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THE USE OF SEROLOGICAL TECHNIQUES TO DEMONSTRATE FREEDOM FROM JOHNE'S DISEASE IN THE AUSTRALIAN SHEEP INDUSTRY

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La maladie de Johne, due à une infection à Mycobacterium paratuberculosis, a été diagnostiquée pour la première fois dans les troupeaux de moutons australiens en 1980. Depuis, la maladie a été identifiée dans trois Etats australiens (Nouvelles Galles du Sud, Victoria et Tasmanie). La maladie clinique n'a pas été mise en évidence dans les autres Etats d'Australie hébergeant des élevages de moutons (Queensland, Australie méridionale, Australie occidentale). Il a été proposé que ces Etats lancent un programme de surveillance pour démontrer qu'ils sont indemnes de l'infection à M. paratuberculosis. Cependant, la mise en évidence du caractère indemne est compliquée du fait de la faible sensibilité des tests sérologiques disponibles, de la longue période d'incubation de la maladie, et de la nature des signes cliniques observés dans les troupeaux de moutons infectés. Nous présentons ici une option sérologique pour démontrer le caractère indemne de l'infection à M. paratuberculosis pour laquelle la réduction de la taille de l'échantillon et des coûts afférents a été permise par l'utilisation conjointe de différents tests.

En maximisant la sensibilité au niveau du troupeau, nous estimons qu'une réduction de 18% du nombre de moutons à tester peut être atteinte, soit une économie potentielle de 130 000 dollars. L'exemple présenté démontre qu'un programme de surveillance de la maladie de Johne peut être mis en oeuvre d'une manière rentable si les objectifs des programmes de surveillance sont bien assimilés et si l'on tire parti du comportement des tests sérologiques au sein des populations de bétail.

INTRODUCTION

Johne's disease in sheep, caused by infection with *Mycobacterium paratuberculosis*, is a slowly progressive disease characterised by ill-thrift and wasting. The history of ovine Johne's disease in Australia has been reviewed by Denholm et al. (1997). The disease was first diagnosed in 1980 in a flock of sheep in New South Wales. Johne's disease was detected in Victorian sheep flocks in 1995, and in Tasmanian flocks in 1996. To date, nearly 200 sheep flocks in Australia are known to be infected. An eradication campaign has commenced in Victoria, with control plans in place in New South Wales. In addition, a national market assurance program has been developed. The spread of Johne's disease in Australia has been primarily through the movement of infected sheep. Nearly half of the total Australian flock of 120 million sheep are potentially at-risk of infection.

In order to define the extent of Johne's disease in the Australian sheep flock, sheep-raising States in which Johne's disease has not been diagnosed (Queensland, South Australia and Western Australia) are investigating methods to demonstrate freedom of their flocks from *M paratuberculosis* infection. However, claims of freedom are complicated by the poor sensitivity of available serological tests, the long incubation period of the disease, and the nature of clinical signs observed in infected sheep flocks. In Australia, an absorbed enzyme-linked immunosorbent assay (ELISA) is the most commonly used test for serological assessment of sheep. Although the sensitivity of this ELISA is estimated to be only about 50% in non-clinical cases, it is highly specific (Eamens, 1995). Despite suboptimal test sensitivty, the ELISA is still a potentially important tool in the control of Johne's disease in the Australian sheep flock. In particular, if the aim of a program is to demonstrate freedom from *M paratuberculosis* infection, the use of the ELISA on a flock basis may be appropriate if flock-level test characteristics are used to advantage.

Demonstration of freedom from Johne's disease in States in which clinical disease has not been recognised has two distinct components - individual testing of sheep and testing of sheep flocks. At the individual level, serological tests applied have a poor negative predictive value because of poor test sensitivity. However, the aim of surveillance programs in States in which Johne's disease has never been diagnosed is to demonstrate freedom of the State flock. In this situation, the status of individual sheep flocks is irrelevant. Recognising the intrinsic difference between demonstrating freedom of a state or national flock and applying a disease status to an individual flock within an endemic Johne's disease area allows serological tests to be used more efficiently.

In this paper we describe a serological approach to demonstrating freedom from ovine Johne's disease. The behaviour of the ELISA when used as a flock test is discussed, and methods to increase the efficiency of surveillance programs are examined.

METHODOLOGY

Sample size calculations were performed assuming an average State sheep population of 5000 flocks. South Australia and Western Australia have more flocks than this (9050 and 9310, respectively) and Queensland has less (2840) (Australian Bureau of Statistics, 1993). An average flock size of 2500 sheep was assumed. The

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ELISA was assumed to have a sensitivity of 50% and a specificity of 99% at the individual animal level. On a flock basis, the system involves follow-up (post-mortem and histopathological examination) of all sheep reacting in the ELISA, and was assumed to have a flock specificity of 100%. An animal prevalence within flocks of 5%, and a flock prevalence of 2%, was assumed.

