*J. Hyg., Camb.* (1986), **96**, 403–413
*Printed in Great Britain*



# Comparison of the brucellin skin test with the lymphocyte transformation test in bovine brucellosis

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(*Received 18 December 1984; accepted 28 May 1985*)

## SUMMARY

The brucellin skin test and the lymphocyte transformation test were compared in heifers infected with virulent *Brucella abortus* strain 544, heifers vaccinated against brucellosis and unexposed cattle. Results of the *in vitro* lymphocyte transformation test were consistently positive for all 9 *Brucella*-infected heifers while the skin test was consistently positive for 6 of the 9 heifers. In 7 heifers repeatedly vaccinated with *B. abortus* strain-19 vaccine the *in vitro* test classified 3 animals as positive whereas the skin test identified all the animals as infected during most of the experimental period. Four heifers injected with a single dose of *B. abortus* strain 19 were consistently negative to the lymphocyte transformation test while the skin test classified all the animals as infected during most of the experimental period. The skin test gave strong reactions indicative of *Brucella* infection in heifers vaccinated with 'Duphavac' and 'Abortox' vaccines whereas the lymphocyte transformation test was consistently negative with these vaccines. The two tests were negative in unexposed cattle. It was concluded that the *in vitro* test correlated better with *Brucella* isolation than the *in vivo* test did and that the lack of agreement between the results of the two tests is likely to be due to the different antigens used in the assays.

## INTRODUCTION

The classical test for the diagnosis of tuberculosis in cattle is the tuberculin skin test. This *in vivo* test is regarded as a measure of cell-mediated immunity (CMI) (Rook, 1978). Delayed hypersensitivity reaction to *Brucella* organisms may be developed as a result of infection or vaccination. This similarity between *Brucella* hypersensitivity and tuberculin sensitivity prompted the use of *Brucella* allergens in the diagnosis of brucellosis.

Various allergens have been developed for this purpose (Alton, Jones & Pietz, 1975). Unfortunately some of these allergens were found to be unsuitable (Alton *et al.* 1975) as they induced antibody production, thereby making interpretation of serological test results very difficult. An important advance was the work of Jones, Diaz & Taylor (1973), who described the preparation of allergens from rough

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strains of *Brucella* that do not have this disadvantage. Fensterbank (1977) and Cunningham *et al.* (1980) used the newer type of allergens and observed that they detected more infected animals than serological tests. It is important to evaluate these new allergens before they are introduced for extensive field work.

Since the discovery by Nowell (1960) that phytohaemagglutinin could induce blast transformation in an *in vitro* culture system, much attention has been focused on the study of lymphocytes. The observations by Swiderska, Osuch & Brzoska, (1971) and Bascoul *et al.* (1976) that lymphocytes from human patients suffering from brucellosis underwent immunologically specific transformation into blast cells when exposed to a *Brucella* antigen have prompted the development of an *in vitro* assay for the diagnosis of bovine brucellosis (Kaneene *et al.* 1978*a*, *b*). Klesius *et al.* (1978) reported good correlation between the delayed hypersensitivity skin test (DHT) and lymphocyte transformation test (LTT) responses in cattle inoculated with *Brucella abortus* strain 19 (S19) vaccine. The LTT has been observed to be very useful in the diagnosis of bovine brucellosis (Kaneene *et al.* 1978*a–d*).

As of now, little information is available regarding studies using both *in vivo* and *in vitro* assays of CMI as diagnostic tools in bovine brucellosis. The purpose in the present study was to compare the brucellin skin test and *in vitro* lymphocyte transformation test in cattle infected with *B. abortus*, cattle vaccinated against brucellosis and unexposed animals.

## MATERIALS AND METHODS

### *Experimental animals*

All the animals in groups 1–4 were commercial Hereford-cross heifers 12–18 months old. There were 9 heifers in group 1, 7 in group 2 and 4 each in groups 3 and 4. The 4 animals in group 5 were all Kerry cows 36–40 months old. All the experimental animals were purchased from brucellosis-free herds and were serologically negative to standard *Brucella* tests (Rose Bengal plate test, serum agglutination test and complement fixation test) on arrival and before vaccination. They were supplied with food (lucerne silage and dry concentrate) and water *ad libitum*.

