

MELIOIDOSIS IN ANIMALS IN NORTH QUEENSLAND. VI. SEROLOGICAL METHODS OF DIAGNOSIS IN GOATS, PIGS, CATTLE, HORSES AND MAN

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SUMMARY

Sera were examined by complement fixation (C.F.) and haemagglutination (H.A.) tests for melioidosis. All the animals and all except one human patient were in tropical Queensland. In addition, the cattle sera were examined by an agglutination test and the goats using an intradermal melioidin test.

C.F. antibodies were detected in 11 and haemagglutinins in 13 of the 14 goats from which *Ps. pseudomallei* was recovered at autopsy. One of the infected goats negative to the C.F. test had been noticed sick for only 24 hours before death. *Ps. pseudomallei* was not recovered from the other 24 goats autopsied. Three of the 24 goats in which infection was not confirmed had reactions (1 positive, 2 suspicious) to the C.F. test and 2 of these 3 goats had abscesses in organs or lymph nodes. Eleven of the 24 goats in which infection was not confirmed were positive to the H.A. test and 7 of these 11 goats had sterile abscesses in organs or lymph nodes. Limited observations suggested haemagglutinins were developed earlier than C.F. antibodies.

Thirty-four of the 38 goats were examined by the intradermal melioidin test in the caudal fold. Nine of the 11 infected goats gave positive melioidin reactions and 4 of the 23 goats in which infection was not confirmed had positive melioidin reactions. Three of these four goats had bacteriologically sterile abscesses in organs or lymph nodes. One infected goat gave negative reactions to the C.F., H.A. and melioidin tests.

Ps. pseudomallei was recovered from 10 of 330 pigs examined. Only 1 of the 10 infected pigs had a positive C.F. reaction and there were no detectable C.F. antibodies in the sera of the other 320 pigs. The sera of 9 of the infected pigs were examined by the H.A. test. Five of the 9 had positive haemagglutinin titres. Only 1 of the other 320 pigs had a positive H.A. reaction. At autopsy of this pig *Brucella suis* was recovered from an abscess in a lymph node.

No C.F. antibodies were detected in the sera of two infected cattle (one experimentally infected, the other a natural infection). From the limited tests done both the agglutination and haemagglutination tests may be useful for the diagnosis of melioidosis in cattle.

The serum of a horse which had been noticed sick for only 24 hours before it died of peracute melioidosis was negative to the C.F. test.

Complement fixing antibodies were detected in the sera of six human patients and *Ps. pseudomallei* had been recovered from five of them. One of these strains was antigenically different from the other 4 strains.

I. INTRODUCTION

Since *Ps. pseudomallei* was first recovered from sheep in Queensland in 1949 (Cottew 1950), melioidosis has been diagnosed in goats, pigs, cattle, a horse and an orang-outang. The disease has been reported also in man (Rimington 1962).

Olds and Lewis (1954) reported that in goats the agglutination test was unreliable but that the intradermal melioidin test was far more accurate. D. F. Mahoney (personal communication) had false negative melioidin reactions in infected goats and false positive melioidin reactions in pigs. G. S. Cottew (personal communication) stated that the agglutinin titre in apparently healthy pigs was often higher than that recorded in infected pigs.

Laws (1967) reported the use of a complement fixation (C.F.) test and a haemagglutination (H.A.) test for the diagnosis of melioidosis in sheep. This paper reports the use of these tests for the diagnosis of melioidosis in goats, pigs, cattle, horse and man. The results are also compared with those of the agglutination test in cattle and the intradermal melioidin test in goats.

II. MATERIALS AND METHODS

(a) Source of Serum Samples

(1) Goats

(a) *Herd goats—farm A, 20 miles south of Townsville.*—On March 1, 1960, a sick goat (D136) was submitted to the laboratory for examination. Blood samples were collected from it the day it arrived at the laboratory and 9 days later when it was killed for autopsy.

When the 42 goats in this herd were bled on March 22, 1960, 13 had reactions to the C.F. test, so the owner donated the herd to the laboratory. This included 2 sick goats (T881 and D212) and 34 clinically normal goats. Six goats had been killed by the owner and were unavailable for examination.

