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Bovine Tuberculosis in Cattle Destined for Human Consumption in Maroua-Cameroon: Prevalence, Economic Losses and Risk Factors to Meat Handlers

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Abstract: Bovine tuberculosis (bovine Tb) is widespread in Cameroon and tuberculin skin test positive reactors associated with detection of Tb lesions have been found in the Maroua abattoir which supplies meat to the Maroua city and its environs. However, there is paucity of information on the epidemiology of bovine Tb, risk to meat handlers and economic losses of condemnation of meat due to Tb. This study was carried out to determine the diagnose of bovine Tb in cattle in Maroua abattoir based on single intradermal tuberculin test (SITT) using detection of Tb lesions and acid fast bacilli in the Tb lesions as gold tests and estimate economic losses using predefined methods while questionnaire survey was used to obtain information on the risk factors of meat handlers. The study revealed that 22.28% of cattle slaughtered in Maroua showed Tb lesions were detected in at meat inspection. Prevalence rates computed against detection of Tb lesions and detection of Tb lesions plus acid fast bacilli showed similar values at all the studied SITT cutoff points (≥3, ≥3.5 & ≥4mm) of 32.9 and 22.3%, respectively. However, higher sensitivity and predictive values were obtained at severe interpretations of SITT bovine Tb responses. The sensitivity (82.1%) of SITT at ≥ 3 mm and ≥ 3.5 mm cut-off points were not significantly higher (P>0.05) than the sensitivity at (71.4%) ≥ 4 mm cut off against detection of Tb lesions but sensitivity (100%) at ≥ 3 and ≥ 3.5 mm cut-off points were significantly higher (P<0.05) than the sensitivity at (89.5%) ≥ 4 mm cut off against detection of Tb lesions plus acid fast bacilli. Consumption of raw meat, unpasteurized milk, aerosol during meat manipulation, longevity as abattoir workers and poor educational level were significant risks of meat handlers to bovine Tb in the study. The financial loss related to organ and carcass rejections due to Tb the sampled cattle was estimated at over 880,000 FCFA equivalent to more than 25,001,460 FCFA/year. Bovine Tb prevalence is high in slaughtered cattle in the Maroua abattoir and given the epidemiological and environmental context of the region, the best performance of SITT bovine Tb was at ≥3.5mm cut-off points and not at the OIE recommended ≥ 4 mm cut off value.

Key words: Bovine Tuberculosis · Tuberculin Skin Test Cutoff Points · Post Mortem Inspection · Diagnostic Performance · Financial Losses · Maroua Abattoir-Cameroon

INTRODUCTION

Bovine tuberculosis (bovine Tb) is a highly prevalent zoonosis in cattle in most African countries [1] including Cameroon where it is among the top most neglected diseases [2]. In affected countries, bovine Tb is estimated to be one of the most important causes of socio-economic losses in cattle production such as losses in productive time on the part of farmers, low productivity due to mortality, losses of amount of money from condemnation of carcasses at slaughter, limitations in the trade of animals and their products as well as public health-related impacts including loss of human live [3, 4]. Though bovine Tb is endemic in most of Cameroon, a working national program for bovine Tb surveillance and control, to obtain accurate data on bovine Tb prevalence, has not been implemented. There are no effective measures in the country for controlling and preventing transmission of zoonotic diseases and the epidemiology of bovine Tb has little or no use in the control of human tuberculosis. Surveillance and control programs based on tuberculin skin test (TST) and slaughter of positive cattle are not

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done in Cameroon due to political, technical and economic reasons such as the inability to replace or compensate slaughtered animals [2].

The Maroua abattoir of the Far North region of Cameroon supplies meat to the Maroua city and its environs. The Maroua area is positive for bovine Tb according to the findings of positive comparative intradermal (2.7%) and single intradermal (10.6%) TST bovine Tb reactors [5] associated with detection of Tb lesions (13.1%) in the Maroua abattoir [6]. TST bovine Tb rates ranging from 3 to15% have also been recorded in other parts of the country [5, 7 & 8]. However, it is essential to know more information on the prevalence of bovine Tb, the main risk factors contributing to the spread of the disease and its role in human tuberculosis. In many areas in Cameroon, there is consumption of untreated milk, direct contact between people and livestock, together with malnutrition and a high prevalence rate of HIV infection, all of which constitute risk factors for this zoonosis [2, 9].

The diagnosis of bovine Tb in cattle is mainly through tuberculin testing, culture and molecular genotyping [10, 11]. However, monitoring bovine Tb by mycobacteriology and molecular genotyping are not practicable in resource poor countries like Cameroon because assays are costly and time consuming and laboratories are not well equipped [12]. Routine diagnosis of bovine Tb is based on the identification of characteristic Tb lesions in Cameroon [7, 13] and results from post mortem examinations are limited in information and prone to subjectivities of the examiners [7]. However, meat inspection provides useful insight into the epidemiology of bovine tuberculosis and plays an important role in both quality assurance and control [7, 13]. Abattoir meat inspection also provides improvement in animal and human health with regard to consumer protection as well as the surveillance, control and eradication of zoonotic tuberculosis in sub-Saharan Africa [12].

