

# MELIOIDOSIS IN ANIMALS IN NORTH QUEENSLAND

## 1. INCIDENCE AND PATHOLOGY, WITH SPECIAL REFERENCE TO CENTRAL NERVOUS SYSTEM LESIONS

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### SUMMARY

Between June 1956 and July 1961, 194 strains of *Pseudomonas pseudomallei* were recovered from 116 animals in North Queensland, comprising 75 pigs, 23 sheep, 14 goats, 3 cattle and a horse. Most of the material was obtained near Townsville and most of the isolations were from that area. The infected animals were from 21 different properties.

One hundred and four of the animals showed no symptoms, and the majority of isolations were made from abscesses detected during routine meat inspection. Abscesses were most commonly found in the mandibular lymph node, spleen and lung with associated lymph nodes in pigs, lung and mediastinal lymph node in sheep and lung, mediastinal lymph node and spleen of goats. *Ps. pseudomallei* was the most common organism recovered from abscesses in pigs, and was recovered from 30 per cent. of the abscesses examined. In sheep, this organism was recovered from only 11 per cent. of abscesses, while *Corynebacterium ovis* was recovered from 47 per cent.

Melioidosis abscesses were not characteristic, and varied from a barely visible caseous lesion to a quite extensive suppurating mass. The abscesses were quite poorly or quite well encapsulated. Abscesses caused by *Ps. pseudomallei* were often indistinguishable macroscopically from abscesses from which *Streptococcus* spp., *Corynebacterium* spp. *Salmonella* spp., *Brucella suis*, *Erysipelothrix insidiosa*, *Chromobacterium violaceum* or *Pasteurella septica* were isolated.

Only 12 of the 116 naturally infected animals showed clinical evidence of disease. These were 3 pigs, 3 sheep, 3 cattle, 2 goats and a horse. Four of these, 2 cows, 1 goat and the horse, had infection of the central nervous system. Involvement of the C.N.S. was also seen in 6 out of 10 experimentally infected sheep. The lesions in the C.N.S. were usually not seen macroscopically. Microscopically they were a mixed purulent and non-purulent lesion involving the leptomeninges and grey and white matter of one or several areas of the C.N.S.

### I. INTRODUCTION

The occurrence of melioidosis in animals in Queensland has been described in sheep by Cottew (1950) and Cottew, Sutherland, and Meehan (1952), in sheep and a goat by Lewis and Olds (1952), in goats by Olds and Lewis (1954) and in a pig by Olds and Lewis (1955).

The investigations into melioidosis in North Queensland during the period June 1956 to July 1961 can be described under four headings, viz. incidence and pathology of the disease, bacteriology, epidemiology, and serological methods of diagnosis. The detailed bacteriology, epidemiological studies and serological

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methods of diagnosis will be published separately. This paper reports the observations in this tropical area on the incidence and pathology of melioidosis in pigs, sheep and goats. The bacteriological examination of 360 abscesses from pigs, 399 abscesses from sheep and 62 abscesses from goats is included because these abscesses were macroscopically indistinguishable from those from which *Pseudomonas pseudomallei* was recovered. Also described is the natural infection of the central nervous system (C.N.S.) of two cattle, a horse and a goat. One calf and 10 sheep were experimentally infected. As six of the sheep had symptoms of lesions of the central nervous system they are described in detail.

## II. MATERIALS AND METHODS

(i) *Source of Lesions.*—Material from the various species of domestic animals was obtained from both natural and experimental infections. That from natural infection comprised:

- (a) Abscesses found during routine meat inspection of pigs slaughtered at the local abattoirs.
- (b) Pigs submitted for necropsy. These included a pig examined after its serum had tested positive to a complement fixation (C.F.) test (Laws 1963, in press).
- (c) Abscesses found in sheep during routine meat inspection at the local abattoirs. These sheep were from various areas of Queensland, but before going to slaughter were held on properties in the vicinity of Townsville for periods up to one month.
- (d) Sheep from the Oonoonba Animal Health Station flock. These were bled each month and a C.F. test for melioidosis (Laws, in press) was carried out. Those which were positive were killed and necropsied, as were any which died or were killed in other experiments. In October and November 1959, half of the flock (21 sheep) was killed. In June and July 1960, the remaining 21 sheep were killed for examination. All of these latter animals were negative to the serum C.F. test.
- (e) Sheep from a flock 10 miles south of Townsville. Sheep in the flock were bled on three occasions and positive reactors to the C.F. test submitted for examination.
- (f) A domestic herd of 38 goats.
- (g) Three infected cows and one infected horse.