On arrival at the laboratory, blood samples were collected from all goats, the two sick goats were killed for autopsy and the other 34 goats were inoculated with melioidin. Blood samples were collected from these 34 goats when they were killed for autopsy between 5 and 16 days later.

(b) *Herd goats—farm B, 15 miles north-west of Townsville.*—All goats in this herd were bled on three occasions. The dates they were bled and the numbers bled were: September 6, 1960 (26); January 24, 1961 (24); May 23, 1961 (17). One goat was killed for autopsy on February 8, 1961.

(2) Pigs

(a) *Pigs killed at the Townsville District Abattoir.*—All pigs had been reared on farms in the vicinity of Townsville. Two hundred and thirty-eight blood samples were collected at the time of slaughter during August, September and October, 1960, and 90 samples during May and June, 1961.

(b) *Herd pigs.*—

(i) In October 1958, 17 blood samples were examined from the breeding animals on farm C near Townsville. One of these samples tested positive and the animal was rebled in December.

(ii) In October 1960, following isolation of *Ps. pseudomallei* from an abscess in a pig on farm M near Cairns, 144 blood samples were collected from pigs on this farm. Further samples were collected again from 6 pigs on this farm in November 1960.

(iii) In May 1961, 59 blood samples were collected from pigs on farm BL near Townsville.

(3) Cattle

(a) *Experimental infection.*—A 5-month-old calf 901 was inoculated subcutaneously with 1 ml of a 24-hr broth culture of *Ps. pseudomallei* strain A234 (Laws and Hall 1963). Blood samples were collected at the time of inoculation, days 3 and 7 post inoculation, weekly until week 21 (except weeks 7 and 14), fortnightly until week 29, then at weeks 35, 66 and 67. The animal was killed for autopsy at week 67.

(b) *Natural infection.*—A blood sample was collected from cow A234 when she was killed for autopsy (Laws and Hall 1963). Serum samples were received from 8 other ataxic cattle. The laboratory herd of 23 cattle was examined.

(4) Horses

Blood samples were collected from horse C119 (Laws and Hall 1963) the day before death and at autopsy. Blood samples were collected from a horse on the same property 7 days and 1 day before it died from traumatic splenitis.

(5) Human patients

Serum samples were examined from Rimington's (1962) cases IV, V and VI, two cases reported by Rao (1965) and one case reported by Woolford (1966).

Eleven serum samples from patients with pyrexia of unknown origin were examined.

(b) Collection of Samples

Blood samples from live goats, cattle and horses were obtained by venipuncture of the jugular vein and from pigs by incision of a marginal ear vein or the ventral coccygeal vessels. All samples were collected into sterile 1-oz glass pomade jars or 1-oz sterile pill bottles.

Sheep, goats and pigs were killed for autopsy by exsanguination. Cattle were killed by exsanguination after stunning. In all animals samples of carotid blood were collected in 4-oz pomade jars.

At the abattoir, pigs were killed by exsanguination after stunning. Blood samples were collected in labelled sterile 1-oz bottles. Following scalding and dressing, the viscera and carcass were examined and lesions placed in labelled sterile bottles.

All human sera were submitted by the medical practitioner.

(c) Examination of Tissues

The techniques used for bacteriological and histopathological examination of tissues, the identification of strains of *Ps. pseudomallei* recovered and the classification of lesions have been described by Laws and Hall (1963) and Laws (1964).

(d) Techniques of the Serological Tests

The techniques of the C.F. and H.A. tests have been described by Laws (1967). Unless otherwise stated, all tests were done with "O" strain antigen. Antigens prepared from *Ps. pseudomallei* strains J53, G91 and N.C.T.C. 8018 (Laws 1967) were also used.

The agglutination test was similar to that described by Olds and Lewis (1954), with the following modifications: the antigen was standardized until its turbidity corresponded to No. 6 tube in Burroughs Wellcome opacity tubes; the initial serum dilution was 1 in 4; the test was incubated at 56°C for 16-20 hr and read immediately on cooling; the agglutinin titre was expressed as the reciprocal of the highest dilution of serum in which there was complete agglutination. The dilution expressed was the final dilution of the serum in the tube after the addition of antigen.

(e) Technique of the Melioidin Test

The melioidin was Synthetic Medium Melioidin prepared by Olds and Lewis (1954) and stored at the laboratory. The goats were inoculated subconjunctivally in the right eye and intradermally in the caudal fold. The sites were examined on days 3 and 4 and were recorded positive and negative as described by Olds and Lewis (1954).

