Scientific Research Publishing

Advances in Microbiology, 2024, 14, 105-114
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Molecular Identification of Mycobacterium Strains Responsible of Bovine Tuberculosis Cases in Bobo-Dioulasso Slaughterhouse, Burkina Faso

Mariétou Konate[1*], Aminata Fofana[2*#], Yacouba Kouadima[3], Aboubacar Sidiki Ouattara[4], Adama Sanou[5]

[1]UFR/Sciences et Techniques, Université Catholique de l'Afrique de l'Ouest, Unité Universitaire à Bobo (UCAO/UUB), Bobo-Dioulasso, Burkina Faso
[2]Departement Biomédical et Santé Publique (BioMed/SP), Institut de Recherche en Sciences de la Santé (IRSS), Bobo-Dioulasso, Burkina Faso
[3]Departement de Biochimie-Microbiologie, UFR Sciences de la Vie et de la Terre, Université Nazi BONI, Bobo-Dioulasso, Burkina Faso
[4]Centre de Recherche en Sciences Biologiques, Alimentaires et Nutritionnelles (CRSBAN), Université Joseph KI-ZERBO, Ouagadougou, Burkina Faso
[5]Laboratoire de Mycobactériologie, Centre MURAZ, Institut National de Santé Publique, Bobo-Dioulasso, Burkina Faso
Email: konamarie94@gmail.com, #aminatafofana920@gmail.com, yackbill@yahoo.fr, as.ouattara@yahoo.fr, sanouadama2000@yahoo.fr

How to cite this paper: Konate, M., Fofana, A., Kouadima, Y., Ouattara, A.S. and Sanou, A. (2024) Molecular Identification of Mycobacterium Strains Responsible of Bovine Tuberculosis Cases in Bobo-Dioulasso Slaughterhouse, Burkina Faso. *Advances in Microbiology*, **14**, 105-114.
https://doi.org/10.4236/aim.2024.142008

Received: January 9, 2024
Accepted: January 30, 2024
Published: February 2, 2024

Abstract

Bovine tuberculosis (bTB) is an endemic zoonosis significantly affects animal health in Burkina Faso. The primary causative agent is *Mycobacterium tuberculosis* (*M. tuberculosis*) complex, mainly *M. bovis*. Cattle are considered as natural reservoir of *M. bovis*. However, in Burkina Faso, the circulation of these strains remains poorly understood and documented. This study aimed to identify and characterize *Mycobacterium* strains from suspected carcasses during routine meat inspection at Bobo-Dioulasso refrigerated slaughterhouse. A prospective cross-sectional study was conducted from January 2021 to December 2022 on cases of seizures linked to suspected bovine tuberculosis. Microbiological and molecular analyzes were used for mycobacterial strain isolation and characterization. Out of 50 samples, 24% tested positive by microscopy and 12% by culture. Molecular analysis identified 6 strains of Mycobacteria, exclusively *Mycobacterium bovis* specifically the subspecies *bovis* (*Mycobacterium bovis* subsp *bovis*). In conclusion, *M. bovis* subsp *bovis* is the primary agent responsible for bovine tuberculosis in Bobo-Dioulasso. Continuous monitoring of mycobacterial strains is therefore

*These authors have contributed equally to this work.
#Corresponding author.

necessary for the effective control of this pathology in the local cattle population.

Keywords

Bovine Tuberculosis, *Mycobacterium bovis*, Molecular Identification, Cattle Population, Burkina Faso

