph node were also positive for the presence of acid-fast bacilli (Table 1). Four (4) out of the seven (7) cattle which provided two (2) or three (3) tissue samples each at necropsy with suspicious lesions tested positive for the presence of acid-fast bacilli.

Culture results

Of the 155 samples (from suspicious lesions) processed and inoculated onto LJ slants for culture, 85.8% (n = 133; 133/155) grew successfully, 5.2% (n = 8; 8/155) were contaminated and 9.0% (n = 14; 14/155) did not show any growths. The 133 successful growths yielded 97 and 27 isolates each from the Accra and Tema, respectively. Colony morphological features of growths observed ranged from dry, rough, lumpy and irregular to smooth and moist colonies. Coloration ranged from creamy white to dull yellow. Out of the 133 culture isolates, 71.4% (n = 95; 95/133) were from pre-scapular lymph nodes, 12.8% (n = 17; 17/133) from lung tissues, 6.8% (n = 9; 9/133) from liver, 2.3% (n = 3; 3/133) from supra-mammary and retro-pharyngeal lymph nodes and 1.5% (n = 2; 2/133) from bronchial lymph nodes. An isolate each that is, 0.6% (n = 1; 1/133) from the heart (cardiac tissue), head, mammary (udder) and mesenteric lymph node were also recorded (Table 2). A comparison of the Pre-culture ZN microscopy (that is, of the inocula) and culture results, using the latter as a test to assess the proficiency of visual slaughter inspection (Table 3), revealed that only 43 out of the 133 which successfully grew on culture were ZN-positive prior to culture.

Table 3. Comparison of Ziehl-Neelsen microscopy results with Culture as “Gold standard”.

ZN Results	Positive	Negative
Positive	43	2
Negative	90	12
Total	133	14

ZN=Ziehl-Neelsen. Of the 155 samples (from suspicious lesions) processed and inoculated onto LJ slants for culture, 85.8% (n=133; 133/155) grew successfully, 5.2% (n=8; 8/155) were contaminated and 9.0% (n=14; 14/155) did not show any growths.

Necropsy: Tissue samples with visible lesions at the Turaku abattoir

As an emergency abattoir, it was observed that the Turaku abattoirs provided a fewer number of suspicious tissue samples. Of the total number of 155 suspicious tissue samples collected, only 11 samples (or 7.2%) emanated from the Turaku abattoir. A total of 133 (out of the 155) suspicious samples produced growths after culture on LJ media, 9 of which originated from the Turaku abattoir.

Confirmatory microscopy of isolates obtained from culture

Confirmatory ZN microscopy was carried out on the 133 isolates obtained from culture. The rationale for a post-

culture microscopy was to ascertain the proficiency of beef inspections in abattoirs and thereby improve beef quality. A total of 127 isolates were confirmed to be acid-fast bacilli while in 6 no acid-fast bacilli were found. Five (5) of these 6 isolates in which no acid-fast bacilli were found, were all found in pre-scapular lymph node lesions of bulls slaughtered at the Accra abattoir. One originated from the udder of a cow slaughtered at the Tema Abattoir. A total of 104 isolates emanated from lymph lesions of which 99 were acid-fast. All 17 isolates obtained from cardiac lesions contained acid-fast bacilli.

DISCUSSION

Despite the serious public health concern associated with BTB infection, little resources have been committed to screen and control this disease in Ghana. In the current study, we screened bovine tissue samples with lesions suggestive of mycobacterial infection from three sites in Accra using Ziehl-Neelsen microscopy and compared the results with those of culture as “Gold standard” and then determined the apparent lesion prevalence of the disease. It must be noted that culture results are not being taken as a ‘Gold Standard’ for diagnostic sensitivity but as a test for assessing the proficiency of visual inspections at beef slaughters. The apparent animal prevalence with lesions suggestive of TB of 5.0% (145/2886) in this study was comparable to published results from other parts of Africa (Ameni and Wudei, 2003; Teklu et al., 2004; Stefan et al., 2009). A distribution of lesions by organs shows that lymph nodes were the most infected 73.1% (or 106/145) followed by lung tissue 16.6% (or 24/145) and liver 8.3% (or 12/145). Fewer lesions were found in the head, mammary (udder) and the cardiac (heart) region. Even though bovine tuberculous lesions are often found in the pulmonary region, other organs can equally be affected (Guitierrez et al., 1993; Pritchard, 1988). The high percentage (or fraction) of samples obtained from the lymphatic system 74.2% (or 115/155) is also common (Milian-Suazo et al., 2000).

