Simultaneous Detection of *Mycobacterium bovis* and *Mycobacterium tuberculosis* in Human Cerebrospinal Fluid

In the April 2006 issue of the *Journal of Clinical Microbiology*, Shah et al. reported the simultaneous presence of *Mycobacterium tuberculosis* and *Mycobacterium bovis* in 22 human cerebrospinal fluid specimens (5). The method used to reveal the alleged coinfection was a novel nested PCR (N-PCR) described by Mishra et al. (3). In that original study, N-PCR correlated as poorly with culture (85/189 bovine specimens) as it did with phenotypical speciation (9/18 positive cultures). Specificity for bovine tuberculosis (TB) diagnosis in relation to tuberculin testing was as low as 22.2%.

Based on those—at the least questionable—results, Shah et al. applied this N-PCR to the diagnosis of human tuberculous meningitis (TBM). N-PCR gave positive results in 15/25 and 5/44 cases with TBM and non-TBM, respectively, which suggests a rather low diagnostic predictive value for that population. A higher correlation with conventional diagnostic methods should be expected from the proposed tool.

In view of the high proportion (34/212) of TBM cases attributed to *M. bovis* by N-PCR, the authors suggested that the differential protection conferred by *M. bovis* BCG against TBM indirectly indicates the role of *M. bovis* as a causative agent of pediatric TBM. We could argue that BCG proved highly protective against TBM in countries like Argentina, where all bacteriologically confirmed TBM cases were caused by *M. tuberculosis* (2).

The number of human specimens with dual *M. bovis*/*M. tuberculosis* N-PCR amplification was noticeably high. To our knowledge, this association has not been reported previously. Precisely, in the study cited by Shah et al. to support this finding, all specimens initially showing PCR amplification for both *M. tuberculosis* and *M. bovis* were in fact negative for *M. bovis* when tested with a more specific PCR assay (1). Although human mixed *M. tuberculosis*/*M. bovis* infection is not improbable in a setting such as the one in this study, the evidence presented by Shah et al. is weak. Inexplicably, culture was not used to confirm their findings. Simultaneous infection with these closely related microorganisms cannot be granted unless viable bacilli of both species are identified.

We agree that the identification to the species level is relevant to assess the weight of zoonotic tuberculosis and to adjust control strategies in vast areas of the world. This is particularly true for Asian, African, and Latin American countries with high prevalences of bovine TB and scarce information about human disease caused by *M. bovis*. The issue was raised in the early nineties, when the first nucleic acid amplification techniques became available for detection/differentiation of the main members of the *M. tuberculosis* complex (4). Expectations were renewed with every new molecular tool proposed. To date, however, none of those techniques led to a better understanding of the problem, probably due to insufficient assay reliability.

Compliance with rigorous standardization protocols and robustness are essential for validating molecular diagnostic tools. Meanwhile, a criterion based on the combination of clinical, bacteriological, biochemical, and radiological data is still more reliable for timely detection of TBM and medical decision making, which are critical for its prognosis.

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Viviana Ritacco*
CONICET-INEI ANLIS Malbran
Velez Sarsfield 563
1281 Buenos Aires, Argentina

Isabel N. de Kantor
Tuberculosis Consultants Panel
World Health Organization
Buenos Aires, Argentina

*Phone and fax: 54 (11) 4302 7635
E-mail: vritacco@anlis.gov.ar

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