Evaluation of four serological tests for the diagnosis of caprine melioidosis

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SUMMARY: A complement fixation (CF) test, 2 indirect haemagglutination (IHA-A; IHA-L) tests which differed in antigen preparation and technique, and a microtitre agglutination (MA) test were compared in the serodiagnosis of melioidosis in goats. One hundred and eighteen experimental sera and 3143 field sera from goats in endemic and non-endemic areas of north Queensland were used in the evaluation. Culture of samples for Pseudomonas pseudomallei from 112 goats provided substantiating evidence of infection. The IHA-A test was the most sensitive, and the CF test the most specific. We advocate the use of the IHA-A as a screening test followed by the CF test for confirmation of active melioidosis. The IHA-A test is the better indicator of past infection.

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Introduction

Pseudomonas pseudomallei, the causative agent of melioidosis, is a soil saprophyte and opportunistic pathogen of man and animals (Chambon 1955; Howe et al 1971; Thomas et al 1979). In northern Queensland, there has been an increase in the human consumption of unpasteurised goats' milk. As P. pseudomallei has been isolated from both normal and mastitic goats’ milk (Lewis and Olds 1952; Omar 1963; Thomas and Forbes-Paulkner, unpublished data), there is a potential public health risk.

Registration of dairy goat herds for freedom from melioidosis requires a reliable, quick and economic serological diagnosis. The serum agglutination test has been frequently used in the past, but its reliability has been questioned (Olds and Lewis 1954; Fournier and Chambon 1958; Ileri 1965; Malizia et al 1969). Ileri (1965) developed an indirect haemagglutination (IHA) test for goats, and Laws (1967) developed complement fixation (CF) and IHA tests for melioidosis in animals, including goats. These tests gave reliable results in naturally infected animals, but were not extensively evaluated.

This work was undertaken to evaluate 4 serological tests for melioidosis, using experimentally and naturally infected goats, and to examine the serological response of goats to experimental infection with P. pseudomallei.

Materials and Methods

For the purposes of this paper, goats from which P. pseudomallei has been isolated will be described as "culture positive" and goats from which P. pseudomallei was not isolated as "culture negative".

Goats

Experimental — Thirty-seven adult Saanen goats were used in a trial conducted at the Oonoomba Veterinary Laboratory to observe clinical and bacteriological reactions to subcutaneous infection with varying doses of P. pseudomallei (Thomas et al 1988). Eleven control goats were given injections of sterile saline. Twenty-six goats were inoculated with doses of P. pseudomallei ranging from 90 bacilli to 5x10^5 bacilli. Of these, 15 goats (Table 1) developed clinical signs of disease and P. pseudomallei was isolated from 13 of these when killed for necropsy, 28 to 156 days after inoculation. The other 2 goats developing clinical signs of disease were culture negative at necropsy, 96 and 156 days after inoculation, although sterile lesions were found in the spleen and right precapular lymph nodes (Thomas et al 1988). The remaining 11 inoculated goats and the 11 control goats showed no clinical signs of melioidosis and were culture negative at necropsy.

Field — Goats from domestic herds were distributed throughout areas of north Queensland endemic or non-endemic for melioidosis (Figure 1). Feral goat sera were sent from the Prince of Wales Island. A domestic herd from Gayndah in southern Queensland was also tested.

Bacteriology — Methods for culture of clinical samples and identification of isolates have been previously described (Thomas et al 1988).

Seroogy

Tests — Serological tests evaluated were: the indirect haemagglutination (IHA-A) and complement fixation (CF) tests of Alexander et al (1970); the indirect haemagglutination (IHA-L) test of Laws (1967); and the microtitre agglutination (MA) test of Ettrel et al (1979). All sera were tested by the 4 tests using doubling dilutions. The arbitrary minimum positive values used were 1/8 for the CF test, 1/40 for the IHA-A test, 1/10 for the IHA-L test, and 1/160 for the MA test. Titres were graded 0, 1 to 4, depending upon the amount of haemolysis and/or buttoning at the bottom of the microtitre well in the CF, IHA-A and MA tests. In comparing the tests, only positive and negative results were used. Suspicious values were recorded as negative for this study, although values for suspicious readings are reported in routine field testing.

