*Full Length Research Paper*

# Prevalence of *Tuberculosis* spp. species in bovine carcasses in two slaughterhouses of Burkina Faso

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**The current prevalence of bovine tuberculosis (BTB) is unknown in Burkina Faso. Effort in controlling this zoonosis is widely based on macroscopic meat inspection at slaughterhouses, while confirmatory laboratory-based diagnostic tests are rarely used. This study aimed at identifying BTB lesions and isolating tuberculosis (TB) strains in bovine carcasses inspected at the two main slaughterhouses of the country. Sample of lymph nodes and other organs were collected from suspected carcasses in two slaughterhouses located in Ouagadougou and Bobo-Dioulasso and cultured in Lowenstein-Jensen medium for isolation. From 1499 inspected carcasses, 102 (73 female and 29 male bovines) had suspicious BTB lesions, representing a prevalence of 6.8% (95% CI, 5.6%-8.2%). The 102 carcasses showed 208 tubercular lesions, of which over 92.2% were found in the lymph nodes or organs, while 7.8% of lesions were generalized into the entire carcass. Mycobacteria were isolated in 48.0% (49/102) of the carcasses. The biochemical testing showed 31/49 (62.2%) strains from *Mycobacterium tuberculosis* complex (MTBC) and 18/49 (36.7%) strains which belonged to non tuberculous mycobacteria. Among the strains of MTBC, 26/31 (83.8%) strains were *Mycobacterium bovis*, 3/31 (9.6%) strains were *Mycobacterium africanum* and 2/31 (6.4%) strains were *Mycobacterium tuberculosis*. BTB represents an emerging infectious disease for both human and domestic animal in our country.**

**Key words:** Bovine tuberculosis, slaughterhouse inspections, *Mycobacterium tuberculosis* complex, nontuberculous mycobacteria, emerging zoonosis, Burkina Faso.

## INTRODUCTION

Bovine tuberculosis (BTB) is a highly infectious zoonotic disease caused by *Mycobacterium spp.* This agent can infect almost all mammals including humans. The mycobacteria are classified into two main groups: the *Mycobacterium tuberculosis* complex (MTBC) and the group of atypical mycobacteria. *Mycobacterium bovis* is part of the MTBC which also includes: *M. tuberculosis, M. bovis BCG, M. africanum, M. microti,* and *M. canetti* (Wayne, 1982; Van Soolingen et al., 1997). Organisms of the MTBC are responsible for significant morbidity and mortality in humans. Whereas *M. tuberculosis* is the most

common MTBC pathogen isolated from humans, *M. bovis* may be transmitted from infected animals to humans; *M.*

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**ABBREVIATIONS:** *Mycobacterium tuberculosis* complex, **MTBC**; nontuberculous mycobacteria, **NTM**; Lowenstein Jensen, **LJ**.

*africanum* causes pulmonary tuberculosis (TB) among humans in tropical Africa and *M. microti* primarily infects animals (Muller et al., 2013). *M. tuberculosis*, *M. bovis and M. africanum* are the main pathogens for human and animals (Jenkins et al., 2011). Mycobacteria from the MTBC usually reside in lymph nodes and may spread to other organs, especially to the lungs (Ayele et al., 2004; Sahraoui et al., 2009). BTB grows slowly in affected animals and common symptoms include weakness, anorexia, weight loss and oscillating fever (Wilkins et al., 1986). When lungs are highly affected, the animals may develop an intermittent cough (Martinho et al., 2013; Cosivi et al., 1998).

