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Editorial

A hup B gene based PCR for the diagnosis of pediatric tuberculous meningitis

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Tuberculosis (TB) caused by *Mycobacterium bovis* is clinically indistinguishable from disease caused by *Mycobacterium tuberculosis*. In much of the developing world, bovine TB is uncontrolled placing young children who drink contaminated milk at risk. In many countries, its occurrence is reported to be sporadic and control measures based on test-and-slaughter policies and disease notification are not applied [1]. Despite efforts in certain areas to quantify the extent of zoonotic tuberculosis, the prevalence of *M. bovis* infection in humans throughout the developing world is essentially unknown.

Tuberculous meningitis (TBM) results in considerable morbidity and mortality without prompt diagnosis and treatment. Recent work has focused on finding a rapid means to differentiate meningitis caused by *M. bovis* from that due to *M. tuberculosis* using a hup B DNA targeted polymerase chain reaction (PCR) assay [2]. Such a tool would be invaluable in guiding therapy and public health control efforts, particularly in developing nations with limited laboratory facilities for culture and typing of tubercle bacilli.

While historically it has been assumed that the vast majority of cases of TBM are due to *M. tuberculosis*, a recent study by Shah et al. [3] suggests that *M. bovis* may be the causative agent in a disturbingly large proportion of cases in India. In this study, which included 100 pediatric patients with clinical signs and symptoms of TBM, cerebral spinal fluid (CSF) samples from 27 children were found to be positive for *M. tuberculosis* or *M. bovis* by N-PCR assay for a hup B DNA target. Of the 27 cases, three were positive for *M. tuberculosis*, 17 were positive for *M. bovis* and seven were positive for both species of mycobacteria [3]. In this issue of the Journal of Pediatric Infectious Diseases, Nambam et al. [4] employ a similar strategy of using a hup B gene based diagnostic PCR to detect the presence of *M. bovis* in the CSF of pediatric patients with signs and symptoms suggestive of TBM. Their findings which are comparable to those reported by Shah et al. [3] suggest that *M. bovis* is the causative agent in 35% (8/23) of cases and mixed infection is present in 9% (2/23) of cases.

There are a limited number of studies in the literature that have looked at the relative contribution of the two pathogens in active cases of tuberculosis. Most used culture-confirmed disease as the gold standard and found the incidence of *M. bovis* infection to be quite low. A review of zoonotic TB studies performed in countries throughout the world found the proportion of human cases due to *M. bovis* to be 3.1% of all forms of tuberculosis and 9.4% of extrapulmonary disease [5]. There is even less information available regarding the incidence of *M. bovis* TBM. One report from the Baja California region of the United States reported that

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M. bovis accounted for 33.9% of all culture positive cases of TB (15.8% of all central nervous system infections) [6]. Interestingly, the studies by Shah et al. [3] and Nambam et al. [4] which employ the hup B gene PCR assay, suggest that the incidence of *M. bovis* TBM is considerably higher than previously reported in the literature.

In multiple studies, including those examining cattle and humans, the hup B gene based PCR detected the presence of mixed infection which raises some questions as to the specificity of the test [7]. Although in endemic regions, it is certainly plausible that persons are latently infected with multiple species of mycobacteria, it seems improbable that such a high percentage of cases of TBM would be due to coinfection. In order for this to occur, both species of mycobacteria would need to gain access to the subarachnoid space at essentially the same time and cause clinical disease. On the other hand, it may be the case that infection with one species disrupts the blood brain barrier and predisposes the central nervous system to infection with the other [8].

A PCR result that suggests the presence of a mixed infection is difficult to interpret in the setting of a culture negative sample. Shah et al. [3] compared the hup B gene based PCR to microscopy, but no human studies to date have described the performance of the PCR in culture positive specimens. A study of bovine samples compared PCR results to culture and found that mixed infection was detected by a hup B gene based PCR in 22 animals, whereas culture detected mixed infection in only one animal [9].

In the Nambam et al. [4] study, a modified version of the Ahuja's criteria was used to select cases of probable TBM with the gold standard being response to therapy [10]. All cultures of CSF in those patients with probable TBM were negative. The criteria, which albeit may be clinically relevant given the difficulty of culturing mycobacteria from the CSF of pediatric patients, make it difficult to evaluate the performance of the test and even more difficult to interpret the significance of a mixed infection. Unless *M. bovis* has a predilection for the CSF in this pediatric population, it would seem that *M. bovis* infection would also be seen at other body sites. If this is true, it may be easier to test the hup B gene PCR on culture positive specimens from these sites in order to verify the specificity of the test. Alternatively, the specimens with mixed infection could be investigated further by employing alternative PCR techniques that differentiate between *M. tuberculosis* and *M. bovis*. Confirmatory testing was used in a similar study of a novel PCR developed to identify the causative agent in TB lymphadentitis. When the assay detected mixed infection with *M. bovis* and *M. tuberculosis* in fine needle aspirates, secondary testing did not confirm the presence of two species of mycobacteria in the specimens [11].

TBM due to *M. bovis* or a mixed infection creates a dilemma for the clinician. Currently, empiric treatment for a pediatric patient with probable TBM is combination therapy consisting of isoniazid, rifampin, ethambutol and pyrazinamide. The use of this regimen is based on the presumption that the etiologic agent is *M. tuberculosis* and would be potentially less efficacious for the treatment of disease caused by *M. bovis* which is intrinsically resistant to pyrazinamide and in some cases, other first line drugs [12,13]. If a larger proportion of cases of TBM were proven to be due to *M. bovis* or a mixed infection, a revision of current empiric treatment guidelines may be warranted in endemic areas.

A diagnostic PCR with the ability to differentiate between *M. tuberculosis* and *M. bovis* would be an important addition to the current laboratory tools available to rapidly diagnose TBM in the pediatric population. However, studies examining the hup B gene based PCR's performance on culture positive specimens are needed to determine the specificity of the test. The concept of TBM due to two species of mycobacteria is new and also needs to be further investigated. If additional research identifies mixed infection to be a relatively common entity, the current understanding of the pathophysiology of TBM and its treatment will need to be revisited.

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