1. Introduction

Bovine tuberculosis (bTB) is a highly contagious disease affecting both humans and animal. The causative agent is *Mycobacterium bovis* (*M. bovis*), belongs to the *M. tuberculosis* complex (MTBc), which also includes various species such as *M. tuberculosis*, *M. africanum*, *M. microti*, *M. canettii*, *M. caprae*, *M. pinnipedii* and *M. mungi* [1]. Infections with atypical opportunists or saprophytes mycobacteria are capable to interfere with bTB screening or diagnosis through false-positive interpretations [2]. The main reservoir of *M. bovis* is cattle [1] [3]. However, wild species such as wild boars, African buffaloes, or deer, among others can be contaminated by cattle which can also infect them during close contact, thus maintaining the epidemiological cycle [2] [4]. In developing countries, infection control in animals and the lack of systematic dairy products pasteurization increase the risks of transmission to humans [4]. In Burkina Faso, as in most developing countries, the disease remains a concern despite the implementation of control measures [5]. Bovine tuberculosis impacts (bTB) occupies an important place in term of public health and economic. Approximately 10% of cases recorded in the human population were due to zoonotic transmission globally [6]. In addition, the World Health Organization attributed 143,000 cases of dental tuberculosis to *M. bovis* worldwide in 2018 [3]. However, molecular epidemiology in endemic areas of sub-Saharan Africa is not sufficiently known to implement surveillance approaches to better understand local dynamics [7]. Burkina Faso is a landlocked country with more than 70% of its population living in rural areas. The country's economy is mainly based on agriculture and livestock, which makes it particularly vulnerable to zoonotic diseases. Statistics from the Ministry of Agriculture, Animal, and Fisheries Resources indicated that in 2022, Burkina Faso had 9,720,615 cattle, 11,129,114 sheep, 10,750,406 goats, 1,416,342 pigs, 1,509,159 donkeys, 166,289 horses, 28,365 camels, and 35,803,843 poultry [8]. The close daily proximity of humans to these animals increases the risk of disease transmission, occurring through direct contact with infected animals. In fact, humans become infected through direct contact with infected animals or through consumption of contaminated meat and unpasteurized milk [3]. Although Burkina Faso notifies the World Organisation for Animal Health (OIE) every year of the presence of bTB in the country, the lack of comprehensive data prevents an accurate assessment of the prevalence and hinders understanding the incidence of human TB due to *M. bovis*, as well as the economic

burden of the disease beyond slaughterhouse seizures. To date, there is very little data on the molecular and cultural characteristics of *M. bovis* in Burkina Faso. The available information does not cover the entire territory [7] [9] [10]. The study aims to address this gap by characterizing MTBc isolates from carcasses of slaughtered cattle suspected of TB at the Bobo-Dioulasso Refrigerated slaughterhouse.

2. Methodology

2.1. Study Design

We conducted a prospective cross sectional study at the refrigerated slaughterhouse in Bobo-Dioulasso located in the Houet province, Hauts-Bassins region of Burkina Faso between January 2021 and December 2022. The samples were collected with the approval of Regional Animal Resources Direction. The selection of Bobo-Dioulasso as the study site was based on several factors, including the frequency of bTB frequency cases, the geographic accessibility and the overall security situation in the country. The samples collected were transported according to the cold chain protocols to maintain their quality during transit. The subsequent analyses were performed at the Mycobacteriology Laboratory of Centre MURAZ. Throughout the study period, only bovine organs and meat seized under suspicion TB by veterinary services were considered for microscopic, microbiological and molecular analyses. The biological material was mainly composed of lymph nodes and nodules which presented lesions suggestive of TB as identified during routine inspection. Samples were individually collected in sterile tubes and accompanied by a collection sheet developed for the study providing essential contextual information.

2.2. Sample Size

We carried out an exhaustive census focusing all seizure including carcass and different organs inspected for macroscopic bTB lesions during the study period.

2.3. Samples Collection

Following a routine meat inspection at slaughterhouse, the study focused on the carcasses seized from slaughtered cattle declared unfit for consumption due to bTB suspicion. The inspection involved thorough examination of the entire carcass, evaluating its overall condition, including color and presence of abnormal shapes. Additionally, scrutinizing target organs, particularly lungs, and all others, searching for lesions suggestive of TB, such as nodules or abscesses. Samples were extrated from tissues of lymph nodes, lungs, liver, kidneys and muscles from carcasses with suspected bTB lesions. A sterile scalpel was used to make an incision in the suspected part/area. Each collected sample, approximately 70 g in size was placed in a labeled sterile tube, then placed in a cooler maintained at +4˚C and transported to the laboratory for further analyses. **Figure 1** in the study illustrates some of the collected samples from cattle with bTB lesions.