Though bovine Tb is widespread in Cameroon [6, 7, 9, 13 & 14], there is paucity of information on the economic losses of condemnation of meat due to Tb lesions and the epidemiology of bovine Tb in the country is not fully understood. This study was carried out to determine the prevalence rate of bovine Tb in cattle slaughtered in Maroua abattoir, estimate the direct financial loss due to rejection of carcasses due to Tb lesions during slaughter meat inspection and determine the levels of awareness, knowledge and practices of the various meat handlers on bovine Tb.

MATERIALS AND METHODS

Study Area and Sample Size: Cattle from the livestock markets in the environs of Maroua destined for slaughter at the Maroua abattoir were sampled. About twenty cattle are slaughtered daily in the abattoir which provides beef to inhabitants of Maroua city and neighbouring areas (10°30" - 10°40"'N and 14°20" - 14°30" E). Bovine Tb prevalence rate based on the detection of Tb lesions in slaughtered cattle [0.46% (0.43% - 0.50%)] and tuberculin skin tests of live cattle [4.67% (3.89% - 5.44%)] recorded by Awah-Ndukum *et al*. [7] in the highlands of Cameroon was used to estimate the number of cattle required to detect at least one positive reactor with 95% confidence and a desired precision of ≥5% as previously described [15]. The selection of cattle for the study was based on haphazard arrival of animals at the abattoir and on random-number generation method of cattle owners from the daily abattoir records whose animals were judged as fit to be slaughtered. However, cattle used for the tuberculin skin test performance study were animals that were judged fit for slaughtered and were not slaughtered until at least 72 hours stay at the abattoir.

Detection of Bovine Tuberculosis: The sample size for this study was calculated to be 7 for Tb lesions detection and 68 for TST bovine Tb testing. However, during November 2013 to March 2014, a total of 736 cattle slaughtered at Maroua (123 males, 613 females; 133 animals were 5 to 10 years old while 597 animals were over 10 years old ; 302 Peulh of Sahel Zebu, 242 Mbororo/Fulani Zebu and 192 Toupouri-Massa Zebu) were used in this study. Single intradermal tuberculin test (SITT) in the middle neck region was done on 85 randomly selected cattle according to standard diagnostic procedure [1] that were slaughtered at least 72 hours days later. Following slaughter the 736 selected animals including the 85 SITT bovine Tb test cattle, intensive meat inspections were carried out based on the government's legislation regulating veterinary health inspection and notification of contagious animal diseases [16]. Evidence of pathologies was also supported by postmortem examination of carcasses as earlier described [17-19]. Briefly, the inspection procedure employed visual examination and palpation of the lungs, liver and kidneys, lymph nodes of the thoracic and head regions, the mesenteric lymph nodes and other lymph nodes of the body and various other parts / organs of the carcass.

A total of 68 tissues specimens, with suspicious TB lesions, (53 thoracic and 7 abdominal lymph nodes and 8 liver tissues) from the 85 SITT bovine Tb test cattle of 736 slaughtered zebu cattle in the study were collected into sterile plastic containers and also stored at -20°C for up to two months before analysis. Individual animal information such as age estimated by examining the incisors [20], sex, breed [16, 21] and body condition scores [22] were recorded during SITT bovine Tb testing. Grinding of TB lesions [23] and direct smear microscopy with Ziehl-Neelsen (ZN) staining for confirmation of acid-fast tubercle bacilli [1, 24-26] was also done.

Questionnaire Survey of Meat Handlers: Risk factors to meat handlers from bovine Tb in the Maroua abattoir were examined. A questionnaire was survey conducted to collect information on a range of variables relating to the lifestyle and level of awareness, knowledge and practices of 136 meat handlers on bovine Tb. Briefly, abattoir workers, butchers, meat traders and cattle owners who visited the abattoir during the study period and willing to participate were included in the survey. The meat handlers of the abattoir were often disorderly, illiterate and also from various cultural communities in the city and environs of Maroua with distinct vernaculars. Considerable time and patience were needed to obtain their maximum cooperation; and where necessary a trusted and knowledgeable intermediary was engaged.