Antigen preparation and techniques — A local, serotype I isolate from a goat was used for inoculation of the experimental goats and in preparation of antigens. Local isolates, both serotype I, from a bird and a sheep, were also used to prepare antigens. Antigens and sensitised erythrocytes were prepared according to the respective authors listed in the previous paragraph. The 2 IHA tests were chosen to compare the difference in antigen preparations. The IHA-A antigen is prepared by growth of the isolate in a synthetic protein-free medium. The IHA-L antigen is grown on nutrient agar.

Techniques were used as recommended by the authors except for the CF test which was adapted to microtitre plates.

Serums

Serological response to melioidosis in goats — Serums (822) were collected from all 37 experimental goats. They were taken 3 times weekly for the first 3 weeks and then twice weekly until death or necropsy. Ninety sera from the 13 culture positive goats were used to determine the mean serological responses in goats to infection with P. pseudomallei. Mean titres were calculated on 13 sera on day 0 and at 7, 14, 21

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TABLE 1
Details of dose rate of *Pseudomonas pseudomallei* and number of trial days until necropsy for 15 goats showing clinical signs of melioidosis

<table>
<thead>
<tr>
<th>Goat Number</th>
<th>Dose Rate</th>
<th>Necropsy (days after inoculation)</th>
<th>Number of tissues with Culture positive lesions</th>
<th>Culture negative lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$5 \times 10^6$</td>
<td>60</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>$6 \times 10^5$</td>
<td>51</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>104</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>34</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>34</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>500</td>
<td>34</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>500</td>
<td>96</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>500</td>
<td>28</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>225</td>
<td>31</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>225</td>
<td>156</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>225</td>
<td>31</td>
<td>9</td>
<td>0</td>
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<td>14</td>
<td>225</td>
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<td>5</td>
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<td>17</td>
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<td>0</td>
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<td>18</td>
<td>90</td>
<td>156</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>19</td>
<td>90</td>
<td>71</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

and 28 days after inoculation; 5 serums at 35, 42 and 49 days after inoculation; 4 serums at 56 days after inoculation and 3 serums at 63 and 70 days after inoculation.

*Evaluation of the 4 serological tests* — Sensitivity and specificity values were calculated using the criteria of Fletcher et al. (1982). The criteria for infected animals was culture positive lesions and not just clinical signs of melioidosis.

*Sensitivity* — Fifty-one necropsy serums (13 from experimentally infected goats and 38 from naturally infected goats) were used to determine diagnostic sensitivity values for the 4 tests. To determine the diagnostic sensitivity values of the 4 tests at the time of active infection, serums were collected from the 13 experimentally infected goats 28 days after inoculation.

*Specificity* — For the determination of diagnostic specificity, serums were collected at necropsy from 11 control goats inoculated with sterile saline and 48 field goats showing no signs of disease.

*Comparison of the 4 tests* — Five hundred and seventy-seven serums from goats with unknown melioidosis status, but from areas where melioidosis is endemic, were used to compare the 4 tests.

*Choosing a serological regimen for melioidosis* — Serums from 2,489 goats pastured in areas endemic or non-endemic for melioidosis were used to evaluate the most sensitive and the most specific of the 4 tests with a view to using them for routine screening and confirmation, respectively.

*Comparison of goat, bird and sheep isolates of *P. pseudomallei* as antigens* — Of the 237 positive serums collected from 13 experimentally infected goats, 130 were selected for retesting by all 4 tests using the antigens prepared from the sheep and bird isolates.

**Results**

Figure 2 shows the mean serological responses of the 13 goats experimentally infected with *P. pseudomallei*. Antibodies were detected by the CF, IHA-A and MA tests within 1 day of inoculation in goats receiving $\geq 500$ bacilli and within 16 days of inoculation in goats receiving 90 to 225 bacilli. There was one exception. One goat that aborted 9 days after inoculation did not develop a positive titre in any test until 21 days after inoculation. Antibody detection by the IHA-L test was generally delayed until 16 to 25 days after inoculation. There was no IHA-L antibody response in 2 of the infected goats. Ten of the experimentally infected goats had an acute form of the disease and were all culture positive when killed for necropsy, 28 to 60 days after inoculation. CF and IHA-A titres were positive in all 10 goats; IHA-L and MA titres were positive in 8. The remaining 3 goats were killed in the chronic stage of the disease, 71 to 156 days after inoculation. *P. pseudomallei* was isolated from all. IHA-A, IHA-L and MA titres were positive in all 3 goats but the CF titre was positive in only 1.