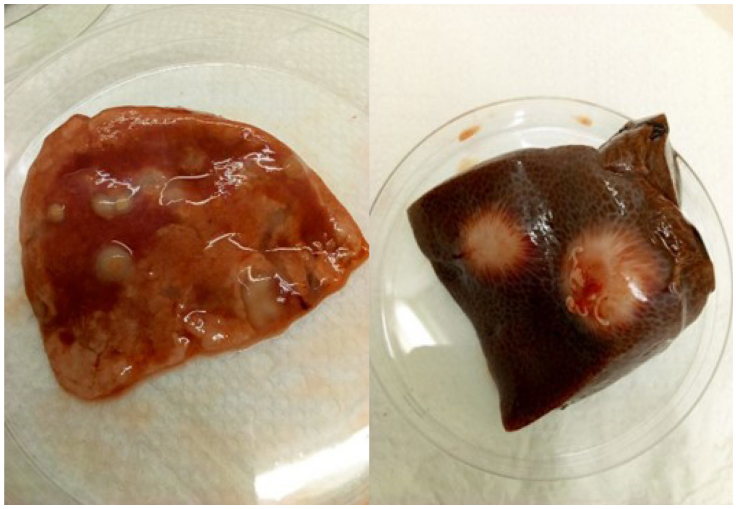

Figure 1. Picture showing a macroscopic granulomatous lesion suggestive bTB from organ of cattle slaughtered in Bobo-Dioulasso abattoir. Credit Picture: Arsène Ouédraogo.

2.4. Sample Preparation and Analysis

Once in the laboratory, the samples were processed using different techniques such as decontamination, microscopy, culture and molecular identification following the standard methods used at the Centre Muraz Mycobacteriology Laboratory.

2.4.1. Decontamination

Decontamination was carried out using protocol described by Sanou *et al.* [9]. Samples were cut into small pieces and then cleaned with sterile distilled water. The nodular parts on the tissue fragments were finely ground in a sterile mortar containing sterile sea sand. The ground material was then recovered in 10 ml of sterile distilled water. The suspension liquid obtained was collected in a first 50 ml Falcon tube and allowed to decant for 5 minutes. Two (2) ml of the supernatant was then transferred into a second 50 ml Falcon tube for the decontamination step according to the PETROFF method.

During the first decontamination step, 10 ml of NaOH was added to the 2 ml of the collected supernatant and homogenized vigorously for 30 seconds by vortexing, then for 20 minutes using a Kahn shaker. The suspension obtained was supplemented with sterile distilled water until an average volume of 45 ml was obtained and homogenized again by vortexing for 30 seconds. After this series of agitations, the suspension was centrifuged successively three times at a rate of 3000 revolutions per minute for 20 minutes. After the last centrifugation, the supernatant was removed and 1.5 ml of sterile distilled water was added to the pellet. The whole was vortexed until a homogeneous mixture was obtained, ready for bascilloscopy and seeding.

2.4.2. Microscopy

On a previously identified and cleaned slide, a drop of the homogenized centri-

fugation pellet was spread and dried at room temperature. Smear was fixed to the Bunsen burner flame for approximately 4 seconds, five times in a row while keeping the spread turned upwards. The fixed smears were then stained using the Ziehl-Neelsen heat method. The reading was made using an optical microscope (100 objective and immersion oil). A standard optical microscope with a 100× objective under immersion oil was used for microscopic examination.

Results were recorded as positive or negative smears. Positive smears were characterized by the presence of Acid Fast Bacilli (AFB) during the reading and graded as +/−, +, ++, or +++, depending on sample bacillary load. Any absence of AFB was considered as negative smear. A bTB case was defined by at least one smear positive for AFB at microscopic examination. A bTB negative one was defined by the absence of AFB in all the smears of a sample.

2.4.3. Isolation of Germs

The pellet obtained after decontamination was double inoculated on the Lowenstein-Jensen (LJ) culture medium using a glycerinated tube and a non-glycerinated one which, was also supplemented with pyruvate (0.5%). To do this, a volume of 0.2 ml of the homogenized pellet was used to inoculate three different tubes, two containing the glycerinated (LJ) medium, and one containing non-glycerinated (LJ) medium was supplemented with 0.5% pyruvate. The seeded tubes were then incubated at 37˚C in an incubator for 90 days. The cultures were observed on the third day of incubation to ensure that there was no contamination, then successively on days 7, 21, 28, 42, 60 and 90 to check the growth of mycobacteria.