In developed countries, successful disease control has stemmed from the implementation of TB control programs and screening of animals to detect infection at slaughter. In these countries, routine microbiological surveillance complements the macroscopic inspection of carcasses (Heckert et al., 2001). This improved surveillance has led to successfully reduce the prevalence of BTB to less than 1% (Etter et al., 2006) in developed countries. Unfortunately, BTB remains endemic in many developing countries, due to weak control programs and a large proportion of animals being slaughtered without inspection (clandestine slaughtering). In addition, the surveillance is usually limited to macroscopic inspection of the meat without complementary microbiologic analysis (Etter et al., 2006; Hambolu et al., 2013). In the last decades, human TB has re-emerged in many developing countries, partly due to the human immunodeficiency virus (HIV) epidemic. While human to human transmission of TB may be controlled through the use of vaccination and aggressive treatment of symptomatic cases, the impact of *Mycobacterium bovis* transmission from infected animal to human remains largely neglected (Malkin et al., 1997). In a previous study conducted in 199 carcasses inspected in the only official slaughter house of Bobo-Dioulasso, 38 (19%) had morphologic lesions suggestive of BTB (Vekemans et al., 1999). There is no large scale study on the prevalence and burden BTB. However, efforts in controlling human TB in developing countries includes preventing infection through consumption of contaminated milk and meat (Adesokan et al., 2012). The aim of this study was to investigate the presence of BTB lesions among bovine carcasses inspected at two major slaughterhouses of the country.

## MATERIALS AND METHODS

### Ethical consideration

This pilot study is a part of a study on TB in human and animals conducted by the Centre Muraz which received permission from institutional ethic committee of Centre Muraz by 15 June 2010/ N°A012-2010/CE-CM and from the Ethic Committee for Health Research of the Ministry of Health by 07 July 2010/Deliberation, N°2010-49 to

carry out the project. Before sampling, we obtained the informed consents from the farmers or the owners of each sampled animal as well as that of the people in charge of each slaughterhouse. The study has been performed according to Good Laboratory Practice.

### Study design

We conducted a cross-sectional study in the only two frigorific slaughterhouses of Burkina Faso, located in Ouagadougou (central region) and Bobo-Dioulasso (western region).

### Sampling strategy

In the two slaughterhouses, animals are slaughtered in the night. In Ouagadougou, during the first and third weeks and in Bobo-Dioulasso during the second and fourth week of May and June, 2011, we inspected all bovine carcasses on each Monday and Tuesday (a mean of 100 bovine carcasses per day in Ouagadougou and 86 carcasses per day in Bobo-Dioulasso). All bovine carcasses with macroscopic tuberculous lesions in any lymph node were eligible for inclusion in the study if the owner consented to give all information (sex, age, origin of the animal) according to our standard questionnaire. Bovine carcasses, whose owners refused to participate in the study, were not included.

### Assessment of macroscopic lesions of bovine tuberculosis

All carcasses were inspected by two veterinarians and five veterinary technicians in Ouagadougou and by two other veterinarians and four veterinary technicians in Bobo-Dioulasso. The inspectors were instructed to estimate the weight of the animal and to carefully inspect the lungs, mediastinal and thoracic lymph nodes, as well as all other lymph nodes of the carcass for the presence of lesions. A macroscopic TB lesion was defined as the presence of pus or tuberculous casein in a lymph node or in any other organ.

### Measurement of characteristics of the cattle with tuberculosis-like lesions

Owners of cattle showing possible BTB lesions at inspection were asked to answer a short questionnaire regarding the race, age and sex of their animal. If the age of the animal was not known, it was estimated using a method previously described (Whiting et al., 2013). According to this method, we classified the age of the animals into young bovines (age 1 ≤ 2 years), young adult bovines (2 years < age 2 ≤ 6 years) and adults bovines (age 3 > 6 years).

### Handling of samples

A 200g sample of each lymph node or organ with

macroscopic tuberculous lesion was taken and placed into an individual sterile box. All samples were kept between 0°C to +8°C and transported by bus from the slaughterhouse in Ouagadougou once a week and daily from the slaughterhouse in Bobo-Dioulasso to the Centre Muraz located in Bobo-Dioulasso, the national reference laboratory for TB. All samples were transported using triple package according to OIE recommendations. Upon receipt, all samples were stored at +4°C for a maximum of 24 h until the performance of the assays.

