

Mycobacterium bovis Prevalence in Humans Does Not Differ between Regions in Burkina Faso

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Abstract

Background: In 1996, tuberculosis detection rate was higher in Oudalan (Sahel region) than in other provinces in Burkina Faso. Cattle breeding, a major activity of the local population, may favor exposure of humans to bovine tuberculosis.

Objectives: (i) to isolate and identify *M. bovis* among new cases of tuberculosis with positive culture in the Sahel (Gorom-Gorom and Dori); the Center (Ouagadougou) and the Western (Bobo-Dioulasso) regions of the country; (ii) to investigate susceptibility of isolated strains to anti-tuberculosis.

Methods: (i) collection of sputum from newly TB patients with a positive smear during a prospective study carried out in 1998 in the North, Central and Western region of the country and transferred at the Mycobacteriology Laboratory, (ii) optimization of operational procedures: (iii) mycobacteria strains's isolation on 3 different media (Loweinstein Jensen (LJ), LJ without glycerine and LJ supplemented with pyruvate; (iv) identification of the tuberculosis complex according to criteria such as: bacillus growth time, colony appearance, biochemical test results, bacilli growth with pyrazinamide or thiophene carboxylate hydrazide, or D-cycloserine; (v) performing antibiograms.

Results: Among the isolated strains, *M. bovis* represented 6.2% (2/30) in Gorom-Gorom, 0% (0/18) in Dori, 2.6% (3/109) in Ouagadougou and 2.9% (2/65) in Bobo-Dioulasso. Sixty nine percent (31/45) of strains were susceptible to the main anti-tuberculosis drugs, 13 strains presented single or

combined resistance and one strain was multidrug-resistant.

Conclusion: We showed that *M. bovis* disease is prevalent in all the studied regions but at a relatively low rate. The transhumance of from the north to the west practiced by the breeders could explain why it was found *M. bovis* in a similar rate as in Ouagadougou and Bobo-Dioulasso where livestock activity is less intense than in Gorom-Gorom.

Keywords: Tuberculosis; *Mycobacterium bovis* prevalence; Anti-tuberculosis drugs; Burkina Faso Sahel; Central; West Regions

Introduction

In humans, tuberculosis is a pulmonary and systemic disease caused by species of the *M. tuberculosis* complex, mainly *Mycobacterium tuberculosis*. It spreads from person to person by aerial transmission of droplets with a diameter of 1-5 µm. Several factors determine the probability of transmission. They include (i) infectivity of source patients - smear positive sputums for acid fast bacilli (AFB) or cavities on thoracic radiographs being strongly associated with infectivity, (ii) the susceptibility of contact subjects, (iii) the duration of exposure, (iv) the in which exposure occurs - small, poorly ventilated spaces present the highest risks. Tuberculosis is the leading cause of death attributable to a single infectious agent, with 10.4 million new cases and 1.4 million deaths in 2015 [1]. Knowledge of the responsibility of *Mycobacterium bovis* in human tuberculosis is longstanding [2]. It would occur in parallel with tuberculosis in livestock and a human-to-human transmission of *M. bovis*, albeit

