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# First Insight into the Current Prevalence of Bovine Tuberculosis in Cattle Slaughtered in Cameroon: the Case of Main Abattoirs of Yaoundé and Douala

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors FXE, SE, FKK conceived and designed the experiments. Authors SE, FKK, AFN performed the experiments. Authors FKK, B, EF performed postmortem examination of carcasses. Authors FXE, SE, FKK, AFN, analyzed the data. Author FKK prepared the first draft of paper and designed figures. All authors provided critical input and approved the manuscript.*

***Short Communication***

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## ABSTRACT

**Aim:** The aim of this study was to evaluate the current prevalence of bovine tuberculosis at Yaoundé and Douala abattoirs.
**Study Design:** Many investigations confirmed that bovine tuberculosis is prevalent in cattle destined for consumption in Cameroon but the magnitude and the distribution of animal tuberculosis in the country are unknown.
**Place and Duration of Study:** Sampling was made during routine meat inspection, in the Yaoundé and Douala abattoirs located in the Central and Littoral regions of Cameroon respectively. Sampling was successively carried out from November 2010 to April 2011. Sample processing (culture, acid-fast staining and spoligotyping) was made at

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Mycobacteriology Reference Laboratory in Centre Pasteur of Cameroon.
**Methods:** About 16,316 slaughtered cattle, were successively inspected for tuberculosis during this study. Among them 9,127 and 7,189 were slaughtered in Yaoundé and Douala abattoirs respectively. Evidence of pathology was supported by postmortem examination of carcasses using visual examination and palpation of lungs, livers, hearts, internal bodies and lymph nodes. The prevalence was calculated as the number of cattle with suspected TB lesions divided by the number of cattle examined at post mortem within the specified period. Ziehl-Neelsen staining, culture on solid medium and spoligotyping were made to identify acid-fast Bacilli and *Mycobacterium bovis*.
**Results:** The overall apparent prevalence of bovine tuberculosis based on suggestive macroscopic lesions induced by tuberculosis was 1.03%. This prevalence was split to 0.81% and 1.3% in Yaoundé and Douala abattoirs respectively. *Mycobacterium bovis* accounted for 47.62% of the tuberculous lesions and its prevalence was 0.49%.
**Conclusion:** This result show that bovine tuberculosis is still prevalent in cattle destined for human consumption in Cameroon and highlighted the contribution of *M. bovis* as the leading cause of bovine tuberculosis in Cameroon.

*Keywords: Bovine tuberculosis; prevalence; Mycobacterium bovis; tuberculous lesion.*

## 1. INTRODUCTION

Bovine tuberculosis (bTB) is an endemic infectious disease of cattle. It is mainly caused by *Mycobacterium bovis* which can also cause disease in human and in a variety of domestic and wild animals. This disease is considered as a major zoonosis [1,2]. Aerosol exposure to *M. bovis* is considered to be the most frequent route of infection of cattle, but infection by ingestion of contaminated material also occurs [3]. Bovine tuberculosis is still common in developing countries where it causes economic losses from livestock deaths, chronic disease and trade restrictions.

Tuberculosis due to *M. bovis* is still a neglected disease in animals as well as in human populations in the sub-Saharan countries [4]**,** where it is less studied. While transmission of *M. bovis* to humans constitutes a public health problem as this specie is naturally resistant to pyrazinamide [5], one of anti tuberculosis drug use to cure tuberculosis.

In Cameroon the breeding sector is composed mainly of cattle and it is very important as it provides income to about 30% of the rural population [6] as well as a principal source of animal protein for the general population. Despite its usefulness, this sector suffers from many diseases, principally tuberculosis, which is endemic and the third cause of seizures in abattoir after distomatosis and cysticercosis [7]**.** Many reports of the Ministry of Livestock, Fisheries and Animal Industries, showed that, in 2004, 16% of bullock slaughtered in Cameroon suffered from tuberculosis. Moreover, all the ten regions are concerned by bovine tuberculosis [6] but their current bovine tuberculosis prevalence and the impact of zoonotic transmission to humans is unknown.

Many methods exist to diagnose cattle suffering from tuberculosis. Among them, *In vivo* test like tuberculin test, the cellular test based on the quantification of gamma interferon, post mortem diagnosis of macroscopic lesions of tuberculosis, microscopic examination of lesion, culture, biochemical characterization and molecular methods [3]. In Cameroon's slaughterhouses, the diagnosis of tuberculosis is mainly based on the screening of typical tuberculous macroscopic lesions of the organs rather than on Ziehl-Neelsen stained smears,

culture, biochemical and molecular methods. True diagnosis of bTB is based on microscopy, culture and identification of the organism with biochemical and molecular methods [8]**.**

The aim of this study was to assess for the first time, the prevalence of bTB in the largest Yaoundé abattoir in Center and once again in the Douala abattoir in littoral regions of Cameroon. This was done in order to complete the paucity of information on bovine TB in the study area so that proper control/preventive measures could be put in place.

