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PERFORMANCE OF AN ELISA IN DETERMINING THE *MYCOBACTERIUM BOVIS* STATUS OF BADGER (*MELES MELES*) SETTS

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On a commencé un essai en 1994 dans le sud-ouest de l'Angleterre pour comparer deux méthodes de contrôle de blaireaux (Meles meles) sur l'incidence de la tuberculose bovine. Dans un cas tous les blaireaux d'une propriété ayant eu un foyer sont piégés et éliminés, dans l'autre, on ne retire que les animaux positifs à un test ELISA indirect, dans un terrier où sont présents des animaux infectés.
Dans ce dernier cas, l'opération a donné une sensibilité individuelle de 40,7 % et une spécificité individuelle de 94,3 % au test ELISA et des chiffres respectivement de 72,9 % et 81,2 % au niveau population.
Sur le terrain d'étude, l'organisation spatiale des groupes de blaireaux était redéfinie chaque année. La zone était divisée en cinq secteurs de piégeage et chaque secteur était piégé 4 fois par an. Les échantillons cliniques (raclage, trachées, fèces, urine) étaient mis en culture. Une prise de sang était testée sur ELISA indirect.
Selon le critère de positivité, l'ELISA, comme outil de dépistage pour l'infection d'un terrier donnait une sensibilité de 34,9 % à 67,2 % et une spécificité de 91,0 % à 91,8 %.
Les raisons des erreurs de diagnostic sont discutées avec une référence particulière pour la sensibilité de l'échantillonnage comme outil pour définir un statut d'infection et selon la campagne de piégeage.

INTRODUCTION

Badgers (*Meles meles*) are now implicated in approximately 90% of cattle tuberculosis breakdowns in south west England. Since 1986, efforts to decrease the risk of transmission from this wild life species have involved trapping and killing badgers, with removal operations limited to the land where herd infection with *Mycobacterium bovis* originally occurred. Although not proven, the increase in herd breakdowns since implementation of this strategy has been ascribed in part to the circumscribed nature of these removal operations.
A trial commenced in 1994 (Report, 1994) in south west England comparing the effects of two approaches to badger control. The first approach continued the strategy started in 1986, whereas the second, based on an indirect ELISA, developed in response to recommendations in the Dunnet report (Dunnet et al, 1986), for detecting *M.bovis*-specific antibodies in blood taken from the live badger (Goodger et al, 1994), aimed firstly at identifying setts containing infected animals and then restricting badger removal to those setts. In this way, it was considered that unnecessary disturbance of social groups could be avoided and removal of uninfected badgers reduced while extending trapping to infected setts beyond the geographical boundaries of the index case.
The ELISA was assessed prior to inception of the trial in terms of both the practicalities of using the test under field conditions and its performance. Approximately 2000 blood samples were tested from badgers taken in over 200 statutory removal operations during 1991-1993 (Clifton-Hadley et al, 1995). The results indicated a sensitivity of 40.7% and a specificity of 94.3% for individual animal samples. When used as a screening test for badger 'populations' grouped by removal operation, the equivalent figures were 72.9% and 81.2%. Since badgers could not be reliably ascribed to particular setts, test performance based on aggregated data from animals trapped at specific setts could not be derived from the same database.
During the course of a prospective study of a naturally infected badger population on the Cotswold escarpment in south west England, blood samples have been regularly taken for testing with the ELISA. In this study the sett related to each capture is recorded and it was thought that additional information on test performance might be derived from this dataset.

MATERIALS AND METHODS

The study area comprises approximately 9 km^2, with one of the highest recorded adult badger densities in the country. Results from 32 social groups, delineated principally by an annual bait marking exercise in spring, were considered in the current analysis. A capture-mark-recapture programme provided population structure data and the opportunity for collecting samples for bacterial isolation and serology. Details of the study methodology have been described elsewhere (Cheeseman et al, 1988).
At first capture the sex of each animal and social group and sett location of capture were recorded. At each capture, clinical samples were collected under ketamine hydrochloride anaesthesia for bacterial isolation of *M.bovis*. These included samples of urine and faeces, tracheal aspirates or laryngeal swabs, and needle biopsies or swabs of abscesses and bite wounds. Blood samples were taken for serological examination using the indirect ELISA. If animals were known to be excreting *M.bovis* from a previous sampling, they were radio-tagged to increase the probability of recovering the carcase for *post-mortem* examination.