2.4.4. Identification of Mycobacteria Isolates

Identification of mycobacteria was carried out using the Genotype Mycobacteria MBTC kit (Hain Life-science GmbH, Nehren, Germany) and according to the manufacturer's instructions. For DNA extraction, 1 to 2 colonies were collected from a solid medium culture and suspended in 100 µl of lysis buffer (A-LYS). The suspension was then incubated at 95˚C for 5 min then centrifuged at 10,000 ×g for 15 min at 37˚C. The supernatant was discarded and the resulting pellet was suspended in 100 µl of neutralization buffer (A-NB), vortexed for 5 minutes. and the mixture obtained was centrifuged for 5 minutes and stored at 4˚C before carrying out the PCR. PCR was carried out using 45 µL of two mixtures (AM-A, AM-B) provided with the GenoTypeMTBDC kit, and 5 µL of the heat-inactivated suspension giving a final volume of 50 µL for amplification. The details of the mix composition and the primers sequences are listed in Table 1. The amplification

Table 1. AM-A and AM-B amplification mixture.

Reagents	Reaction Volume	
	1×	13×
AM-A Solution	10 µl	130 µl
AM-B Solution	35 µl	455 µl

protocol included 15 min of denaturation at 95˚C, followed by 10 cycles including 30 s at 95˚C and 2 min at 65˚C. A phase of 20 additional cycles including 25 s at 95˚C, 40 s at 50˚C and 40 s at 70˚C, and a final extension at 70˚C for 8 min was then carried out.

Hybridization and detection were performed using a wash and shake device (TwinCubator). The program was started by putting 20 µL amplification products added to 20 µL of denaturing reagent (provided with the kit) into separate cuvettes of a plastic well and incubated for 5 min at room temperature. One (1) ml of pre-warmed hybridization buffer was then added, followed by stopping to drop a strip into each well. The hybridization procedure was carried out at 45˚C for 30 min, followed by adding a strip into each well. The hybridization procedure was carried out at 45˚C for 30 min, followed by two washing steps. For revelation, hybridized amplicons for revelation, hybridized amplicons, alkaline phosphatase-conjugated streptavidin, and substrate buffer were added. In the final wash, the strips were air dried and fixed on paper.

2.5. Data Analysis

Data was entered using Excel 2016 software. Descriptive analysis was used to present the results due to the low number of positive bTB cases.

3. Results

3.1. Prevalence and Location of Tuberculosis Lesions

In total, 10,641 cattle carcasses were examined during our study. Among them, 50 exhibited lesions suspicious for TB, resulting in a prevalence of 0.47% (50/10,641) with a confidence interval of [0.37% - 0.57%].

Upon stratifying the carcasses by gender/sex, it was observed that 49% (n = 19) of the cases with suspected TB lesion were females while 40% (n = 20) were males. The gender of the remaining 11 carcasses was not provided. The male/female sex ratio was estimated at 1.05 (20/19), suggesting a relatively balanced distribution of the suspected TB lesions between male and female cattle. Among the carcasses with suspicious lesions, the majority 48% (n = 24) exhibited lesions in the lungs, followed by 34% (n = 17) with lesion in the lymph nodes, and 10% (n = 5) with lesions in the breasts (Table 2).

3.2. Microscopic Analysis of bTB Suspected Cases

In the microscopic analysis revealed that 24% (n = 12) of the samples tested positive for Acid Fast Bacilli (AFB) compared to 76% who tested negative (n = 38) (Table 3).

3.3. Isolation and Identification of Mycobacteria Species

Among the total samples cultured (n = 50), 12% (n = 6) yielded positive results, with 86% (n = 43) testing negative, and 2% (n = 1) being contaminated (Table 4). Within the samples that tested positive by culture, 4 were also positive by

Table 2. Organs seized frequency for TBb suspicion.

Organ	Frequency	Percentage (%)
Liver	3	6.00
Nodes	17	34.00
Intestines	1	2.00
Udders	5	10.00
Lungs	24	48.00
Total	50	100

Table 3. Microscopic analysis result of samples.

Microscopic result	Frequency	Percentage (%)
Negative	38	76.00
Positive	12	24.00
Total	50	100

Table 4. Result of Mycobacteria growth on LJ media.