Assessment of Direct Financial Losses: The assessment of economic loss was based on annual slaughter capacity of the Maroua abattoir, average market price of each organ in Maroua town and condemnation rate of carcasses and organs. The average market price was determined by interviewing butchers and consulting with officials of the Chamber of Trade and Commerce of Cameroon. The economic loss due to condemnation was estimated as previously described by Ogunrinade and Ogunrinade [27] as follows:

$$EL = \Sigma srx \times Coy \times Roz.$$

where: EL = Annual economic loss estimated due to organ condemnation; Σ srx = Annual number of cattle slaughtered at the abattoir; Coy = Average cost of each organ; Roz = Condemnation rate of each organ. The average cost of a live animal destined for slaughter (350,000 FCFA) was used as the average cost of a whole condemned carcass

For the estimation of the direct financial losses, the total number and weights of whole or partially condemned carcasses and organs and costs per kilogram of whole carcass and affected carcass parts in Maroua were used. The mean cost per kilogram of meat, viscera and other parts were obtained through interviews with butchers and officials of the Chamber of Trade and Commerce of Cameroon. The immediate financial losses following inspection were calculated according to Mbaya *et al*. [28] as follows:

$$DFL = nW * Av.P/kg$$

where: DFL = the Direct Financial Losses; n = number of condemned carcasses and or organs; W = weight of organs and or carcasses; Av.P/kg = average price per kilogram of meat and or giblets.

Risk assessments of the project were performed by the researchers to avoid hazards to all persons and animals involved in the project. Ethical clearances were obtained from the required authorities before carrying out the study. The purpose of the study was explained to the targeted participants usually with the assistance of resident veterinarians, local leaders at the abattoir and or trusted intermediaries. An animal was tested after an informed consent was given by the owner. Apart from intradermal injections of bovine tuberculin and procedural restraining manipulations for safety purposes, the animals were not subjected to suffering. Slaughtering and dressing of cattle carcasses were done as described by the Cameroon veterinary services [16]. All laboratory analyses including ZN staining were carried out in a laboratory equipped with a category II Biosafety cabinet.

Data Analysis: The data were entered into Microsoft Excel and then transferred to SPSS 20 and R software. Frequency distributions of bovine Tb were generated for the different diagnostic techniques. The Chi-square test was used to evaluate the sensitivity of SITT bovine Tb and assess various associations. The ROC (*Receiving Operating Characteristic*) analysis was also used to evaluate diagnostic performance of SITT bovine Tb at different cutoff points [15].

RESULTS

Prevalence of Bovine Tuberculosis: Tb lesions were detected at meat inspection in 164 of 736 (22.28%) cattle slaughtered at Maroua during the study period equivalent

to 1.63% male animals, 20.65% female animals, 5.98% animals aged 5 to 10 years old, 16.30% animals aged over 10 years old, 8.42% Peulh of Sahel Zebu, 11.68% Mbororo/Fulani Zebu and 2.17% Toupouri-Massa Zebu.

However, single intradermal tuberculin test (SITT) done on 85 of these 736 cattle showed 28 (32.94%, 95% CI: 22.9 – 42.9); 26 (30.59%, 95% CI: 20.8 – 40.4) and 22 (25.88%, 95% CI: 16.6 – 35.2) positive reactors at ≥ 3, ≥ 3.5 and ≥ 4 mm cut-off points, respectively. Of the 85 animals, Tb lesions and Tb lesions plus acid fast bacilli were detected in 28 animals (32.95%, 95% CI: 22.95 – 42.93) and 19 animals (22.35%, 95% CI: 13.5 – 31.2), respectively. The affected cases showed localised lesions in the pulmonary region (57%), retropharyngeal (28.6%), pre scapular (7.2%) and pre parotid (3.6%) regions while 3.6% of cases showed generalized lesions.

The performances of SITT technique at various cutoff points to diagnosis bovine Tb in cattle in Maroua Cameroon using detection of Tb lesions and detection of Tb lesions accompanied with acid fast bacilli in the lesions as references for defining the status disease are shown in Table 1. Based on computed sensitivity and specificity values of SITT compared to detection of Tb lesions and Tb lesions plus acid fast bacilli, severe interpretations of SITT tests detected more disease cases. The sensitivity of SITT at ≥ 3 and ≥ 3.5 mm cut-off points compared to the sensitivity at ≥ 4 mm cut off were not significantly higher (P>0.05) against detection of Tb lesions but significantly higher (P<0.05) against detection of Tb lesions plus acid fast bacilli to define disease status.

It is worth mentioning that overall, the predictive values were usually superior at SITT ≥ 3 mm and ≥ 3.5 mm cut off points compared the OIE recommended (≥ 4 mm) cut-off point. Indeed, the performance of SITT against detection of Tb lesions revealed positive predictive values of 82.1 (73.9 – 90.2); 88.5 (81.6 – 95.2); & 90.9 (84.7 – 97.0) and negative predictive values of 91.2 (85.1 – 97.2); 91.5 (85.5 – 97.4); & 87.3 (80.2 – 94.3) at reactors at ≥ 3, ≥3.5 and ≥ 4 mm cut-off points, respectively. Accordingly, the performance of SITT against detection of Tb lesions plus acid fast bacilli revealed positive predictive values of 67.9 (57.97 – 77.82); 73.1 (63.67 – 82.52); & 77.3 (68.4 - 86.2) and negative predictive values of 100; 100 & 96.8 (93 - 100).

