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Abstract

Background: The need to understand the contribution of bovine tuberculosis (BTB) to the general tuberculosis burden in a poor resource setting is paramount. The aim of this study is to determine the burden of BTB among herdsmen and cattle in the North Tongu district of Volta Region in Ghana.

Materials and Methods: A cross-sectional study was conducted in the North Tongu District of the Volta Region between the period of October 2011- March 2012. A well-structured questionnaire was used to collect socio-demographic information and possible risk factor information on cattle from participants. Sputum samples from 68 herdsmen and blood samples from 200 cattle belonging to these herdsmen were also collected. Sputum samples were analyzed using Ziehl- Neelsen staining while Anigen Rapid BTB Test was used for Cattle blood samples.

Results: Ninety percent (61/68) of respondents were also found to consume fresh milk while 84% (57/68) do not use protective clothing. Of a total of 1580 cattle owned by the herdsmen, 200 cattle consisting of 14 bulls and 186 cows were screened where the prevalence of bovine TB was 19% (38/200) and those affected were all females. All (100%) human sample tested negative for Acid-Fast Bacilli (AFB). However, the seropositivity of cattle and kraal density were statistically associated ($p=0.001$).

Conclusion: Bovine TB is prevalent in cattle in North Tongu district. Although herdsmen indulge in risky lifestyles that expose them to BTB, a zero prevalence of BTB was observed, further study is envisaged using a larger sample size.

Key words: Bovine tuberculosis, Ghana, Herdsmen, North Tongu.

Introduction

Tuberculosis (TB) is an infectious disease of warm-blooded animals caused by *Mycobacterium tuberculosis* (*MTB*) complex which includes *Mycobacterium tuberculosis* complex, *Mycobacterium bovis*, *Mycobacterium tuberculosis*, *Mycobacterium avium* (Kaneene and Theon, 2004). The rate of decline of incidence of the disease is very slow due to the emergence of the Multi- Drug Resistance TB (MDR-TB) (Cosivi *et al.*, 1998). There is also the problem of bovine tuberculosis (BTB) whose numerical contribution currently to the general TB burden is unknown (Ayele *et al.*, 2004). Human *M. bovis* infection was a major public health risk and an important source of TB in humans in the 1930s as a result of a high prevalence of bovine tuberculosis in cattle but the practice of the test and slaughter programme as well as the introduction of pasteurisation of milk reduced the incidence (Shitaye *et al.*, 2007). Currently bovine tuberculosis is mainly endemic in developing countries including sub-Saharan Africa where the test and slaughter policy has not been properly implemented (Cosivi *et al.*, 1998). As a result, bovine TB is either only partially controlled or not controlled at all which makes people working with cattle such as herdsmen, veterinarians and livestock workers to be at high risk of BTB infection (Georghiou *et al.*, 1989).

Humans often get exposed to *Mycobacterium bovis* the causative agent of BTB through direct inhalation from animals, consumption of uncooked infected meat or infected unpasteurized milk (Ayele *et al.*, 2004). The close co-existence of farmers and animals is exemplified by the herdsmen, who live their entire lives with their animals, offering ample opportunity for zoonotic transmission of infection.

At the peripheral level of the health system in the Ghana, the Ziehl-Neelsen method is used for the diagnoses of tuberculosis. Detection of BTB in cattle in the country is also carried out most commonly on the basis of tuberculin skin testing using the Purified Protein Derivative (PPD), abattoir meat inspection and rarely on bacteriological techniques (Abubakar *et al.*, 2007). In recent years, there are many diagnostic methods for screening cattle for BTB. One of such methods is the Anigen Rapid BTB Ab Test Kit which saw a tremendous patronage over the years in other countries due to its specificity (98 %), sensitivity (85%), as well as being easy to use (Danbirni *et al.*, 2010).

