Characterization of Mycobacterium orygis, Mycobacterium bovis, and Mycobacterium caprae Infections in Humans in Western Canada

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Epidemiologic research on zoonotic tuberculosis historically used Mycobacterium bovis as a surrogate measure; however, increased reports of human tuberculosis caused by other animal-associated Mycobacterium tuberculosis complex members like Mycobacterium orygis necessitates their inclusion. We performed a retrospective cohort study including persons infected with any animal-lineage M tuberculosis complex species in Alberta, Canada, from January 1995 to July 2021, identifying 42 patients (20 M bovis, 21 M orygis, 1 M caprae). Demographic, epidemiologic, and clinical characteristics were compared against persons with culture-confirmed M tuberculosis infection. The proportion of culture-positive infections caused by M orygis increased continuously from 2016 to 2020. Significantly more females at a higher median age were impacted by M orygis, with all patients originating from South Asia. Mycobacterium bovis caused significantly more extrapulmonary disease and disproportionately impacted young females, particularly those pregnant or postpartum. All infections were acquired abroad. These findings can aid in developing targeted public health interventions.

Keywords. tuberculosis; Mycobacterium bovis; bovine tuberculosis; zoonoses; animal diseases; Mycobacterium orygis.

Tuberculosis (TB) remains an important public health issue, with >10 million new cases and 1.6 million deaths globally each year [1]. TB is caused by members of the Mycobacterium tuberculosis complex (MTBC), some of which can infect both humans and animals. Zoonotic TB (zTB) is human TB acquired directly or indirectly from animal exposure and is caused by MTBC members with established animal reservoirs. More recently, zTB has been recognized as an important but understudied component of the global TB epidemic, and the first-ever “Roadmap for Zoonotic Tuberculosis” was launched in 2017 by multiple international entities including the World Health Organization (WHO) [2, 3]. This roadmap called for detailed epidemiologic investigation of zTB. The prevalence of zTB continues to pose a significant burden to public health, and despite these recommendations, its origin and epidemiology remain poorly understood worldwide including in Canada [4–6].

Members of the MTBC that cause zTB include Mycobacterium bovis (predominantly cattle), Mycobacterium caprae (sheep and goats), Mycobacterium microti (rodents), and Mycobacterium pinnipedi (seals and sea lions) [7, 8]. In 2019 it was estimated that 1.4% of new TB infections (approximately 140 000 cases) were attributable to M bovis, with an estimated 11 400 deaths worldwide [9]. The true burden of zTB is likely higher than this, given that mycobacterial species causing zoonotic disease are often not identified.

Mycobacterium orygis is an increasingly recognized cause of TB. First identified in a captive East African oryx, it has since been found most commonly in the domestic cattle of South Asia (Bos indicus) and humans [2, 10–12]. Mycobacterium orygis has also been recovered from various other species including waterbucks, black bucks, deer, rhesus monkeys, and...
rhinoceroses; thus its reservoir host and host range remain unknown [12, 13]. Aside from 1 case of documented M orygis transmission from human to cattle during calf rearing, there have been no other studies outlining mode of transmission [14]. It is presumed to be a zoonotic disease, though there is no definitive evidence for either animal-to-human or human-to-human transmission. However, given the potential for zoonotic transmission, the argument to operationally include M orygis in studies assessing zTB epidemiology has been made [10]. The disease characteristics and epidemiology of M orygis infections are also poorly described, and thus further studies are needed.

This study describes the epidemiologic, demographic, and clinical characteristics of all persons infected with any animal-lineage MTBC species in the province of Alberta over 25 years. Alberta is one of the 4 major immigrant-receiving provinces in Canada alongside Quebec, Ontario, and British Columbia. These provinces account for >75% of all patients with TB in Canada, and >80% of these patients are foreign-born [15].

**METHODS**

We performed a retrospective cohort study of persons infected with animal-lineage MTBC species in Alberta, Canada, from January 1995 to July 2021. Culture-confirmed *M tuberculosis* sensu stricto cases from 1995–2020 were used for comparison.

**Mycobacterial Species Identification**

Alberta’s mycobacterial diagnostics are centralized in the Alberta Provincial Public Health Laboratory (APHL). Animal-lineage MTBC species were identified by the APHL from clinical specimens, and forwarded to Canada’s National Microbiology Laboratory (NML) for further characterization. From 2014 to 2021, all newly identified MTBC isolates were DNA fingerprinted using 24-loci mycobacterial interspersed repetitive units–variable number tandem repeats (MIRU-VNTR) [16].

