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Preliminary Investigation of Bovine Tuberculosis in Suspected Beef from a Metropolitan Abattoir in Ghana with Ziehl-Neelsen Microscopy

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Abstract: Bovine tuberculosis is an important zoonotic disease transmissible through aerosols inhalation and the ingestion of contaminated milk and meat from cattle. Abattoirs in Ghana mainly depend on post-mortem examinations as means of diagnosing the presence of mycobacterium in meat (beef). A Ziehl-Neelsen microscopy was used to investigate the presence of *Mycobacterium bovis* as Acid-Fast Bacilli (AFBs) in beef samples from the Kumasi Metropolitan abattoir; thereby vetting post-mortem examinations at the abattoir. Lesioned lung tissues and calcified or puss-filled thoracic lymph nodes were collected at post-mortem as directed by an expert veterinarian. A total of 159 samples from 130 cattle (bulls and cows) were used in this study from April to July 2006. Ninety-five (i.e., 73.1%) of the 130 cattle sampled were positive for AFBs, whilst the remaining thirty-five (26.9%) were negative. Out of the total 159 individual samples specimen collected, 114 (71.7%) were found with AFBs. A total of 64 lung tissues and 95 lymph nodes were collected, respectively. Interestingly, 70.3% of the lung tissues were AFB-positive with 69 (72.6%) out of the 95 lymph nodes, also being positive. The ZN microscopy was effective in detecting the presence of mycobacteria, as 73.1% of the suspected samples were AFB-positive. It presupposes that, abattoir post-mortem examinations were also efficient however; the lapses of non-detection of asymptomatic carcasses could also pose a serious health risk to consumers. Also, lack of a functional on-site laboratory and a practical monitoring system was found to be unfavourable to the maintenance of meat quality. Detailed laboratory examinations (such as culture, PCR and other biochemical tests) to augment ZN microscopy is recommended for thorough detection of bovine tuberculosis.

Key words: Ziehl-Neelsen microscopy, bovine tuberculosis, *Mycobacterium bovis*, acid-fast bacilli

INTRODUCTION

Available data of Tuberculosis (TB) in Ghana indicates that the disease burden is high and TB remains an important cause of major disability and death in the country. With the country's population of over 20 million, the World Health Organization (WHO) estimates that, there will be 44,041 new cases of all forms of TB in Ghana corresponding to a TB incidence rate of 211 per 100,000 inhabitants of whom 19,285 are smear positive cases (WHO, 2005).

Mycobacterium bovis, known to be the main cause of tuberculosis otherwise known as bovine tuberculosis in cattle causes similar disease to that by *M. tuberculosis* in humans (Ayele *et al*., 2004; Biet *et al*., 2005). Reported facts indicate a broader host spectrum that includes other livestock such as sheep and goats and wild animals like badgers (*Meles meles*), possums and buffalo (Ayele *et al*., 2004; Leite *et al*., 2003).

Bovine tuberculosis is an important zoonotic disease in that it is transmissible to man through aerosols and the ingestion of contaminated meat and unpasteurised milk (which often lead to extra-pulmonary infections) (Office International des Epizooties, 2005). The disease is present in almost all African countries (Ayele *et al*., 2004). It is known to be prevalent in about 33 (80%) of the 43 African

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member countries of the regional commission of the Office International des Epizooties (Daborn *et al*., 1994; Raviglione *et al*., 1995).

In Ghana, a study in the Ho district of the Volta Region revealed a prevalence rate of 3.1% infection in cattle and 5.9% within a cluster. The study also revealed an increase in the prevalence of bovine tuberculosis from 0.9-1.5% for the period under review (Ankugah, 2000). Similarly, in another study in the Dangme-West District of Ghana, an overall prevalence of 13.8% was recorded with as high as 50% prevalence in some kraals (Akanmori *et al*., 2000).

Man's susceptibility to this infection poses a great health risk especially to consumers of beef and milk as well as veterinarians and abattoir workers. This is because TB caused by *M. bovis* is clinically indistinguishable from TB caused by *M. tuberculosis* (Ayele *et al*., 2004). Also, although the risks of transmissions of bovine tuberculosis to humans are real, there is little or no published evidence establishing an epidemiological association between TB in cows and bovine tuberculosis in humans in West Africa (Pia, 2001; Raviglione *et al*., 1995).