The serum of goat 4 showed a transient negative titre (<1/40) to the IHA-A test between 10 and 21 days after inoculation. Serums of goats 6 and 11 had transient negative

![Figure 1: Distribution of melioidosis in Queensland.](image)

![Figure 2: Mean serological responses to 2 indirect haemagglutination (IHA-A; IHA-L), complement fixation (CF) and microtitre agglutination (MA) tests for melioidosis in experimentally infected goats. As the goats were necropsied at various intervals during the trial, the mean response was calculated on 13 goats on day 0 and at 7, 14, 21 and 26 days after inoculation; 5 goats at 35, 42 and 49 days after inoculation; 4 goats at 56 days after inoculation and 3 goats at 63 and 70 days after inoculation.](image)


<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Sensitivity and specificity values for 2 indirect haemagglutination (IHA-A; IHA-L), microtitre agglutination (MA) and complement fixation (CF) tests for caprine melioidosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>28 days</strong></td>
</tr>
<tr>
<td><strong>Test</strong></td>
<td><strong>SEN±</strong></td>
</tr>
<tr>
<td>IHA-A</td>
<td>100</td>
</tr>
<tr>
<td>IHA-L</td>
<td>84.6</td>
</tr>
<tr>
<td>MA</td>
<td>84.6</td>
</tr>
<tr>
<td>CF</td>
<td>100</td>
</tr>
</tbody>
</table>

* sera collected from 13 experimentally infected goats during active infection (sensitivity) or from 11 control goats at a similar period (specificity)
† sera collected from 13 experimentally and 38 naturally infected goats (sensitivity) and from 11 experimental and 48 field culture negative goats (specificity)
‡ SEN = Sensitivity; SPEC = specificity
§ number of sera

≥2/320 titres were culture negative at necropsy, but had sterile lesions in the spleen and/or precapillary lymph nodes. Regression of melioidosis lesions with eventual sterility of abscesses is not uncommon in goats (Lewis and Olda 1952; Laws 1967; Thomas et al 1988). From our findings, IHA-A titres persist longer in the majority of surviving goats than do the CF titres, and the IHA-A test is a better indicator of past infection with *P. pseudomallei*.

Nigg (1963), Johnson (1967) and Laws (1967) reported that CF antibody did not persist for very long after melioidosis infection was resolved, and that persistence of a CF titre indicated persistence of infection (Nigg 1963). Howe et al (1971) suggested that the CF test was a highly sensitive and specific test for the diagnosis of active infection and subclinical disease in man. Our work has shown that the CF test indicates active infection. All 10 experimentally infected goats killed during the acute stage of the disease had positive CF titres. Only one of the 3 experimentally infected goats killed during the chronic stage of infection had a positive CF titre, although all 3 were CF positive at 28 days after inoculation. This result contributed to the difference between the sensitivity values for the CF test in Table 2. Melioidosis in goats in the field is most often a chronic disease (Laws and Hul 1963).

Agreement between the IHA-A test and the other 3 tests was high (Table 3) when evaluating 577 field sera. This was not unexpected as the majority of these sera were from clinically negative goats, even though the herds were from areas endemic for melioidosis. As seen in Table 2, the specificity values for all 4 tests were relatively high.

We would not recommend either the IHA-L or MA tests for routine diagnostic testing. Sensitivity values for both tests were low (Table 2) and this could be due to antigen preparation. Neither of these antigens were prepared in protein-free media and protein extracts can reduce sensitivity and/or specificity values (Malizia et al 1969; Alexander et al 1970). The IHA-L titres were delayed when compared to the other 3 tests, although they did tend to persist in the longer surviving goats as observed by Laws (1967). The IHA-L test is done on a glass slide with a rocking technique and can be time-consuming when dealing with large numbers of sera. The MA test is easy to perform and requires no addition of red cells. However, it is an overnight cold test which delays results and there are problems with subjectivity. Both sensitivity and specificity values are low (Table 2).