III. RESULTS

(a) Goats

(1) Herd Goats—Farm A

At autopsy, each of the three sick goats (D136, T881 and D212) had lesions from which *Ps. pseudomallei* was recovered. Goat D212 has lesions of the central nervous system only and had been noticed sick only 24 hours before death. The results of the serological tests on these three goats are given in Table 1. The results of the examination of the 11 clinically normal goats from which *Ps. pseudomallei* was recovered are given in Table 2. The serum of goat T878 of April 30, 1960, was tested with all four antigens in the H.A. test. No haemagglutinins were detected with any antigen.

TABLE 1

SEROLOGICAL RESULTS OF THE 3 SICK GOATS FROM FARM A, FROM WHICH *Ps. pseudomallei* WAS RECOVERED AT AUTOPSY

| Goat No. | Serology, First Sample | | Days to Autopsy | Serology at Autopsy | |
|----------|------------------------|------|-----------------|---------------------|------|
| | C.F. | H.A. | | C.F. | H.A. |
| D136 | 40 | 64 | 9 | 40 | 512 |
| T881 | 40* | N.D. | 5 | N.D. | 4 |
| D212 | — | — | 9 | — | 4 |

* Higher dilution not tested

N.D. = Not tested

— = Negative result

TABLE 2

RESULTS OF THE C.F., H.A. AND MELIOIDIN TESTS ON 11 CLINICALLY NORMAL FARM A GOATS FROM WHICH *Ps. pseudomallei* WAS RECOVERED AT AUTOPSY BETWEEN 6 AND 16 DAYS AFTER INOCULATION WITH MELIOIDIN

| Goat No. | Serology | | Melioidin Test | |
|----------|----------|-------|----------------|-------------|
| | C.F.* | H.A.† | Sub. Con. | Caudal Fold |
| T858 | 40 | 16 | + | + |
| T860 | 40 | 64 | — | + |
| T862 | 40 | 4 | — | + |
| T863 | 40 | 32 | — | — |
| T869 | — | 64 | — | + |
| T872 | 40 | 4 | + | + |
| T873 | 40 | 16 | + | + |
| T878 | — | — | — | — |
| T880 | 40 | 16 | + | + |
| T886 | 40 | 16 | + | + |
| T897 | 40 | 32 | + | + |

* Highest serum dilution tested 1 : 40

† = Highest serum dilution tested 1 : 64

— = Negative reaction

Sub. Con. = Subconjunctival inoculation

Caudal Fold = Intradermal inoculation into caudal fold

No *Ps. pseudomallei* was recovered from the other 23 clinically normal goats. Seven of them had negative C.F., H.A. and melioidin reactions and had no visible lesions at autopsy. Five other goats had one or more calcified abscesses containing inspissated pus in internal organs and gave negative reactions to the C.F., H.A. and melioidin tests. The other 11 goats gave positive reactions to one or more of the diagnostic tests and the results of the examination of them are given in Table 3.

Serum samples collected from uninfected goats after they had been inoculated with melioidin showed significant rises in C.F. titres. The results of the examination of the sera collected at autopsy of all goats which had been inoculated with melioidin are not shown.

(2) Herd Goats—Farm B

All 26 goats bled on September 6, 1960, were negative to the C.F. test, but one (T841) had a haemagglutinin titre of 4. All 24 goats bled on January 24, 1961, were negative to the C.F. test; three had titres of 2, one a titre of 4 and one (T841) a titre of 16 to the H.A. test.

Goat T841 was killed on February 8, 1961, when its C.F. reaction was negative and H.A. titre was 16. No lesions were detected and no *Ps. pseudomallei* was recovered from the organs or lymph nodes.

All 12 goats bled on May 23, 1961, were negative to the C.F. test. One had a H.A. titre of 2. It was not autopsied.

(b) Pigs

(1) Pigs Killed at the Townsville District Abattoir

Ps. pseudomallei was recovered from 9 of the 328 pigs killed at the abattoir. The sera of these 9 pigs gave negative C.F. reactions. Five showed haemagglutinin titres of 4, 8, 8, 32 and 64 respectively, and 4 were negative to the H.A. test. No haemagglutinins were demonstrated in one of these sera tested with all four antigens. One farmer owned 8 of these infected pigs, including the 5 with H.A. titres, and the ninth belonged to a second farmer.