Sources of material from experimental infection were:

- (a) A calf (901) inoculated subcutaneously with 1 ml of a 24-hr broth culture of *Ps. pseudomallei*, Strain A234 from a cow.
- (b) Ten sheep which were each inoculated subcutaneously with 1 ml of a 24-hr broth culture of *Ps. pseudomallei*. Sheep 1 to 6 were inoculated with Strain A234, Sheep 7 and 8 with Strain C119 from

a horse and Sheep 9 and 10, respectively, with Strains G91 and J53 from sheep. As these sheep were being used in serological investigations, five of them were treated in an endeavour to prevent a fulminating infection. The drugs, dose rates and routes of administration were: chloromycetin (microcrystalline, Parke Davis), 0.5 g intramuscularly; sodium sulphadiazine (May and Baker), 4.0 g intravenously; tetracycline (polyotic, Lederle Laboratories), 0.5 g intramuscularly. Sheep No. 3 was given chloromycetin and sodium sulphadiazine on the 9th, 10th and 11th days post-inoculation, sodium sulphadiazine on the 15th and 19th days and tetracycline on the 22nd and 23rd days. Sheep No. 4 had chloromycetin and sodium sulphadiazine on the 8th, 9th and 10th days and sodium sulphadiazine on the 15th, 19th, 20th and 21st days. Sheep No. 5 was treated with sodium sulphadiazine on the 19th day, Sheep No. 9 with tetracycline on the 5th day and Sheep No. 10 with tetracycline on the 4th day.

(ii) *Histology*.—Tissues for histological examination were fixed in 10 per cent. formalin in 0.85 per cent. saline, embedded in paraffin wax, sections cut  $6\mu$  and stained with haematoxylin and eosin. Some sections were also stained with Giemsa.

The affected part of the central nervous system was postulated from the symptoms displayed by the animal. This part was taken for histological examination and additional sites were also selected.

(iii) *Bacteriology*.—For bacteriological examinations, material from affected viscera, lymph nodes or central nervous system tissue was sown onto 10 per cent. sheep blood agar and MacConkey agar and/or inoculated intraperitoneally into guinea pigs. Before inoculation the tissue was macerated with sterile 0.85 per cent. saline and sand. The media were incubated aerobically at 37°C.

Bacteriological examinations were done on sites of the central nervous system adjacent to those selected for histological examination.

In order to minimize the number of guinea pigs used, some of the specimens were held under refrigeration at 1–3°C for up to 5 days and then inoculated into guinea pigs only if cultures were negative. Generally, very small or very dry abscesses were not cultured, but inoculated into a guinea pig.

Guinea pigs that died were necropsied and pieces of affected tissues smeared onto 10 per cent. sheep blood agar and MacConkey agar. Guinea pigs that survived were killed and examined 3–4 weeks after inoculation. Abnormal tissues were examined bacteriologically.

Motility was determined by dark-ground microscopy of a drop of a nutrient broth culture which had been incubated at 37°C for 24 hr.

All cultures were examined by a slide agglutination test with an antiserum prepared in rabbits, using "Ooonoba" strain of *Ps. pseudomallei*. This strain was isolated from Sheep case 1 (Lewis and Olds 1952).

### III. RESULTS

#### (a) Identification of *Ps. pseudomallei*

All cultures of *Ps. pseudomallei* had the characteristic earthy aromatic odour which was well developed after 24 hours' incubation. The organisms occurred as short rods, and were Gram-negative, with marked bipolarity. All strains were actively motile. On primary isolation, most of the strains had the colonial form of Cottew's (1950) "type A". A high percentage of these colonies showed concentric rings of growth. After prolonged growth on both blood and MacConkey agar, radiate wrinkling appeared occasionally. This is described by Cottew as occurring on glycerol agar. All of the "type A" colonies were of glutinous consistency. They were opaque, frosty white on blood agar and pink on MacConkey agar. Several strains produced round, smooth, moist, mucoid colonies with an entire edge. They were translucent on blood agar and red on MacConkey agar.