The presence of insertion sequence 6110 and region of difference 9 was confirmed by real-time polymerase chain reaction to detect MTBC and *M tuberculosis*, respectively. In older specimens, *M bovis* was identified using gyrB and mptra sequencing, or by a specific mutation in pncA (His57Asp). *Mycobacterium caprae* was identified using partial gyrB gene-specific nucleotide sequencing. Since 2018, the NML has used whole genome sequencing (WGS) and the BioHansel bioinformatic tool for identification of MTBC species including *M bovis* and *M caprae* [17].

For *M orygis*, the gyrB gene single-nucleotide polymorphisms (SNPs) incorporated in k-mer-based BioHansel could only identify this species as an animal-lineage member of the MTBC with low confidence. The WGS-based identification technique has recently been improved by expanding the gene targets used for SNP analysis to include gyrB, PPE55, Rv2042c, leuS, mmpL6, and mmpS6 to differentiate MTBC animal lineages, including 10 unique SNPs for *M orygis* [18]. We used this improved WGS-based SNP approach to identify *M orygis* with high confidence. We also reviewed results of MIRU-VNTR, and isolates with an undetectable locus 2163b (typical *M orygis* MIRU-VNTR) were selected for testing [8, 19]. *Mycobacterium africanum* isolates from 2007 onward underwent WGS-based SNP phylogenetic analysis, given that older identification methods would misclassify *M orygis* as *M africanum* due to their shared gyrB1450 (G → T) mutation [8]. Notably, our methods would not have detected *M orygis* before 2007. Thirteen patients with *M africanum* (3 identified pre-2007 unconfirmed by WGS, 6 WGS-confirmed, 4 failed to grow from frozen stock) and 19 patients with iatrogenic *M bovis* bacillus Calmette-Guérin (BCG) strain were excluded from analysis.

**Antimycobacterial Susceptibility Assays**

From 1995 to 2010, all antimycobacterial susceptibility testing was performed using the BACTEC 460TB radiometric system (Becton Dickinson) and from 2010 to 2021, the BACTEC 960 MGIT system (Becton Dickinson). All MTBC isolates were assayed against the first-line antimycobacterials ethambutol, isoniazid, rifampin, and pyrazinamide.

**Patient Characteristics**

Epidemiologic, demographic, and clinical variables analyzed included age at TB diagnosis, sex, birth region, human immunodeficiency virus (HIV) status, end-stage renal disease (ESRD), medical immunosuppression, treatment outcome, and, among foreign-born persons, years in Canada prior to TB diagnosis. Birth regions were defined using the United Nations “Standard Country or Area Codes for Statistical Use” [20]. ESRD was defined as receiving dialysis. Diabetic status was defined as hemoglobin A1c >6.4% or diabetic medication use. Medical immunosuppression was defined as use of tumor necrosis factor inhibitors, transplant-related immunosuppressants, and long-term corticosteroid use (>1 month of prednisone 15 mg/day or equivalent). Treatment outcomes were reported as cure/treatment completion, death before/during treatment, or other (transfer out of province, nonadherence, unknown) with standards outlined by the Pan-Canadian Public Health Network [21].

The annual percentage of culture-confirmed TB infections attributable to animal-lineage MTBC members from 1995 to 2020 was measured. Disease characteristics assessed included site of infection, sputum smear status, cavitary disease, and antimicrobial susceptibility. *International Classification of Diseases* codes were used to determine infection sites and are reviewed by a physician [22]. To further characterize patients infected with animal-associated MTBC species, chart review was performed to determine pregnancy/postpartum status, travel to endemic regions, and consumption of unpasteurized dairy products. Referre
data from Immigration, Refugees and Citizenship Canada (IRCC) was also collected for these patients (migrants to Canada with history of TB or chest radiograph abnormalities consistent with prior TB are referred by IRCC for a TB surveillance medical evaluation). Contact tracing data for close contacts (defined by 2014 Canadian Tuberculosis Standards) of patients with pulmonary involvement were reviewed for secondary cases, and for rates of tuberculin skin test (TST)/interferon-γ release assay (IGRA) positivity and conversions [23].

Data were abstracted from the Alberta Provincial Tuberculosis Registry, with chart review performed for each patient with an animal-associated mycobacterial species using multiple health information systems.

Descriptors of interest were compared to culture-confirmed *M tuberculosis* cases using 2-tailed *t* tests assuming unequal variance for continuous variables, as well as Fisher exact test for most categorical data, and for rates of tuberculosis skin test (TST)/interferon-γ release assay (IGRA) positivity and conversions [23].