## Preparation of samples

The stored specimens were washed using distilled water and cut into small pieces using sterile blades, according to the method previously reported by Cosivi et al. (1998) and Sahraoui et al. (2009). Briefly, samples were dissected and manually homogenized using a mortar. Samples were decontaminated by addition of 4 mL of 4% $H_2SO_4$ and neutralized with 6% NaOH. A total of 2 mL from the supernatant was then mixed by vortex using a Cahn shaker for 20 min. The suspension was then filled up to 45 mL of distilled water and centrifuged for 20 min at 3000 rpm. The centrifugation process was repeated three times. A total of 1.5 mL of sterile distilled water was added to the resulting pellet, and the solution was mixed using a vortex until a homogeneous mixture was obtained.

## Microscopic examination

A smear was made from the decontaminated homogeneous mixture prepared above and stained using Ziehl-Neelsen acid-fast staining method. The slides were read under an optical microscope ($\times$ 100 magnification with oil immersion objective) according to the standard operating procedure used by Gathogo et al. (2012).

## Media preparation and culture

### Media preparation

We prepared the basic of Lowenstein Jensen (LJ) medium using the following components: L-Asparagine and Potato flour to provide sources of nitrogen and vitamins in Lowenstein-Jensen Medium; monopotassium phosphate and magnesium sulfate as buffers to enhance microorganism growth; glycerol and the egg suspension to provide fatty acids and proteins required for the metabolism of mycobacteria. The coagulation of the egg albumin during sterilization solidifies the medium for inoculation purposes. Sodium citrate and malachite green are selective agents that prevent growth of most contaminants and allow early growth of mycobacteria. The following components per liter were used to prepare the basic of LJ medium: L-Asparagine (3.6 g), monopotassium phosphate (2.5 g), magnesium sulfate (0.24 g), sodium citrate (0.6 g) and malachite green (0.4

g).

### Preparation of classic Lowenstein Jensen ordinary medium (medium 1)

For LJ ordinary solid medium preparation, we dissolved 37.3 g of the basic of LJ medium in 600 mL of purified water containing 12 mL of glycerol (Becton DickinsonDifco™ Glycerol) and heated with frequent agitation to completely dissolve the medium; then we autoclaved the obtained mixture at 121°C for 15 min. We prepared 1000 mL of a uniform suspension of fresh eggs (from chicken farm Cado in Bobo-Dioulasso) under aseptic conditions, avoiding whipping air into suspension during the collection and mixing. The 1000 mL of egg suspension was aseptically mixed with 600 mL of the sterile LJ medium cooled to 50 - 60°C, avoiding air bubbles. Finally, the finished medium 1 (classic ordinary LJ medium containing 0.75% of glycerol) was dispensed into sterile screw-cap test tubes, and placed in a slanted position and heated at 85°C for 45 min. The test tubes containing medium 1 were stored at +4°C in a refrigerator for further use.

### Preparation of Lowenstein Jensen solid medium enriched by 0.2% of sodium pyruvate (medium 2)

We added 18 mL of 20% sodium pyruvate to medium 1 (classic ordinary LJ medium containing 0.75% of glycerol), after which the finished medium 2 was dispensed into sterile screw-cap test tubes and placed in a slanted position and heated at 85°C for 45 min. The test tubes containing medium 2 were stored at +4°C in a refrigerator for further use.

### Preparation of Lowenstein Jensen solid medium enriched by 0.5% sodium pyruvate without glycerol (medium 3)

We added 1200 mL of the basic of LJ medium (containing suspension of fresh eggs) before its solidification into 30 mL of 20% sodium pyruvate. We dispensed the finished medium 3 into sterile screw-cap test tubes, after which the tubes were placed in a slanted position and heated at 85°C for 45 min. The test tubes containing medium 3 were stored at +4°C in a refrigerator for further use.

### Culture

The decontaminated homogeneous mixture of the 102 available specimens was inoculated into each of the three prepared media as well as into the liquid medium "Mycobacteria Growth Indicator Tube" (MGIT Bactec 960, Singapore) to enhance the recovery of mycobacteria species. While Medium 1 is more suitable for *M. tuberculosis* growth, Medium 2 and Medium 3 are more suitable for both *M. bovis* and *M. africanum* growth.