The remaining 319 sera were negative to the C.F. test; 10 had haemagglutinin titres of 2. No lesions were detected in any of these 10 pigs. However, abscesses were detected in 18 of the other 309 pigs with negative C.F. and H.A. reactions and 2 others had subperitoneal spargana. Ten of the 18 abscesses were bacteriologically sterile and the following bacteria were recovered from the other 8: *Pasteurella septica* (2), *Corynebacterium* sp. (2), *C. equi* (1), *C. pyogenes* (1), *Salmonella* Gp C (1), *Erysipelothrix insidiosa* (1).

(2) Herd Pigs

One of the 17 sera collected on October 2, 1958, from pigs on Farm C had a C.F. titre of 80. The titre was 80 when the pig was bled on December 18, 1958. *Ps. pseudomallei* was recovered from splenic abscesses and abscesses in the gastro-hepatic lymph node when the pig was killed on March 21, 1959. Serum was not retained when the pig was killed.

All 114 samples from Farm M in October, 1960, were negative to the C.F. test. Seven showed haemagglutinin titres of 2, 2, 2, 4, 8, 8 and 8 respectively. Between the times the pigs were bled in October and November one pig (titre 8) was killed by the owner. When the 6 remaining pigs were bled in November all had negative C.F. reactions, 5 had negative H.A. reactions but one which had a persistent haemagglutinin titre of 8 was killed for autopsy and *Brucella suis* was recovered from an abscess in the gastro-hepatic lymph node.

All 59 samples from pigs on farm BL were negative to the C.F. and H.A. tests. No pigs were killed for examination.

(c) Cattle

(1) Experimental Infection

The course of the disease and the autopsy findings of calf 901 experimentally infected have been described by Laws and Hall (1963). No C.F. antibodies were detected in any of the serum samples of this calf. Haemagglutinins were first detected 1 week post inoculation (titre 2), had reached a maximum of 32 by week 9 but had decreased to 8 by week 35. Agglutinins were first detected at week 5 post inoculation (titre 8), had reached a maximum of 32 at week 12 but had decreased to 16 by week 35. Both the haemagglutination and the agglutination reactions were negative on the sera collected at weeks 66 and 67. *Ps. pseudomallei* was recovered from pus aspirated from a subcutaneous abscess at week 6. No *Ps. pseudomallei* was recovered from tissues examined at autopsy of the calf in week 67.

(2) Herd Cattle

No C.F. antibodies were detected in the serum of cow A234, which had abscesses due to *Ps. pseudomallei* in the spleen and perirenal fat and a purulent and non-purulent myelitis. Its haemagglutinin and agglutinin titres were both 128.

None of the 23 laboratory cattle or 8 ataxic cattle had serum haemagglutinins. There was no evidence of melioidosis at autopsy of the 8 ataxic cattle. The laboratory cattle were not autopsied.

(d) Horses

Neither of the serum samples from horse C119 (collected the day before it died and at autopsy) showed C.F. antibodies but in each the haemagglutinin titre was 4.

The horse showed nervous signs for only 24 hr before it died. At autopsy *Ps. pseudomallei* was recovered from the brain.

A suspicious C.F. reaction was recorded in both blood samples from the horse which died from traumatic splenitis. Both samples were negative to the H.A. test. This horse had such extensive lesions of the abdominal viscera that satisfactory bacteriological examination was not possible. A beta haemolytic streptococcus was recovered from the liver, gastro-hepatic lymph node and spleen.

(e) Human Patients

The results of the examination of the human patients were as follows:

Rimington (1962) Case IV.—When the patient had been sick a week *Ps. pseudomallei* was recovered from culture of blood and the C.F. titre was 40.

Rimington (1962) Case V.—When the patient had been sick 12 days *Ps. pseudomallei* was recovered from culture of blood; the C.F. titre was 20 and the H.A. reaction was negative.