## 2. MATERIALS AND METHODS

### 2.1 Slaughterhouse Sampling

The abattoirs of Yaoundé in Central and Douala in Littoral regions of Cameroon were chosen to screen slaughtered cattle. We made the choice on the basis of the following; these abattoirs receive cattle coming from almost all the breeding regions in Cameroon and are located in the regions those are known to have high incidence of human tuberculosis (TB) in Cameroon. We also found it easier to transport samples to the Mycobacteriology National Reference Laboratory (Centre Pasteur du Cameroun).

### 2.2 Sampling

Sampling for tuberculosis lesions were made during routine inspection by veterinaries surgeon from the Ministry of Livestock, Fisheries and Animal Industries (MINEPIA) and us. This inspection was done according to the government's legislation regulating veterinary health inspection and notification of contagious animal diseases [9]**.** Sampling was done from November 2010 to April 2011. About 16, 316 cattle slaughtered, were successively inspected for tuberculosis during this study. Among them 9,127 and 7, 189 were slaughtered in Yaoundé and Douala abattoir respectively.

Evidence of tuberculous lesions were supported by postmortem examination of carcasses as earlier described in [10]. Briefly, the inspection procedure employs visual examination of the entire body of each slaughtered cattle and palpation of the lungs, liver, and kidneys, for the research of caseous nodules or by making an incision on lymph nodes of the thoracic and head regions, the mesenteric lymph nodes, and other lymph nodes of the body of the carcass for the research of tuberculous granuloma with caseous center.

### 2.3 Lesions Collection

A caseous granuloma sample was collected from the suspected infected organ or a fragment of lymph node presenting a caseous granuloma with or purulence. The lesion sample was taken from the most infected organ in any suspected animals and transported dry in sterile plastic containers under controlled conditions and conveyed immediately to the Mycobacteriology National Reference Laboratory of Centre Pasteur du Cameroun, Cameroon - Pasteur Institute International Network, Yaoundé, Cameroon.

### 2.4 Processing of Samples

The processing of lesions samples was based on grinding and decontamination procedure using sodium lauryl sulphate [11]. Briefly, the samples were first carved on sterile Petri dish using a sterile scalpel, then ground in a mortar by using a pestle before addition of sterile

distilled water. Using a sterile 50 ml centrifuge tube with a screw cap, equal amounts of specimen and sodium lauryl sulfate were added. The centrifuge tube was capped and its content was vortexed until the specimen was liquefied. The mixture was allowed to stand at room temperature for 45 min with permanent gentle shaking by Khan Shaker. Prepared phosphate buffer was added to the mixture in the centrifuge tube and mixed, then centrifuged for 20 min at 3,000 *g*. The supernatant was carefully decanted and 2 ml of sterile distilled water was added to re-suspend the sediment. The suspension was inoculated onto Lowenstein-Jensen slopes with pyruvate and/or glycerol and incubated at 37ºC for 8 to 12 weeks. All smears were stained by the conventional Ziehl-Neelsen method for the presence of acid-fast bacilli (AFB) and observed under a light microscope [12].

## 2.5 Spoligotyping Analysis

Spoligotyping of isolates was performed as described by Kamerbeek and colleagues [13] and was used to identify directly the members of *M. tuberculosis* complex responsible of bTB.

## 2.6 Data Analysis

The prevalence was calculated as the number of cattle with suspected TB lesions divided by the number of cattle examined at post mortem within the specified period.

The Pearson and McNemar's Chi-square test were respectively use to estimate the association between the affected organ or tissue and Ziehl-Neelsen or culture results and to compare Ziehl-Neelsen and culture test using SPSS statistic software version 17.0.

# 3. RESULTS

## 3.1 Postmortem Inspection Findings

The cattle screened during this period of study belonged to three main breeds in Cameroon, the Zebu Mbororo, Zebu Goudali and Zebu Akou. A total of 168 cattle presented organs with the typical macroscopic lesions of tuberculosis, mainly represented by caseous tubercles with a yellowish appearance. (Fig.1a,1b). The lymph node sometime appears hypertrophied with purulent appearance (Fig. 1c)


**Fig. 1. Example of some tuberculous lesions identified in the abattoirs**

a. Caseous nodular lesions on the cattle body in the milliary tuberculosis in abattoir of Yaoundé (Cameroon)
b. Nodular granuloma on the cattle liver suffering from tuberculosis in the Yaoundé abattoir;
c. Hypertrophied axillary lymph node with purulent aspect of a cattle suffering from tuberculosis in abattoir of Douala (Cameroon)

The organs and tissues from which a sample was taken were, lungs (32.7%), lymph nodes (28%), liver (13.7%), body cavity (8.33%), heart (5. 35%), Diaphragm (4.8%), spleen (0.6%), intestine (0.6%) and others (6%). (Table: 1).

Ziehl-Neelsen staining confirmed bTB in 97(58%) (Table 1) lesions while bacterial culture positive for *M. tuberculosis* complex strains was 80 (48%). Twenty two (13%) positive cultures were obtained from negative stained smear.