rare, is possible. There are also consistent findings on the relative frequency of the location of tuberculosis due to *M. bovis* in humans. Where the consumption of unpasteurized milk is frequent *M. bovis* strains are found in the mesenteric and cervical ganglia. Locations were more frequently described in subjects living in close contact with infected cattle [3]. In Europe, before systematic slaughter of infected cattle and pasteurization of milk, human tuberculosis due to *M. bovis* represented one to 10% of all human tuberculosis cases: 1.5% in France [4] and 10% in England [5]. In 1995, 38 cases of human tuberculosis due to epizootic *M. bovis* were recorded in France [6]. The localization of tuberculosis was known for 32 cases: 17 (53%) in the lung, 14 (44%) extrapulmonary and one (3%) had a mixed localization. In developing countries, especially in West Africa, tuberculosis called "Sogo-Sogo Gwé" (the "white cough" in the Bambara language), was always dreaded by the populations. In Burkina Faso, epidemiological surveys carried out between 1950 and 1980 showed that high levels of tuberculosis were detected at slaughterhouses. Indeed, the percentage of seizures for tuberculosis at Bobo-Dioulasso slaughterhouse was 19.5% in some period of 1953 [7]. There was also a correlation between the rates of seizures for tuberculosis in slaughterhouses and the rate of positive reactions rates on animals following humanobovine tuberculin injection. In villages where highest rates of positive tuberculin skin test reactions in children were showed, the rates of positive reactions in animals were also very high. Forty years after these major epidemiological surveys data in Burkina Faso, 1996, health statistics and previous years' estimates suggested that there were more cases of human tuberculosis in the Sahel region (Gorom-Gorom and Dori) than in the other regions of the country, particularly those of the center (Ouagadougou) or and the west (Bobo-Dioulasso) regions. One of the hypotheses put forward to explain this situation was the high breeding activity practiced by the populations in the Sahel region which could expose them to *M. bovis* tuberculosis (by ingestion of unpasteurized milk, or following a closed contact with infected cattle).

The management of patients with *M. bovis* disease is critical because some strains of *M. bovis* are known to be less sensitive than *M. tuberculosis* to anti-tuberculosis drugs [8]: para-aminosalicylic acid, streptomycin, cycloserine and isoniazid; This last antibiotic is among the most important one in the drug regimen of human tuberculosis in Burkina Faso.

The main objective of the study was to analyze the relative frequency of *M. bovis* among mycobacterial strains isolated from positive culture tuberculosis cases. The second objective was to study the chemosensitivity of all isolated strains to the anti-tuberculosis drugs used by Burkina Faso's National Tuberculosis Program (NTP).

The analysis of drug resistance, against *M. bovis* in this study, became a therapeutic emergency since the findings of nosocomial transmission of multidrug-resistant tuberculosis among HIV-positive subjects [9-11].

Material and Methods

Type and sites of the study

A prospective study was carried out from January to December 1998 in Sahel (Gorom-Gorom, Dori), Central (Ouagadougou) and Western (Bobo-Dioulasso) regions in Burkina Faso (**Figure 1**). Tuberculosis screening centers in these regions transferred to Center Muraz, Bobo-Dioulasso the sputum of newly TB infected person with a positive smear.

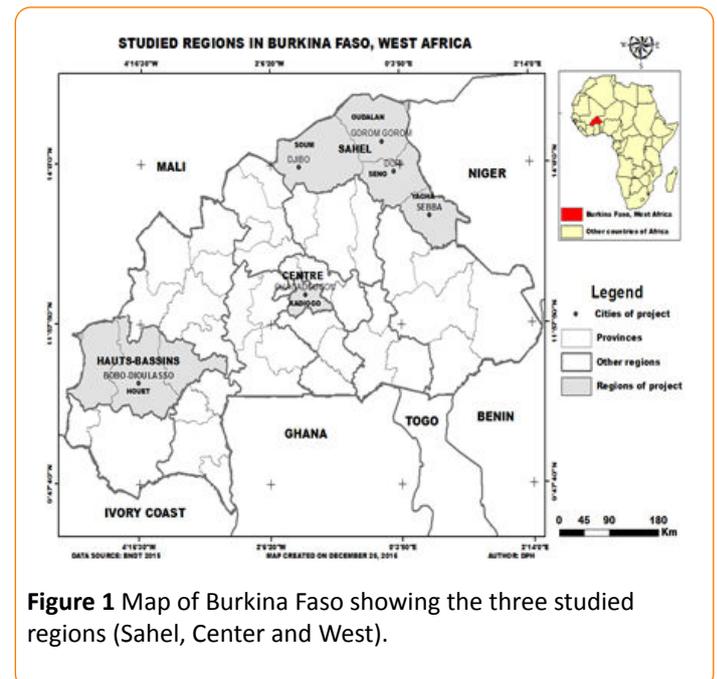


Figure 1 Map of Burkina Faso showing the three studied regions (Sahel, Center and West).