Cultures were incubated at 37°C for up to 90 days, with weekly observation for discernible growth. Grown colonies were put onto a smear, and stained using the Ziehl-Neelsen method for confirmation of the presence of *Mycobacterium* spp growth (Ayele et al., 2004; Gathogo et al., 2012).

### Identification of Mycobacterium species

The initial identification of *Mycobacterium* species was based on the presence of the colonies on the slant and their morphological aspects. The nontuberculous mycobacteria (NTM) appeared on the slant after one to three weeks and were all pigmented. Antigenic and biochemical identifications were conducted. The presence of MPT64 antigens specific to MTBC was determined using the immunochromatographic rapid test developed by SD Bioline (SD Bioline TB Ag MPT64Kit, South Korea), while the identification of MTBC species was conducted using conventional biochemical tests including thiophen-2-carboxylic acid hydrazide (TCH) resistance or sensitivity, catalase thermolabile activity at 68°C and 22°C, nitrate reduction and niacin production tests (Heckert et al., 2001). *M. tuberculosis* was identified when colonies grew on LJ ordinary medium (medium 1), TCH resistance was found, catalase activity was present at 22°C but absent at 68°C, and nitrate reduction and niacin production tests were both positive. *M. bovis* was identified based on the appearance of colonies after 4 and 13 weeks on medium 2 or 3. They were small and shiny; the nitrate reduction test was negative. After Zielh-Neelsen (ZN) coloration, *M. bovis* species appeared as corded bacilli in the liquid medium. *M. africanum* isolates grew as smooth, dysgonic, non-pigmented colonies. All *M. africanum* strains had no catalase activity at 68°C, produced no color change on bromocresol medium, and displayed dysgonic growth on medium 2 or 3.

### Statistical analyses

A specimen was considered to be positive when a mycobacterium was isolated on any of the cultures seeded. Double entry was used to enter data in Excel software. Statistical analysis was performed using STATA 11.0 (Stata Corporation 12.1, MP Parallel Edition, College station, TX, USA). Descriptive analyses comprised assessing the frequency distributions and proportions for each categorical variable. Logistic regression analysis was performed to measure the association between the presence of mycobacterium species and each independent variable (age, sex). Ninety-five percent confidence intervals (CIs) were calculated. P values <0.05 were considered to be statistically significant.

### RESULTS

Of the 1499 carcasses inspected, 102 (6.8%; 95% CI:

5.6%-8.2%) showed suspicious lesions of BTB, with 53 (6.6%, 95% CI: 5.0%-8.5%) found among the 806 carcasses inspected in the Central region (Ouagadougou), and 49 (7.1%; 95% CI: 5.3%-9.2%) among the 693 carcasses inspected in the Western Region (Bobo-Dioulasso). The macroscopic examination focused on tubercle-like lesion showing arrow-head which is completely absent in healthy animals' organs (Figure 1A). Infected carcasses showed granulomatous lesions and highly infected animals usually had multiple organs infected (Figure 1B and C). Among the carcasses with lesions, eight (7.8%) had generalized lesions (milliary) and 94 (92.2%) showed localized lesions, mostly located in the lymph nodes and the lungs. Table 1 shows the distribution of all 208 lesions found in the 102 carcasses with TB-like lesions.

### Direct microscopy

*Mycobacterium* spp bacteria were detected from 38 smears, 37.3% (95%CI: 27.9-47.4), prepared from 102 suspected animals with TB lesions. The percentage of confirmed TB using direct microscopy was slightly larger in Ouagadougou, 48.1% (95%CI: 34.0-62.4), than in Bobo-Dioulasso, 26.4% (95%CI: 27.9-47.4), but this difference was not significant (p>0.05).

