A theoretical basis for the use of a skin test for brucellosis surveillance in extensively-managed cattle herds

S.C. MACDIARMID *

Summary: The theoretical basis for a system based on a delayed hypersensitivity skin test for screening extensively managed cattle herds for the presence of brucellosis is discussed. Calculations based on the hypergeometric distribution and test sensitivity are used to calculate the probabilities with which the skin test may identify herds in which infection is present at different prevalence. It is concluded that despite the relatively low sensitivity of the skin test the probability of its identifying a herd as infected is sufficiently high for it to be used as a low-cost screening test.

KEYWORDS: Brucellosis - Cattle diseases - Delayed type hypersensitivity - Disease surveillance - Skin test.

While whole-of-herd serological screening is an essential tool for the eradication of bovine brucellosis, there remains a need for a cheaper form of monitoring, both to locate infected herds in the early phase of a national eradication programme and also to maintain surveillance over districts or countries from which brucellosis is believed to have been eradicated.

In areas from which bovine brucellosis has been eradicated, surveillance for the disease needs to be maintained for a number of years to ensure that there is no recrudescence of possible latent infections (13), to monitor for the possible reintroduction of infection, and to satisfy the requirements for international recognition of brucellosis-free status (17). The question confronting veterinary authorities is which available surveillance method is the most cost-effective.

The New Zealand Ministry of Agriculture and Fisheries has been examining the use of a delayed hypersensitivity skin test (14, 15).

SKIN TEST ALLERGENS

Because hypersensitivity to Brucella antigens is similar to that to tuberculin, it has been proposed from time to time that a delayed hypersensitivity (DH) skin test could be used for the detection of bovine brucellosis (2, 18).

However, various shortcomings have hindered the widespread adoption of DH tests. In the past, the allergen preparations available were usually poorly purified extracts of Brucella and contained large quantities of lipopolysaccharide (LPS)
The presence of LPS in allergen preparations led to several problems, including non-specific reactions, the induction of serological responses and toxic reactions (7, 9, 12, 20).

Various workers realised that if the shortcomings associated with the presence of LPS could be overcome, a DH skin test could be applied as a low-cost surveillance test aimed at identifying herds infected with brucellosis (7, 8). A simple test procedure, based on the intradermal injection of a suitable allergen, could be carried out at the same time as tuberculin testing and would eliminate the need for the collection, processing and testing of large numbers of blood samples. Such a test would find favour in situations where herds are grazed extensively, as cattle could be returned to distant grazing as soon as the test was read, rather than being held near the yards until laboratory results were known (15).

In 1970, Bhongbhibhat and co-workers (4) published a method of preparing a LPS-free protein allergen from Brucella. Such purified protein allergens do not cause non-specific inflammatory reactions at the injection site, nor do they induce a serological response (7, 9, 11, 12, 16). The majority of protein antigens are common to all species of the genus Brucella and so an allergen prepared from one species may be used as a diagnostic aid for the detection of infection by any of the Brucella species (12).

The most widely studied of the various purified protein allergens is known as brucellin INRA (22)*. Prepared from cultures of B. melitensis (11), brucellin INRA has been reported to have a sensitivity (19) of between 70% and 75% when used to detect B. abortus infection in cattle (8, 9, 10).

The specificity (19) of intradermal tests using purified protein allergens is generally very high (between 95% and 100%) (7, 15, 21) although previous vaccination with adjuvanted killed strain 45/20 vaccine (but not living strain 19) will result in positive test results (6, 11, 21).

The relatively low sensitivity of the brucellin INRA skin test limits its usefulness as a tool for the eradication of brucellosis from an infected herd. However, it would be suitable for the low-cost screening of a cattle population to identify those herds which are infected with brucellosis (7, 8, 15). Skin tests are used in a similar fashion in a number of countries to screen flocks of small ruminants for Brucella melitensis infection (3, 10).

THE PROBABILITY OF DETECTING INFECTION

To assess the suitability of a surveillance system based on the use of a DH skin test, two approaches may be taken. Firstly, one may calculate the probability that the system will detect at least one infected animal in a herd in which infection is present at a stated prevalence. Alternatively, one may calculate the minimum prevalence of infection which may be detected with a stated confidence level. Both these calculations can be made from equations based on the hypergeometric distribution modified to take into account test sensitivity**.

* Brucellergen, Rhône-Mérieux, Lyons, France.
** Equation 1 was derived by R.M. Cannon of the Australian Bureau of Rural Science. A paper describing the derivation of Mr Cannon's equation is in preparation. Previously published equations either fail to take into account test sensitivity (e.g., ref. 5) or are based on the binomial, rather than the hypergeometric, distribution (e.g., ref. 1).
The probability that a given test will identify a herd as infected may be calculated as $1 - \beta$, where $\beta$ is the probability that there are no test-positive animals found in the sample given that there is a true infection prevalence in the herd of $p$. $\beta$ is calculated as:

$$\beta = (1 - \frac{n \times Se}{N}) \times pN$$  [equation 1]

Where:

Se = sensitivity of the test
N = herd size
n = sample size

By rearranging equation 1, it is possible to calculate the minimum prevalence of true infection, $p$, which must be present in a herd for a given test system to identify at least one reactor with a nominated confidence level equal to $(1 - \beta_m)$.

$$p = \frac{\log \beta_m}{N \times \log (1 - \frac{n \times Se}{N})}$$  [equation 2]

Where: $\beta_m$ = nominated probability that there will be no test-positive animals in the sample.

In other words, if the testing protocol detects no reactor animals in a sample of size $n$ from a herd of size $N$, then we can be 95% confident (or whatever level we nominate for $1 - \beta_m$) that the herd is either free of infection or has a prevalence less than $p$.