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All badger carcases found in or, if captured previously, near the study area were examined for the cause of death and subjected to *post-mortem* examination. Tuberculous lesions were recorded if present and tissue samples from all lymph nodes and major internal organs taken for bacteriological examination.
A record was compiled for each badger consisting of its date of birth where known, its sex, each capture date and location of trapping, the clinical samples taken at each capture and culture results for each sample, the serological results from ELISA tests when blood samples had been taken, and the cause of death and results of any *post-mortem* examination.
From October 1987 the site was divided into five sectors which were trapped in rotation on four occasions each year. Each occasion represented two consecutive nights' trapping. In the present study, analysis was restricted to the whole years 1988 to 1995. Each year was split into quarters (quarter 1 = Jan-Mar etc). Individual capture records with ELISA results were used in the analysis provided that there were four or more captures within a sector in any quarter. This ensured that only capture data collected on main trapping occasions were considered. From this database the number of captures with ELISA results and the infection status at each sett over any specified time period could be calculated.
The performance of the test was investigated using two scenarios based on the criteria for classifying a badger as infected with *M.bovis*. 1) Badgers were designated infected if *M.bovis* was isolated from one or more clinical samples during their lifetimes. 2) Badgers, which during their lifetimes had a single, *M.bovis* positive clinical sample followed by two or more captures where all samples were negative, and which were negative at any *post-mortem* examination, were excluded from the positive category.
For this analysis, a badger was positive from the first positive sample date, unless it had a previous capture record, in which case it was counted as positive from half way between its first positive sampling and its previous negative sampling. If a positive badger was caught in different setts it counted as positive to mid-way between the captures in its original sett and then in the second sett from that point on.

RESULTS

The sensitivity, specificity and predictive values of the indirect ELISA for determining sett infection status, under the two scenarios, are summarised in Tables I a and I b.

Table I a
Scenario 1. Badgers considered infected if *M.bovis* cultured from any sample

	Sensitivity %	Specificity %	Predictive value of a positive test	Predictive value of a negative test
Overall	34.9 (29.8-40.3)	91.8 (89.9-93.4)	57.3 (50.1-64.2)	81.7 (79.4-83.9)
Quarter:				
1	23.9 (14.9-35.8)	95.3 (91.7-97.4)	58.6 (39.1-75.9)	81.8 (76.8-85.9)
2	43.0 (32.1-54.6)	93.7 (89.4-96.4)	70.8 (55.7-82.6)	82.2 (76.8-86.6)
3	30.8 (22.3-40.7)	87.8 (83.5-91.1)	45.7 (33.9-58.0)	79.2 (74.5-83.3)
4	42.5 (31.2-54.6)	91.6 (87.5-94.6)	59.6 (45.1-72.7)	84.5 (79.5-88.5)
ELISAs per sett:				
1-2	30.8 (22.3-40.7)	95.5 (93.3-97.0)	57.1 (43.3-70.0)	87.6 (84.5-90.1)
3-5	34.7 (26.5-43.8)	90.7 (86.9-93.5)	58.9 (46.8-70.1)	78.3 (73.7-82.3)
6+	39.4 (29.9-49.7)	83.2 (76.8-88.1)	55.7 (43.4-67.4)	71.8 (65.2-77.7)

( ) 95% confidence interval

DISCUSSION

Results from animals with single culture-positive samples, which are then not confirmed at subsequent captures, had a major influence on ELISA performance and led us to reassess the data, excluding these animals from the infected category. These single positive records may represent several possible events such as transient infection or initial infection with subsequent latency (although no infection was found in several of these badgers at *post-mortem* examination). By filtering out such records, the emphasis then was on examining how the ELISA would perform in setts where infection was represented by badgers in which not only could the infection status be relied on but also disease was more likely to be progressive.
Under both scenarios the sensitivity of the ELISA was lowest in the first quarter of the year. Although not statistically significant, larger numbers might confirm this finding. Certainly, the difficulties of trapping during the early part of the year are greater than at other times, reducing the chances of catching any ELISA-positive, culture-positive animal, so contributing to this result.
Contrary to expectations, there was no significant increase in sensitivity as the number of captures/sett increased in scenario 1. However, there was under scenario 2. This suggests that under scenario 1 there may be an effect from badgers labelled as culture-positive where subsequent history suggests that, at least for some of them, this may not represent their true status.