Descriptive statistics were compared to culture-confirmed *M tuberculosis* cases using 2-tailed *t* tests assuming unequal variance for continuous variables, as well as Fisher exact test for most categorical data, and for rates of tuberculosis skin test (TST)/interferon-γ release assay (IGRA) positivity and conversions [23].

Ethics approval was obtained from the University of Alberta’s Health Research Ethics Board (Study ID: Pro00108521).

**RESULTS**

We identified 20 patients infected with *M bovis*, 21 with *M orygis*, and 1 with *M caprae* and compared them to 3557 patients with culture-confirmed *M tuberculosis* infection.

**Mycobacterium orygis**

From 2014 onward, there were 17 patients infected with *M orygis* identified by MIRU-VNTR screening of MTBC isolates followed by WGS, or by WGS of isolates identified as *M africanum*. Initially, 10 of these isolates were correctly identified as *M orygis*, and 7 were misclassified as *M africanum*. From 2007 to 2013 based on WGS of *M africanum* isolates, there were 4 patients with *M orygis*, all reported from 2007 to 2010. There were no patients with *M orygis* from 2011 to 2015. The proportion of culture-confirmed TB cases secondary to *M orygis* increased from 2016 to 2020 (Figure 1). Within the first half of 2021 there were already 3 documented cases. This trend was also observed when analysis was restricted to patients originating from Bangladesh, India, Nepal, or Pakistan, and from 2007 to 2010 *M orygis* also caused a high proportion of the infections in this population (Figure 2A).

Concerning treatment outcome, HIV status, presence of ESRD, and diabetes mellitus, patients infected with *M orygis* did not differ...
significantly from those with *M. tuberculosis* (Table 1). All *M. oryges* infections occurred in foreign-born individuals from India or Pakistan (Table 2). Two of 21 *M. oryges* cases were referred to TB services from IRCC screening (Table 2).

Regarding disease presentation, 52% of patients with *M. oryges* had extrapulmonary involvement compared to 37% with *M. tuberculosis*; however, the results were not statistically significant (*P* = .082; Table 3). Of those with extrapulmonary
were seen in patients with *M. orygis* and *M. tuberculosis* (75% and 76%, respectively). There was no significant difference in sputum smear positivity or cavitary disease in patients with *M. orygis* with pulmonary involvement. All isolates were fully susceptible to first-line antimycobacterial drugs. Among 119 close contacts to patients with pulmonary involvement, there were no secondary cases and 3 TST/IGRA conversions (Table 4). WGS and MIRU-VNTR genotyping did not identify any secondary cases from *M. orygis*.

### Mycobacterium bovis

The proportion of culture-confirmed TB cases secondary to *M. bovis* has remained unchanged for 25 years, fluctuating...
between zero to 2 cases annually causing 0%–2.2% of culture-confirmed TB infections (Figure 1). More females than males were infected with \textit{M. bovis} compared to \textit{M. tuberculosis} (60% vs 45%, respectively, \(P = .26\)), and they were generally diagnosed at a younger median age (35 vs 49.5 years, respectively, \(P = .26\)), though these differences were not statistically significant (Table 1). Thirty-three percent of female patients diagnosed with \textit{M. bovis} infection were pregnant or recently postpartum within the last 2 years. No significant differences in treatment outcome, HIV status, ESRD, diabetes mellitus, or medical immunosuppression were seen between \textit{M. tuberculosis} and \textit{M. bovis} patients (Table 1).

Compared to those with \textit{M. tuberculosis}, patients with \textit{M. bovis} were more likely to originate from Northern Africa (10% vs 0%), Western Asia (15% vs 0%), Latin America (10% vs 1%), and Western Europe (10% vs 0%) (Table 1). Only 1 case of \textit{M. bovis} infection was identified in a patient who migrated from India (Table 2). No patients infected with \textit{M. bovis} were referred to TB services through IRCC screening (Table 2).

Patients with \textit{M. bovis} infection had significantly more extrapulmonary involvement compared to those with \textit{M. tuberculosis} (80% vs 37% respectively, \(P < .001\); Table 3). Of those with extrapulmonary disease, 69% had peripheral lymphadenitis, with the next most common manifestation being gastrointestinal disease at 19% (Table 3). Patients infected with \textit{M. bovis} were significantly less likely to have pulmonary involvement compared to those with \textit{M. tuberculosis} (45% vs 74% respectively, \(P < .001\); Table 3), and these cases with pulmonary involvement were significantly less likely to be sputum smear positive (11% vs 42% respectively, \(P = .040\); Table 3). All \textit{M. bovis} isolates were resistant to pyrazinamide, with 1 isolate being extensively drug-resistant (XDR). Among 37 close contacts to patients with pulmonary involvement, there were no secondary cases identified and no TST/IGRA conversions (Table 4).