Rimington (1962) Case VI.—*Ps. pseudomallei* was recovered from pleuritic effusion 22 days after the patient was admitted to hospital. When first tested 15 days later, his serum had a C.F. titre of 80 and a H.A. titre of 64 (highest dilutions tested) and 2 months after this the serological reactions were the same. At this time bilateral cervical abscesses which had developed were surgically opened and *Ps. pseudomallei* was recovered from the pus. Following surgery the abscesses healed. Three weeks after surgery the C.F. titre was 80 and the H.A. titre 128. During the next 7 months the patient showed no evidence of melioidosis and the C.F. titre was then 20 and the H.A. titre 32. No further samples were examined.

Rao (1965) Case 1.—*Ps. pseudomallei* was recovered from exudates or blood of this patient on three occasions during a period of 1 month. Commencing 1 month before the first bacteriological recovery and continuing for 2 months after the last recovery, the patient received intermittent therapy with several antibiotics. He was then placed on sulphonamide therapy. A serum sample was examined 3 months after the last recovery of *Ps. pseudomallei*. This gave a negative C.F. reaction to "O" strain antigen but there was a C.F. titre of 160 to J53 antigen and a titre of 80 to G91 antigen.

Rao (1965) Case 2.—It is not known how long this patient had been sick, but 3 weeks after his admission to hospital *Ps. pseudomallei* was recovered from pus from a subperitoneal lesion. Serum samples were examined from this patient on the day the pus was cultured, the next day and a week later. All had a C.F. titre of 40.

Woolford (1966) Case 1.—This patient was admitted to hospital after being sick for 3 weeks. During the next 3 weeks there was remittent pyrexia and gross liver enlargement with development of a localized anterior bulge (perhaps an abscess). At this time the C.F. titre was 40. On admission to hospital and for the next three months the patient was treated with chloromycetin, penicillin, tetracycline and sulphadiazine. The patient was then placed on sulphadiazine therapy. Following the first positive C.F. reaction, serum samples were examined at monthly intervals for 3 months. Each sample had a C.F. titre of 20. By the time the last serum sample was collected the liver was not readily palpable and the anterior bulge had decreased in size. No bacteriological examination of this patient was attempted.

The other 11 human sera from patients with pyrexia of unknown origin gave negative reactions to the C.F. test.

IV. DISCUSSION

The interpretation of the results of the C.F. and H.A. tests in goats was the same as for sheep (Laws 1967). The sera of infected goats showed a high percentage of positive reactions to the C.F. test. Infection could not be confirmed in 3 goats with reactions to the C.F. test (Table 3). At autopsy, 2 of these 3 goats had sterile abscesses in organs or lymph nodes and it would seem that the C.F. titres were persisting following recovery from *Ps. pseudomallei* infection. The third goat had no visible lesions at autopsy. Laws (1967) reported similar reactions in sheep. As was the case with sheep, these goats were from a flock with active melioidosis.

TABLE 3

RESULTS OF THE C.F., H.A. AND MELIOIDIN TESTS AND THE AUTOPSY EXAMINATION OF THE 11 CLINICALLY NORMAL FARM A GOATS FROM WHICH *Ps. pseudomallei* WAS NOT RECOVERED BUT WHICH GAVE POSITIVE RESULTS TO ONE OR MORE OF THE DIAGNOSTIC TESTS. THE GOATS WERE AUTOPSIED BETWEEN 5 AND 16 DAYS AFTER INOCULATION WITH MELIOIDIN

| Goat No. | Serology | | Melioidin Test | | Pathology |
|----------|----------|------|----------------|-------------|---|
| | C.F. | H.A. | Sub. Con. | Caudal Fold | |
| T866 | — | — | — | + | <i>Corynebacterium</i> sp. lung abscess |
| T867 | S | 32 | — | — | Abscesses in spleen, liver, bronchial and supra-pharyngeal LNS |
| T874 | — | 4 | — | — | Multiple abscesses in liver and mesenteric L.N. All inspissated pus |
| T875 | — | 8 | + | + | Abscesses in both supratharyngeal lymph nodes, one liver abscess |
| T879 | — | 8 | — | — | No visible lesions |
| T889 | 20 | 16 | + | + | Abscesses in spleen, omentum and lung |
| T890 | — | 8 | — | + | Pleural adhesions. Two liver abscesses |
| T892 | — | 4 | — | — | Liver lesions— <i>Oesophagostomum</i> sp. |
| T893 | — | 4 | — | — | Calcified liver abscess |
| T894 | — | 8 | — | — | Liver abscess, inspissated pus |
| T899 | S | 4 | — | — | No visible lesions |

