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M. bovis Infection: High Requirment for Surgical Interventions in HIV-Infected Subjects

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Abstract: Background: Zoonotic Mycobacterium bovis infection continues to occur, particularly in regions where there is no surveillance for bovine tuberculosis and raw milk consumption is common, or dairy products, including artisanal cheeses, are marketed unpasteurized. We describe the clinical and microbiological characteristics, procedures and treatment outcomes of subjects with *M. bovis* infection in HIV-infected individuals. **Methods:** A retrospective study was conducted obtaining the sociodemographic and clinical data, microbiological characteristics, CT findings and outcomes of 12 subjects with *M. bovis* infection which were compared these characteristics of 14 individuals with *M. tuberculosis* infection in HIV-infected subjects in the same period. **Results:** We found a significantly increased risk of *M. bovis* transmission due to consumption of unpasteurized dairy products and higher CD4+ T cells count in subjects with *M. bovis* infection vs M. tuberculosis infection (p <0.0001 and 0.01 respectively). All subjects with *M. bovis* infection had extrapulmonary involvement. CT findings in *M. bovis* infection that were significantly more frequent vs *M tuberculosis* infection were retroperitoneal lymphadenopathy, hepatosplenomegaly, and splenic abscesses. The site of microbiological identification was extrapulmonary in all *M. bovis*-infected subjects. Surgical interventions such as surgical drainage of abscesses or splenectomy were required significantly more frequently in subjects with *M. bovis* infection (p =0.0003). **Conclusions:** Extrapulmonary involvement, particularly with abdominal involvement, is routinely present in *M. bovis* infection in HIV-infected individuals. Surgical interventions are frequently required for diagnosis and management. Efforts to identify *M. bovis* should be made, particularly in high-burden regions.

Keywords: *Mycobacterium bovis*; Zoonotic tuberculosis; *Mycobacterium tuberculosis*; HIV infection

1. Introduction

Zoonotic tuberculosis (zTB), a form of human tuberculosis is caused primarily by M. bovis, a mycobacterial specie belonging to the Mycobacterium tuberculosis complex (MTBC) [1]. Estimates of the number of cases and deaths from zTB are imprecise. According to WHO, around 140,000 new cases and 11,400 deaths were due to zTB worldwide in 2019 [2]. Globally, the prevalence of zTB is around 1.4% among all tuberculosis (TB) cases, however, the disease is underreported mostly in resource-limited countries due to lack of systematic surveillance and limited diagnostic capacities to distinguish TB caused by *M. tuberculosis* or *M. bovis* which requires mycobacterial culture and subsequent use of biochemical or molecular diagnostic methods [3–5].

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Consumption of unpasteurized milk and dairy products represents the main route of transmission of *M. bovis* [6,7]. In middle and low-income countries, pasteurization is less implemented. In Mexico, almost 30% of the milk produced is sold unpasteurized, including that used for making artisanal cheese[8]; and in many African countries, pasteurization of milk is not carried out regularly and 80-90% of the milk produced is sold by small daily farms and pastoral communities[9]. The artisan cheese making process, which usually uses raw milk, does not eliminate the *M. bovis* bacillus and several studies have found the presence of *M. bovis* in artisanal cheeses through culture and molecular methods [10–13].

Bovine TB is endemic in several countries, data from 119 countries report having *M. bovis* circulating in their cattle in 59% of them, but only 10% have implemented zTB surveillance activities [14]. The lack of surveillance of bovine TB in countries with a high burden and the absence of test-and-slaughter policies in infected cattle increases transmission to humans [15].

Although human-to-human transmission of *M. bovis* might be less effective than *M. tuberculosis* due to three mutations affecting the two-component virulence regulation system PhoP/PhoR [16], outbreaks of *M. bovis* infection have been reported including multidrug resistant, especially in HIV-positive patients associated to high mortality [17–19].