Description of the study

The implementation of this study required a strong collaboration between Gorom-Gorom Medical Center, Dori Regional Hospital Center (for samples collection from the Sahel region), the NTP Ouagadougou (for samples collection from the Central and Western regions) and the Center Muraz, Bobo-Dioulasso (for bacteriological analyzes). The main stakeholders benefited from theoretical and practical training adapted to their missions: direct microscopy training for laboratory technicians and training on epidemiology, clinical and bronchial fibroscopy of adults and children for physicians and nurses in charge of patients sorting.

Optimization of laboratory operational procedures

The method of decontamination with sodium hydroxide was used to decontaminate pathological products before AFB culture. The Dubos medium with cetyl pyridinium chloride was used to conserve bacilli in pathological products for delayed mycobacteriological analysis over several days. The sputum sample collected in well identified vials were then transported in a cold cool box (+4°C) to the Centre Muraz mycobacteriology Laboratory in Bobo-Dioulasso.

Isolation of strains of tuberculous mycobacteria

The sputum samples received in the Center Muraz, Mycobacteriology Laboratory, were immediately centrifuged at 3000 rpm for 15 minutes. The pellet was used for smear, then decontaminated by the Petroff method and inoculated three different solid media (Loweinstein Jensen (LJ), Loweinstein Jensen without glycerine and Loweinstein Jensen supplemented with pyruvate to stimulate growth of *M. bovis*). The cultures incubated at 37°C were examined after 48 hours and then weekly for three months.

A special effort has been made to investigate all *M. bovis* specimens taking into account its specific morphology and growth characteristics in order to allow its isolation and identification. The growth of *M. bovis* was slow or incomplete on media usually used for *M. tuberculosis* [12]. On LJ medium, the colonies of *M. bovis* grow very slowly (more than a month for isolation). Glycerin has an adverse effect on the growth of *M. bovis*. Conversely, pyruvate at a concentration of 0.3 to 0.5% stimulated its growth but inhibited the action of isoniazid, cycloserine and thiophene-2-carboxylic acid and decreased the activity of kanamycin and ethambutol [12,13]. On the contrary, pyruvate potentiated the activity of ethionamide [12]. *M. bovis* did not produce niacin and did not reduce nitrates to nitrites; it was resistant to pyrazinamide, but sensitive to 5 µg of T₂H/ml.

Mycobacterium strains identification and antibiograms

The identification of the tuberculosis complex was carried using basic standard criteria: bacilli growth time, colonies appearance and biochemical tests results: niacin test, nitrate reductase, urease. Growth with pyrazinamide or hydrazide of thiophene carboxylate (TCH), or D-cycloserine was also used as an identification criterion.

The antibiograms were carried out using the principle of the proportions method defined by Canetti, Rist and Grosset [14], on a single strain isolate for each patient. The antibiotics used by the National Tuberculosis Program of Burkina Faso were tested: isoniazid (INH), streptomycin (SM), rifampicin (RFP), ethambutol (EMB) and pyrazinamide (PZA). The interpretation took into

account the usual resistance criteria, ie 1% for all drugs. Three bacillary dilutions were seeded from the bacillary suspension at 1 mg/mL of 10⁻¹, 10⁻³, 10⁻⁵ bacilli. Each dilution was seeded on 2 control tubes without antibiotics for harvesting the total population and on one tube of each concentration retained for each antibiotic. The tube were incubated at 37°C and examined after 48 hours once a week and then weekly for 3 months.

Quality control and quality assurance

The standard strain H37Rv was used as a reference strain for the internal quality control of LJ culture media and anti-tuberculosis drugs. The National Reference Laboratory of Cotonou, Benin, provided external quality control. A concordance threshold of 90% or greater was established for isoniazid and rifampicin.