### *Animal vaccinations*

In group 1, heifer No. AB 18 was immunized with killed *B. abortus* strain 45/20 adjuvant (K45/20A) vaccine (Philips-Duphar, Amsterdam, Netherlands): 2 ml of the vaccine was given by deep intramuscular injection in front of the shoulder high on the side of the neck. A second injection was given 6 weeks later.

Heifers AB 38, AB 43, AB 45, AB 57 and AB 59 were vaccinated with 'Abortox' vaccine (*B. abortus* killed 45/20 in an oil emulsion obtained from Iffa Merieux, Lyon, France). Two injections of 3 ml each of Abortox vaccine were given with an interval of 4 weeks between the injections.

Group 2 animals were inoculated with S19 vaccine every fortnight during the experimental period. Freeze-dried vaccine, obtained from the Central Veterinary Laboratory, Weybridge, England, was reconstituted with sterile phosphate-buffered saline and cultured on Albimi agar (Albimi Laboratories New York, USA) at 37 °C for 48 h. *Brucella* colonies were washed off with sterile phos-

phate-buffered saline which was used as diluent. The vaccine suspension was adjusted to contain $10^9$ viable *B. abortus* organisms/ml. Every animal in this group was injected each time with $5 \times 10^9$ viable organisms subcutaneously into the dewlap area.

Group 3 heifers were injected with a single dose of S19 vaccine ($5 \times 10^9$ live organisms each) while group 4 animals were vaccinated with K45/20A (Duphavac) vaccine (Philips-Duphar, Amsterdam, Netherlands) as described for heifer no. AB 18 in group 1. The Kerry cows (group 5) were used as unexposed controls.

*Artificial insemination*

All the heifers in group 1 were artificially inseminated about 1 month after vaccination. They were examined for pregnancy by rectal palpation and confirmed as pregnant.

*Animal challenge*

Freeze-dried form of *B. abortus* strain 544 (Central Veterinary Laboratory, Weybridge, England) was cultured on Albimi agar as described for S19 vaccine above. Sterile phosphate-buffered saline was used as diluent. The challenge suspension was adjusted to contain $2 \times 10^8$ viable organisms/ml and 0·1 ml of the diluent containing $2 \times 10^7$ live *Brucella* organisms was deposited in the conjunctival sac of each animal in group 1 during the sixth month of gestation. The infected heifers were placed in separate boxes after challenge while the other animals were housed together.

*Isolation and identification of* Brucella abortus

The following examinations were carried out on heifers in group 1 that calved or aborted. Cultures were made from fetal stomach contents of all aborted fetuses, vaginal discharges and milk on Albimi agar with antibiotic supplement. The rest of the isolation and identification procedure was conducted according to the methods of Alton, Jones & Pietz (1975).

*Blood collection*

Blood for LTT was collected in sterile vacutainers containing appropriate amounts of sterile preservative-free heparin (50 units/ml) obtained from Leo Laboratories Limited, Dublin, Republic of Ireland. Five millilitres of blood was collected from each animal by jugular venipuncture.

*Lymphocyte transformation test antigen, medium and mitogen*

The antigen was *Brucella* polypeptide containing some lipopolysaccharide. It was prepared from *B. abortus* strain 99 and kindly donated by the Wellcome Research Laboratories, Kent, England. The antigen was used at a dose of 2·5 $\mu$g of polypeptide in 50 $\mu$l of culture.

The tissue culture medium was RPMI 1640 (GIBCO Europe, United Kingdom) with HEPES buffer, supplemented with L-glutamine, penicillin (100 units/ml) and streptomycin (100 $\mu$g/ml).

The mitogen was concanavalin A (ConA) purchased from Calbiochem-Behring Corporation Limited, La Jolla, California, U.S.A. One microgramme was added to each 50 $\mu$l of culture. (Kaneene *et al.* 1978*a*).

