Validation of the BrockTB Stat-Pak Assay for Detection of Tuberculosis in Eurasian Badgers (Meles meles) and Influence of Disease Severity on Diagnostic Accuracy

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A lateral-flow immunoassay (BrockTB Stat-Pak) for detecting tuberculosis in Eurasian badgers was 49% sensitive and 93% specific against culture for M. bovis (n = 1,464) at necropsy. However, the sensitivity was significantly higher (66% to 78%) in animals with more severe tuberculosis, indicating that the BrockTB Stat-Pak may be useful for the detection of badgers with the greatest risk of transmitting disease.

Despite attempts to control bovine tuberculosis (TB), the average incidence of disease in cattle in Great Britain increased an estimated 225% between the years 1996 and 2006 (7), causing considerable economic loss to the government and the farming community. In parts of Great Britain and Ireland, Eurasian badger (Meles meles) populations constitute a reservoir of infection with Mycobacterium bovis and a potential source of infection to cattle (8, 14, 15). In an effort to simplify the serodetection of badger TB, we developed a lateral-flow immunoassay (13) that is now manufactured as the BrockTB Stat-Pak assay (Chembio Diagnostic Systems, Inc., Medford, NY). We report here the results of ongoing evaluation of the assay, in particular the influence of disease severity on diagnostic accuracy.

Badger sera were obtained in Great Britain from two sources and stored frozen at −20°C until testing (14.8%) were either hemolyzed or lipemic. Although previously reported by us with a smaller sample size (13) and are equivalent to the performance of the existing serum enzyme-linked immunosorbent assay-based test for TB (Brock Test) (2, 5, 12). Although the specificity was relatively high, this is likely to be an underestimate. The badgers were sampled from parts of the country where TB is endemic, and it is likely that true infection was missed in some cases despite the use of an extensive postmortem culture protocol.

During the present study, samples that were hemolyzed and/or lipemic were identified prior to testing. Although previous work showed that the condition of these samples did not influence the Brock Test (unpublished data), the potential effect on the performance of the BrockTB Stat-Pak was unknown. A total of 216 RBCT serum samples from the 1,464 submitted to testing (14.8%) were either hemolyzed or lipemic. Compared to “normal” samples, those that were hemolyzed and/or lipemic exhibited a statistically significant reduction in sensitivity (P = 0.003, Fisher exact test), but no difference in specificity (P = 0.613, Fisher exact test) (Table 1).

Subdivision of culture-positive badgers based on criteria as-
associated with disease severity revealed an increased ability of the BrockTB Stat-Pak to detect infected badgers with more severe (progressed, disseminated) disease (Table 2). By inference, these individuals may pose a greater risk of infecting other badgers and cattle through repeated and/or greater excretion of *M. bovis* (4, 10). However, it must be borne in mind that the relationship between pathology in the badger and the likelihood of onward transmission of TB is unknown. Two criteria were used to identify such animals: either their “excretor status” (for WP badgers, which were live sampled and then released) or presentation of gross lesions at necropsy (for RBCT badgers). The BrockTB Stat-Pak detected 25 of 32 so-called “super-excretor” WP badgers, representing a sensitivity of 78.1% (95% CI = 60.0 to 90.7%). An animal was classified as a “super-excretor” after its second consecutive culture positive result or after it had provided two positive culture results from different samples (e.g., feces and urine) on its last sampling occasion (6). In addition, “super-excretors” have a higher mortality rate (17) and so probably represent animals with progressive TB that does not resolve. In contrast, “excretors” exhibit intermittent shedding or may apparently cease bacterial excretion altogether (6). The BrockTB Stat-Pak detected 15 of 36 “excretor” badgers, representing a sensitivity of 41.7% (95% CI = 25.5 to 59.3%). The difference in the sensitivity of TB detection between “super-excretor” and “excretor” badgers was highly significant (*P* = 0.003, Fisher exact test), a finding consistent with our previous observation that seropositivity in badgers was associated with more severe disease (1). Similarly, the BrockTB Stat-Pak detected 66.1% of culture-positive badgers with visible lesions suspicious of TB at necropsy (VL) compared to only 34.4% of those with no suspect TB lesions at necropsy (NVL) (Table 2). The difference in sensitivity was again highly significant (*P* < 0.0001, Fisher exact test). We have recently demonstrated improved detection rates of TB in the badger using quantitative real-time PCR (16) and an enzyme immunoassay (5) to detect the antigen-specific production of gamma interferon (IFN-γ). For both the IFN-γ enzyme immunoassay and the Brock Test, the sensitivity was also significantly higher in badgers with VL than in those with NVL (5). However, the Brock Test and both IFN-γ tests require specialist laboratory facilities and equipment and take 3 and 48 h, respectively, to complete. In contrast, the BrockTB Stat-Pak can be performed anywhere and produces a result in no more than 20 min.

The BrockTB Stat-Pak had the lowest sensitivity in culture-positive NVL badgers, which would be consistent with the suggestion that this group is proportionally less likely to be excreting *M. bovis* (10). Moreover, not all VL badgers will excrete *M. bovis*, since this will depend on the anatomical location of the lesion, its structure, and the quantity of bacteria contained therein (10, 11). This is consistent with the lower sensitivity of the BrockTB Stat-Pak for VL badgers than that seen for “super-excretors.” Overall, these data are consistent with a positive BrockTB Stat-Pak test result being more frequently associated with advanced TB, especially that which gives rise to more extensive or persistent shedding of bacteria.

In summary, the BrockTB Stat-Pak is an easily executed and rapid test for the detection of TB in badgers. The BrockTB Stat-Pak could be performed directly in the field, although consideration should be given to the quality of the blood sample so that test sensitivity is not adversely affected. With a sensitivity of 49% compared to the “gold standard” of necropsy tissue culture, the BrockTB Stat-Pak is unlikely to be sufficiently sensitive for routine TB surveillance. However, the sensitivity was significantly higher in animals with more severe TB, classified by more frequent excretion of *M. bovis* or the presence of visible lesions at necropsy. This raises the possibility that the BrockTB Stat-Pak could be of use where a simple tool is required to detect badgers more likely to be at advanced stages of disease.

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**REFERENCES**