Ethical considerations

The tuberculosis national program of Burkina Faso Health department obtained all necessary authorizations for clarification of high screening rate of tuberculosis in the Sahel region of the country.

Statistical analysis

The chi-square (p<0.05) exact test was used to compare tuberculosis proportions in the three regions.

Results

A total of 460 sputum samples were sent to the Centre Muraz's Mycobacteriology Laboratory, from which: 132 specimens from the Sahel region (Gorom-Gorom Medical Center, 80 sputum specimens; and the Regional Hospital Center of Dori, 52 sputum specimens); the Tuberculosis Centers of Ouagadougou and Bobo-Dioulasso sent 186 and 142 specimens respectively. Laboratory results were available for 317 specimens (68%). **Table 1** shows the distribution of mycobacterial strains isolated from patients' sputum according to the region of the country: Sahel (Gorom + Dori), Center (Ouagadougou) and West (Bobo-Dioulasso).

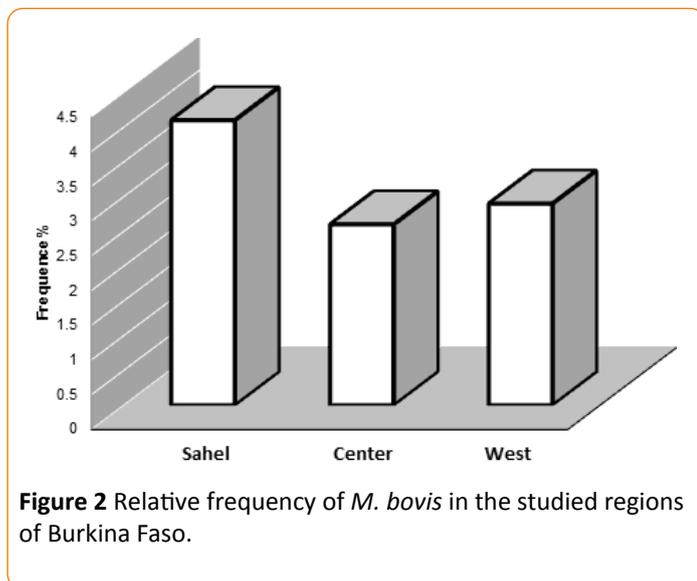
Table 1 Results of cultures and identification of *M. tuberculosis* complex in sputum according to the studied region.

Cultures	SAHEL		CENTER	WEST	TOTAL
	Gorom- Gorom	Dori	Ouagadougou	Bobo-Dioulasso	
<i>M. tuberculosis</i>	30	18	109	65	222
<i>M. bovis</i>	2	0	3	2	7
<i>Non tuberculous mycobacteriae</i>	0	0	0	2	2
Negative cultures	17	15	23	31	86
TOTAL	49	33	135	100	317

Two strains of atypical mycobacteria have been identified among Bobo-Dioulasso patients. No strain of *Mycobacterium africanum* was identified during this study.

The relative frequency of *Mycobacterium bovis* is shown in **Figure 2**. Four percent of strains isolated from Sahel region (Gorom + Dori) were *M. bovis*; with 6.2% for Gorom-Gorom. *M.*

bovis rates for Central and West regions were 2.6 and 2.9%, respectively, but the differences were not statistically significant ($p \geq 0.05$).