Based on microscopy alone, 44 (or 30.3%) cattle out of the 145 with suspicious lesions were positive for the presence of acid-fast bacilli pre-culture. In terms of tissue distribution, although the number of lesions seen in lymph nodes was higher than those in the lungs and liver, the fractions of acid-fast bacilli, pre-culture, in lung tissue (14/24 or 58.33%) and liver (8/13 or 61.5%) were higher than that in lymph nodes (26/115 or 22.61%). A possible explanation for the low ZN-positive results in the lymph nodes, which were different from results found in the thoracic region, is the low rate of survival of mycobacteria in the central caseation environment of the lymph node (Cassidy, 2006) or the loss of bacterial structure as a result of some immune reactions that occur in response to infection by mycobacteria, a condition which is evident by the inflammation of the granuloma (Guitierrez et al., 1993). A breakdown of the ZN microscopy results of all lymph node samples indicated that lymph nodes of the thoracic region (bronchial and retropharyngeal lymph nodes) all tested positive for acid-fast bacilli (Table 1). It must be noted that the presence of visible lesions in an organ may not always be linked to mycobacterial infections since lesions with similar pathologies could also be caused by other parasites or intracellular agents and this could potentially lead a meat inspector to proffer an erroneous judgement (Asseged et al., 2004).

It must be noted that the efficiency of any routine abattoir meat inspection is largely dependent on the time, work load and diligence on the part of the meat inspector (Corner et al., 1990; Aylate et al., 2012; Shitaye et al., 2006; Bekele and Belay, 2011). Pre-culture microscopy identified 32.3% (or 55 samples) as acid-fast out of the 155 lesioned specimens identified and sampled. This was however lower than the 71.7% reported in a similar study in the Kumasi Metropolitan Abattoir, Kumasi, Ghana (Adu-Bobi et al., 2009). It is important to note that pre-culture microscopy was able to correctly identify 40 (or 31.5%) out of the 127 isolates microscopically identified as acid-fast from the 133 isolates which successfully grew on culture. The findings of the current study however give some insight into the efficiency of necropsy at the three abattoirs. It reveals that the Turaku abattoir had the highest fraction of beef containing acid-fast bacilli followed by the Tema Abattoir and then the Accra Abattoir. The Turaku abattoir, located at the Turaku Livestock Market, is an emergency transitional slaughter facility, though certified, ostensibly set up by resident cattle brokers on the promptings of itinerant merchants. Thus, the urge by unscrupulous dealers to separate out and slaughter weak and very sick animals and promptly offer the carcasses for sale at the Turaku abattoir cannot be resisted. Indeed, microscopic examination of slides of specimens taken from the Turaku abattoir were consistently scored highest for the presence of acid-fast bacilli (data not shown) implying a greater mycobacterial load of inocula, probably emanating from very sick animals.

Growths were successful in 133 out of the 155 tissue samples which were cultured representing 85.8% (Table 2). This result is higher than the 60% culture yield obtained from cultured bovine tissue samples from abattoirs in Brazil (Nassar et al., 2007) and in Britain (Liebana et al., 2008). This may indicate that the disease is more endemic in the geographical areas from where the cattle were procured or, at the worst, point to the application of a very stringent decontamination procedure. It has been suggested that the rate of culture yield largely depends on the type of decontamination

procedure used (Haddad et al., 2004). In terms of cultured tissue distribution, out of the 133 successful culture isolates obtained, 95 (or 71.4%) were from lymph node lesions. This was less than 84% of culture isolates obtained by Milian-Suazo and co-workers (Milian-Suazo et al., 2000). The same study showed that all nine pre-scapular lymph nodes yielded isolates on culture, indicating a high culture yield associated with pre-scapular lymph nodes. The high proportion of pre-scapular lymph nodes with lesions suggestive of tuberculosis indicates that most routes of infection could be through aerosol infection of superficial neck injuries or some other supercutaneous ulcers on the neck or shoulders or even the surfaces of the thorax or chest cavity (Henderson, 1946; Sisson and Grossman, 1938).

It has been demonstrated that the pre-scapular lymph node receives afferent vessels from the skin enclosing the neck and shoulder and even muscles like the pre-scapular muscle (Henderson, 1946; Sisson and Grossman, 1938). These findings are consistent with those of other studies where it was also observed that lymph nodes are the organs in cattle most frequently affected by tuberculous lesions (Tammemagi et al.,, 1974; Lepper and Pearson, 1973; Corner, 1994). It is significant to note that, macroscopic or visual inspection pre-culture revealed that a high number of lymph nodes harboured gross lesions that is, 115 lymph samples (or 74.2%) out of 155 samples. Twenty-six (26) out of these macroscopically observed 115 lesioned lymph nodes were positive for acid-fast bacilli pre-culture. Twenty-two (22) or 84.6% of the 26 acid-fast-positive bacilli successfully grew on culture. Recovering or isolating mycobacteria from bovine tissues, in the context of slaughter surveillance or carcass inspection, as was the case in this study can be difficult because there should first exist gross visible lesions in the opinion of the inspector. It must also be noted that not all infected bovines may exhibit gross lesions. Lesions embedded in the deep recesses of organs or altogether not fully developed are likely to be invisible to the meat inspector.