### Bacterial culture

Inoculation of suspected samples onto appropriate TB media revealed that 49 out of 102 (48%) samples were positive regarding *Mycobacterium* spp after 7, 21, or 90 days post inoculation; nine samples were contaminated (Figure 2). However, among carcasses with TB-like lesions, the percentage with successful bacterial culture was higher in Ouagadougou, 65.5% (95%CI: 47-74.7) as compared to Bobo-Dioulasso, 34.0% (95%CI: 21.2-48.8) and this difference was statistically very significant (p=0.0053) (Table 2).

### Cultures and biochemical identification of mycobacterium strains

The biochemical identification technique was performed on all 49 samples where the bacteria could be cultured. Eighteen (36.7%) samples showed pigmented colonies on the media about 1-3 weeks and after biochemical tests were nontuberculous mycobacteria (NTM), while 31 (62.2%) samples showed strains belonged to MTBC. Further analysis of the MTBC group revealed 26 (83.8%) strains of *M. bovis*, 3 (9.6%) strains of *M. africanum* and 2 (6.4%) strains of *M. tuberculosis*. After investigation of the distribution of mycobacteria strains according to the age and the sex of animals, 22/31 (71%) cases of MTBC occurred in female bovines against 9/31 (29%) in male bovines; 4/31 (12.9%), 6/31 (19.3%) and 21/31 (67.7%) cases of MTBC were detected in bovines which ages
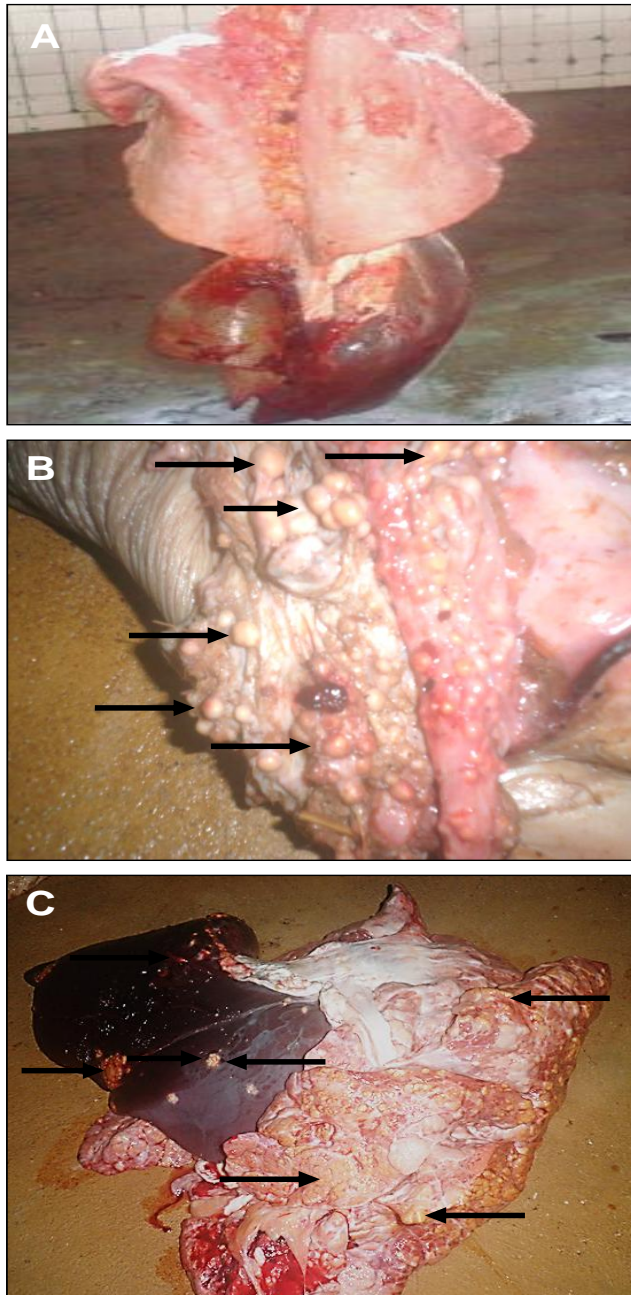
**Figure 1.** Characteristic granulomatous lesions of tuberculosis in bovine organs. (A) Bovine organs (lungs and liver) without tuberculosis lesions. (B) Granulomatous lesions of tuberculosis in bovine mesenteric lymph nodes. (C) Disseminated granulomatous lesions of tuberculosis in bovine lungs and liver.

were ≤ 2 years, < age ≤ 6 years and > 6 years respectively (Table 3).