In many regions of the world, especially in low-income countries, the diagnosis of TB is based on sputum smear microscopy or rapid assays as Xpert MTB/RIF®, this diagnostic tool recommended by the WHO since 2010, is considered an important breakthrough in the fight against tuberculosis globally and despite its solid evidence supporting its wide use in the detection of pulmonary and extrapulmonary tuberculosis, as well as mutations in the rpoB gene that confers rifampicin resistance [20], the assay identifies MBTC but cannot differentiate *M. tuberculosis* from *M. bovis*, so if the diagnosis is based only on Xpert MTB/RIF® positivity without identifying the species either by culture to molecular methods leaves M. bovis infection underdiagnosed and misclassified [21].

The aim of this study is to describe the clinical, microbiological characteristics, CT findings, surgical intervention and treatment outcomes of subjects with *M. bovis* infection compared with individuals with *M. tuberculosis* infection in HIV-infected subjects during the same period.

2. Materials and Methods

2.1. Design

This was a retrospective study conducted in Guadalajara, Jalisco, México from January 2019 to March 2023. Data were obtained from the database of the HIV Unit of the Hospital Civil de Guadalajara, approved by the hospital's Ethics Committee.

Sociodemographic data, comorbidities, clinical and microbiological characteristics (symptoms, anatomical site of infection, site of isolation/identification, diagnostic method), computed tomography (CT) imaging findings, surgical interventions performed, and treatment outcomes from HIV-infected subjects diagnosed with *M. bovis* infection through culture and/or polymerase chain reaction (PCR) were collected and compared with cases of *M. tuberculosis* infection, diagnosed by culture and PCR in HIV-infected individuals during the same period. Patients with *M. tuberculosis* infection received treatment according to World Health Organization tuberculosis treatment guidelines[22]. For patients with *M. bovis* infection, a fluroquinolone (levofloxacin) was included in the regimen, due to the intrinsic resistance of *M. bovis* to pyrazinamide.

2.2. Statistical Analysis

Categoric variables were summarized using counts and percentages and medians and interquartile range or mean, and standard deviation were used for continuous variables. Qualitative variables were analyzed using Fisher's exact test. Quantitative variables were analyzed using Student's t-test for parametric data and the Mann-Whitney U test for nonparametric data. Data was analyzed using SPSS version 20 A p value <0.05 was considered significant.

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3. Results

Twelve cases of *M. bovis* infection were diagnosed in the period evaluated, which were initially classified as *M. tuberculosis* infection since they presented a positive Xpert MTB/RIF® result of some anatomical site, but all of them were later diagnosed as *M. bovis* infection by multiplex PCR assay or culture. Characteristics were compared with fourteen cases of *M. tuberculosis* infection diagnosed by culture in the same hospital during the same period. Sociodemographic characteristics showed two significant differences between both groups, the habitual consumption of unpasteurized dairy products (a risk factor for M. bovis infection) and a higher median CD4 T cell count in subjects with *M. bovis* infection vs. *M. tuberculosis* infection (p< 0.0001 and <0.01 respectively) **(Table 1).**

Table 1. Sociodemographic characteristics of patients with *M. bovis* vs. *M. tuberculosis* infection in HIV-infected subjects.

Characteristics	*M. bovis* n=12	*M. tuberculosis* n=14	*p* value
Mean age, years (SD)	39.64±8.86	38.3±9.25	ns
Gender Male Female	10 2	14 0	ns
Regular consumption of unpasteurized dairy products (milk, artisan cheeses)	9	0	<0.0001
Recent contact with people infected with tuberculosis	1	2	ns
Drinking alcohol	6	7	ns
Current smoking	4	10	ns
Diabetes mellitus	1	0	ns
Charlson Index	6.25	6.2	ns
Absolute CD4+ T Cell count/µl, median (IQR)	102.5 (38-165)	20.5 (14.5-43)	0.01
HIV-1 RNA (copies/mL), median (IQR)	176260 (229-300750)	154000 (74600-683264)	ns

Abbreviations: SD, standard deviation; IQR, interquartile range; ns, not significant. Qualitative variables were analyzed using Fisher's exact test. Quantitative variables were analyzed using Student's t-test for parametric data (age) and Mann-Whitney U tests for nonparametric data (CD4 and HIV-1 RNA). A *p* value <0.05 was considered significant.