The Kumasi abattoir situated in Kaase, a suburb of Kumasi, serves as the main metropolitan abattoir in the Ashanti Region. It has the capacity of slaughtering about 1000 cattle per day excluding other animals such as sheep, goats and pigs. On the average, a total of 600 cattle are slaughtered daily in the two highly mechanized slaughter-carcass haul and meat examination sections.

Mycobacterium bovis can be detected microscopically on direct smear, from clinical samples and on prepared tissue materials (Ayele *et al*., 2004). Tissue smears from affected organs (in this case from abnormal thoracic lymph nodes and lung tissues) stained by the Ziehl-Neelsen (ZN) method can be used to detect the presence of acid-fast bacilli which appears as red or pink rods with a blue background (Ayele *et al*., 2004). Ziehl-Neelsen staining is cheap, relatively simple to perform and a useful preliminary diagnostic step especially in developing countries where laboratory facilities are not available (Pritchard, 1988).

In this study, we describe a preliminary investigation of bovine tuberculosis with ZN staining and microscopy based on post-mortem examinations carried out by veterinary experts at the Kumasi abattoir. The main objective was to use the ZN microscopy to identify the presence of mycobacteria as acid fast bacilli in suspected beef samples at post-mortem in the abattoir.

MATERIALS AND METHODS

A total of 130 cattle presented for slaughter between April and July, 2006, after inspection were enrolled. From them, 159 samples, consisting of lung tissues and thoracic lymph nodes were collected. Most of the cattle were from local livestock markets in the upper East and Northern regions of Ghana and neighbouring countries such as the Burkina Faso and Chad.

The study population comprised both bulls and cows whose carcasses presented gross abscesses and tubercle-like lesions, suggestive of possible classical mycobacterial infection (Fig. 1, 2). When necessary, a maximum of two samples were obtained from each animal otherwise a sample was taken. These samples were taken under the prevailing hygienic conditions which are strained due to the overcrowding and insufficient resources.

Samples collected were washed with sterile distilled water and stored in small plastic containers with lids and transported to the laboratory on ice and frozen immediately at -20°C until worked on.

Ziehl-Neelsen microscopy: The ZN staining procedure was employed as a preliminary diagnostic tool to detect acid-fast bacilli. Smears were prepared from pus-filled or

Fig. 1: A Lung with multiple abscesses

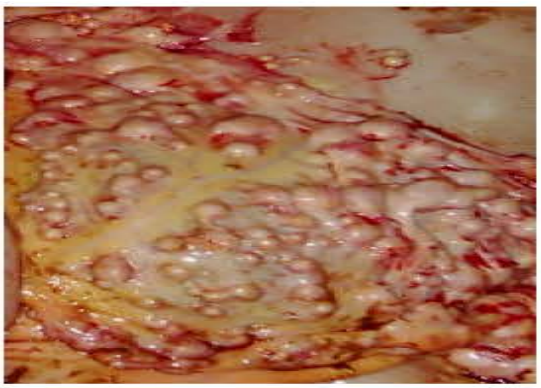

Fig. 2: Reverse side of the above organ showing multiple abscesses

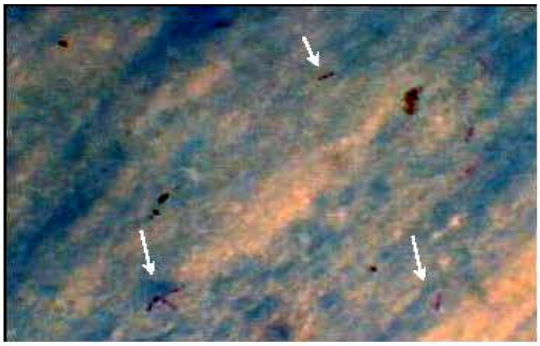

Fig. 3: Ziehl-Neelsen stained slides showing acid fast bacilli as single pale red rods

Fig. 4: Ziehl-Neelsen Stained slide showing acid fast bacilli as pale red rods in a cluster

Table 1: Results of Ziehl-Neelsen microscopy on all suspected 130 cattle

Results	Positive	Negative	Total
No. of samples	95.0	35.0	130
Percentage	73.1	26.9	100

calcified tissue samples. The Ziehl-Neelsen technique is as follows: staining with 0.3% carbol fuchsin for 5 min, 20% sulphuric acid as a decolouriser for 5 min and 0.3% methylene blue as a counter-stain for 1 min. (Vijayan, 2002). Stained-slides (Fig. 3, 4) were air-dried and examined under a light microscope with a 100x oil-immersion objective to identify the presence (or absence) of acid-fast bacilli.