*Lymphocyte transformation test procedure*

The LTT was carried out using the whole-blood lymphocyte stimulation assay according to the method described by Kaneene *et al.* (1978*a*) with slight modifications. The modifications included the use of 2·5 $\mu$g of *Brucella* polypeptide per culture, the addition of sterile distilled water to empty wells in microtitration culture plates to prevent the cultures from drying up, the use of [6-$^3$H]thymidine (2 Ci/mmol) and the processing of cultures, which was performed according to the method described by Muscoplat *et al.* (1974): 0·5 $\mu$Ci of the [6-$^3$H]thymidine (Radiochemical Centre, Amersham, England) was added to each culture. After incubation for a further 18 h the cultures were filtered under suction and cells on glass-fibre filters were washed with isotonic saline. Washing with isotonic saline alone has been observed to give results identical with washing with saline, trichloroacetic acid and methanol (Hartzman *et al.* 1972). Each filter was dried in an incubator at 37 °C for 20 h. It was then placed in a scintillation vial containing 10 ml of scintillation liquid obtained from the Radiochemical Centre, Amersham, England. Radioactivity was counted in a liquid scintillation counter (SL 3000; Intertechnique, France).

*Delayed hypersensitivity skin test with brucellin*

The DHT was carried out essentially according to the method described by Cunningham *et al.* (1980). The *Brucella* allergen was prepared from *B. abortus* strain 45/20 and contained no lipopolysaccharide. It was kindly donated by the Wellcome Research Laboratories, Kent, England. Briefly, 0·1 ml of the allergen containing 100 $\mu$g of *Brucella* polypeptide was injected intradermally in the anterior two-thirds of the neck of each animal. The increase in skin thickness was determined by measuring the skin fold with a pair of callipers before injection and at 72 h after injection; the right and left sides of each animal were alternated for the injections.

## RESULTS

*Results of* Brucella abortus *strain 544 challenge*

Heifers nos. AB 18, AB 38, AB 43, AB 45 and AB 59 in group I had live calves but *B. abortus* was isolated from their milk and vagina. Animals nos. AB 22, AB 54, AB 57 and AB 66, also in group I, aborted and *B. abortus* was isolated from their milk, vagina and from the fetal stomach contents of their aborted fetuses.

*Expression of DHT and LTT results*

For the DHT an increase of skin thickness of 3 mm or greater was considered to indicate infection whereas any skin reaction less than 3 mm was regarded as negative (Cunningham *et al.* 1980).

The LTT was expressed as:

$$\text{stimulation index (SI)} = \frac{\text{mean c.p.m. triplicate cultures with mitogen or specific antigen}}{\text{mean c.p.m. triplicate cultures without mitogen or specific antigen}}.$$

Table 1. *Comparison of brucellin skin-test reactivity and responses of lymphocytes of cattle (group I) infected with virulent* Brucella abortus *strain* 544

(Increase in skin thickness measured in millimetres while lymphocyte transformation was expressed as stimulation index (SI). These tests were carried out at 3 weeks between tests.)

| Animal no. | | 1st test | 2nd test | 3rd test | 4th test |
|---|---|---|---|---|---|
| AB 18 | Skin | 2 | 2 | 2 | 2 |
| | BA | 3·8 | 5·6 | 3·4 | 6·9 |
| | Con A | 66 | 42 | 18·2 | 30·2 |
| AB 38 | Skin | 16 | 10 | 14 | 12 |
| | BA | 5·1 | 7·3 | 4·1 | 5·8 |
| | Con A | 45 | 21·9 | 35·6 | 14·5 |
| AB 43 | Skin | 14 | 14 | 16 | 9 |
| | BA | 6·7 | 4·8 | 3·6 | 8·3 |
| | Con A | 18·5 | 33·3 | 15·8 | 28·4 |
| AB 45 | Skin | 15 | 11 | 10 | 7 |
| | BA | 6·1 | 7·2 | 5·30 | 6·7 |
| | Con A | 16·9 | 21·7 | 25·9 | 38·1 |
| AB 57 | Skin | 10 | 8 | 7 | 5 |
| | BA | 5·3 | 3·7 | 5·7 | 4·2 |
| | Con A | 41·3 | 27·8 | 18·1 | 23·4 |
| AB 59 | Skin | 12 | 16 | 14 | 10 |
| | BA | 6·3 | 3·4 | 4·7 | 3·3 |
| | Con A | 21·9 | 35 | 17·8 | 20·5 |
| AB 22 | Skin | 1 | 1 | 1 | 1 |
| | BA | 4·2 | 3·2 | 3·7 | 4·7 |
| | Con A | 51·8 | 13 | 23 | 30·2 |
| AB 54 | Skin | 3 | 3 | 3 | 5 |
| | BA | 6·2 | 6·4 | 9·8 | 6·8 |
| | Con A | 41 | 17·9 | 27·1 | 66 |
| AB 66 | Skin | 2 | 2 | 2 | 4 |
| | BA | 3·9 | 5·7 | 4·7 | 6·7 |
| | Con A | 14·1 | 52 | 25·7 | 16·1 |

