JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2007, p. 3434–3435 Vol. 45, No. 10
0095-1137/07/$08.00+0 doi:10.1128/JCM.00682-07

Evaluation of the BD ProbeTec ET System for Direct Detection of *Mycobacterium bovis* in Veterinary Specimens▽

David C. Bean,[1,2] Andrea Hills,[1] Terry Ryan,[3] and John Aitken[1]*

Inward Bound Ltd., Christchurch, New Zealand[1]; Centre for Infectious Disease, Barts and The London School of Medicine and Dentistry, Queen Mary, University of London, London, United Kingdom[2]; and New Zealand Food Safety Authority, Hamilton, New Zealand[3]

Received 27 March 2007/Returned for modification 9 May 2007/Accepted 2 August 2007

We describe the application of the BD ProbeTec ET direct tuberculosis system for the detection of *Mycobacterium bovis* in bovine and cervine lymph node tissues. Compared to traditional culture, the overall sensitivity, specificity, and positive and negative predictive values of the BD ProbeTec were 87, 100, 100, and 87%, respectively.

Bovine tuberculosis (Tb) is New Zealand's most serious animal health problem. It affects the throat, lungs, and associated lymph nodes of both cattle and deer and can develop into a chronic condition causing wasting and death. Although the potential for transfer to humans is low, the economic consequences of this disease are huge; dairy products and meat are New Zealand's two principal exports (accounting for 19.1 and 14.3% of total exports, respectively), worth a combined total of 10.9 billion New Zealand dollars in 2006 (11). The cost of trade barriers and restricted export markets would have severe implications for New Zealand trade.

Consequently, a great deal of research into the epidemiology and control of bovine Tb has been conducted. In many countries, bovine Tb is effectively controlled by a test-and-slaughter technique. In New Zealand, control is complicated by the presence of a number of wildlife vectors, the most important being the brushtail possum (*Trichosurus vulpecula*) (4, 8). Therefore, most strategies proposed to control bovine Tb in New Zealand concern intervening in the transmission of Tb by the eradication of the vector animals. While controlling the spread of bovine Tb is the ultimate goal of such projects, they are hampered by the lack of a fast and accurate system for the detection of *Mycobacterium bovis*. The current standard, the skin test, has been shown to have low sensitivity (10), and traditional culture takes many weeks to perform. Herein we describe the adaptation of an existing rapid diagnostic test for human Tb, the BD ProbeTec ET direct Tb (DTB) system, and assess its usefulness for the detection of bovine Tb.

The study ran through 2003 and 2004, during which 322 animals from various locations on the South Island of New Zealand were included. Of these, 160 (49.7%) were cattle and 162 (50.3%) were deer. To ensure approximately even numbers of animals with and without Tb, inclusion in the study was based on the result of a skin test. The animals with positive results (reactors) included 87 cattle (54.4%) and 85 deer (52.5%). Lymph node specimens were taken at the time of slaughter and split for culture and DTB analysis. Specimens were taken from multiple lines (a "line" is a group of animals scheduled to be slaughtered on a given day[s]) to ensure a random distribution of animals. For the reactors, samples from all deer and a maximum of eight cattle per line were obtained. For the animals with negative results (nonreactors), samples

TABLE 1. Characteristics of BD ProbeTec ET system compared to culture[a]

Animal species (no. of samples)	BD ProbeTec result	No. with culture result of:		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		Positive	Negative				
Cattle (160)	Positive	80	0	92	100	100	91
	Negative	7	73				
Deer (162)	Positive	70	0	82	100	100	84
	Negative	15	77				
Total (322)	Positive	150	0	87	100	100	87
	Negative	22	150				

[a] PPV, positive predictive value; NPV, negative predictive value.

* Corresponding author. Mailing address: Inward Bound Ltd., Christchurch, New Zealand. Phone: 64 03 331 6629. Fax: 64 03 325 7095. E-mail: jaitk@attglobal.net.

▽ Published ahead of print on 15 August 2007.

from a maximum of 10 animals per line were collected. All specimens were cultured on two Lowenstein-Jensen slopes (with and without pyruvate), and all reactor specimens were verified byculture at an independent laboratory using the BD BACTEC 460 broth culture system (Becton Dickinson). The second lymph node sample was prepared for DTB analysis; tissue was dissected, mechanically macerated, and subjected to decontamination by a standard *N*-acetyl-L-cysteine digestion procedure. Postdecontamination, the sediment pellet was resuspended and sonicated for 20 min. Supernatant from this procedure was used as a template for the DTB assay. The BD ProbeTec ET system was operated according to the instructions of the manufacturer (Becton Dickinson).

Culture determined 87 (54.4%) of the specimens from cattle and 85 (52.5%) of those from deer to be positive (Table 1). Of the culture-positive specimens, 80 from cattle and 70 from deer were positive by the DTB assay. These results yielded sensitivities of 92 and 82% for bovine and cervine specimens, respectively, and an overall sensitivity of 87%. There were no false positives for either species, giving an overall specificity of 100%. These results compare with those of other studies evaluating the DTB assay for human respiratory specimens (1–3, 9). Furthermore, the DTB system had positive and negative predictive values of 100 and 93%, respectively. The internal amplification control showed no evidence of inhibition in any of the 322 specimens.