Sample preparation involved the manual maceration and homogenisation of tissue before decontamination. Thus, culture which was taken as the test standard, depended on the efficiency of visual perception in correctly identifying a lesion and that of manipulation of lesioned specimens to release acid-fast bacilli. It must be noted that culture results are not being taken as a ' test standard' for diagnostic sensitivity but as test for assessing the proficiency of visual inspections at beef slaughters. Also, slants (pyruvate impregnated and/or glycerol impregnated) were classified as negative for mycobacterial growths after 8 weeks, though the observation period could have been slightly longer (Sahraoui et al., 2008, 2009).

Out of the 133 samples which eventually grew on culture, only 43 were deemed to harbour acid-fast bacilli by ZN microscopy pre-culture. A low mycobacterial load in tissue samples can lead to poor detection by microscopy. Since ante-mortem examinations (data not shown) ensured the slaughter of apparently healthy animals tissue samples with low mycobacterial loads may have resulted from recent primary infections in the cattle. Out of the 14 samples which did not grow on culture, 12 (or 85.7%) were also negative by microscopy pre-culture. This is an indication that ZN microscopy is quite good at correctly identifying samples that are truly negative for the disease. Thus, in other words, the high specificity of ZN microscopy as a test indicates that a sample returning a positive ZN microscopy result is likely to contain acid-fast bacilli.

A noteworthy observation is that post-culture microscopy of the 133 successful growths revealed that 127 isolates contained acid-fast bacilli giving an 'apparent' prevalence estimate of 95.5% (127/133). Contextually, slaughter point inspection revealed that 5.0% (145/2886) of the cattle slaughtered exhibited lesions suggestive of tuberculosis. It should be noted that the 133 positive cultures were obtained from lesioned samples obtained from these 145 carcasses, and that the 'apparent' prevalence of 95.5% (127/133) refers to the fraction of positive cultures which was also microscopically confirmed as acid-fast. This 'apparent' prevalence translates into a 'real' prevalence of 4.6% (133/2886) in terms of positive cultures or a 'real' prevalence of 4.4% (127/2886) in terms of positive cultures which are also acid-fast.

Conclusion

The results of the study reveal that there is sub-clinical mycobacterial infection of some slaughtered cattle at the Accra and Tema abattoirs since all cattle passed for slaughter were apparently healthy ante-mortem. This study also reveals that it is relevant to consider the apparent lesion prevalence of cattle with lesions observed at necropsy at the three abattoirs when designing more effective control and management protocols for slaughter surveillance for mycobacterial disease in meat inspecttions in Ghana. Samples were only taken from lesions which presented with patho-morphological features suggestive of and consistent with tuberculosis. Culture of these suspected lesions and subsequent microscopic examination of isolates confirmed that majority of these were acid-fast and very probably mycobacterial infections even though pre-culture ZN microscopy detected only a small fraction (that is, 33.2% or 43/133).

Taking into account the results of culture and post-culture microscopy, the study reveals that pre-culture microscopy correctly identified only 40 cattle as harbouring lesions positive for acid-fast bacilli whereas

visual inspection correctly identified 127. This implies that pre-culture microscopy confirmed only a small fraction of visually identified TB-like lesions. On the other hand, only 6 out of the 93 samples found not acid-fast by pre-culture microscopy were successful on culture and also acid-fast (that is, microscopy revealed 87 false-negatives). Thus, it is entirely justified for the continued use of visual inspection as a quality control measure in abattoirs in resource-stretched settings, so long as this can be done with a high level of proficiency. The fact that pre-culture microscopy could confirm a high fraction of samples which did not grow on culture (that is, 12/14; Table 3) as not having acid-fast bacilli indicates it could be a useful screening tool. Microscopy may, thus, be employed to augment visual inspection in very difficult cases. Further work, at the molecular level, needs to be initiated to characterise these mycobacterial species and also to investigate the risk of transmission of BTB from cattle not only to the abattoir workers, other cattle handlers and the general consumer but also to other domestic animals such as pets (Kaneene et al., 2002). As is the practice elsewhere (Olea-Popelka et al., 2012), a regime of continuous slaughter surveillance at necropsy is being recommended for Ghanaian abattoirs to improve the quality of beef and also the introduction of a programme of routine intradermal tuberculin skin testing to screen herds of cattle being reared (Probst et al., 2011).

ABBREVIATIONS

BLN, Bronchial lymph node; **BTB,** bovine tuberculosis; **AFB,** acid-fast bacilli; **MLN,** mesenteric lymph node; **MTBC,** *Mycobacterium tuberculosis*-complex; **NALC,** N-acetyl-L-cysteine; **PSLN,** pre-scapular lymph node; **RTPLN,** retro-pharyngeal lymph node; **SMLN,** supra-mammary lymph node; **TB,** tuberculosis; **WASH,** West African Short-Horn; **w/v,** ratio of weight to volume; **ZN,** Ziehl-Neelsen.

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Conflict of Interest

The authors declare that they have no conflict of interests.

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