If it is assumed, on the basis of published studies (8, 9, 10), that brucellin INRA has a sensitivity of 70%, then a whole-of-herd test using this allergen has a 70% probability of identifying that herd as infected when it contains a single infected animal. Using equation 1 it can be calculated that when the prevalence of infection is 1%, the probability of identifying a herd as infected increases with increasing herd size, exceeding 99% in herds of 400 or more animals (Fig. 1). Even where within-herd prevalence is only 0.5%, the probability of identifying the herd as infected is greater than 90% for herds containing 400 or more animals (Fig. 1).

It may be considered that whole-of-herd testing is too expensive in larger herds, in which case a sample of animals only could be tested. Figure 2 shows the probability of identifying a herd as infected where true prevalence exceeds 1% and where the maximum number of animals tested is 250 and 300. It can be seen that where 300 animals are tested, the probability of identifying a 1,000-head herd as infected is approximately 90%.

The maximum sample size selected for a surveillance system based on brucellin INRA would be determined by the distribution of herd sizes in the area under surveillance. Where there are a large number of big herds in an area, the sample size selected would need to be greater than in an area where most herds were relatively small. For instance, if few herds exceeded 400 head, a sample of 250 might be considered sufficient. On the other hand, if a significant number of herds contained 900 to 1,000 animals, a sample size of 300 might be preferable.

If one is satisfied that a 95% confidence level is sufficient, then using equation 2 one may calculate the minimum prevalence of infection to be detected by a
The probabilities of a surveillance system based on brucellin INRA detecting bovine brucellosis in different sized herds in which 1% and 0.5% of animals are infected. Sensitivity of brucellin INRA taken as 70%.

**FIG. 1**

The probabilities of a surveillance system based on brucellin INRA detecting bovine brucellosis in different sized herds where 1% of animals are infected and number tested equals: (a) whole of herd; (b) maximum of 250; (c) maximum of 300.
surveillance system based on brucellin INRA (Fig. 3). For example, it can be seen that the minimum prevalence which can be detected in a 400-animal herd is 0.5%. If a 99% confidence level is required, then the minimum prevalence detected in a 400-animal herd is 1%. At a 90% confidence level, 0.5% is the minimum prevalence detected in the same-sized herd.

![Graph showing the minimum prevalence of brucellosis likely to be detected in herds of different sizes by a surveillance system based on brucellin INRA, sensitivity taken as 70%]

**Fig. 3**

The minimum prevalence of brucellosis likely to be detected in herds of different sizes by a surveillance system based on brucellin INRA, sensitivity taken as 70%

**THE ADOPTION OF A SKIN TEST**

Substantial financial savings could be expected should a DH skin test for brucellosis be substituted for a serological test in the routine surveillance of extensively-managed cattle herds. However, present international recognition of country freedom from brucellosis requires that each herd should be subject to periodic **serological** tests (17). A barrier, therefore, to the adoption of a surveillance system based on a DH skin test remains the attitude of international veterinary regulatory authorities.

Organisations such as the Office International des Epizooties must be convinced that a system incorporating this test provides satisfactory guarantees of national freedom from brucellosis. An examination of the properties of a surveillance system based on the use of the brucellin INRA skin test shows that such a test is sufficiently sensitive to provide excellent assurances of freedom from infection when larger herds are tested (Figs. 1 & 2). This may also hold true where a sampling system, rather than whole-of-herd testing, is examined (14). In addition, it may be argued (14, 15) that a surveillance system based on a DH skin test is at least as good as one based
on abattoir surveillance. A skin test has the advantage of allowing regular and reliable sampling of all herds, rather than the haphazard sampling inherent in slaughterhouse surveillance.

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FONDEMENTS THÉORIQUES DE L'UTILISATION D'UNE ÉPREUVE CUTANÉE POUR LA SURVEILLANCE DE LA BRUCELLOSE DANS LES TROUPEAUX DE BOVINS EN ÉLEVAGE EXTENSIF. - S.C. MacDiarmid.

Résumé : L'auteur étudie les fondements théoriques d'un système reposant sur une réaction cutanée d'hypersensibilité retardée pour le dépistage de la brucellose dans les troupeaux de bovins en élevage extensif. Il utilise une méthode de calcul fondée sur la distribution hypergéométrique et la sensibilité de l'épreuve pour déterminer le degré de probabilité pour que la réaction cutanée identifie les troupeaux dans lesquels l'infection est présente avec une prévalence variable. La conclusion de l'étude est que, malgré la sensibilité relativement peu élevée de la réaction cutanée, la probabilité qu'elle identifie un troupeau comme étant infecté est suffisamment grande pour qu'on puisse l'employer comme épreuve de dépistage peu coûteuse.


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FUNDAMENTOS TEÓRICOS DEL USO DE UNA PRUEBA CUTÁNEA PARA VIGILANCIA DE LA BRUCELOSIS EN CRÍA EXTENSIVA DE REBAÑOS DE BOVINOS. - S.C. MacDiarmid.

Resumen: El autor estudia los fundamentos teóricos de un sistema basado en una reacción cutánea de hipersensibilidad retardada para la detección de la brucelosis en rebaños bovinos en cría extensiva. Utiliza un método de cálculo a partir de la distribución hipergeométrica y de la sensibilidad de la prueba capaz de determinar el grado de probabilidad de que la reacción cutánea identifique los rebaños en que la infección se presenta con prevalencia variable. La conclusión es que, a pesar de la sensibilidad relativamente poco elevada de la reacción cutánea, las probabilidades de identificación de un rebaño infectado que ésta permite son suficientes para que su empleo como prueba de detección poco costosa sea conveniente.

PALABRAS CLAVE: Brucelosis - Enfermedades bovinas - Hipersensibilidad retardada - Prueba cutánea - Vigilancia epidemiológica.
REFERENCES