S = Suspicious C.F. reaction

— = Negative reaction

Sub. Con. = Subconjunctival inoculation

Caudal Fold = Intradermal inoculation into the caudal fold

Three infected goats showed false negative C.F. reactions. One of these (D212) had been noticed sick for only 24 hr and it would seem there was too little time for C.F. antibody production. In experimentally infected goats, Laws (1956) had demonstrated the appearance of C.F. antibodies at approximately 7 days after the inoculation of cultures. Although the proof that they were not infected with heterologous strains was not conclusive, it appeared that no C.F. antibodies had developed in the other 2 infected goats with false negative C.F. reactions.

Haemagglutinins were demonstrated in the sera of a high percentage of infected goats. The only false negative reaction was T878, which was negative also to the C.F. and melioidin tests.

Eleven of the 24 goats in which infection was not confirmed had positive H.A. reactions. In 7 of these there were sterile abscesses in viscera or lymph nodes and this suggests persistence of haemagglutinins following recovery from infection. Although haemagglutinins persisted for a longer time than C.F. antibodies the results of the examination of the serum from goat D212 indicated earlier haemagglutinin development. In sheep, Laws (1967) demonstrated slower haemagglutinin development.

The results suggested that C.F. and H.A. tests in parallel would not detect a higher percentage of infected goats than the H.A. test alone and that the C.F. test could not be used with certainty to determine false positive reactions to the H.A. test.

Lewis and Olds (1954) reported on the accuracy of the melioidin test in goats and stated that the caudal fold was a better inoculation site than the eye. The results recorded in the present study support the findings of Lewis and Olds (1954). Two of the 11 infected goats (T863 and T878) had false negative melioidin reactions. One of these was positive and the other negative to both serological tests. There is no information concerning the stage in the course of the disease that the melioidin test becomes positive. The lesions in both goats appeared to be of some standing.

Four of the 23 uninfected goats showed false positive melioidin reactions. Three of these 4 goats had bacteriologically sterile abscesses in the internal organs or lymph nodes.

The time intervals at which the melioidin test can be repeated are not known. Olds and Lewis (1954) in one herd repeat-tested goats after 12 weeks. None of the goats on the second test showed reactions and none were killed for post-mortem examination. These authors showed a rise in agglutinin titre following inoculation with melioidin and in the present work there was a rise in serum C.F. antibody titres following inoculation with melioidin.

There was poor correlation between C.F. antibody titre, haemagglutinin titre and recovery of *Ps. pseudomallei* in pigs. This agrees with the results of Nguyen-Ba-Luong (1956) who found poor correlation between the results of the C.F., H.A. and agglutination tests on serum samples of incontact pigs from a property where melioidosis had been diagnosed.

There were no false positive reactions to the C.F. test but only 1 of 10 infected pigs showed a C.F. antibody titre. Although no tests were done with homologous antigen, limited tests with the four antigens indicated that the false negative sera did not contain detectable C.F. antibodies.

The lowest haemagglutinin titre in an infected pig was 4 and several uninfected pigs had a titre of 2. If a haemagglutinin titre of 4 in a single serum sample was indicative of infection then only 5 of the nine infected pigs showed a positive reaction. Three of these pigs with a false reaction were from one farm and the other pig was from a second farm. The sera of these pigs were not tested with homologous antigens. All five infected pigs with haemagglutinin titres were from the first farm. No haemagglutinins were detected in the serum of the pig from the second farm when it was tested with the four antigens. This suggests that the false negative reactions were due not to variant antigenicity but to lack of detectable haemagglutinin production.

The limited tests on cattle sera showed that the C.F. test was of no value for the diagnosis of melioidosis in cattle but that both a haemagglutination and an agglutination test could be of value. These results suggested that a haemagglutinin titre of 4 and an agglutinin titre of 8 indicated current or recent infection. It seemed haemagglutinins developed earlier in the disease than agglutinins, but both persisted after the animal overcame infection.