Furthermore, the ROC (*Receiving Operating Characteristic*) analysis showed that the area under the curve was significantly higher at cut off points < 4 mm; particularly at ≥3.5 mm cut off point according to detection of Tb lesions [0.884 (0.794-0.975)] and detection of Tb lesions plus acid fast bacilli in the lesions [0.95 (0.90-0.99)] (Table 2). For the ROC curves according to detection of detection of Tb lesions plus acid fast bacilli in the lesions, the SITT cut off points ≥ 3.5 and ≥ 4 mm were between 0.9 – 1 indicating that these cut off values were very informative for the detection of bovine Tb. However, SITT at ≥ 3 and ≥ 3.5 mm cut off points showed significantly higher (P<0.001) discriminatory power compared to SITT at ≥ 4 mm cut off point. Therefore, the ROC findings also confirmed severe interpretations of SITT bovine Tb detection (particularly ≥ 3.5 mm cut off points) as for sensitivity and specificity evaluations.

Table 1: Performance of Single intradermal tuberculin test (SITT) at various cutoff points to diagnose bovine tuberculosis in zebu cattle in Maroua Cameroon using detection of tuberculosis lesions and detection of tuberculosis lesions accompanied with acid fast bacilli in lesions to define disease status

	Detection of tuberculosis lesions to define disease status				Detection of tuberculosis lesions and acid fast bacilli in lesions to define disease status			
	Sensitivity		Specificity		Sensitivity		Specificity	
SICCT cut off point	Value, % (95 % CI)	X2 (P value)	Value, % (95 % CI)	X2 (P value)	Value, % (95 % CI)	X2 (P value)	Value, % (95 % CI)	X2 (P value)
≥ 3.0 mm	82.1 (73.9 - 90.2)	3.2 -0.073	91.2 (85.1 - 97.2)	2.43 -0.118	100	11.08 (0.000)*	86.4 (79.1 - 93.7)	1.89 -0.168
≥ 3.5 mm	82.1 (73.9 - 90.2)	3.2 -0.073	94.7 (89.9 - 99.4)	0.38 -0.534	100	11.08 (0.000)*	89.4 (82.85 - 95.94)	0.54 -0.46
≥ 4 mm	71.4 (61.8 - 81)	/	96.5 (92.6 - 100)	/	89.5 (82.8 - 95.9)	/	92.4 (86.76 - 98.03)	/

*Significantly different (P<0.05) when compared to ≥ 4 mm cut off point

Table 2: Area under the ROC (*Receiving Operating Characteristic*) curve for analysis of the performance of SITT bovine Tb detection in zebu cattle in Maroua at various cutoff points

Classification of the Single Intradermal Tuberculin test (SITT) cut-off point performance with detection of tuberculous lesions as reference test			Classification of the Single Intradermal Tuberculin test (SITT) cut-off point performance with detection of tuberculous lesions and Acid Fast Bacilli as reference test.		
Skin Cut-off points	Area Under Curve (CI: 95 %)	Asymptomatic Significant	Skin Cut-off points	Area Under Curve (CI : 95 %)	Asymptomatic Significant
SITT ≥ 3 mm	0.867 (0.773-0.960)	0	SITT ≥ 3 mm	0.93 (0.88-0.98)	0
SITT ≥ 3.5 mm	0.884 (0.794-0.975)	0	SITT ≥ 3.5 mm	0.95 (0.90-0.99)	0
SITT ≥ 4 mm	0.840 (0.733-0.946)	0	SITT ≥ 4 mm	0.91 (0.82-0.99)	0

Tableau 3: Distribution of true prevalence of SITT bovine Tb at various cutoff points according to breed, sex, age and body condition score