Once more than one sheep is tested for *M paratuberculosis* infection within a flock, a new test attribute needs to be considered - aggregate test sensitivity (which will be referred to as flock-level sensitivity). Flock-level sensitivity can be defined as the proportion of truly infected flocks that are classified as infected by a test procedure applied to the flock (Jordan, 1996). Flock-level sensitivity depends on the probability of obtaining one or more test positive results (p[T+]) from testing a number (n) of individual sheep:

$$\text{flock-level sensitivity} = 1 - (1 - p[T+])^{n} \qquad (1)$$

where the probability of obtaining one or more test positive results depends on the prevalence of disease in the flock being tested and individual test characteristics (Jordan, 1996):

$$p[T+] = \text{prevalence} * \text{sensitivity} + (1 - \text{prevalence}) * (1 - \text{specificity}) \qquad (2)$$

In a program to demonstrate freedom from Johne's disease, flock-level sensitivity has more importance than flock-level specificity from an industry perspective because an assurance must be given that the chance is negligible of potentially infected flocks being classified as test-negative, and therefore that results from a surveillance program are credible.

The number of sheep to be sampled to obtain a flock-level sensitivity of 100% was calculated using the program FreeCalc (Cameron and Baldock, 1997), for flock sizes in the range 500 to 5000. Sampling without replacement (hypergeometric distribution) was assumed. Sample size calculations were also performed to determine the number of sheep to be tested per flock using the conventional method of setting type I error (1 - flock sensitivity, α) and type II error (1 - flock sensitivity, β) to 0.05. Using flock specificity of 100% (assuming all sheep reacting to the ELISA are followed-up and shown to be false-positive reactors) and flock sensitivty of either 100 or 95%, the number of flocks to be tested was calculated (Cameron and Baldock, 1997).

RESULTS

The number of sheep which need to be tested to achieve a flock-level sensitivity of 100%, or a flock-level sensitivity and specificity of 95%, for various flock sizes is shown in Table I.

Table I

The number of sheep per flock which need to be tested with a Johne's disease absorbed ELISA sensitivity 50%, specificity 99%) to achieve a flock-level sensitivity of 100%, or a flock-level sensitivity and specificity of 95%, for various flock sizes assuming a prevalence of infection of 5% within flocks

Flock size	Flock sensitivity 100%	Flock sensitivity/specificity 95%
500	321	292
750	367	307
1000	378	328
1250	378	328
2500	378	328
5000	378	328

Assuming that follow-up of all sheep reacting to the ELISA provides a flock-level specificity of 100%, it was calculated that 149 flocks would need to be tested if the flock-level sensitivity in each flock tested is maintained at 100%. If flock-level sensitivity is 95%, then 157 flocks would need to be tested. The total number of sheep which would need to be examined using the two methods was calculated to be 48,872 and 59,346, respectively. Therefore, it was estimated that a reduction of 18% in sample size could be achieved if a sampling method is used which maximises flock-level sensitivity.

DISCUSSION

Recognising the different objectives of control and surveillance programs enables efficiencies to be made in the number of animals which must be examined to detect disease. In the example presented, a reduction of 18% in the total number of sheep tested could be achieved if flock-level sensitivity is maximised, rather than attempting to optimise both flock-level sensitivity and specificity. The objective in disease control programs is to determine, within the constraints of sampling and test imperfection, the disease status of individual flocks within a defined zone, state or nation. However, in surveillance programs for diseases which have not been previously recognised and where demonstration of freedom is desired for trade and market advantage, information on the disease status of individual flocks is not of primary concern. In surveillance programs, the objective is to assess the entire population within the zone, state or nation.

Test sensitivity is the critical factor in surveillance programs, because falsely declaring a flock to be uninfected (reduced flock sensitivity) has a greater cost associated with it for livestock industries than falsely declaring a flock to be infected (reduced flock specificity). Reduced flock sensitivity leading to a false declaration of disease freedom may undermine confidence in future declarations. In addition, the cost of allowing Johne's disease to spread to uninfected flocks needs to be considered. In contrast, follow-up post-mortem investigations to clarify positive test reactions are relatively inexpensive in the Australian sheep industry, compared to the cost of failing to detect infected flocks. The affect of follow-up of serological test reactors on test specificity and therefore flock-level sensitivity must also be considered in such a program.

It is the fundamental difference in objectives of surveillance, compared to control programs, which enables cost efficiencies to be made. In surveillance programs, cost is an important factor, particularly when the perceived benefit of demonstrating freedom is difficult to measure. In the case of ovine Johne's disease surveillance in Australia, costings can be estimated. We estimate that it costs approximately $9.50 per sheep for serological testing, including laboratory costs, disposables, labour and travel, and $280 per serological reactor which is followed-up by post-mortem and histopathological examination. Thus, using the conventional method, the cost of demonstrating freedom in a 5000-flock population would be approximately $730,000. In contrast, the cost associated with using the method to maximise flock sensitivity would be $600,000, a cost saving of $130,000.

The recognition of Johne's disease in Australian sheep flocks within the past 17 years has challenged veterinary epidemiologists to design and implement disease control and surveillance programs. Early detection of Johne's disease within a population is the key to successfully eradicating the disease. With eradication and market assurance programs already initiated in infected States, States in which the disease has not been recognised must implement surveillance programs to maintain market access. We believe that a successful surveillance program can be implemented despite dealing with a disease which has a long incubation period, non-specific clinical signs, and for which imperfect serological tests are available. Although declaration of freedom from Johne's disease can not be solely based on active serological surveillance programs, but also needs to take into account factors such as veterinary infrastructure and passive surveillance information, taking into account the behaviour of tests available within sheep populations will assist the process of gathering data to demonstrate freedom from Johne's disease.

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