No differences were found in symptoms, except for neurological symptoms, which were significantly more frequent in *M. tuberculosis* infection vs *M. bovis* infection (p <0.03). Extrapulmonary involvement was significantly more frequent in *M. bovis* infection, while pulmonary involvement was significantly more frequent in *M. tuberculosis* infection (p <0.01). All cases (100%) of *M. bovis* infection presented extrapulmonary disease. Several abdominal CT findings were significantly more frequently found in *M. bovis* infection vs. *M. tuberculosis* infection, including retroperitoneal lymphadenopathy (p=0 0.01), hepatomegaly (p=0.001), splenomegaly (p<0.001), and splenic abscesses (p=0.004). The site of isolation/molecular identification was significantly more frequent extrapulmonary in *M. bovis* infection (mainly from cervical or retroperitoneal lymph nodes samples, and spleen or psoas abscesses) vs *M. tuberculosis*, meanwhile the site of isolation was more frequent pulmonary in *M. tuberculosis* infection (p =0.01) **(Table 2).**

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Table 2. Clinical, imaging and microbiological characteristics of patients with *M. bovis* vs *M. tuberculosis* infection in HIV-infected subjects.

Characteristics	*M. bovis* n=12	*M. tuberculosis* n=14	*p* Value
Presenting symptoms			
Fever	10	8	ns
Cough	8	6	ns
Weight loss (>10%)	7	11	ns
Cervical lymphadenopathy	11	10	ns
Gastrointestinal (abdominal pain, diarrhea, vomiting)	8	12	ns
Neurological	1	7	0.03
Anatomical sites of involvement			
Pulmonary	1	6	0.01
Extrapulmonary	12	8	0.01
Pulmonary and extrapulmonary	1	2	ns
Pulmonary CT findings			ns
Miliary	0	5	0.01
Cavitations	1	3	ns
Bronchiectasis	1	1	ns
Abdominal CT findings			
Retroperitoneal lymphadenopathy	9	3	0.01
Psoas abscess	4	2	ns
Hepatomegaly	7	0	0.001
Splenomegaly	8	0	<0.001
Splenic abscesses	6	0	0.004
Site of isolation/molecular identification			
Pulmonary†	0	12	<0.0001
Neck lymph nodes	9	2	0.04
Abdominal‡	5	0	0.01
Genitourinary	1	0	ns

Abbreviations: CT, computed tomography; ns, not significant. Qualitative variables were analyzed using Fisher's exact test. A p value <0.05 was considered significant. †Includes sputum, bronchoalveolar lavage, gastric aspirate. ‡ Includes peritoneal/retroperitoneal, liver, spleen, psoas and stool samples.

Positive cultures were found in only 4 cases (33.3%) of *M. bovis* infection, the remaining were identified by molecular methods, while the 14 cases of *M. tuberculosis* infection were identified by positive cultures. Due to the sites of involvement of *M. bovis* infection, a surgical procedure (percutaneous catheter placement (n=2), open surgery (n=6), or splenectomy n=5)) was performed in 8 patients (66.6%) vs none in *M. tuberculosis* infection (p <0.0003) for drainage/removal of abdominal abscesses with diagnostic and treatment purposes. No differences were found in treatment failure, relapse or mortality between both groups **(Table 3).**

Table 3. Surgical procedures and outcomes of patients with *M. bovis* vs. *M. tuberculosis* infection in HIV-infected subjects.

Characteristics	*M. bovis* n=12	*M. tuberculosis* n=14	*p* value
Surgical procedure†	8	0	0.0003
Outcomes			
Cured	8	7	ns
Treatment failed	2	2	ns

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Lost to follow-up	1	3	ns
Died	1	2	ns

Qualitative variables were analyzed using Fisher's exact test. A p value <0.05 was considered significant. † A surgical procedure was performed for diagnostic and treatment purposes, either percutaneous catheter placement, open surgery, or splenectomy for drainage/removal of abdominal abscesses.

4. Discussion

We examined the sociodemographic, clinical, microbiological characteristics, and outcomes in a series of cases of *M. bovis* infection and compared these characteristics with those of M. tuberculosis infection in HIV-infected subjects. Overall, we found a significantly increased risk of *M. bovis* transmission due to consumption of unpasteurized dairy products and higher CD4+ T cells count in subjects with *M. bovis* infection. There was a significantly greater incidence of extrapulmonary involvement, with more frequent retroperitoneal involvement, hepatosplenomegaly, and splenic abscesses, in subjects with *M. bovis* infection. Indeed, all subjects with *M. bovis* infection had extrapulmonary involvement, and the site of microbiological identification was extrapulmonary in all subjects. Surgical interventions such as surgical drainage of abscesses or splenectomy were required significantly more frequently in subjects with *M. bovis* infection.