Results of Ziehl-Neelsen microscopy: The 73.1% (i.e., 95/130) of samples examined microscopically, tested positive for AFBs (Table 1). However, considering the overall 159 samples that were collected (in which case some animals contributed more than one specimen) 71.70% (114/159) of the samples were positive for AFBs with 28.3% (45/159) being negative for AFB's. Table 2 shows the number of lymph nodes and lungs found to be a haven for the acid-fast bacilli. On the whole more lymph nodes were collected. From the same table, 69 (i.e., 72.6%) of the lymph nodes were AFB positive as against 45 (i.e., 70.3%) of lung tissues. Figure 3 and 4 show ZN-stained slides with AFBs in a cluster and single rod, respectively.

Table 2: Results of Ziehl-Neelsen microscopy of the tissue types from the total 159 tissue samples collected

Tissue type	ZN-Positive	ZN-negative	Total
Lung tissue	45 (70.3)	19 (29.7)	64
Lymph node	69 (72.6)	26 (27.4)	95
Total	114 (100)	45 (100)	159

Values in brackets are percentage

DISCUSSION

The present study confirms the efficiency of routine post-mortem examination by veterinarians at the abattoir for bovine tuberculosis in carcasses. The significant number of suspected beef that turned out positive at microscopy in fact proves the efficiency of the ZN process as a first line diagnostic tool. However, it is possible that some cattle, which were asymptomatic before slaughter (in this case have no visible lesions or abscesses) could possibly harbour *M. bovis*. These carcasses could have been eventually passed on as fit for consumption. From our data, it was evident that ZN microscopy as a diagnostic tool was useful as it detected 73.1% of the suspected carcasses as harbouring AFBs.

The high figure of 95/130 cattle (Table 1) being AFB positive is consistent with the high prevalence rates within kraals as recorded by Ankugah (2000) and Akanmori *et al*. (2000) which translates to high infection rates especially with temporary or permanent kraal were cattle are kept before slaughter.

Another significant finding was the total number of thoracic lymph nodes isolated at post-mortem as against the lung tissues and the resulting high percentages especially for the lymph nodes (Table 2). Though this trend may seem inconsistent with a similar study by McIlroy *et al*. (1986) confirming the presence of *M. bovis* in 73.0% of tuberculous lung samples and thus indicates active infection through aerosol means among cattle. However, it is being emphasized that, all lymph nodes collected were only from the thoracic region of the cattle and hence buttress the report that the active infection were by aerosol routes.

This study shows that whilst post-mortem examinations at the abattoir are effective, there is still the need for more rigorous tests to capture carcasses that may not present lesions or abscesses. Also, the non-compliance to good hygienic and good quality control practices within the abattoir may continue to provide conditions that promote contamination between condemned carcasses and wholesome ones. These poor practices within the examination line could also pose a serious risk of TB infection to persons within and in the immediate vicinity of the abattoir.

Post-mortem examinations alone may add to cost incurred by cattle dealers because 73.1% of cattle (Table 1) labelled as suspected were actually positive for AFBs. It is worth mentioning that a trim-off system (i.e., a system where lesions/abscesses are physically removed when they should have been totally discarded) exists as a minor way of mitigating huge losses by cattle owners. However, the lapses in abattoir policies, may pose a serious public health threat. The total rejection policy cannot fully be implemented when the abattoir management is in no position to bear the cost of a condemned carcass. This will leave cattle owners at a loss. Also, the setting-up of an on-site laboratory would help with the early diagnosis of cattle at ante-mortem.

CONCLUSION

We conclude that, post-mortem examinations at the Kumasi abattoir are efficient but could be enhanced with other on-site cost effective laboratory tests such as ZN microscopy and possibly culture and PCR. It is recommended also that, ante-mortem examinations be made plausible and routine since they are mainly conducted when an animal shows signs of illness. Another study, could also be conducted at ante-mortem; targeting the live animals with or without disease symptoms. This will help reduce losses after post-mortem as more animals could be isolated and treated before slaughter.

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