There was no significant association between the organ or tissue lesions origin and neither Ziehl-Neelsen nor culture result in this study. Difference between occurrence of TB in Zielh-Neelsen and culture positive was also not significant statistically.

**Table 1. Zielh-Neelsen and Bacterial culture results according to the origin of lesion**

| Organe or Tissue lesion origin | Zielh-Neelsen results | | | | Bacterial culture results | | | |
|---|---|---|---|---|---|---|---|---|
| | Positive | % | Negative | % | Positive | % | Negative | % |
| Intestine | 1 | 100 | 0 | 0.0 | 1 | 100 | 0 | 0.0 |
| Spleen | 1 | 100 | 0 | 0.0 | 1 | 100 | 0 | 0.0 |
| Diaphragm | 7 | 87.5 | 1 | 12.5 | 4 | 50.0 | 4 | 50.0 |
| Heart | 7 | 77.8 | 2 | 22.2 | 3 | 33.3 | 6 | 66.7 |
| Body Cavity | 9 | 64.3 | 5 | 35.7 | 10 | 71.4 | 4 | 28.6 |
| Liver | 9 | 39.1 | 14 | 60.9 | 8 | 34.8 | 15 | 65.2 |
| Lymph nodes | 39 | 83.0 | 8 | 17.0 | 25 | 53.2 | 22 | 46.8 |
| Lung | 20 | 36.4 | 35 | 63.6 | 25 | 45.4 | 30 | 54.6 |
| Others | 4 | 40.0 | 6 | 60 | 3 | 30.0 | 7 | 70 |
| Total | 97 | | 71 | | 80 | | 88 | |

## 3.2 Molecular Identification

Spoligotyping analysis of the 80 positive culture strains identified all as *M. bovis* (100%).

## 3.3 Evaluation of Current Prevalence

The overall prevalence of cattle with macroscopic tuberculous lesions in the two principal abattoirs was 1.03%. This prevalence was split into 0.81% and 1.3% in the abattoir of Yaoundé and Douala respectively.

The overall prevalence of bTB base on Ziehl Neelsen smears results was 0.6% and from positive culture was 0.49%.

The proportion of *M. bovis* isolates in macroscopic TB lesions of suspected cattle with tuberculous lesions during this period of study was 47.62% but when looking at the overall prevalence of *M. bovis* in cattle slaughtered in this two abattoirs we found a value of 0.49%.

## 4. DISCUSSION

Considered as a major Zoonosis [1,2], tuberculosis due to *M. bovis* is still a neglected disease either in human being or in animal in Sub-Saharan countries [4], where it is less studied so far. Information about the current prevalence of bTB in Sub-Saharan countries are scares [14]. The difficulties to determine the true prevalence of bTB in sub-Saharian Africa, are due to the absence of test and slaughter policy, and poorly equipped laboratories. Abattoir inspection still remains therefor the best option for monitoring bovine TB prevalence. [15] For this period study, the overall prevalence of cattle with macroscopic tuberculous lesions in the two principal abattoirs was 1.03%. This finding was close to that found earlier in Cameroon, based also on abattoir record. [16,17]. Others studies in various parts of African countries also report the nearby results among which Togo [18], and Ethiopia [19]. However our findings were low compared to that found in the neighboring countries of Cameroon such as Nigeria were the reported prevalence value were 2.8%, 4.1%, 8.8% [20] [15,21] and Chad with the macroscopic tuberculous lesions prevalence of 7.3% [22]. All these differences observed could be intriguing because there is frequent cattle movement between Cameroon and these countries, which can lead to the strain dissemination and transmission of bovine tuberculosis. The tentative explanation might be the fact that only the vigorous and “healthy” cattle were often concerned by this movement and then ill and old animal were slaughtered in each country prior to save money and buy supply for the journey [7].

Twenty two negative Ziehl - Neelsen result were recovered by culture in this study. This result can be explained by low detection rate of the Ziehl-Neelsen method which is incapable of detecting bacilli that are fewer than 10,000 in number per slide or per ml of specimen [23], [24]. As lesions are often paucibacillary.

To the best of our Knowledge, this study is the first to provide data on the proportion of *M. bovis* in macroscopic tuberculous lesion and its prevalence in cattle slaughtered in the abattoir of Yaoundé in the Center region of Cameroon and in Douala in Littoral region of Cameroon. This result highlighted the contribution of *M. bovis* as the leading cause of bTB in slaughtered cattle in Cameroon. These results might be underestimated because of the difficulty of growing of *M. bovis* and *M. africanum* [25,26].

## 5. CONCLUSION

This study establish that bTB is still prevalent in cattle slaughtered in Cameroon, with a prevalence of bovine tuberculosis of 0.81% and 1.3% in abattoirs of Yaoundé in Center region and Douala Littoral region of Cameroon respectively. *M. bovis* was found to be the leading cause of the tuberculous lesions. The prevalence was low when compared with those of other neighbouring countries. Therefore there is need to improve quarantine services in order to control movement of cattle between Cameroon and its neighboring countries through a proper legislation, and also test and slaughtered policy of infected cattle.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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