Table I b
Scenario 2. Badgers with a single *M.bovis* positive sample excluded from the infected category

	Sensitivity %	Specificity %	Predictive value of positive test	Predictive value of a negative test
Overall	67.2 (58.4-75.0)	91.0 (89.2-92.5)	44.2 (37.3-51.4)	96.3 (95.0-97.3)
Quarter:				
1	48.1 (29.2-67.6)	94.6 (91.3-96.8)	44.8 (27.0-64.0)	95.3 (92.0-97.3)
2	75.8 (57.4-88.3)	91.4 (87.2-94.4)	52.1 (37.4-66.5)	96.8 (93.6-98.5)
3	66.7 (49.7-80.4)	88.3 (84.5-91.3)	37.1 (26.1-49.6)	96.2 (93.5-97.9)
4	75.0 (56.2-87.9)	90.4 (86.2-93.4)	46.2 (32.5-60.4)	97.0 (94.0-98.6)
ELISAs per sett:				
1-2	60.4 (45.3-73.9)	95.4 (93.3-96.9)	51.8 (38.2-65.2)	96.7 (94.8-98.0)
3-5	57.1 (42.3-70.9)	88.7 (85.1-91.6)	38.4 (27.4-50.5)	94.4 (91.4-96.4)
6+	91.2 (75.2-97.7)	84.3 (79.1-88.5)	44.3 (32.6-56.6)	98.6 (95.6-99.6)

( ) 95% confidence interval

Clinical sampling cannot be considered as sensitive a method of determining a badger's infection status as *post-mortem* examination, especially given the intermittent nature of bacterial shedding (Clifton-Hadley et al, 1993). Therefore, misclassification of infected badgers as uninfected will occur and will tend to depress the ELISA's specificity. Further misclassification of sett infection status will have occurred by designating every day of a badger's life 'uninfected' or 'infected' by using discrete trapping records. In this way, for any one quarter, a sett may be classified as infected without the infected badger being trapped in that quarter. This will in effect act to depress the test's sensitivity.

There was a significant difference ($P<0.001$) in sensitivity (34.9% v 67.2%) when the results using scenario 1 or 2 were compared, while specificity remained similar (91.8% v 91.0%). These specificity figures suggest that under both scenarios there is misclassification of animals occurring by labelling animals as seroconverting while infection was not detectable. This could be accounted for both by false positive ELISA results and by failure to detect infection from clinical samples resulting from the intermittent nature of *M.bovis* excretion. Conservatively, about 50% of animals with culture-positive results at *post-mortem* examination have not been detected as infected from previous clinical samples taken within one year of an animal's death. Therefore, a proportion of the apparent false positive ELISA results will in fact be correct results.

From these points it can be seen that any extrapolation of test performance from the current study to the field trial is unjustified since it is likely that, using this dataset, the performance of the ELISA at the sett level will be underestimated both in terms of its sensitivity and specificity.

BIBLIOGRAPHY

Cheeseman C.L., Wilesmith J.W., Stuart F.A., Mallinson P.J., 1988. Dynamics of tuberculosis in a naturally infected badger population. Mammal Review 18 (1), 61-72.

Clifton-Hadley R.S., Sayers A.R., Stock M.P., 1995. Evaluation of an ELISA for *Mycobacterium bovis* infection in badgers (*Meles meles*). Veterinary Record 137, 555-8.

Clifton-Hadley R.S., Wilesmith J.W., Stuart F.A., 1993. *Mycobacterium bovis* in the European badger (*Meles meles*): Epidemiological findings in tuberculous badgers from a naturally infected population. Epidemiology and Infection 111 (1), 9-19.

Dunnet G.M., Jones D.M., McInerney J.P., 1986. Badgers and Bovine Tuberculosis - Review of Policy. London: HMSO. 73 pages.

Goodger J., Nolan A., Russell W.P., Dalley D.J., Thorns C.J., Stuart F.A., Croston P., Newell D.G., 1994. Serodiagnosis of *Mycobacterium bovis* infection in badgers: development of an indirect ELISA using a 25 kDa antigen. Veterinary Record 135, 82-5.

Report, 1994. Bovine tuberculosis in badgers. Seventeenth report by the Ministry of Agriculture, Fisheries and Food. London: MAFF Publications. 37 pages.