			True prevalence computed against detection of Tb lesions to define disease status; % (95% CI)			True prevalence computed against detection of tuberculosis lesions and acid fast bacilli in lesions to define disease status; % (95% CI)		
Variable		Number	≥ 3 mm	≥ 3,5 mm	≥ 4 mm	≥ 3 mm	≥ 3,5 mm	≥ 4 mm
Breed	Mbororo Zebu	9	63.85 (32.45-95.23)	65.49 (34.43-96.55)	60.23 (28.26-92.21)	48.61 (15.95-81.26)	50.33 (17.66-83.00)	44.93 (12.43-77.43)
	Peulh of Sahel Zebu	28	51.29 (32.78-69.81)	53.51 (35.04-71.99)	52.72 (34.23-71.21)	37.96 (19.98-55.93)	40.04 (21.89-58.19)	38.7 (20.66-56.74)
	Toupouri-Massa Zebu	48	16.37 (5.90-26.83)	14.84 (4.78-24.90)	16.34 (5.88-26.80)	8.33 (0.51-16.15)	6.82 (0 - 13.95)	8.54 (0.63-16.45)
	X2 (P)		49.16 (0.000)*	56.71 (0.000)*	44.94 (0.000)*	40.28 (0.000)*	47.22 (0.000)*	35.59 (0.000)*
Sex	Male	21	13.91 (0 - 28.71)	17.84 (1.46-34.21)	15.9 (0.26-31.54)	6.25 (0 - 16.60)	9.39 (0 - 21.87)	8.18 (0 - 19.90)
	Female	64	39.15 (27.19-51.11)	37.89 (26.00-49.77)	38.58 (26.65-50.51)	27.66 (16.70-38.62)	26.62 (15.79-37.45)	26.98 (16.10-37.85)
	X2 (P)		16.34 (0.000)*	9.99 (0.001)*	12.97 (0.000)*	16.27 (0.000)*	10.05 (0.001)*	12.19 (0.000)*
Age (years)	< 5	11	22.1 (0 - 46.62)	25.65 (0 - 51.45)	19.44 (0 - 42.82)	13.19 (0 - 33.19)	16.11 (0 - 37.83)	11.11 (0 - 29.68)
	5≤X< 10	37	33.42 (18.22-48.62)	32.94 (17.79-48.08)	35.78 (20.34-51.23)	22.8 (9.28-36.32)	22.37 (8.94-35.79)	24.66 (10.77-38.55)
	≥ 10	37	35.88 (20.42-51.33)	35.28 (19.88-50.68)	34.61 (19.28-49.93)	24.88 (10.95-38.81)	24.38 (10.54-38.22)	23.68 (9.98-37.38)
	X2 (P)		5.09 (0.078)	2.34 (0.31)	7.92 (0.019)*	4.81 (0.09)	2.24 (0.32)	7.18 (0.027)*
Body condition score	2 (thin)	17	36.15 (13.31-58.99)	39.06 (15.86-62.25)	38.14 (15.05-61.23)	25.11 (4.49-45.73)	27.63 (6.37-48.88)	26.61 (5.60-47.62)
	3 (fat)	68	32.19 (21.09-43.30)	31.38 (20.35-42.40)	31.66 (20.60-42.72)	21.76 (11.95-31.56)	21.03 (11.34-30.71)	21.24 (11.52-30.96)
	X2 (P)		0.35 (0.55)	1.29 (0.25)	0.92 (0.33)	0.31 (0.57)	1.27 (0.26)	0.79 (0.37)
	Total	85	32.9 (22.89-42.86)	32.9 (22.95-42.93)	32.98 (22.99-42.98)	22.34 (13.48-31.19)	22.37 (13.51-31.23)	22.34 (13.48-31.19)

*Significantly different (P<0.05) for variables compared at a particular cut off point

Calculated true SITT bovine Tb prevalence based on the sensitivity and specificity values of SITT obtained in the study at < 4 mm and the OIE-recommended ≥ 4 mm cut off values showed no significant difference (P>0.05) between cut off points (Table 3). True prevalence computed against detection of Tb lesions and detection of Tb lesions plus acid fast bacilli in lesions as gold tests showed similar values at all the studied SITT cutoff points of about 32.9 and 22.3%. However, evaluation of the distribution of true prevalence of SITT bovine Tb revealed that breed, sex and age were significant (P<0.05) influencing factors (Table 3). The ≥ 5 years age ((X^2: 7.38; p < 0.05)), Mbororo zebus ((X^2: 47.22; p < 0.001)) and females ((X^2: 10.05; p <0.01)) were more affected than others. The body condition score of the animals had no effect on prevalence of SITT bovine Tb.

Questionnaire Survey of Meat Handlers: A total of 136 persons contacted for the study accepted to respond to the questionnaire Survey. The respondents were composed of 87.5% men aged 16 to 77 years, single / not married (21%) and married (79%; (58% monogamy and 21% polygamy)). The respondents had not been to a formal school (36.1%) while the others had at least primary (48.5%) and secondary (15.4%) school education. The respondents were Christians (50%), Muslims (42.6%), neither Christians nor Muslims (7.4%) and also butchers

including butcher aids (87.5%) and restaurant operators' (12.5%) who were all females. Over 38.8% of the meat handlers in the study had less than 10 years of experience in the profession, 32.4% had 11 to 20 years of experience, 14.7% had 21 to 30 years and 14.7% had more than 30 years of experience. Also, a high and widespread contact rate (70.4%) between respondents and live animals (cattle and or other domestic animals) was recorded in the survey. The contact included restraining, health care and feeding of animals.

Questionnaire survey showed that **o**ver 74.3% of respondents knew that bovine Tb is zoonotic while 25.7%, particularly female meat handlers (6.61%) and respondents who were uneducated or had below secondary education (13.97%), were either ignorant (12.5%) or falsely stated that bovine Tb is not zoonosis (13.2%). However, the respondents who knew that bovine Tb is zoonotic named at least one mode common vehicles of transmitting bovine Tb to man including aerosol from infected animal and manipulation of Tb lesions (19.8%) as well as consumption of raw meat (76.2%) and unpasteurized milk (5%) from infected animal.