Discussion

Very few studies in Africa were focused on the ecology of mycobacteria or the prevalence of *M. bovis* tuberculosis. According to the Tuberculosis Technical Guide of the National Tuberculosis Program of Burkina Faso, the management of tuberculosis requires microscopic examination of AFB sputum. These characteristics are common to the *M. tuberculosis* complex. Very rarely NTPs have been interested in describing the species involved in human tuberculosis. However, in the years 1996, the NTP in Burkina Faso observed high rates of tuberculosis in the province of Gorom-Gorom compared to the other provinces wished to understand the reasons. Gorom-Gorom is a breeding province, and as consequence, some populations living there are likely to develop human tuberculosis due to *M. bovis* by ingestion of unpasteurized milk or being in closed contact with infected cattle. In order to determine the frequency of *M. bovis* in human tuberculosis case, this study attempted to isolate and identify *M. bovis* strains in culture positive recruited consecutively in Gorom-Gorom and Dori (Sahel region) compared to the central region of Ouagadougou and the western region of Bobo-Dioulasso. Our results showed that the prevalence of *M. bovis* was 6.2% (2/30) in Gorom-Gorom, 0% (0/18) in Dori, 2.6% (3/109) in Ouagadougou and 2.9% (2/65) in Bobo-Dioulasso.

A total of 45 strains were completely tested for antibiotics (Table 2), from which 31 were susceptible to the main antibiotics (INH, SM, RFP, EMB) and 13 strains showed a single and/or combined resistance; one strain was resistant to both isoniazid and rifampicin.

Table 2 Resistance to the main anti-tuberculosis drugs observed by region.

	Ouagadougou	Bobo-Dioulasso	Dori	Gorom-Gorom	TOTAL
INH	6 2 Single 4 Combined	4 2 Single 2 Combined	1 Combined	0	11 4 Single 7 Combined
SM	2 Combined	3 1 Single 2 Combined	1 Combined	0	6 1 Single 5 Combined
RFP	3 Combined	0	1 Combined	0	4 Combined
EMB	1 Combined	0	1 Combined	0	2 Combined
PZA	3 Combined	1 Single	1 Combined	0	5 1 Single 4 Combined

INH : isoniazide, SM : streptomycine, RFP : rifampicine, EMB : ethambutol, PZA : pyrazinamide
 Single: resistance to one antibiotic, Combined: resistance to at least two antibiotics. 18 strains sensitive to antibiotics (INH, MS, RFP, EMB, PZA), 13 strains sensitive to 4 antibiotics (INH, SM, RFP, EMB), 13 strains with resistance to a single antibiotic or combined

These results showed that there was a trend toward higher prevalence of *M. bovis* disease in the Northern part of the country. However the prevalence of *M. bovis* disease was not statistically significant between the regions ($p \geq 0.05$). The fact that the difference is not significant is probably linked to the small sample size and the low power of this study to identify a difference. These prevalences remain low compared to our expectations. And very strangely, in Dori, a city close to Gorom-Gorom with a similar population and environment we did not identify any strain of *M. bovis*. A low prevalence of *M. bovis* has

been reported by Rey et al., where only one strain of *M. bovis* (1.8%) was identified from 55 strains of mycobacteria isolated from sputum samples obtained in the Sahel department. The other strains were *M. tuberculosis* (27/55) and *M. africanum* (27/55) [15]. Paradoxically, in the same period, the prevalence of bovine tuberculosis (tuberculin test positive) in cattle at Dori (Sahel region) was 6% [16]. The transhumance of livestock practiced by the breeders may play a role in exposure to *M. bovis* at distance from breeding areas in Burkina. Indeed, the

(from North to West) and between the neighboring countries (from Mali and Northern Burkina Faso to Ivory Coast and Ghana) [17,18].