**DISCUSSION**

This study is the first to describe the epidemiologic characteristics of zTB infection in Canada, with inclusion of the animal-associated MTBC species \textit{M. orygis}. We found that while the annual proportion of culture-confirmed TB in Alberta attributable to \textit{M. bovis} was stable from 1995 to 2020, ranging from 0% to 2.2%, the proportion of culture-confirmed TB infections...
caused by *M. orygis* has increased annually since 2016, peaking at 2.3% in 2020 (Figure 1). The proportion of culture-confirmed TB cases caused by animal-adapted MTBC species is also increasing in Alberta, largely due to this increase in *M. orygis* infections. This is unlikely to be driven by changes in immigration patterns, as the 2016–2020 trend is also observed within migrants from *M. orygis*-endemic regions (Figure 2A). Increasing transmission occurring in endemic regions could explain this increase; however, because our identification methods were unable to detect *M. orygis* before 2007, this trend could be artifactual. The years 2007, 2009, and 2010 also saw high proportions of South Asian migrants infected with *M. orygis*; however, this represents only 4 cases, and there were no cases detected from 2011 to 2015 (Figure 2A). Given these findings, the possibility that *M. orygis* is an emerging pathogen in South Asia requires further investigation [24–26].

Reporting on the epidemiologic and clinical characteristics of *M. orygis* infections in humans remains sparse. Lavender et al assessed TB infections in the Australian state of Victoria from 2005 to 2010, identifying that *M. orygis* caused 0.45% of culture-confirmed TB cases corresponding to 8 patients, all originating from India with 7 being female [27]. Marcos et al found that 8 of 6322 isolates (0.13%) submitted to the New York State Department of Health between 2005 and 2016 were *M. orygis*, all in patients from India, Pakistan, or Nepal [28]. Lipworth et al used SNP analysis on 3128 clinical isolates in the United Kingdom and found 24 *M. orygis* isolates (0.77%) despite no cases being described in the UK in the extant literature [24]. Eldholm et al assessed *M. africanum* transmission in Norway from 2010 to 2020 and identified 5 isolates of *M. orygis* by WGS that were initially misclassified as *M. africanum*, all in patients born in South Asia [29]. To date, Duffy et al is the only study that has assessed prevalence of *M. orygis* infection in India using molecular diagnostics, where 7 of 940 cultures of MTBC from hospitalized patients were *M. orygis* [10]. Sumanth et al characterized these patients, with 1 additional case, and found all 8 patients had extrapulmonary disease, with only 2 having concurrent pulmonary involvement [30]. Five patients were female, and 3 were aged <18 years [30].

Countries of origin differed between those infected with *M. orygis* and *M. bovis* in this study. Patients infected with *M. bovis* more frequently originated from Morocco, Lebanon, Iraq, Mexico, and the Netherlands, while 100% of patients infected with *M. orygis* were born in either India or Pakistan (Table 2). This is consistent with literature identifying *M. orygis* as the predominant animal-associated MTBC species affecting humans in South Asia [10]. Taking a One Health approach involving countries with endemic zTB is necessary to reduce disease burden globally [3, 31].

Patients infected with *M. orygis* were significantly more likely to be female than those with *M. tuberculosis*, a finding also observed by Lavender et al [27]. Furthermore, 17.5% of culture-confirmed MTBC infections diagnosed in 2019–2020 in female patients from *M. orygis*-endemic countries were caused by this organism, indicating much higher prevalence in this subpopulation than previously reported, possibly due to difficulty with species-level identification (Figure 2B). Those with *M. orygis* also developed disease at a significantly older age than those with *M. tuberculosis*. There is no apparent explanation for why elderly females were more significantly affected by *M. orygis*, and this notably differed from what was observed by Sumanth et al. This could be explained by the difference in clinical presentation of TB in migrants compared to patients living in endemic regions. Fifty-two percent of patients with *M. orygis* had extrapulmonary involvement (predominantly peripheral lymphadenitis and gastrointestinal sites) compared to only 37% of patients with *M. tuberculosis*, suggesting a nonrespiratory mode of transmission such as foodborne via unpasteurized dairy as hypothesized by Brites et al [11].