The BD ProbeTec ET is a semiautomated real-time system that allows simultaneous amplification of a gene sequence and detection of *M. tuberculosis*. Its amplification target is the mycobacterium-specific insertion sequence IS*6110*. *M. bovis* is a member of the *M. tuberculosis* complex and has a genome sequence >99.95% identical to that of *M. tuberculosis* (5). Despite this high level of genetic similarity, *M. bovis* tends to have far fewer copies of the IS*6110*, typically containing only one or a few copies of this insertion sequence (6). Despite the lower copy number, the BD ProbeTec ET proved to be very sensitive for the detection of *M. bovis*.

The BD ProbeTec ET, although designed initially for the detection of *M. tuberculosis* in respiratory specimens, has also proven useful for the detection of Tb bacteria in nonpulmonary samples (7). In the present study, we extended this observation to show that the DTB assay is also effective for nonhuman, nonpulmonary specimens. The DTB system performed very well compared to traditional culture; the time taken to get a result is reduced from 4 to 8 weeks by culture to within 5 h of the receipt of the specimen. The rapid turnaround time of this assay may in turn reduce the reliance on skin tests and allow farmers to make confident interventions within 24 h of submitting a specimen, and the test thus offers the maximum possibility of limiting dissemination. Overall, the BD ProbeTec ET system provides a rapid and cost-effective way to monitor the spread of *M. bovis* in a veterinary environment, which in turn may potentially help prevent the spread of Tb and its associated cost.

We are grateful to Kevin Crews of the New Zealand Animal Health Board for coordination of specimen collection for this study, to Christian Beaumont, QMUL, for his comments on the manuscript, and to AgResearch, New Zealand, for their cooperation.

This study was jointly funded by DeeResearch Ltd. and the New Zealand Animal Health Board (project R80570).

REFERENCES

1. **Barrett, A., J. G. Magee, and R. Freeman.** 2002. An evaluation of the BD ProbeTec ET system for the direct detection of *Mycobacterium tuberculosis* in respiratory samples. J. Med. Microbiol. **51:**895–898.
2. **Bergmann, J. S., W. E. Keating, and G. L. Woods.** 2000. Clinical evaluation of the BD ProbeTec ET system for rapid detection of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **38:**863–865.
3. **Bergmann, J. S., and G. L. Woods.** 1998. Clinical evaluation of the BD ProbeTec strand displacement amplification assay for rapid diagnosis of tuberculosis. J. Clin. Microbiol. **36:**2766–2768.
4. **Coleman, J. D., and M. M. Cooke.** 2001. *Mycobacterium bovis* infection in wildlife in New Zealand. Tuberculosis (Edinburgh) **81:**191–202.
5. **Garnier, T., K. Eiglmeier, J. C. Camus, N. Medina, H. Mansoor, M. Pryor, S. Duthoy, S. Grondin, C. Lacroix, C. Monsempe, S. Simon, B. Harris, R. Atkin, J. Doggett, R. Mayes, L. Keating, P. R. Wheeler, J. Parkhill, B. G. Barrell, S. T. Cole, S. V. Gordon, and R. G. Hewinson.** 2003. The complete genome sequence of *Mycobacterium bovis*. Proc. Natl. Acad. Sci. USA **100:**7877–7882.
6. **Haddad, N., M. Masselot, and B. Durand.** 2004. Molecular differentiation of *Mycobacterium bovis* isolates. Review of main techniques and applications. Res. Vet. Sci. **76:**1–18.
7. **McHugh, T. D., C. F. Pope, C. L. Ling, S. Patel, O. J. Billington, R. D. Gosling, M. C. Lipman, and S. H. Gillespie.** 2004. Prospective evaluation of BD ProbeTec strand displacement amplification (SDA) system for diagnosis of tuberculosis in non-respiratory and respiratory samples. J. Med. Microbiol. **53:**1215–1219.
8. **Morris, R. S., and D. U. Pfeiffer.** 1995. Directions and issues in bovine tuberculosis epidemiology and control in New Zealand. N. Z. Vet. J. **43:**256–265.
9. **Rusch-Gerdes, S., and E. Richter.** 2004. Clinical evaluation of the semiautomated BD ProbeTec ET system for the detection of *Mycobacterium tuberculosis* in respiratory and nonrespiratory specimens. Diagn. Microbiol. Infect. Dis. **48:**265–270.
10. **Scacchia, M., R. Lelli, A. Petrini, V. Prencipe, P. Calistri, and A. Giovannini.** 2000. Use of innovative methods in the eradication of bovine tuberculosis. J. Vet. Med. B **47:**321–327.
11. **Statistics New Zealand.** 20 March 2007, revision date. New Zealand external trade statistics, December 2006. Statistics New Zealand, Auckland. http://www.stats.govt.nz/products-and-services/ext-trade-stats/default.htm.