Epidemiol. Infect. (2003), **130**, 235–237. © 2003 Cambridge University Press
DOI: 10.1017/S0950268802008105 Printed in the United Kingdom

Audit of scope and culture techniques applied to samples for the diagnosis of *Mycobacterium bovis* by hospital laboratories in England and Wales

F. DROBNIEWSKI[1]*, M. STRUTT[1], G. SMITH[2], J. MAGEE[3] AND P. FLANAGAN[4]

[1] *PHLS Mycobacterium Reference Unit and Regional Centre for Mycobacteriology, South London Public Health Laboratory and Department of Infection Guy's, King's & St. Thomas' School of Medicine, Kings College Hospital (Dulwich), East Dulwich Grove, London SE22 8QF, UK*
[2] *Regional Centre for Mycobacteriology, Birmingham Heartlands Hospital, UK*
[3] *Regional Centre for Mycobacteriology, Newcastle General Hospital, UK*
[4] *Regional Centre for Mycobacteriology, University Hospital of Wales, Cardiff, UK*

(*Accepted 31 October 2002*)

SUMMARY

This audit examines the ability of English and Welsh laboratories to diagnose *Mycobacterium bovis* infection. All 164 clinical laboratories submitting samples to the PHLS Mycobacterium Reference Unit and Regional Centres for Mycobacteriology were surveyed. Twenty per cent of responding centres did not use a pyruvate-containing medium or incubate for the minimum recommended period of 8 weeks. This study demonstrates the potential for the underdiagnosis of *M. bovis* infection in England and Wales. Possible reasons for underdiagnosis are discussed together with strategies to optimize recovery of *M. bovis*.

INTRODUCTION

The number of herds with *Mycobacterium bovis* infection reported annually in England and Wales has increased since the 1980s. From 1988 to 1998 affected herds increased from 122 to over 700 with spread from Wales and the south-west of England to the west Midlands [1]. Fortunately, human disease caused by *M. bovis* still appears to be uncommon with between 40 and 45 cases, representing about 1–1·3% of bacteriologically proven cases of tuberculosis each year [2]. Most of these have occurred in older UK-born citizens reflecting past exposure, probably through the consumption of contaminated unpasteurized milk. However, outbreaks of *M. bovis* infection have been reported in a hospital setting elsewhere [3].

Although the number of patients with clinical disease remains small there are several reasons why the figure might be higher. Clinically *M. bovis* infection often has an extrapulmonary presentation without pathognomomic features. Samples may be taken in formalin for histological examination, which are subsequently not suitable for microbiological culture. Optimal culture of the organism requires extended incubation on pyruvate-containing media. Speciation of isolates is difficult, as *M. bovis* and *M. tuberculosis* are genetically almost identical, and requires specialized phenotypic and molecular techniques not available to routine laboratories. Commercial molecular amplification methods and DNA probes do not distinguish between the two organisms. For example, the first reported published outbreak of multi-drug resistant *M. bovis* in a Paris hospital was subsequently shown to be due to *M. tuberculosis* [4, 5]. A previous national audit of laboratory diagnosis of tuberculosis and other mycobacterial diseases did not specifically focus on the ability of laboratories to culture *M. bovis* [6].

This audit examines to what extent the above factors might influence the accuracy of current figures on the incidence of human *M. bovis* disease in England and Wales. A comparable audit is taking place in Scotland.

* Author for correspondence.

METHODOLOGY

All clinical laboratories currently sending mycobacterial cultures to the PHLS Mycobacterium Reference Unit and Regional Centres for Mycobacteriology were sent a questionnaire addressed to the Consultant Medical Microbiologist during December 1999. The questionnaire asked whether the sputum and/or lymph nodes were inoculated onto Lowenstein–Jensen medium containing glycerol, pyruvate or both types, what other solid or liquid culture media were employed, including automated systems, and the duration of incubation of samples. Respondents were also asked whether specimens were already in formalin when received by the microbiology laboratories and how often samples were lost for culture because of this. Finally the questionnaire asked what, if any, molecular diagnostic methods were employed.

RESULTS

One hundred and sixty-four questionnaires were sent out and 88 returned: a response rate of 54%.

Culture medium

Both glycerol- and pyruvate-containing media were employed for sputa by 76/88 (86·4%) of respondents with only 68 (77·3%) of the laboratories using pyruvate-containing media to culture lymph nodes. Of the 18 laboratories that specifically reported that they did not, 6 used an automated liquid system. In general, 24 (27·3%) and 53 (60·2%) used the liquid culture for sputum and lymph nodes respectively.

Duration of culture

Sputa and lymph nodes were cultured on Lowenstein–Jensen media for at least 8 weeks in 51 (80%) out of the 64 laboratories which responded to this question. A 12-week incubation was performed by 20 (31·3%) laboratories.