In Ghana, cattle testing by the tuberculin skin test is sporadic. Between the period of 2005 to 2010, 516 TB cases were diagnosed at abattoirs and slaughter houses in Ghana (Veterinary monthly summary reports, 2010). Currently, the contribution of BTB to human tuberculosis is unknown in Ghana. In a study conducted at the Korle-bu Teaching Hospital, 3% of TB positive human samples were *M. bovis* (Addo *et al.*, 2007).

Although bovine tuberculosis cases were reported in cattle at slaughter (2005-2009) according to the Veterinary monthly summary reports in 2010, an unknown proportion of cattle in the country is still infected with bovine TB because the test and slaughter policy for the control of the disease has sporadic and not well articulated. Since people who attend to these animals such as

herdsmen and veterinarians as well as the general public who consume fresh milk or infected meat are exposed to bovine TB from cattle (Bilal et al., 2010). There is therefore the need to ascertain the burden of BTB in cattle and in human in Ghana using the field approach, rather than abattoir and hospital based approaches targeting herdsmen in rural settings. This study therefore evaluates the burden of BTB among herdsmen and cattle in the North Tongu district of the Volta Region.

Study Area

A cross-sectional study was conducted between the periods of October 2011 to March 2012 in the North Tongu District of the Volta Region. It lies within latitude 5° 47' North to 6° North and longitude 0° 5' East. The total area of the district is 1460 square km, which is about 7.1% of the Volta Region. The district lies within the Tropical Savannah Grassland zone. The total population of the area is 130,388. Livestock productions in the district include large ruminants (cattle), small ruminants (sheep and goats) and monogastrics (pigs, rabbits) as well as poultry. Cattle production is mainly done by the semi-intensive system where animals are herded by local cattle boys and Fulani herdsmen for grazing and watering in the mornings and return in late afternoon. Most kraals are situated in the communities and in close proximity to households to minimize theft.

North Tongu was purposively chosen for the study based on the fact that it is the main cattle producing area in the Volta Region and from Veterinary monthly summary reports, 2010, 2% (9/516) of national bovine TB cases are observed at slaughter. According to the District profile of the area, TB is among the top five human diseases (Malaria, HIV/AIDS, Cardiovascular Accident, Anaemia and Tuberculosis) in North Tongu and also a high human and cattle interaction alongside risky lifestyle was observed in the area.

Variables

The dependent variables in the study were positive/ negative test result for bovine tuberculosis in both human and cattle. The independent variables in the human group were socio-demographic characteristics such as age, sex, educational level, religion, ethnicity and occupation as well as occupational characteristics such as experience (years), consumption of raw milk and overcrowding while the independent variables in cattle were age, sex, breed, herd size, antimicrobial usage, and husbandry practices.

Sampling of Cattle

The study population for cattle reared in the North Tongu District which was 34,564 (projected livestock census, 2010) and all cattle of age 6 months between October 2011 and March 2012 were included in the study. All cattle that were moved from elsewhere to the study location after the commencement of the study were excluded.

The sample size was 200 using the following formula; $N = \frac{z^2 p(1-p)}{d^2}$, where N= sample size,
z= risk of Type 1 error (=1.96 at 95% confidence level)
p= prevalence of BTB =13.8%
d= precision (allowable error) = 5% = 0.05

Using an estimated prevalence of BTB in cattle of value 13.8 % (Bonsu *et al.*, 2003) a minimum sample size of 183 was computed and rounded up to 200.

The population of cattle herdsmen in the North Tongu District which was estimated to be 1182 constituted the study population for humans. All herdsmen of 10 years and above in North Tongu District between October 2011 to March 2012. People who are physically and mentally unable to give consent were excluded from the study.

The sample size for herdsmen which was 80 was computed using the formula; $N = \frac{z^2 p(1-p)}{d^2}$, where N= sample size,
z = risk of Type 1 error (=1.96 at 95% confidence level)
p= prevalence of BTB in human = 3%
d= absolute precision = 5% = 0.05

Using a prevalence of BTB in human of 3% found in a study done by Addo and others in Ghana (Addo *et al.*, 2007) a minimum sample size of 44 was computed and rounded up to 80.