Over 70.6% of meat handlers reported contact with tuberculosis cattle and or Tb lesions in the abattoir, the proportion being significantly higher ($P<0.05$) in males (68.4%) and butcher (68.4%). Over 15.4% of respondents, all of whom were males butchers who had been ($P<0.05$) in the profession for over 10 years (12.5%) consumed raw meat. All categories of respondents regularly drink unpasteurized milk (44.9%) especially respondents who were ($P<0.05$) males butchers (41.91%), uneducated or had below secondary education (36.08%) and had been handling meat in the abattoir for over 10 years (28%).

The respondents stated that at farm level, they would attempt to treat (chemotherapy, traditional pastoral husbandry and ethno-Veterinary practices) bovine Tb infected animals (52.2%), slaughter for human consumption (22.1%) and or notify the veterinary service (13.2%) while others would not know what to do (12.5%) with a suspected case of bovine Tb in cattle. However, at the level of the abattoir, they stated that the management of suspected bovine Tb case depends on the veterinary service (15.4%), some (52.2%) recommended exclusion from the food chain and destruction and some did not know what to do (11.8%) while others (20.6%) opted for consumption of the suspected meat. Lack of knowledge of managing or reporting suspected bovine Tb cases to the veterinary service were significantly ($P<0.05$) higher among the uneducated respondents (27.94%), butchers (41.0%) and respondents who had spent at least 10 years on the professional in the abattoir (28.0%).

Furthermore, 44.9% of meat handlers were not attentive of the professional/occupational hazards of bovine Tb; due to ignorance (19.2%) or false declaration that it does not constitute any professional danger (25.7%). As for precautions to take some respondents opted for good hygiene and routine hospital visits (22.1%) at least once or twice a year and or avoid manipulating and consuming suspected Tb tissue (28.7%) while others did not know what to do and or would not advocate any action (39.3%). A wide range of respondents reported contacts and interactions with human Tb patients (39%); who were their family members, colleagues and or persons in their neighborhood.

Assessment of Direct Financial Losses: The annual slaughter rate of the abattoir was estimated to be 5,400 cattle based on the abattoir records giving an annual estimated economic loss of 25,001,460 FCFA per annum year (approximately 38,463.78 € at an estimated rate of 1€ = 650 FCFA) (Table 6). For the 85 animals used in this study, 16 (40 kg at 1500 FCFA/kg) lungs, 9 (59 kg at 1500 FCFA/kg) heads, 2 (15 kg at 2500 FCFA/kg) fore limbs and 1 (250 kg at 2500 FCFA/kg) whole carcass were condemned and valued at direct money losses of 880,000 FCFA (approximately 1353.84 €) were condemned during the November 2013 to March 2014 study period (Table 2). Whole carcass (71.02%) condemnations took the highest proportion of all the losses followed by the heads (12.27%), limbs (8.52%) and lungs (8.18%). However, excluding analysis of condemnations of whole carcasses for these periods, the direct financial losses due to organ condemnations alone were 255,000 FCFA (approximately 392.30 €) and the annual estimated economic losses due to organ condemnations alone were 2,699,460FCFA (approximately 4,153.01 €).

DISCUSSION

Prevalence of Bovine Tuberculosis: The Tb lesions detection rates in slaughtered cattle (22.28%) is higher in Maroua abattoir than rate (13.1%) reported in the same abattoir by Egbe *et al.* [6]. The value is also higher than rates (<1 to 7.7%) reported in abattoirs in the Littoral, Centre, West, Southwest and Northwest regions of Cameroon [6, 7, 13 & 29] and similar to values (21.3%) in Garoua abattoir of North Region of Cameroon [6]. Though a lower Tb lesion detection rate (15%) at meat inspection was reported and *M. bovis* was confirmed by acid fast staining and PCR in the Bauchi State of Nigeria [30], similar to the findings of this study, a higher prevalence rate was observed female than male cattle and a

significant association between detection of bovine Tb and the age of cattle. Also, significantly higher SITT bovine Tb prevalence estimates (25.88%) at the OIE recommended ≥ 4 mm cut off point were obtained compared to 12.21% [7] and 18.35% [31] recorded in the highland regions. However, higher sensitivity and predictive values were obtained at severe (< 4mm) interpretations of SITT bovine Tb responses and better performances were obtained at ≥ 3 and ≥3.5 mm cut off points.