Formalized specimens

This question was used to determine the extent to which specimens treated with formalin were received in microbiology laboratories. Thirty-six of 64 (56·3%) laboratories reported that this happened rarely and one laboratory reported that this never happened. Only four (6·3%) laboratories reported this as occurring often. While this question does not quantify the problem it does indicate it is widespread.

Molecular methods

Only two laboratories were performing their own molecular analysis, and these were also sending isolates to the PHLS reference units, which would also perform conventional phenotypic identification.

DISCUSSION

The survey of laboratory methods supports the potential for under or misdiagnosis of *M. bovis* infection. Twenty per cent of laboratories surveyed did not use a pyruvate-containing media and 20% did not incubate slopes for the minimum recommended period of 8 weeks. Within the survey group only two laboratories performed their own molecular analysis. Fortunately in the England and Wales the PHLS network of regional mycobacterial reference centres and the Mycobacterial Reference Unit undertakes free identification of mycobacterial isolates using phenotypic methods. This reduces the incentive for laboratories to perform costly molecular identification in-house. However, laboratories which did not submit isolates to the PHLS for identification were not included in the survey. Commercial assays cannot distinguish between *M. tuberculosis* and *M. bovis* although non-commercial genotypic methods are arguably the best means of distinguishing the two species.

Within the PHLS, standardized operating procedures for mycobacterial culture are based on methodology devised by the Mycobacterial Reference Unit and regional centres. This should ensure the ability of PHLS laboratories to culture *M. bovis*. The Mycobacterial Reference Unit and regional centres also advise NHS laboratories on appropriate techniques. It is possible that the trend towards automated liquid culture systems may enhance the capabilities of diagnostic laboratories to isolate this organism. However, there are no data on the performance of commercial liquid systems in isolating *M. bovis*. Published comparisons of the continuous automated liquid systems with conventional culture methods have not specifically included samples from patients with *M. bovis* infection [7–10].

Incidence and prevalence data based on laboratory-confirmed cases represent an underestimate of the true prevalence and incidence, taking into consideration the difficulties of diagnosis and deficiencies

in laboratory practice delineated above. But the extent of this underestimation remains speculative. This study has only audited the practice of 54% of laboratories in England and Wales sending isolates to the PHLS. This may have biased the results; it is possible that laboratories that perceived their techniques for the isolation of *M. bovis* to be inadequate would have been less inclined to return the questionnaire. Using techniques currently available it might be possible to obtain prevalence data based on culture of gut-associated lymph nodes removed at surgery for other reasons.

REFERENCES

1. Anonymous. The incidence of TB in cattle – Great Britain. London: Department for Environment, Food and Rural Affairs and National Statistics, 2001.
2. Irish C, Herbert J, Bennett D, et al. Database study of antibiotic resistant tuberculosis in the United Kingdom, 1994–6. BMJ 1999; **318**: 497–8.
3. Rivero A, Marquez M, Santos J, et al. High rate of tuberculosis re-infection during a nosocomial outbreak of multidrug-resistant tuberculosis caused by *Mycobacterium bovis* strain B. Clin Infect Dis 2001; **32**: 159–61.
4. Gutierrez MC, Galan JC, Blazquez J, et al. Molecular markers demonstrate that the first described multidrug-resistant *Mycobacterium bovis* outbreak was due to *Mycobacterium tuberculosis*. J Clin Microbiol 1999; **37**: 971–5.
5. Gutiérrez MC, Bouvet E, Blazquez J, et al. Identification as *Mycobacterium tuberculosis* of previously described *M. bovis* multidrug-resistant strains. Lancet 1998; **351**: 758.
6. Watt B, Smith EG, Magee JG, et al. A national audit of the laboratory diagnosis of tuberculosis and other mycobacterial diseases within the United Kingdom. J Clin Pathol 1999; **52**: 334–7.
7. Harris G, Rayner A, Blair J, et al. Comparison of three isolation systems for the culture of mycobacteria from respiratory and non-respiratory samples. J Clin Pathol 2000; **53**: 615–8.
8. Benjamin WH, Waites KB, Beverly A, et al. Comparison of the MB/BacT system with a revised antibiotic supplement kit to the BACTEC 460 system for detection of mycobacteria in clinical specimens. J Clin Microbiol 1998; **36**: 3234–8.
9. Rohner P, Ninet B, Metral C, et al. Evaluation of the MB/BacT system and comparison to the BACTEC 460 system and solid media for isolation of mycobacteria from clinical specimens. J Clin Microbiol 1997; **37**: 3127–31.
10. Magee JG, Freeman R, Barrett A. Enhanced speed and sensitivity in the cultural diagnosis of pulmonary tuberculosis with a continuous automated mycobacterial liquid culture (CAMLiC) system. J Med Microbiol 1998; **47**: 547–53.