All persons living in households with the selected kraals who fall within the inclusion criteria and who consented were sampled by simple random sampling using the ballot method. The study protocol was reviewed and approved by the Ethical Review Board of the Ghana Health Service. The protocol was also approved by the Veterinary Services Directorate of the Ministry of Food and Agriculture. The District Health Management Team (DHMT), the District Assembly and traditional rulers also permitted the study. Verbal and written consent was duly sought from the study participants for voluntary participation in the study. Confidentiality was ensured.

Questionnaire administration

Study questionnaire was pre-tested in a community which is similar to the selected area to train the Research assistants. Structured questionnaires were administered to eligible and consenting participants for socio-demographic, risk (predicting) factors and to assess their knowledge on the occurrence of BTB and its control. The owners of the selected cattle were also interviewed on herd composition and risk (predicting) factors of the disease in their animals.

Sample collection, staining techniques and Anigen Rapid Test

3- 5ml of sputum samples were collected from herdsmen for laboratory testing for BTB. About 5 ml of blood was collected from jugular vein of each cattle for laboratory testing for BTB.

For the diagnoses of tuberculosis in human samples, Ziehl-Neelsen staining was used because this is the currently used method for tuberculosis diagnoses in the country at the peripheral levels of the health system as recommended by OIE Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees) (OIE; 2008)

For the diagnoses of BTB in cattle, Anigen Rapid BTB Ab Test Kits was used and the test was as described by Danbirni, et al., (2010). Anigen Rapid Test Kit is a chromatographic immunoassay for the qualitative detection of *Mycobacterium bovis* antibody in plasma and serum which has many advantages over the tuberculin skin test. The Anigen Rapid BTB test kit has a 'T and C' line as Test and control Lines on the surface of the kit. Both the 'T and the C' lines in the result window are invisible before any sample is applied. The 'control Line' is used as the procedural control (Figure 1). Two bands of purple colour within the result window indicated a positive result. A single purple band within the result window indicates a negative result.

Data Analysis

Data was entered into Epi Data, coded and exported into SPSS version 16.0 for analysis. Data was cleaned and descriptive and statistical analysis was done.

Pearson's Chi-Square test was used to test associations between demographic data and dependent variables. Tabulations of frequencies (and percentages) and graphical presentations were done using Statistical Package for Social Sciences software (SPSS, version 16). All statistical tests were declared significant for p-value < 0.05.

Results

Cattle population

A total of 200 cattle were sampled. The prevalence of bovine TB in cattle was found to be 19% (Table 1). All cattle samples that tested positive came from cows.

Table 1: Laboratory result of cattle for bovine TB, North Tongu District, October 2011- March 2012.

Species	Status	Counts	%
Cattle	Positive	38	19
	Negative	162	81

Of the 200 cattle, 14 were bulls and were 186 cows. Ten were exotic breeds, 95 were local breeds, and 95 cross breeds. Ninety nine percent (193/194) of cattle were kept under the semi- intensive system and the rest under the intensive system. Eighty four percent (164/195) of cattle were routinely treated with antibiotics (Table 2).

Table 2: Possible Risk Factors of BTB in cattle, North Tongu District, October 2011- March 2012

Variable	Item	Counts	%
Husbandry System	Semi-intensive	193	99.5
	Intensive	1	0.5
	Total	194	
Breed	Local	95	47.5
	Exotic	10	5.0
	Crossbreed	95	47.5
Antibiotic Usage	Yes	164	84.1
	No	31	15.9
	Total	195	
Sex	Male	14	7.0
	Female	186	93.0
	Total	200	100

Table 3: Seropositivity of cattle to bovine TB and related possible Risk factors, North Tongu District, October 2011-March 2012.