**DISCUSSION**

This pilot study is the first report on the presence of different *Mycobacterium* strains in bovine carcasses

**Table 1.** Tissue samples with TB lesions found during post-mortem inspection on the period of May - October 2011 in Burkina Faso.

| Location of lesions | TB lesions | |
| --- | --- | --- |
| | Number | Percentage |
| Lymph nodes | 87 | 41.8 |
| Lungs | 47 | 22.5 |
| Breasts | 18 | 8.6 |
| Liver | 17 | 8.1 |
| Mesentery | 14 | 6.7 |
| Spleen | 8 | 3.8 |
| Head | 8 | 3.8 |
| Kidney | 6 | 2.8 |
| Heart | 3 | 1.4 |
| Total | 208 | 100 |

inspected in the two largest slaughterhouses of Burkina Faso since 1960. These results were presented to the Ministry of Animal Resources to raise awareness regarding the important risk that BTB is posing to human and animal health in Burkina Faso. In addition, the presence of any contaminated carcass in slaughterhouses limits the possibilities of exporting safe meat to neighboring countries or elsewhere and hence imposes a further burden to the country. Indeed, the fact that the study was conducted in the two best slaughterhouses of the country means that the estimated prevalence of TB-like lesions (6.8%) is likely to be higher among animals slaughtered in villages. The estimated prevalence of TB-like lesions is twice as high as the prevalence reported in two slaughterhouses of Algeria and South Africa (Sahraoui et al., 2009; Muller et al., 2013; Silaigwana et al., 2012) but three times lower than the TB-like prevalence of 18.95% reported in rural abattoir of Kenya (Gathogo et al., 2012).

While the majority of carcasses showed localized tuberculous lesions in organs or tissues, about 10% of the carcasses contained generalized lesion through the entire carcass. This generalized form of tuberculous lesions generally confirmed a severe form of TB (Sahraoui et al., 2009). The estimated prevalence of TB using direct smear microscopy is most likely an underestimate of the true prevalence. Indeed, unless $10^4$ bacilli /mL of acid-fast bacilli (AFB) are present in the sample, the test will be negative (Aung et al., 2001; Awah et al., 2010). Although the direct smear is considered the least sensitive method to diagnose TB (Cosivi et al., 1998), it remains the most commonly used diagnostic methods in slaughterhouses in developing countries due to its speed, ease and low cost.