Against detection of Tb lesions, SITT ≥3 and ≥3.5 mm showed non-significantly (P>0.05) higher sensitivity of 82.1% compared to 71.4% for SITT ≥4 mm and non-significantly (P>0.05)lower specificity values of 91.2 and 94.7% compared to 96.5% for SITT ≥4 mm, respectively. For detection of Tb lesions accompanied with acid fast bacilli in lesions, SITT ≥3 and ≥3.5 mm showed significantly (P<0.05) higher sensitivity of 100% compared to 89.5% for SITT ≥ 4 mm and non-significantly (P>0.05)lower specificity values of 86.4 and 89.4% compared to 92.4% for SITT ≥4 mm, respectively. Overall, the detection of Tb lesions showed lower sensitivity values compared to detection of Tb lesions accompanied with demonstration of acid fast bacilli in the lesions in this study. Detection of Tb lesions and demonstration of acid fast bacilli have also been used by Ameni *et al*. [32] in Ethiopia and Ngandolo *et al*. [33] in Chad to evaluate the diagnostic performances of tuberculin skin tests. Though highest detection of disease cases by SITT tests were detected at ≥ 3.0 mm cut-off point, the overallbest performances was observed at ≥ 3.5 mm cut off points. The study revealed that bovine Tb is highly endemic in cattle in the Maroua area compared to other parts of Cameroon and severe interpretation of TST results is essential for optimal diagnosis of the disease. These findings are similar to those of Ameni *et al*. [32] in Ethiopia, Ngandolo *et al*. [33] in Chad and Awah-Ndukum *et al*. [31] in the highlands of Cameroon who reported that improved diagnostic performances of TST in zebu cattle was obtained at severe interpretation of TST bovine Tb results.

The ROC analysis and sensitivity evaluations support severe interpretation of TST in this study, particularly at ≥3.5 mm cut off points. The performance of TST has also been affected by environmental factors, host factors, (status of immunity, genetics, etc.), prevalence of the disease in the population tested and the nature of the tuberculin used [32, 34-38]. A perfect cut-off point in a specific geographic area may not be so useful at another environment [34, 36] and the ability of the test to accurately predict the true positive disease status depends on its sensitivity, specificity and prevalence of the disease in the population tested [34]. However, excessively high sensitivity of tuberculin skin tests will generate false positive reactions during interpretations of test results. Also, severe interpretations for improved diagnosis have been done in regions or herds where *M. bovis* infection had been confirmed based on the discretion of the veterinarian [36].

This study revealed that severe interpretation of TST, at cut off values less than the OIE recommended cut off value of > 4 mm, is essential for optimal diagnosis of bovine Tb in Zebu cattle in Maroua – Cameroon. The interpretations should be done at ≥3.5 mm cutoff points given the epidemiological and environmental context of the region.

Risk Factors to Meat Handlers from Bovine Tuberculosis in Maroua Abattoir: Tuberculosis due to *M. bovis* is an endemic zoonosis that is neglected worldwide and particularly in many African countries [39]. In countries where bovine Tb is endemic such as Cameroon and there is no control program [2, 7], the risk for human tuberculosis due to *M. bovis* is high [3].

Zoonotic bovine Tb can be transmitted to humans through ingestion of unpasteurized milk from infected cows and consumption of raw or insufficiently cooked contaminated meat from infected animals [2, 3, 7, 34]. In Jigawa state of Nigeria, the habit of milk and meat consumption was found to be affected by occupation while TST bovine Tb reactor cattle were detected in the herds of cattle handlers and professionals suffering from TB or showing clinical signs suggestive of TB [40]. Air borne transmission has also been described particularly where humans work in close proximity to infected animals and or affected carcasses; and also share same micro-environment with infect animals of infected animals [41]. Robinson *et al*. [42] found patients suffering from pulmonary Tb due to *M. bovis* in four different abattoirs in South Australia. They stated that transmission of the disease in these cases was certainly by inhalation and different from the 'classic' form of bovine Tb transmitted by ingestion of infected milk and resulting in extrapulmonary disease. Assessing the levels of awareness, knowledge and practices of the various meat handlers on bovine Tb in the Maroua abattoir revealed high rate of contacts and interactions between human and cattle, the potential reservoir host of zoonotic Tb. Butchers were most at risk, particularly those who had spent more than 10 years in the profession as well as

those with low levels of education (primary) or no formal education. Khattak *et al*. [43] also reported *M. bovis* infection in abattoir workers, not including butchers and livestock farmers and observed that duration of work in abattoir was significantly associated with the prevalence of zoonotic Tb. Constant manipulation of potentially infected cattle and Tb lesions in Maroua was common due to professional motivation, poverty alleviation, joblessness and sometimes the desire to ensure the continued existence tradition being inherited.