Factor	P-Value	Odd Ratio	95% Confidence Interval	
			Lower	Upper
Kraal density	0.001	-	-	-
Age of cattle	0.936	-	-	-
Sex of cattle	0.235	0.306(male/female)	0.039	2.411
Type of breed	0.650	-	-	-
Type of husbandry	0.621	-	-	-
Cough/Running nose	0.552	0.778(yes/no)	0.339	1.784
Treated with antimicrobial	0.313	1.765(yes/no)	0.578	5.388

Kraal density is statistically associated with seropositivity of cattle to bovine TB ($p=0.001$) but all the other risk factors considered were not statistically associated (Table 3).

Cattle between the ages of 4-6years (97- 120months) were most infected with bovine TB (Fig 1).

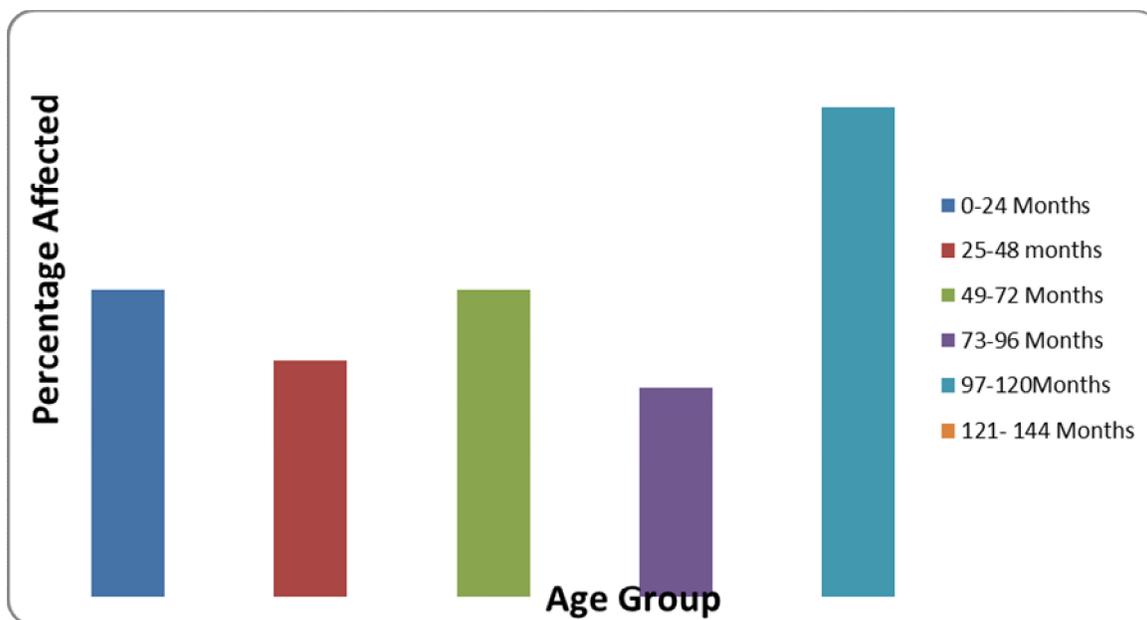


Figure 1: Seropositivity of BTB in cattle by age groups, North Tongu District, October 2011- March 2012.

Human population

A total of 68 herdsmen were sampled. Almost 68% (46/68) of participants were males. All human samples tested Negative for Acid Fast Bacilli (AFB) (Table 4). Majority of respondents were between the ages of 11- 20 years and the median age group of respondents was 31-40 years (Table 5).

Table 4: Laboratory result of humans for TB, North Tongu District, October 2011- March 2012.

Species	Status	Counts	%
Human	Positive	0	0
	Negative	68	100

Table 5: Characteristics of participants, North Tongu District, October 2011- March 2012.

Variable	Item	Count	%
Age	1-10	1	1.5
	11-20	18	26.5
	21-30	5	7.3
	31-40	13	19.1
	41-50	16	24
	51-60	7	10.3
	61-70	5	7.3
	71-80	3	4.0
Sex	Male	46	68
	Female	22	32

The majority of the respondents were illiterates. Among those who had education, middle school/JSS was the highest level of education attained (Table 6).

