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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 over-emphasised. 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

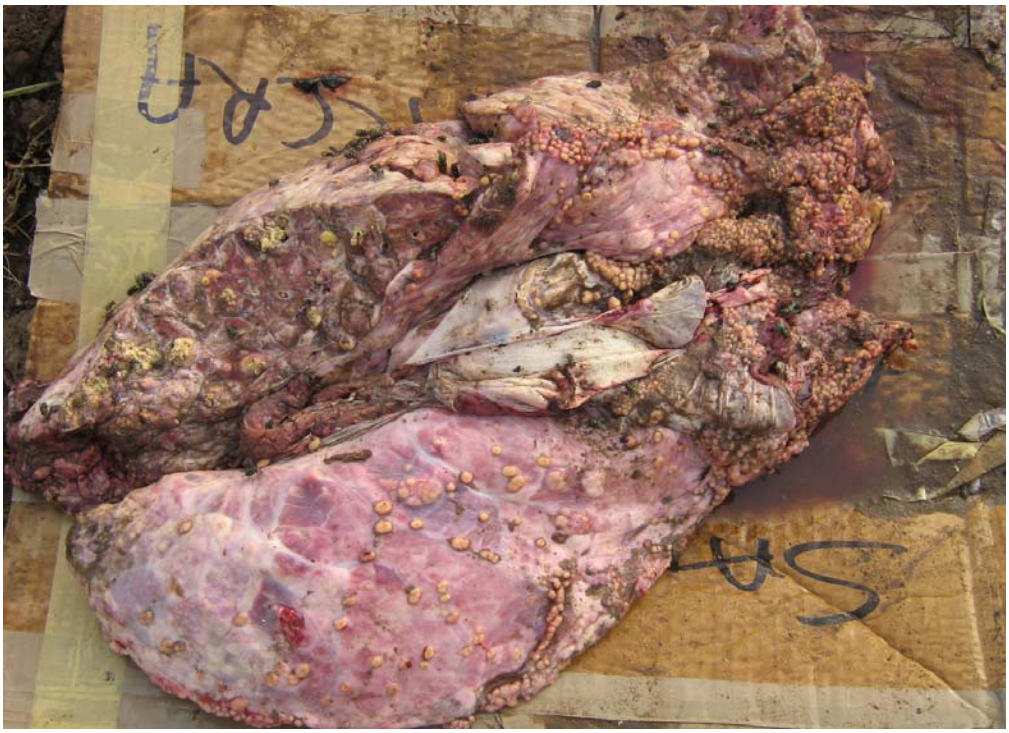

Figure 1. Disseminated Bovine Tuberculosis lesions seen, calcified and invasive, in lungs of a bull carcass at necropsy in the Accra abattoir. Observe the calcified and necrotic granulomas invading the periphery of the lobe in the foreground, and the interior of the sectioned lobe in the background.

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

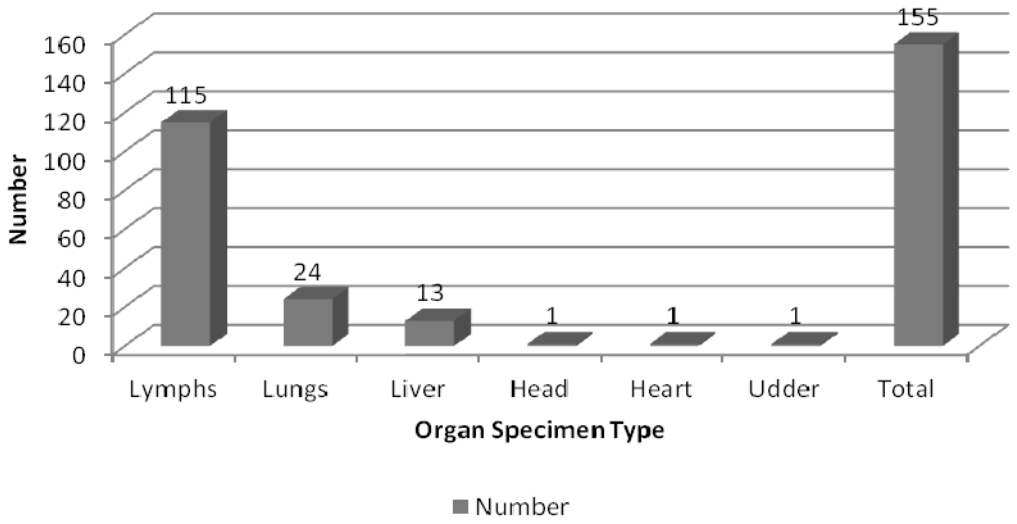

Figure 2. Distribution of organ specimen exhibiting tuberculous lesions at necropsy.

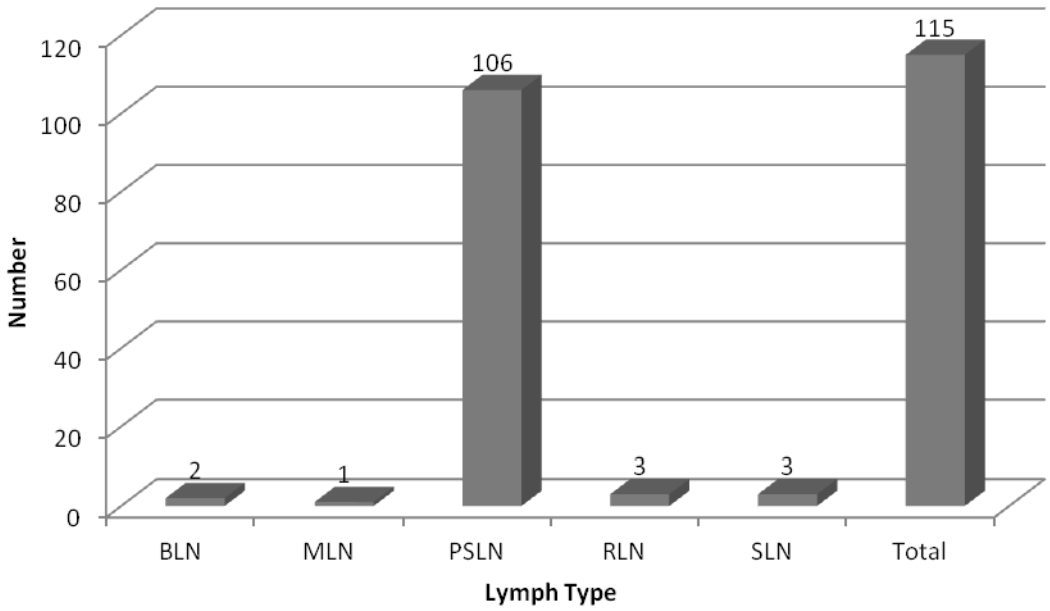

Figure 3. BLN=Bronchial lymph node; MLN=Mesenteric lymph node; PSLN=Pre-scapular lymph node; RLN=Retro-pharyngeal lymph node; SLN=Supra-mammary lymph node**.**

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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