BA, Stimulation index induced by *Brucella* antigen; Con A, stimulation index induced by Concanavalin A; Skin, increase in skin thickness elicited by *Brucella* allergen.

The mitogen was used only as a positive control. A stimulation index of 3 or greater was considered to indicate infection whereas any SI less than 3 was considered negative (Kannene *et al.* 1978*b*).

*Results of the LLT and DHT*

Lymphocytes from animals in all groups were immunologically responsive, as indicated by their ability to undergo non-specific stimulation with the mitogen, ConA (Tables 1–5). It is evident that all the heifers in group 1 had high lymphocyte transformation responses throughout the test period and the LTT correctly classified the heifers as infected (Table 1). The skin test reactions exhibited by the same animals are also shown on Table 1. Six out of the nine *Brucella*-infected heifers gave persistent high skin reactions, indicating that these animals were positive. Two of the infected heifers (AB 18 and AB 22) were persistently negative to the skin test while another infected animal (AB 66) was negative during most of the test period but only became positive at the last test.

Table 2. *Comparison of brucellin skin-test reactivity and lymphocyte transformation in heifers (group 2) with multiple injections of* Brucella abortus *Strain* 19

(Increase in skin thickness was measured in millimetres while lymphocytes transformation was expressed as stimulation index (SI).)

| Animal no. | | Time after primary vaccination | | | | |
|---|---|---|---|---|---|---|
| | | 2 weeks | 6 weeks | 10 weeks | 14 weeks | 18 weeks |
| BW I | Skin | 2 | 2 | 3 | 6 | 5 |
| | BA | 0·5 | 2·4 | 1·8 | 3·9 | 1·8 |
| | Con A | 13·8 | 49·2 | 31·8 | 12·9 | 28·9 |
| BW 2 | Skin | 4 | 6 | 9 | 5 | 7 |
| | BA | 0·9 | 2·5 | 1·2 | 2 | 1·3 |
| | Con A | 10·9 | 20·1 | 15 | 14·6 | 25·9 |
| BW 3 | Skin | 1 | 3 | 6 | 11 | 10 |
| | BA | 1 | 2·6 | 0·8 | 3 | 1·3 |
| | Con A | 21·2 | 17·5 | 13·2 | 26·5 | 22 |
| BW 4 | Skin | 3 | 4 | 3 | 3 | 5 |
| | BA | 1·1 | 0·9 | 1·8 | 2·9 | 1·7 |
| | Con A | 60·5 | 10·8 | 24·1 | 13·2 | 17·5 |
| BW 5 | Skin | 2 | 5 | 3 | 5 | 4 |
| | BA | 1 | 1 | 1·7 | 1·4 | 24·5 |
| | Con A | 23·6 | 25·2 | 11·9 | 32·5 | 24·5 |
| BW 6 | Skin | 1 | 4 | 5 | 9 | 8 |
| | BA | 0·2 | 1·6 | 1·5 | 1·9 | 3·6 |
| | Con A | 10·7 | 20·5 | 16·5 | 21·4 | 21·8 |
| BW 7 | Skin | 4 | 5 | 3 | 3 | 6 |
| | BA | 0·8 | 3·3 | 1·9 | 1·3 | 1·5 |
| | Con A | 37·2 | 30 | 46·5 | 26 | 19·8 |

BA, Stimulation index induced by *Brucella* antigen; Con A, stimulation index induced by Concanavalin A; Skin, increase in skin thickness elicited by *Brucella* allergen.