Table 6: Characteristics of participants, North Tongu District, October 2011- March 2012. Continued.

Variable	Item	Count	%
Educational Level	Primary	16	24
	Middle/JSS	17	25
	Secondary/SSS	2	2.5
	Tertiary	2	2.5
	None	31	46
Ethnic group	Mole Dagbani	13	19
	Ewe	49	72.1
	Krobo	1	1.5
	Other	5	7.4
Religion	Christian	28	41.2
	Moslem	20	29.4
	Traditional	8	11.8
	None	8	11.8
	Other	4	5.8

Most of the participants indulge in risky practices such as consuming fresh milk, sharing bulls for breeding as well work without protective clothing that could expose them to contracting bovine TB (Table.7).

Table 7: Possible risk factors of bovine TB in human, North Tongu District, October 2011- March 2012.

Variable	Response	Count	%
Fresh milk consumption	Yes	61	89.7
	No	7	10.3
Protective clothing usage during herding and milking	Yes	11	16.2
	No	57	83.8
Bull sharing with other farms	Yes	48	70.6
	No	20	29.4

Most of the participants indulge in risky practices such as consuming fresh milk, sharing bulls for breeding as well work without protective clothing that could expose them to contracting bovine TB (Table.7).

Discussion

The need to determine the contribution of bovine tuberculosis (BTB) to the general tuberculosis burden in a poor resource setting is paramount. This study evaluates the burden of BTB among herdsmen and cattle in the North Tongu district of the Volta Region in Ghana.

The 19% prevalence rate of BTB in cattle that was found from this study indicates the existence of the disease in the area. The prevalence found in this study is higher than what was found by other workers whose results was based on routine data from slaughter houses. This could mean that active search other than passive surveillance yielded more results (Bonsu *et al.*, 2003).

The strong association found between seropositivity and the kraal density demonstrates the effect of overcrowding on the degree of transmission of BTB within the flocks and agrees with the findings of Humblet *et al.*, (2010) that seropositivity of cattle to BTB and animal density were statistically significant. The increase in seropositivity of cattle to BTB with age which was though found to be statistically insignificant agrees with the fact that BTB manifests most at the later stage of the disease especially between 4-6 years opined by Kazwala *et al.*, (2001) and Folitse *et al.* (2013).

Although the fulanis usually treat their animals with antimicrobial to reduce the level of infection in the herd, this was not effective probably because the antimicrobials employed were not effective against *M. bovis* which further confirmed that *Mycobacterium bovis* is resistant to many antibiotics (Bilal *et al.*, 2010).

The study result which shows cows were thrice infected as bulls by BTB which agrees with the findings of Bonsu *et al.*, (2003) and Folitse *et al.*, (2013) where cows were twice affected as bulls by BTB. In practice this could mean that cows are more at risk of contracting BTB especially during pregnancy when they have reduced immunity since tuberculosis flourishes well in immuno-compromised host. In addition, for the purpose of increasing production, most cattle farmers have more cows than bulls on their farms which gave room for exposure.

In the human population, the zero prevalence of BTB that was found does not necessarily indicate the absence of the disease, as humans are exposed in diverse ways to bovine TB infection. This can be seen in our results where 90% (61/68) of respondents consume fresh milk and 84% (57/68) do not use protective clothing coupled with close proximity of humans and animals. It could also mean that milk may be infected with *Mycobacterium bovis* but there were other underlying factors that made it impossible for us to detect the pathogen among humans or herdsmen might have strong immune systems that combat the disease.

Conclusion

Bovine TB is prevalent in cattle in North Tongu district. The total number of animals in a kraal (Kraal density) is a risk factor for cattle contracting bovine tuberculosis. Although herdsmen indulge in risky lifestyles that expose them to BTB, a zero prevalence of BTB was found among them. Hence further study is envisaged using a larger sample size and milk from cattle in the area should be tested for *M. bovis*.

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