**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 (Lowenstein 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 antibigrams.

**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 cates 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...
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 (Lowenstein Jensen (LJ), Lowenstein Jensen without glycercine and Lowenstein 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 TzH/ml.

*Mycobacterium* strains identification and antibiograms

The isolation 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 *M. bovis* was resistant to pyrazinamide, but did not produce niacin and did not reduce nitrates to nitrites; it was slow or incomplete on 0.3 to 0.5% concentration of isoniazid, cycloserine and thiophene-2-carboxylic acid and decreased the activity of kanamycin and ethambutol [12,13].

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^(-3), 10^(-2), 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).

| Cultures                   | SAHEL   | CENTER  | WEST    | TOTAL |
|---------------------------|---------|---------|---------|-------|
|                           | Gorom-Gorom | Dori    | Ouagadougou | Bobo-Dioulasso |
| *M. tuberculosis*         | 30      | 18      | 109     | 65    | 222   |
| *M. bovis*                | 2       | 0       | 3       | 2     | 7     |
| Non tuberculous mycobacteria | 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 ≥ 0.05).

Figure 2 Relative frequency of M. bovis in the studied regions of Burkina Faso.

A total of 45 strains were completely tested for antibiotics (Table 2), from which 31 were susceptible to the main antibiotics (INH, MS, 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    | 1 Combined  | 11    |
|                |             | 4 Combined     |      |             | 4 Single |
| SM             | 2 Combined  | 3              | 1    | 1 Combined  | 6     |
|                |             | 2 Combined     |      |             | 1 Single |
| RFP            | 3 Combined  | 0              | 1    | 0           | 4     |
|                |             | 2 Combined     |      |             | 4 Combined |
| EMB            | 1 Combined  | 0              | 1    | 0           | 2     |
|                |             | 2 Combined     |      |             | 2 Combined |
| PZA            | 3 Combined  | 1 Single       | 1    | 0           | 5     |
|                |             | 4 Combined     |      |             | 4 Combined |

INH: isoniazide, SM: streptomycin, 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 ≥ 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 Figure 3 shows the major transhumance routes in the country.

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

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% of 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 found 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 undertook 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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