Culture result	Frequency	Percentage (%)
Contamination	1	2.00
Negative	43	86.00
Positive	6	12.00
Total	50	100

microscopy while 2 were negative. The obtained mycobacteria isolates (n = 6) were identified using the "Genotype Mycobacteria MBTC" test as the *M. bovis* species subspecies *bovis*.

4. Discussion

The objective of this study was to contribute to data collection on the identification of *M. bovis* species from lesions originating in cattle slaughtered at the Bobo-Dioulasso abattoir. A prevalence of 0.47% was observed among the 10,641 inspected carcasses, which is lower than the prevalence reported in previous studies carried out in the country [7] [10] and elsewhere [11] (2.6%, 2.7%, 4.3% respectively) thus confirming a geographic variation in bTB [7] [12]. This variation could be explained by resistance of the Zebu breed in cattle to bTB infections or to the animal production system [11]. The study showed that tuberculosis lesions were much more localized in the lungs compared to the other organs seized. This localization is attributed to the tropism of mycobacteria which often prefer the lungs before spreading to other parts of the animal organism. Aerosols particularly from sputum, constitute the most important source of contagion and mainly come from sputum [12].

Microscopic examination of the lesions showed the presence of AFB in 24% of cases. Culture identified positive isolates in 16% of bTB lesions with *M. bovis* as the only species isolated. These results are consistent with those of previous studies which confirm the circulation of *M. bovis* among cattle slaughtered in Burkina Faso [7] [10]. However, a much higher positivity rate of 54% for microscopic detection of *M. bovis* was reported by Kanyala *et al*. [7]. The lower positivity rate in our study could be explained by incorrect diagnosis of tuberculosis lesions, the influence of decontamination and culture growth conditions [13]. Furthermore, the detection of bTB cases by veterinary services suggests the surveillance of the disease, posing a growing threat in Burkina Faso. It's noteworthy that smear-positive samples can be negative in culture due to low viability of some bacilli. In contrast, smear-negative samples can be positive in culture because of the high sensitivity of culture compared to smear microscopy. In this study, no presence of *M. tuberculosis* was detected, however, its presence/occurrence has been reported in cattle and humans in some studies [9] [14] thus implying a potential risk of zoonotic tuberculosis.

It's essential to acknowledge certain limitations, including the restricted analysis to samples based on tuberculosis lesions at the slaughterhouses, limting a comprehensive estimate of bTB prevalence in Burkina Faso. Furthermore, the exclusive inclusion of slaughtered cattle might bias the overall prevalence rate of Mycobacterium strains in the city's animal population. The low rate of positive samples also hindered the feasibility of statistical analysis, and the absence of reported information on sex and age by veterinary service agents limited the exploration of these parameters reflecting the study population. Therefore, a need for further research and monitoring is necessary to improve our understanding of bTB dynamics in Burkina Faso.

5. Conclusion

This study reveals that Mycobacteria strains were isolated from tuberculosis lesions from cattle carcasses seized due to suspected bTB. Through microbiological and molecular analyses made it possible to identify *Mycobacterium bovis* ssp *bovis* was identified as the causative agent of bTB. Despite a relatively low frequency rate, the identification of the *M. bovis* strain suggests circulation of this pathogen in the cattle population of cattle slaughtered at the Bobo-Dioulasso slaughterhouse. This reinforces the significance of genuine public health issue linked to exposure to this pathogen. This study therefore suggests a continuous and in-depth molecular and bacteriological surveillance of mycobacterial strains contributing to the development and effective TbB control strategies in Burkina Faso.

Acknowledgements

Regional and provincial directorates of Animal and Fishery Resources in the Hauts-Bassin regions as well as the various agents stationed at the Bobo-

Dioulasso refrigerated slaughterhouse, at the Institute of Science and Technology (INSTechs) and at the Centre MURAZ.

Conflicts of Interest

There is no conflict of interest between the authors.