In the C.F. test the sera of pigs and cattle were tested only with guinea pig complement. Hoet, Blomfield and Coombs (1954) tested an imallein sera of man, horse, guinea-pig, rabbit, cat, ox, sheep, goat, ferret, pig, dog and fowl with haemolytic complements from guinea pigs, rabbits and man. The pig antisera gave no reactions with guinea pig complement but titres of 80 with rabbit complement and titres of 10 and 5 with human complement. However, the sera was anti-complementary with rabbit complement. Their bovine antisera gave no titre with guinea pig and human complement but high titres with rabbit complement.

The only infected horse examined had been noticed sick for only 24 hours before death. No C.F. antibodies were detected in sera from this animal collected at autopsy or the day before although the haemagglutinin titre in both was 4. The significance of the haemagglutinin titre was unknown but suggested haemagglutinin development very early in the infection. Using a C.F. antigen prepared in a manner similar to that used here Van der Schaff (personal communication) obtained good results on sera of horses experimentally infected with *Ps. pseudomallei*. Cravitz and Miller (1950) stated that complement fixation tests appeared specific for Malleomyces infection but did not differentiate between glanders and melioidosis. It could be expected that the C.F. test reported here would be satisfactory for the diagnosis of chronic melioidosis in horses, particularly so in Australia where glanders is not present in the horse population.

The results of the C.F. test on the sera of infected patients indicated that this test could be of value for the diagnosis of both acute and chronic melioidosis in man. All infected patients had C.F. titres of 20 or higher. The small number of sera (11) from patients in which there was no melioidosis showed no fixation at a serum dilution of 1 in 10.

Fournier and Chambon (1958) using an antigen prepared by trichloroacetic acid extraction of cultures of *Ps. pseudomallei* found 5.2% false positive reactions in the sera of 537 patients with diseases other than melioidosis. Nigg and Johnston (1961) testing the sera of 47 normal patients had no non-specific reactions with an antigen which was a particle free filtrate of a suspension of disintegrated *Ps. pseudomallei* cells.

Chambon *et al.* (1953) reported that with human sera the haemagglutination test was positive earlier and was more sensitive than the agglutination test, but false positives occurred. In the limited tests reported here no false positive haemagglutination reactions occurred but the haemagglutination test failed to detect infection in Rimington's patient V, 12 days after the onset of illness.

Laws (1967) demonstrated antigenic heterogeneity in strains of *Ps. pseudomallei* recovered from sheep. Serological results indicate that Rao's (1966) Case I was infected with a strain of *Ps. pseudomallei* antigenically different from that of the other patients but similar to one of the strains described by Laws (1967). This strain was recovered from sheep near Townsville. There was evidence that Rao's Case I had become infected while resident on Thursday Island.

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REFERENCES

- CHAMBON, L., FOURNIER, J., DE LAJUDIE, P. and DARRIEUSSECQ, J. (1953).—*Annls Inst. Pasteur, Paris* 85:530.
- COTTEW, G. S. (1950).—*Aust. J. Exp. Biol. Med. Sci.* 28:677.
- CRAVITZ, L., and MILLER, W. R. (1950).—*J. Infect. Dis.* 86:46.
- FOURNIER, J., and CHAMBON, L. (1958).—"La Mélioidose et le Bacille de Whitmore". Collection de L'Institut Pasteur. (Ernest Flammarion: Paris.)
- HOET, J. J., BLOMFIELD, A. M., and COOMBS, R. R. A. (1954).—*Br. J. Exp. Path.* 35:32.
- LAWS, L. (1964).—*Qd J. Agric. Sci.* 21:15.
- LAWS, L. (1967).—*Qd J. Agric. Anim. Sci.* 24:207.
- LAWS, L., and HALL, W. T. K. (1963).—*Qd J. Agric. Sci.* 20:499.
- NGUYEN-BA-LUONG (1956).—*Bull. Soc. Path. Exot.* 49:25.
- NIGG, CLARA, and JOHNSTON, MARGARET M. (1961).—*J. Bact.* 82:159.
- OLDS, R. J., and LEWIS, F. A. (1954).—*Aust. Vet. J.* 30:253.
- RAO, AHALYA (1965).—Unpublished data.
- RIMINGTON, R. A. (1962).—*Med. J. Aust.* 1:50.
- WOOLFORD, H. B. (1966).—Unpublished data.

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