Our study revealed that 44/102 (43.1%) carcasses carrying suspect lesions of TB proved to be negative in the culture. This percentage is high, even if the culture method is recognized to be less sensitive diagnostic tool but remains a routine technique to diagnose BTB and to
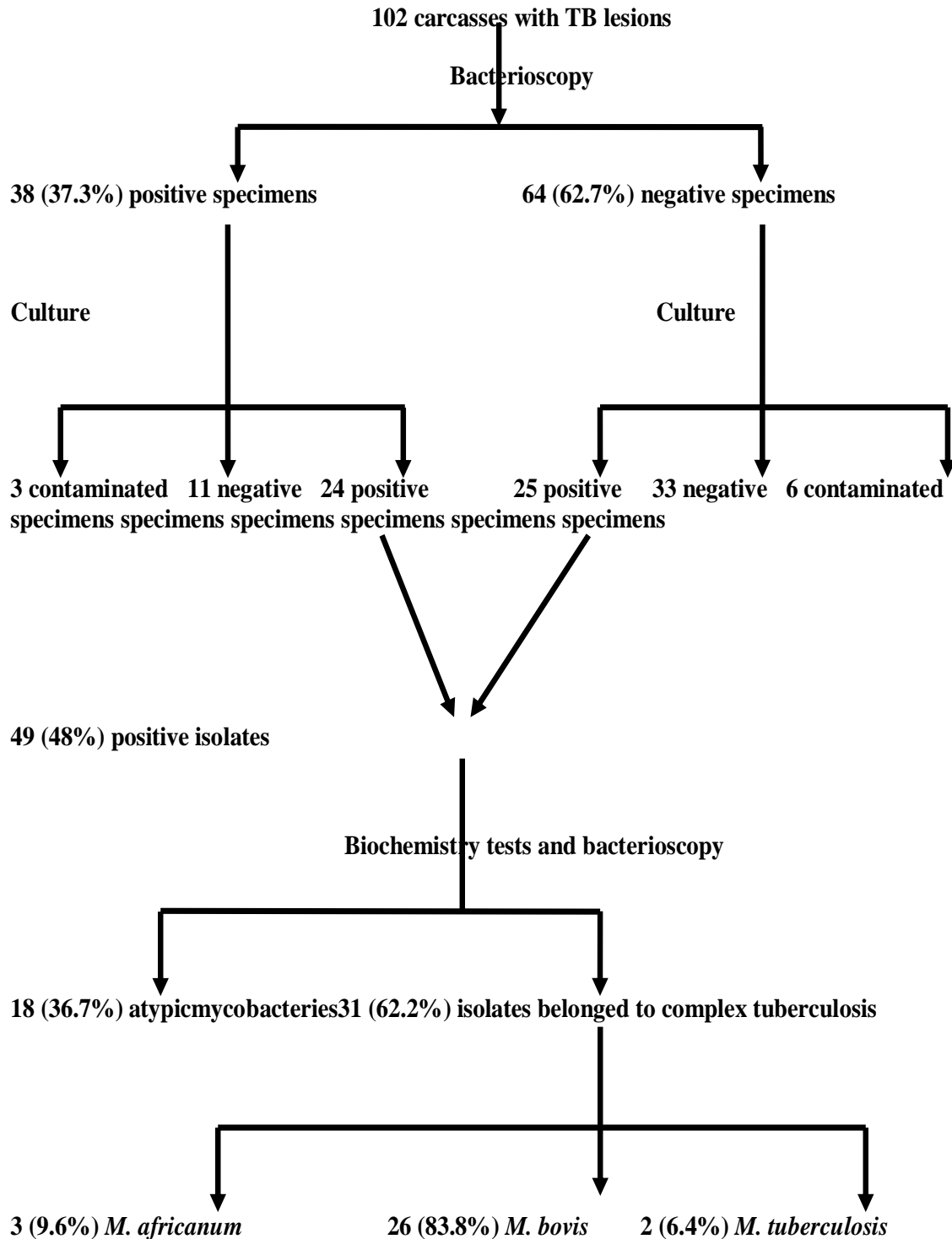
**102 carcasses with TB lesions**

**Bacterioscopy**

**38 (37.3%) positive specimens**                    **64 (62.7%) negative specimens**

**Culture**                                          **Culture**

**3 contaminated   11 negative   24 positive      25 positive    33 negative   6 contaminated**
**specimens specimens specimens   specimens specimens specimens**

**49 (48%) positive isolates**

**Biochemistry tests and bacterioscopy**

**18 (36.7%) atypicmycobacteries31 (62.2%) isolates belonged to complex tuberculosis**

**3 (9.6%) *M. africanum*          26 (83.8%) *M. bovis*     2 (6.4%) *M. tuberculosis***

**Figure 2.** Results of different analyses to isolate and identify mycobacteria from 102 carcasses with tuberculosis – like lesions.

identify various subtypes of mycobacterium (Cosivi et al., 1998). However, the absence of viable bacilli in the calcification lesion can explain these negative results (Teklul et al., 2004). The 49/102 (48%) individual carcasses showing lesions from which *Mycobacterium* spp. strains was successfully cultured clearly represent a medical risk, as the clandestine slaughtering of the bovines has not been eradicated yet in rural as well as in