The zTB burden of all TB cases shows a wide range of proportions globally[5]. The unavailability of appropriate methods for the identification of *M. bovis* in many middle and low-resource countries leads to an underestimation of the prevalence [21]. A recent meta-analysis of 19 studies and 7184 MTBC isolates found a vast difference in proportions of M. bovis infection in individual studies that ranged from 0.42% to 76.7%. The prevalence of studies that used conventional methods for the identification of *M. bovis* was 47.1%, while the prevalence of those that used genotypic methods was 1.4%. This discrepancy in prevalence might indicate an incorrect identification with the methods used [23].

Lowenstein-Jensen (LJ) solid medium for culture of *M. tuberculosis* supplemented with glycerol inhibits the growth of *M. bovis*. Stonebrick medium (supplemented with pyruvate) promotes the most rapid growth of *M. bovis*, however, culture on this medium is not frequently used, nor are biochemical identification methods [24]. However, liquid culture systems such as the BACTEC Mycobacteria Growth Indicator tube (MGIT) 960 which employs a fluorometric detection system overcome the problem with the LJ [25]

Culture and biochemical identification of *M. bovis* is time-consuming and infrequently performed in many laboratories. Several rapid molecular methods PCR-based have been developed to differentiate *M. bovis* from *M. tuberculosis* such as PCR targeting oxyR gene[26], multiplex-PCR based on a 500-bp fragment and the *pncA* gene [27], multiplex-PCR based on simultaneous detection of *pncA 169C > G* change in M. bovis and the IS6110 present in MTBC [28], PCR *pncA*-restriction fragment length polymorphism and PCR based on 3 regions of difference (RD-PCR): RD9, RD4 and RD1 [29]; PCR with confronting two-primers (PCR-CTPP) targeting the lepB gene [30], or that identify eight specific MTBC members using genomic regions of difference (RD1, RD1mic, RD2seal, RD4, RD9 and RD12) [31]. However, these methods are rarely available in most laboratories in low and middle-income countries.

One health approach to reduce the risk transmission of zTB should be implemented globally [9,14], because the consumption of raw milk and artisan cheeses is a very common practice mainly in middle and low-income countries [32]. In the United States, around 90% of *M. bovis* infection cases occur in Hispanic people and are attributed to the consumption of cheese made from unpasteurized milk in Mexico [11,33,34]. In our report, 9/12 (75%) patients with *M. bovis* infection had at least one risk factor for infection, either raw milk consumption or frequent consumption of soft artisanal cheese.

The rate of detection of *M. bovis* in fresh unpasteurized cheeses is variable, Cezar et al. found genetic material of *M. bovis* in 2.8% of 107 artisanal cheeses obtained from grocery stores and markets [10], while Barros de Melo et al. detected *M. bovis* DNA in 17.5% of samples de cheeses confiscated

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from baggage of incoming travelers to Brazil [13]. On the other hand, an experimental study showed that *M. bovis* cultured in raw souring milk samples at high concentrations (107 cfu/mL) can survive for at least 2 weeks at 20°C [35]. In addition, viable bacilli have been found in yogurt and cream cheese made from raw milk for up to 14 days and up to 100 days in butter [36].

Various factors have been associated with *M. bovis* infection. A nationwide US study found 165 (1.4%) of 11,860 human TB cases, were caused by *M. bovis*. The multivariate analysis of the study identified that subjects born outside the United States, Hispanic ethnicity, age <15 years, HIV infection, and having extrapulmonary disease were associated with *M. bovis* infection versus *M. tuberculosis* [33]. In a study in a Mexican population, Torres-Gonzalez et al. found that younger age, use of glucocorticoids, and extrapulmonary disease were independently associated with *M. bovis* infection compared to *M. tuberculosis* infection [37].