Table 3. *Comparison of brucellin skin-test reactivity and lymphocyte transformation in heifers (group 3) given a single injection of* Brucella abortus *S19*

(Increase in skin thickness was expressed in millimetres while lymphocyte stimulation was expressed as stimulation index (SI).)

| Animal no. | | Time after vaccination | | | | |
|---|---|---|---|---|---|---|
| | | 2 weeks | 6 weeks | 10 weeks | 14 weeks | 18 weeks |
| BW 8 | Skin | 2 | 3 | 5 | 2 | 2 |
| | BA | 0·8 | 0·6 | 1·3 | 2·1 | 0·9 |
| | Con A | 17·2 | 12·1 | 11·9 | 16·3 | 33 |
| BW 9 | Skin | 2 | 5 | 3 | 1 | 2 |
| | BA | 1 | 1·2 | 1 | 2 | 1 |
| | Con A | 11 | 15·8 | 17·3 | 20·9 | 19·7 |
| BW 10 | Skin | 3 | 2 | 5 | 2 | 2 |
| | BA | 1 | 1·3 | 1·8 | 1·2 | 1·2 |
| | Con A | 11 | 13·5 | 32 | 21 | 36·8 |
| BW 11 | Skin | 4 | 5 | 3 | 2 | 1 |
| | BA | 0·7 | 1·1 | 1 | 0·4 | 1 |
| | Con A | 20·9 | 12·8 | 13·5 | 14·2 | 22·5 |

Skin, Increase in skin thickness elicited by *Brucella* allergen; BA, stimulation index induced by *Brucella* antigen; Con A, stimulation index induced by Concanavalin A.

https://doi.org/10.1017/S002217240006616X Published online by Cambridge University Press

Table 4. *Comparison of brucellin skin-test and lymphocyte transformation test reactivities in heifers (group 4) vaccinated with* Brucellaabortus *K45/20A vaccine*

(Increase in skin thickness was expressed in millimetres while lymphocyte transformation was expressed as stimulation index (SI).)

| | | Time after vaccination | | | | |
|---|---|---|---|---|---|---|
| Animal no. | | 2 weeks | 6 weeks | 10 weeks | 14 weeks | 18 weeks |
| BW 12 | Skin | 6 | 4 | 4 | 8 | 7 |
| | BA | 0·9 | 1·2 | 1·2 | 1·1 | 1·1 |
| | Con A | 11·8 | 12·7 | 17·2 | 17·8 | 35 |
| BW 13 | Skin | 5 | 6 | 8 | 4 | 6 |
| | BA | 1·1 | 0·8 | 1·2 | 2 | 1·1 |
| | Con A | 14·5 | 12·5 | 18·8 | 14·6 | 20·1 |
| BW 14 | Skin | 4 | 4 | 8 | 6 | 12 |
| | BA | 0·8 | 1·2 | 1·4 | 2·9 | 1·1 |
| | Con A | 11·1 | 20·4 | 39 | 15·7 | 18·4 |
| BW 15 | Skin | 2 | 6 | 7 | 15 | 6 |
| | BA | 1·2 | 0·9 | 1·2 | 2·4 | 2 |
| | Con A | 10·8 | 12·2 | 17·9 | 16·4 | 19·4 |

Skin, Increase in skin thickness elicited by *Brucella* allergen; BA, stimulation index induced by *Brucella* antigen; Con A, stimulation index induced by Concanavalin A.

In group-2 animals inoculated with S19 vaccine every fortnight the LTT induced high lymphocyte transformation responses indicative of sensitization in 4 of the 7 heifers (Table 2). Heifers BW 1 and BW 3 were positive at 14 weeks, BW 6 at 18 weeks and BW 7 at 6 weeks after the primary vaccination. Thereafter all the animals were positive to the test during the rest of the experimental period.

The LTT correctly classified all the heifers in group 3 (inoculated with one dose of S19 vaccine) as not infected (Table 3). However, two animals in this group (BW10 and BW11) had a positive skin test 2 weeks after immunization. Six weeks after vaccination three heifers were positive to the skin test. All four animals were positive at 10 weeks post-immunization but at 14 and 18 weeks all heifers had become negative.