**Table 2.** Results of bacterial culture of samples collected from infected carcasses.

| Sites | Quality | Cultures | | |
|---|---|---|---|---|
| | | Number | Percentage | 95% CI |
| Ouagadougou | Positive | 32 | 65.5 | (47-74.7) |
| | Negative | 16 | 30.8 | (18.7-45.1) |
| | Contaminated | 4 | 7.7 | (2.1-18.5) |
| | Total | 52 | 100 | |
| Bobo-Dioulasso | Positive | 17 | 34.0 | (21.2-48.8) |
| | Negative | 28 | 56.0 | (41.3-70) |
| | Contaminated | 5 | 10.0 | (3.3-21.8) |
| | Total | 50 | 100 | |
| Both sites | Positive | 49 | 48.0 | (38-58.2) |
| | Negative | 44 | 43.1 | (33.4-53.3) |
| | Contaminated | 9 | 8.8 | (4.1-16.1) |
| | Total | 102 | 100 | |

**Table 3.** Nontuberculous Mycobacteria and *Mycobacterium tuberculosis* complex strains culture results.

| Variable | Isolated NTM | | Isolated MTBC | | *M. bovis* | | *M. africanum* | | *M. tuberculosis* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % | Number | % | Number | % |
| **Age** | | | | | | | | | | |
| Age 1≤2years | 4 | 22.2 | 4 | 12.9 | 3 | 11.5 | 0 | 0 | 1 | 50.0 |
| 2<Age 2≤6years | 8 | 44.4 | 6 | 19.3 | 5 | 19.2 | 0 | 0 | 1 | 50.0 |
| Age >6years | 6 | 33.3 | 21 | 67.7 | 18 | 69.2 | 3 | 100 | 0 | 0.0 |
| **Sex** | | | | | | | | | | |
| Female | 12 | 66.7 | 22 | 71.0 | 19 | 73.0 | 1 | 33.3 | 2 | 100.0 |
| Male | 6 | 33.3 | 9 | 29.0 | 7 | 27.0 | 2 | 66.7 | 0 | 0.0 |

urban areas, despite the vigilance of veterinary services in Burkina Faso. Thirty one cultures showing the presence of TB strains belonging to MTBC confirm the circulation of pathogenic mycobacteria strains which can infect both domestic animal and human.

*Mycobacterium africanum* has been reported in human cases by Gomgnimbou et al. (2012) and *M. bovis* has been reported to infect cattle in slaughterhouses of Bobo-Dioulasso (Delafosse et al., 1995). Our results showed for the first time that three subtypes of *Mycobacterium* (*M. bovis, M. africanum* and *M. tuberculosis*) are capable of infecting humans and they were present among cattle slaughtered in the two largest slaughterhouses in Burkina Faso. These results raise the question of their epidemiological importance as a source of infection for humans (Vekemans et al., 1999; Jenkins et al., 2011). The distribution of positive cases by animal gender has shown that over 70% of strains of mycobacteria belonging to the tuberculosis complex were isolated from

females compared to only 29% in male. Since females are fed for meat and for milk, it significantly increases the risk of contamination for both animal and consumers (Silaigwana et al., 2012). The other important concern is that 67.7% of cases infected by MTBC were found in cattle over 6 years compared to 19.2 and 11.5% for cattle aged between 2 and 6 years and less than 2 years, respectively. These results encourage consumption of the meat from young cattle to minimize the risk of being infected.

**Conclusion**

Bovine TB pathogen circulates in Burkina Faso and *M. bovis* is one of the potential causative strains of this disease. Other strains of *Mycobacterium* such as *M. africanum* and *M. tuberculosis* were also identified. Isolation of *M. tuberculosis* from cattle underscores the risk of TB transmission from cattle to human and other

animals. Consequently, there is a need to formulate and implement control programs in order to mitigate cross-transmission between animals and humans.

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