Alexander et al (1970) found transient titres to the CF and IHA-A tests in 19% of human cases, though rarely in the 2 tests simultaneously. Transient titres occurred in our study with the IHA tests. These were recorded in 3 of the 13 experimentally infected goats (23%). Delayed seroconversion in animals after abortion is not uncommon (Christie 1969; Worthington 1982).

Use of a *P. pseudomallei* isolate from goats is not necessary for the diagnosis of caprine melioidosis. Antigens prepared from local sheep and bird isolates of *P. pseudomallei* gave similar results to the goat antigen.

None of the 4 tests gave both high sensitivity and specificity values at 28 days after inoculation and at necropsy. However, use of the sensitive IHA-A test for screening in conjunction

| TABLE 3 | Comparison of indirect haemagglutination (IHA-L), microtitre agglutination (MA) and complement fixation (CF) tests against an indirect haemagglutination (IHA-A) test for caprine melioidosis on 577 sera from goat herds in areas endemic for melioidosis |
|------------------|-------------------|-------------------|-------------------|
|                  | **Tests**         | **IHA-L**         | **MA**            | **CF**            |
|                  | **Pos** | **Neg** | **Pos** | **Neg** | **Pos** | **Neg** |
| IHA-A            | 42      | 34      | 8       | 33      | 9       | 30      | 12      |
|                  | (98.3%) | (98.4%) | (97.7%) |         |         |         |         |

* Pos = positive; Neg = negative; † ( ) = percentage agreement with the IHA-A test.

Discussion

Both sensitivity and specificity are considered when selecting a testing regimen for the serodiagnosis of disease (Fletcher et al 1982). A rapid, specific screening test followed by a highly specific confirmatory test on any positive sera is a system often used (Dohoo et al 1986). Alexander et al (1970) recommended the use of the CF and IHA-A tests for the serodiagnosis of human melioidosis. Our work indicated that the IHA-A test, with a sensitivity of 98.0% (Table 2), might be a useful screening test for caprine melioidosis with the CF test (specificity of 100%) as a confirmatory test. This was examined by testing 2480-field sera, of which 2432 (98.1%) were negative to both tests. Of the 15 (0.6%) sera reacting at positive titre in both tests, 14 were collected from goats that were culture positive. Thirty-three (1.3%) sera were IHA-A positive, CF negative. Most of the IHA-A titres were low — 1/40 to 4/40 — and could have been due to cross-reactions with other pseudomonads or *Salmonella* spp (Nguyen-Ba-Luong 1961). Shelds and Thomas (unpublished data) observed CF negative, IHA-A melioidosis titres from 1/40 to 1/160 in goats with confirmed cases of salmonellosis but culture negative for *P. pseudomallei*. This would explain the lower specificity value for the IHA-A test compared to the CF test (Table 2). One experimentally inoculated goat and 2 goats from domestic herds with CF negative, IHA-A

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with the specific CF test on IHA-A positive sera provides a reliable procedure for the serodiagnosis of active melioidosis in goats. This is important in the accreditation of goat dairy herds. The lower sensitivity of the CF test in chronic infectious must be recognised and goat sera with high IHA-A and negative CF titres should be regarded with suspicion and further testing implemented. Although this was not an epidemiological trial, plotting of our results on a distribution map of Queensland (Figure 1), showed that both tests, the IHA-A test in particular, could be valuable as epidemiological tools in the identification of areas endemic for *P. pseudomallei*.

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**References**


Chamberl L (1955) — *Ann Inst Post (Paris)* 89: 229

Christie TE (1969) *Vet Rec* 85: 258


Fotherill TE, Lewis DH and Gravilles J (1979) — *The Prog Fish-Cult* 41: 55

Fletcher RH, Fletcher SW and Wagner EH (1982) — In *Clinical Epidemiology — The Essentials*, Williams and Wilkins, Sydney


Ilori S (1965) — *Br Vet J* 121: 164


Laws L and Hall WTK (1963) — *Queens J Agric Sci* 20: 499

Lewis FA and Olds RJ (1952) — *Aust Vet J* 28: 145


Nguyen-Be Luong (1961) — *Bull Off Int Epizoot* 56: 964

Nigg C (1963) — *J Immunol* 91: 18

Olds RJ and Lewis FA (1954) — *Aust Vet J* 30: 253

Omar AR (1963) — *J Comp Pathol* 73: 359


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