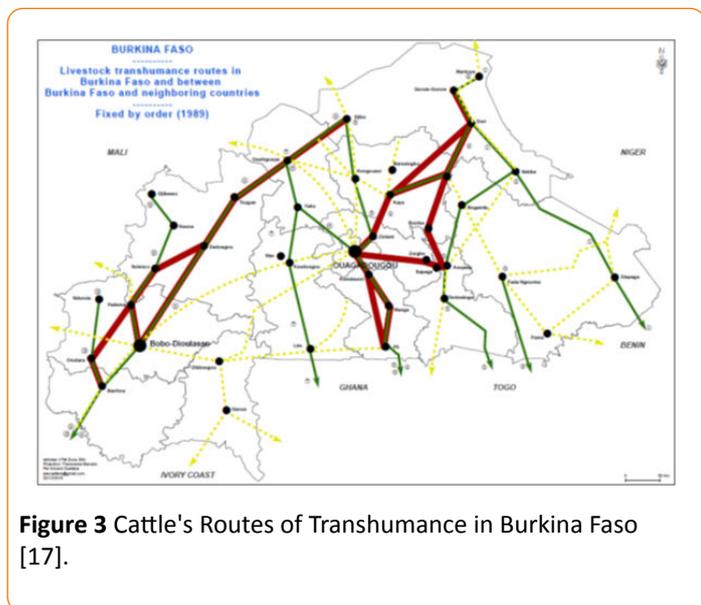


Figure 3 Cattle's Routes of Transhumance in Burkina Faso [17].

The prevalence of tuberculosis due to *M. bovis* is relatively low in some African countries. Indeed, in Nigeria and Ethiopia, *M. bovis* prevalence of 0.2% and 0.4% were respectively found on mycobacterial cultures [19,20]. In Europe and the US, prevalences of 1.4%, 2%, 3% and 7% were found in the Netherlands, France, Ireland and San Diego respectively [21-25].

The transmission of *M. bovis* from livestock to human is possible by ingestion of contaminated milk or by exposure to diseased animals. A study in Mexico showed a high prevalence of pulmonary latent tuberculosis among workers exposed to infected livestock and increases the risk for those working in non-ventilated areas. Thus, a high prevalence of tuberculosis due to *M. bovis* in areas with high bovine tuberculosis prevalence would be expected. These results are in accordance with those founded in Ethiopia where between 2000 and 2006, four cases of *M. bovis* were detected after molecular typing of nearly 1000 isolates and three of these patients (75%) had known regular animal exposure [20]. In China, however, a prevalence of 0.34% of *M. bovis* was found in a region with a high prevalence of bovine tuberculosis [26].

Inter-human airborne transmission in the contribution of spread of *M. bovis* was controversial. The predominant idea is that inter-human transmission of tuberculosis due to *M. bovis* is rare and appears to occur only in people who are particularly susceptible to tuberculosis such as those infected with HIV [9-11,27]. However, previous reports of clusters of cases with social links and molecular epidemiology and evidence from surveys of contact subjects from *M. bovis* pulmonary tuberculosis patients suggested that inter-human transmission occurs even in non-immunocompromised individuals. Several cases of inter-human transmission of tuberculosis to *M. bovis* was well documented [28-32].

M. bovis is naturally resistant to pyrazinamide, the molecule of choice in the treatment of human tuberculosis. *M. bovis*

strains can mutate and lead to multi-resistant tuberculosis and these mutants can maintain (original) pathogenicity for humans. Possibly, livestock and other animals may be re-infected by *M. bovis* bacilli including multidrug resistant strains.

In our study, 31/45 strains of *M. tuberculosis* isolated were sensitive to the main anti-tuberculosis drugs, 13 strains showed resistance alone and/or combined and one strain was multiresistant to isoniazid and rifampicin.

The main limitations of our study were the lack of a joint survey in cattle to determine the prevalence of bovine tuberculosis in the same period and a study on cow milk, the small sample size, the lack of information on diagnosed tuberculosis patients, including living conditions and their occupational activities (Breeders, shepherds) and the absence of antimicrobial susceptibility testing on *M. bovis* isolated strains. A previous study undertaken by our group on 64 samples milk collected in Bobo-Dioulasso shown a prevalence of mycobacterium of 53% [33].

Conclusion

It seems that there is a relatively "weak" circulation of *M. bovis* throughout the country, and more in the Sahel region than elsewhere. There is a trend toward a North-South-West prevalence gradient, suggesting persistent exposure of individuals to *M. bovis* due to breeding and potentially to transhumance activities.

It is necessary that a larger scale study be undertaken throughout the country and over a longer period to include a larger number of patients.

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