Tb caused by *M. bovis* is frequently indistinguishable from *M. tuberculosis*, clinically, radiologically, and pathologically [9]. A systematic review of eight studies found a higher proportion of extrapulmonary involvement in zTB than in cases of *M. tuberculosis* infection (median 63% vs 22%, p = 0.008). Lymph nodes and the genitourinary system were the sites most frequently affected, followed by bones and joints, intestine and peritoneum, and the nervous system [38]. In our case series, all patients with *M. bovis* infection had extrapulmonary disease (100%) with pulmonary involvement in 6 patients (50%). Cervical lymphadenopathy was present in 11 cases (91.6%), retroperitoneal lymphadenopathy in 9 (75%), splenomegaly in 8 patients (66.6%), hepatomegaly in 7 patients (58.3%), spleen abscesses in 6 subjects (50%) and psoas abscesses in 4 cases (33.3%). In a multivariate analysis of a study of HIV and tuberculosis coinfection, it was found that in addition to being male and Hispanic ethnicity, abdominal disease was associated with *M. bovis* infection [39].

The higher association of extrapulmonary involvement of *M. bovis* infection compared with TB caused by *M. tuberculosis* is related to the route of transmission, the alimentary route through the consumption of unpasteurized dairy products leads to extrapulmonary forms of *M. bovis* infection [36]. Adults at professional risk, especially farmers, abattoir workers and veterinarians, are more frequently infected with *M. bovis* by the airborne route through aerosols from infected cattle[40]. Pulmonary disease caused by *M. bovis* has been more frequently found in subjects with occupational vs non-occupational exposure (17/17, 100% vs. 6/9, 67%; P = 0.03, respectively) [41].

Few studies have evaluated the prevalence and clinical characteristics of TB caused by *M. bovis* in HIV-infected individuals. An increased proportion of zTB has been reported in HIV-infected compared to HIV-uninfected subjects in studies from the United States (RR= 2.6-8.3 times higher in HIV positive vs HIV negative) [5].

A study found that of 86 cases of HIV and TB coinfection, where 34.9% were caused by *M. bovis*, a more advanced state of immunosuppression was found in *M. bovis* infection (17.9% of *M. tuberculosis* infection cases had >200 CD4+ T cell count/μL vs 0% of M. bovis infection, p=0.01) [39] Contrary to our report, although we found that 91.6% of subjects with M. bovis infection had CD4+ T cells <200 cells/ μL, subjects with *M. tuberculosis* infection had significantly lower median CD4 T cells than subjects with *M. bovis* infection.

A study reported higher mortality in *M. bovis* infection compared to *M. tuberculosis* infection (15% vs 7%), particularly, higher death rates occurred in HIV-infected subjects with *M. bovis* disease vs *M. tuberculosis* (28% vs 8%, p= 0.006)[42]. Higher mortality in HIV+ vs HIV- (85%, 6/7 vs. 6%, 1/18; P = 0.01) was observed in a study in *M. bovis* infection in Argentina [41] In our report, only 1 patient (8.3%) died of complications related to *M. bovis* infection. It is important to emphasize that aggressive treatment with surgical drainage of abscesses or splenectomy was performed in many subjects infected with *M. bovis* in our study, given the high number of abdominal (including psoas) and splenic abscesses that the subjects presented.

Our study has some limitations. The retrospective design leads to potential selection and reporting biases and challenges in establishing causality. The sample size is small due to the difficulties in diagnosing *M. bovis* infection. The study only included HIV-infected individuals, a state

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of immunosuppression that can lead to different clinical presentations and outcomes than the general population. Therefore, the results cannot be extrapolated to the HIV-negative population.

5. Conclusions

M. bovis infection is underdiagnosed due to lack of routine of zTb and bovine TB surveillance and limited laboratory capacities representing a great challenge in public health. HIV-infected subjects are more susceptible to mycobacterial infections, accordingly, identification of *M. bovis* is particularly important. Extrapulmonary involvement is a key factor in diagnosing *M. bovis* infection in HIV-infected individuals. Surgical interventions are frequently required for diagnosis and management. Efforts to ensure appropriate identification and timely and appropriate management, particularly in regions with limited resources and high burden of *M. bovis* infection.

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