Our findings on *M. bovis* are consistent with previous studies in the United States and the Netherlands, where the proportion of cases attributable to *M. bovis* ranged from 1.3% to 2.0% [32, 33]. In our study, people infected with *M. bovis* were predominantly female (60%) and younger (median age of 35) compared to people with *M. tuberculosis* infection, consistent with trends in the United States [32]. We highlight that 33% of female patients with *M. bovis* infection were pregnant or recently postpartum, which has not been previously described. *Mycobacterium bovis* infections were significantly more likely to be extrapulmonary, in keeping with previous literature and consistent with consumption of contaminated unpasteurized dairy products as the primary mode of transmission [32, 34]. Contrary to the findings of Scott et al [32], patients with pulmonary involvement of *M. bovis* seen in Alberta were significantly less likely to have sputum smear–positive disease than those with *M. tuberculosis* and experienced no significant difference with regard to treatment outcome.

Our study did not find evidence of local transmission for any animal-adapted MTBC species. Close-contact tracing data revealed no secondary cases and lower rates of both new TST/IGRA positivity and conversion for all animal-associated MTBC species when compared to *M. tuberculosis* from 2002 to 2013 in Alberta [35]. None of the isolates shared a matching MIRU-VNTR type. Overall these results suggest that animal-adapted MTBC species are less transmissible from human to human than *M. tuberculosis*, and support the hypothesis that *M. orygis* is transmitted from animal to human. In *M. bovis* infections, the lower likelihood of pulmonary involvement and sputum smear positivity also suggests lower transmissibility. Of the 4 Canadian-born patients with *M. bovis* infection, 3 had traveled to Mexico prior to their diagnoses, with 2 endorsing consumption of unpasteurized dairy products in Mexico. The remaining patient had close contact with a HIV-seropositive patient in Spain with pulmonary XDR *M. bovis* infection [36].
Furthermore, the Canadian bovine TB surveillance system was evaluated as being able to detect a bovine TB prevalence of 0.00028%, essentially indicating TB elimination in Canadian livestock [37]. The last case of transmission of an animal-associated MTBC species in Alberta occurred in 1991, when a farmer was infected with M. bovis from a domesticated elk [38]. Additionally, commercial dairy pasteurization has been compulsory Canada-wide since 1991, making it difficult for Canadians to access unpasteurized products, and neither M. orygis or M. caprae have been isolated from animals in Canada [39]. Thus, it appears all patients with animal-associated MTBC species in Alberta since 1995 acquired their infection abroad.

Of note, minimal animal-adapted MTBC species infections in Alberta were detected as part of IRCC screening, likely due to the predilection of these organisms for extrapulmonary involvement. Thus, current immigration screening practices do not appear to be effective for these species.

A limitation of this study is the small size of the cohort. HIV status, as a function of opt-out testing, was routinely recorded in Alberta beginning in 2003, and ESRD, diabetic status, and medical immunosuppression have only been routinely recorded since 2013, limiting interpretation of these variables. With our study techniques, M. orygis could not be identified before 2007, making it impossible to interpret trends for this organism before that time. Furthermore, 3 M. africanum isolates pre-2007 did not undergo WGS, and 4 M. africanum isolates post-2007 failed to grow, all of which could have been misclassified and were excluded from analysis. The lack of a standardized, accurate, accessible, and affordable test for diagnosis of animal-associated MTBC species remains a barrier to zTB epidemiologic research.

This is the first study to assess the epidemiology of zTB and M. orygis in a Canadian population. The incidence of M. orygis, specifically, has been increasing in Alberta since 2016, leading to animal-adapted MTBC species causing 3.3% of all culture-confirmed TB cases in 2020, the highest recorded proportion during the entire 25-year study period and a high proportion as compared to the extant literature. Consequently, M. orygis case numbers should be monitored closely from a public health perspective. These findings also necessitate the inclusion of M. orygis in future studies assessing the epidemiology of zTB globally, despite the uncertainty surrounding its transmission dynamics. Prospective studies should include a detailed animal exposure history (including unpasteurized dairy consumption), travel history/country of origin, pregnancy status, BCG vaccination status, and contact tracing data, in addition to the disease and clinical characteristics outlined in this study. In patients with M. orygis, the specific region of origin within India/Pakistan should be recorded. Taking a One Health approach focusing on evaluating and improving bovine TB control measures in endemic countries with culturally appropriate interventions is of the utmost importance to reducing the incidence of animal-associated MTBC species infections worldwide. Additionally, targeted education initiatives should also be developed in high-risk groups including travelers to endemic areas.

Notes

Potential conflicts of interest. All authors: No reported conflicts.

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