In group-4 animals injected with *B. abortus* killed 45/20 adjuvant vaccine, the LTT correctly identified all the vaccinates as negative (Table 4). Only one heifer was negative to the skin test 2 weeks after vaccination. Thereafter all the animals became strongly positive to the test.

Both the LTT and DHT correctly identified all the cows in the control group (group 5) as negative (Table 5).

*Correlation between LTT and* B. abortus *isolation*

There was very good correlation between the LTT and the isolation of *B. abortus* from infected cattle. The *in vitro* test correctly identified all the nine heifers with active *Brucella* infection (Table 1).

*Correlation between DHT and* Brucella abortus *isolation*

The DHT was consistently positive in 6 of the 9 heifers infected with virulent *B. abortus* strain 544. This suggests that the DHT would detect 2 out of every 3 infected cattle.


https://doi.org/10.1017/S002217240006616X Published online by Cambridge University Press

Table 5. *Brucellin skin test and lymphocyte transformation reactions in non-exposed control cattle (group 2).*

(Skin increase was measured in millimetres while lymphocyte transformation was expressed as stimulation index (SI). Animals tested 3 weeks apart.)

| Animal no. | | 1st test | 2nd test | 3rd test | 4th test | 5th test |
|---|---|---|---|---|---|---|
| K 229 | Skin | 1 | 1 | 1 | 0 | 0 |
| | BA | 0·6 | 1 | 1 | 0·8 | 1·2 |
| | Con A | 12·3 | 11·5 | 20·5 | 24·1 | 18·1 |
| K 239 | Skin | 1 | 2 | 1 | 1 | 1 |
| | BA | 0·8 | 0·5 | 0·9 | 0·5 | 1·3 |
| | Con A | 20 | 19 | 10·7 | 11·4 | 15 |
| K 230 | Skin | 1 | 1 | 1 | 1 | 1 |
| | BA | 1 | 0·8 | 1·1 | 1 | 0·6 |
| | Con A | 9·9 | 13·1 | 14·8 | 20 | 36·1 |
| K 386 | Skin | 1 | 0 | 1 | 1 | 1 |
| | BA | 1·1 | 1 | 0·6 | 0·9 | 0·4 |
| | Con A | 13·2 | 15 | 16·7 | 12 | 15·6 |

Skin, Increase in skin thickness elicited by *Brucella* allergen; BA, stimulation index induced by *Brucella* antigen; Con A, stimulation index induced by Concanavalin A.

## DISCUSSION

*Brucella* allergens have been employed for the diagnosis and epidemiological studies of human and animal brucellosis for many years. In some countries allergic tests are used in the diagnosis of ovine, caprine, porcine and bovine brucellosis. Many factors, such as the sensitivity of the skin, intradermal injection of inadequate amounts of allergens and incorrect interpretation of results, affect the results of the DHT. Early investigators of the skin test in cattle (Live & Stubbs, 1947) did not find it useful as the test detected fewer infected animals than agglutination tests. However, the simplicity of the test and its ease of application to a large population of animals have prompted some investigators (Jones, Diaz & Taylor 1973; Cunningham *et al.* 1980) to search for suitable allergens. Newer types of allergens have been reported to be useful in the diagnosis of bovine brucellosis (Fensterbank, 1977; Cunningham *et al.* 1980).

Because of the limitations of allergic skin testing for brucellosis (Alton *et al.* 1975), the development of an *in vitro* test with a high degree of reliability would be a useful aid in the diagnosis of bovine brucellosis. The LLT for bovine brucellosis was studied by Kaneene *et al.* (1978*a*, *b*) but has not been widely used. It has been reported to be highly reliable in the diagnosis of bovine brucellosis (Kaneene *et al.* 1978*a–d*, 1980*a*, *b*) although this has been disputed (Baldwin *et al.* 1984). The data obtained in the present investigation indicated clearly that the LTT correlated better with *Brucella* infection than the DHT. In this regard, the LTT was more reliable than the skin test as it correctly identified all *Brucella* positive animals (Table 1). Results of the LTT in the present studies are in good agreement with those of other investigators (Kaneene *et al.* 1980*a*, *b*) who demonstrated that the assay correlated well with *B. abortus* isolation.