Many respondents were ignorant about the zoonotic status of bovine Tb and potential professional / occupational health hazards due to the disease. There was also poor knowledge of transmission of the disease to man such as spread by aerosol and consumption of unpasteurized milk. Lack of health education of abattoir workers and meat handlers in Cameroon including Maroua about zoonoses such as bovine Tb and their public health implications also explained the severe lack of awareness observed in this study. Consumption of raw meat and unpasteurized milk has been reported in the Northwest and Adamawa regions of Cameroon [2, 7, 9]. Inclusion of suspected Tb contaminated meat in the human food chain, non-respect of the decision of veterinary inspectors, non-declaration of bovine Tb at farm level and false or poor knowledge about zoonotic Tb observed in this study have been reported elsewhere [2]. However, prevention of transmission of bovine Tb in milk has offered the best approach for human risk mitigation in the country though it requires strategies that improved risk awareness among producers and consumers [9]. Also, the lack of protective wear, non-respect of standard operating procedures at the abattoirs, unsolicited visitors including consumers of meat to the abattoir and poor sanitation of abattoir environment were noted in the study. This finding agrees with Khattak *et al.* [43] who stated that abattoir workers, including those infected with *M. bovis*, did not use protective equipment and appropriate safe working techniques; and were at high risk of acquiring zoonotic Tb. The study is also in agreement with Sa_idu *et al.* [44] who reported significant association between abattoirs personnel awareness of bovine Tb and their occupational status, age and duration of exposure to cattle carcasses. The study further suggests that incubation of various pathogens could be occurring in the Maroua abattoir including Tb agents which are hazardous to abattoir workers and consumers of slaughtered cattle. Therefore, bovine Tb is a serious professional, occupational and accidental hazard to meat handlers, abattoir workers and visitors of this abattoir. Suitable pre-employment screening programmes for abattoir workers [42], the use of protective wears, equipment and standard abattoir working procedures [17, 18 & 19] and not allowing unsolicited visitors around the abattoir environment should be established at the Maroua abattoir.

Control of bovine Tb in Cameroon is also constrained by lack of compensation after rejection of carcasses or organs during slaughter meat inspection as well as the non-application of existing legislature on the disease [2, 7]. However, political unwillingness such the neglect and little or no bovine Tb control program had been identified as an important socio-economic factor favouring the transmission of the disease to humans [45]. Fonteh *et al*. [29] revealed that over 40% of visually examined carcasses considered safe for consumption were in fact infected with Tb. Routine meat examination should be complemented with laboratory analysis for confirmation [29] as well as enhancing the number and capacity of inspectors [7,13 & 29]. Though these meats were not detected during inspection and passed into the food chain [29], inspectors are usually not empowered by legal and executive commands to contain arising situations and resistance to condemnation from meat handlers particularly butchers. For veterinary inspectors not supported by law keeping officers like police officers would be unable to condemn and seize affected carcasses or organs of unruly meat handlers for proposal disposal.

Assessment of Direct Financial Losses: The study estimated that direct money losses for about 5 months of 880,000 FCFA and annual economic losses analysis of 25,001,460 FCFA was induced by carcass and or organ condemnations due to Tb. Enormous direct money loss abattoirs related to organ condemnations only in Ethiopia ranging from US$9,093.88 - US$13,508.16 (equivalent to 4,546,940 FCFA - 6,754,080 FCFA at US$1 = 500 FCFA) have been estimated [46, 47]. Cadmus and Adesokan [48] revealed a total organ/offal economic value loss of $ 332,904 (USD) with an average economic loss of $110.968 (USD) due to organ condemnation after meat inspection. The main causes of organ condemnation were attributed to pneumonia. Fasciolosis and tuberculosis and mainly the lungs and liver were most affected. It is worth mentioning that the estimated direct financial and annual economic losses in this study were based on post mortem inspection of 85 SITT bovine tested cattle. The estimates could have been much higher if more animals were inspected. In a similar study in Yaoundé Cameroon, enormous direct financial losses of 18,129,600 FCFA and

annual economic losses of 338,914,732 FCFA induced by tuberculosis were estimated in slaughtered cattle [49]. The Tb lesions were detected in additional organs including liver, kidney and the sample size was much big compare to this study. In fact they used 2,400 cattle slaughtered in about four months and 319,475 cattle slaughtered in about seven years to estimate the direct financial and annual economic losses, respectively. Similar to the Yaoundé abattoir study, condemnations of whole carcass, compared organ condemnations, due to Tb was associated with enormous financial implications in the Maroua abattoir. As a matter of fact, Tb has been estimated to singly induce financial losses estimated at 10 million USD / year [39, 50], with public health consequences through consumption of contaminated fresh milk and meat [2, 13].

CONCLUSION

Bovine Tb prevalence is high in the Maroua abattoir. High SITT bovine Tb rates at ante-mortem inspection of live cattle and high Tb lesions detection rates in slaughtered cattle destined for human consumption were recorded in this study. However, given the epidemiological and environmental context of the study region, severe interpretation of tuberculin skin tests at ≥ 3.5 mm cutoff and not at the OIE recommended ≥4 mm cut off value is essential for optimal diagnosis of bovine Tb in Zebu cattle in Maroua – Cameroon.

Also, bovine Tb is a significant health risk among meat handlers and other professionally exposed groups in Maroua of Far North region in Cameroon. The financial loss related to organ and carcass rejections due to detection of Tb lesions at slaughter meat inspection of 85 zebu cattle in the Maroua abattoir was estimated at over 880,000FCFA equivalent to more than 25,001,460 FCFA / year.

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