References

[1] World Organisation for Animal Health (2019). Zoonotic Tuberculosis in Mammals, Including Bovine and Caprine Tuberculosis. Organização Mundial de Saúde Animal (OIE). 1-20. https://www.cfsph.iastate.edu/

[2] Rivière, J. and Riviere, M.J. (2016) Evaluation du dispositif de surveillance de la tuberculose bovine dans la faune sauvage en France à l'aide de méthodes épidémiologique, économique et sociologique. https://theses.hal.science/tel-01362997/document

[3] Thoen, C., LoBue, P. and de Kantor, I. (2006) The Importance of *Mycobacterium bovis* as a Zoonosis. *Veterinary Microbiology*, **112**, 339-345. https://doi.org/10.1016/j.vetmic.2005.11.047

[4] Boussini, H., Traoré, A., Tamboura, H.H., Bessin, R., Boly, H. and Ouédraogo, A. (2012) Prevalence de la fuberculoseef de la brucellose dans les elevages bovins laifiers infra-urbains efperiurbains de la ville d'Ouagadougou au Burkina Faso. *Revue Scientifique et Technique*, **31**, 943-951. https://doi.org/10.20506/rst.31.3.2168

[5] Dibaba, A.B., Daborn, C.J., Cadmus, S. and Michel, A. (2019) Tuberculosis in Animals: An African Perspective. https://doi.org/10.1007/978-3-030-18690-6

[6] Carruth, L., Roess, A.A., Mekonnen, Y.T., Melaku, S.K., Nichter, M. and Salman, M. (2016) Zoonotic Tuberculosis in Africa: Challenges and Ways Forward. *Lancet*, **388**, 2460-2461. https://doi.org/10.1016/S0140-6736(16)32186-9

[7] Kanyala, E., Shuaib, Y.A., Schwarz, N.G., Andres, S., Richter, E., Sawadogo, B., *et al.* (2022) Prevalence and Molecular Characterization of *Mycobacterium bovis* in Slaughtered Cattle Carcasses in Burkina Faso; West Africa. *Microorganisms*, **10**, 1378. https://doi.org/10.3390/microorganisms10071378

[8] DCRP/MARAH (2022) Première enquête nationale sur le cheptel (ENC1): Les résultats validés. https://www.agriculture.bf/jcms/pv10_104263/fr/premiere-enquete-nationale-sur-le-cheptel-enc1-les-resultats-valides

[9] Sanou, A., Dicko, A., Sow, K.R., Djibougou, A., Kabore, A., Diarra, B., *et al.* (2021) Epidemiology and Microscopic Diagnosis of Tuberculosis in Pigs and Small Ruminants Slaughtered at Bobo-Dioulasso Abattoir, Burkina Faso. *Onderstepoort Journal of Veterinary Research*, **88**, 1-6. https://doi.org/10.4102/ojvr.v88i1.1908

[10] Tialla, D. and Ouedraogo, A. (2021) Tuberculose bovine et conséquences associées dans la commune rurale de Tanghin-Dassouri au Burkina Faso. *Science et Technique, Sciences de la Santé*, **44**, 19-29.

[11] Ejo, M., Haile, B., Tariku, T., Nigatu, S., Kebede, E., Bitew, A.B., *et al.* (2021) Bacteriological and Molecular Characterization of *Mycobacterium bovis* Isolates from Tuberculous Lesions Collected among Slaughtered Cattle, Northwest Ethiopia. *BMC Microbiology*, **21**, Article No. 286. https://doi.org/10.1186/s12866-021-02349-1

[12] Sibhat, B., Asmare, K., Demissie, K., Ayelet, G., Ameni, G., Dawa, D., *et al.* (2018) Bovine Tuberculosis in Ethiopia: A Systematic Review and Meta-Analysis. *Preventive Veterinary Medicine*, **147**, 149-157.

https://doi.org/10.1016/j.prevetmed.2017.09.006

[13] Nuru, A., Zewude, A., Mohammed, T., Wondale, B., Teshome, L., Getahun, M., *et al.* (2017) Nontuberculosis Mycobacteria Are the Major Causes of Tuberculosis Like Lesions in Cattle Slaughtered at Bahir Dar Abattoir, Northwestern Ethiopia. *BMC Veterinary Research*, **13**, Article No. 237. https://bmcvetres.biomedcentral.com/articles/10.1186/s12917-017-1168-3 https://doi.org/10.1186/s12917-017-1168-3

[14] Tarnagda, Z., Kanyala, E., Zingué, D., Sidibé, S., Yougbaré, I., Méda, T.K.N., *et al.* (2014) Prevalence of Tuberculosis spp. Species in Bovine Carcasses in Two Slaughterhouses of Burkina Faso. *International Journal of Microbiology and Immunology Research*, **2**, 92-100.