It is disappointing that the DHT could not detect two of the infected heifers

(Table 1, nos. AB 18 and AB 22) and incorrectly classified another (AB 66) on three occasions. False negative reactions in the DHT have been reported in cattle by Cunningham *et al.* (1980). This phenomenon of cutaneous anergy has been observed in some cases of human brucellosis (Thornes, 1977*a*, *b*). The mechanism of anergy is not well defined. The lack of agreement between the results of the DHT and the LTT in these studies is likely to be due to the fact that different preparations were used in the two assays. The antigen used in the DHT was a mixture of *Brucella* proteins containing no LPS and prepared from rough *B. abortus* 45/20 whereas that used in the LTT was *Brucella* protein containing some LPS and made from smooth *B. abortus* S99. It is well recognized that the protein part of micro-organisms is primarily responsible for stimulating CMI (Daniel & Hinz, 1974). Injection of *Brucella* preparations containing LPS stimulates anti-*Brucella* agglutinating antibodies (Alton *et al.* 1975). This would suggest that *Brucella* LPS is responsible for stimulating humoral response. It would appear that the DHT detects sensitization of T-lymphocytes with *Brucella* protein antigens while the LTT, using *Brucella* protein containing LPS as antigen, detects B-lymphocytes sensitized with *Brucella* LPS antigen and T-lymphocytes sensitized with *Brucella* protein antigens.

The strong skin reactions exhibited by animals vaccinated with Abortox vaccine (Table 1) and Duphavac vaccine (Table 4) may have been due to the effect of the mineral-oil adjuvant, which is known to promote DHT reactions. The skin reactions obtained in group-4 animals (Table 4) in the present studies are consistent with those of Fensterbank & Pardon (1977) and Cunningham *et al.* (1980), who observed significant skin reactions in such vaccinates for a long time. However, one heifer (Table 1, no. AB 18) immunized with Duphavac vaccine before infection did not develop any significant reaction in the DHT. Anergy in this animal is difficult to explain. It appears that the DHT would not be suitable for testing cattle inoculated with mineral-oil adjuvant vaccines. The lack of positive reactions to the LTT exhibited by these animals suggests that the test could be used to differentiate *Brucella*-infected cattle from those immunized with mineral-oil adjuvant vaccines.

The strong LTT and DHT reactions exhibited by the animals in group 2 (Table 2) which were repeatedly vaccinated with S19 vaccine are not surprising since the animals were vaccinated many times at relatively short intervals, thereby maintaining continuous antigenic stimulation and providing many secondary stimulations. It is evident that the brucellin elicited positive skin reactions for 10 months in animals given one dose of S19 vaccine (Table 3). The lack of high lymphocyte stimulation in these animals confirms previous reports that the LTT could be used to differentiate cattle infected with virulent *B. abortus* and those immunized with S19 vaccine (Kaneene *et al.* 1978*a*). The difference in the LTT reactions between S19 vaccinates and cattle infected with field strain is most probably due to the difference in pathogenicity of the two strains. *B. abortus* S19 vaccine is attenuated while strain 544 is virulent. The virulent strain would, no doubt, invade more tissues and stimulate more lymphocytes than the attenuated strain. The negative results recorded by both tests with the control animals (Table 5) indicate that they are useful in detecting unexposed cattle.

In conclusion, there is no doubt that the LTT is more reliable than the DHT for detecting cattle with active *Brucella* infection. The LTT has other advantages

over the DHT: it can be performed many times as it needs less than 1 ml of blood and all the antigen and mitogen remain in the cultures available to the cells whereas in the DHT most of the allergen leaves the injection site within a few hours (Oort & Turk, 1963). However, the DHT is cheap, simple and can be applied on a large scale and results are available in less than half the time that it takes to obtain results of the LTT. The LTT is expensive, laborious and time-consuming and needs freshly collected blood. It is doubtful if it could be used as a standard *Brucella* test. It is recommended for research work where a few animals are involved. Nevertheless, if the assay was computerized or automated it could be a useful adjunct for the diagnosis of bovine brucellosis.

I am greatly indebted to Professor B. Cunningham for his guidance. Gratitude is due to Mr P. Nolan for his technical assistance. Finally, I wish to thank the Wellcome Research Laboratories, Kent, England, for supplying the *Brucella* allergens.

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