Of the 165 guinea pigs inoculated with tissues from animals with suspected infection, 43 died with lesions indicative of melioidosis and from which *Ps. pseudomallei* was recovered.

In the slide agglutination test all cultures were agglutinated by the specific rabbit antiserum.

#### (b) Occurrence and Pathology of Natural Infections

The occurrence of *Ps. pseudomallei* in naturally infected animals is shown in Table 1.

TABLE 1  
OCCURRENCE OF *Ps. pseudomallei* IN NATURALLY INFECTED ANIMALS

Site of Infection	75 Pigs	23 Sheep	14 Goats	3 Cattle	1 Horse
Lung .. .. .	10	17	5		
Mediastinal lymph node .. ..	4	4	8		
Bronchial lymph node .. .. .	12	2	4		
Pleura .. .. .	2	1			
Spleen .. .. .	20	5	7	1	
Liver .. .. .	6				
Kidney .. .. .	2	1	2		
Perirenal fat .. .. .				1	
Suprathyroid lymph node .. ..			2		
Prepectoral lymph node .. ..		1			
Prescapular lymph node .. ..		2	1		
Gastro hepatic lymph node .. ..	8				
Mandibular lymph node .. .. .	31				
Supramammary lymph node .. ..	5				
Uterine discharge .. .. .	1				
Urine (bladder of dead animal) ..	1				
Joints .. .. .		2 (lambs)			
Suprarenal gland .. .. .				1	
Nasal discharge .. .. .			1		
Central nervous system .. .. .			1	2	1
Abscess on aorta .. .. .	1				

### (1) Pigs

A total of 360 abscesses from 226 pigs was examined. *Ps. pseudomallei* was recovered from 110 abscesses from 75 pigs. Of these pigs, 72 were killed at the local abattoirs, 2 dead pigs were submitted for necropsy and the other pig was examined after it had given a positive C.F. reaction. The pigs were from 13 piggeries around Townsville, 2 near Ayr (80 miles south of Townsville) and one from Cairns (230 miles north of Townsville).

Only three pigs showed clinical evidence of disease. These were emaciated and at necropsy each had generalized abscesses. The abscesses in pigs varied in location (Table 1), number, size, encapsulation and the consistency of the contents. The most common sites for localization were the mandibular lymph node, spleen and lungs with the associated lymph nodes. Three pigs had generalized abscesses, 30 others had abscesses in more than one organ or lymph node, while 42 had abscesses in one site only, often the mandibular lymph node.

Macroscopically the abscesses varied in diameter from 1 mm to 3 cm and varied in encapsulation from a just visible capsule to a well-defined fibrous band 3 mm thick. The contents varied from a pale green to white semifluid, glistening pus to a dry caseous mass.

The following descriptions of the microscopic changes seen in lesions illustrate the marked variation in the degree of the inflammatory response and the type of cellular infiltration:

*Acute.*—These abscesses had a central necrotic mass in which there were phagocytic nuclei, surrounded by a zone of macrophages and polymorphonuclear leucocytes (polymorphs), then a reticulum of loose fibrous tissue and round cells, all surrounded by a rather poorly developed fibrous tissue capsule.

*Chronic.*—These consisted of a focus of polymorphs surrounded by a well-defined fibrous tissue capsule in which plasma cells were prominent.

From the other 250 abscesses which were macroscopically indistinguishable from those from which *Ps. pseudomallei* was recovered, the following bacteria were isolated—*Pasteurella septica* 30, *Corynebacterium* spp. 26, *Streptococcus* spp. 15, *Brucella suis* 16, *Salmonella* spp. 10, *Chromobacterium violaceum* 4, *Erysipelothrix insidiosus* 2. No pathogenic bacteria were recovered from 147 abscesses.

### (2) Sheep

In all, 399 abscesses from 280 sheep were examined. *Ps. pseudomallei* was recovered from 46 abscesses from 23 sheep. These sheep included 10 killed at the local abattoirs, 11 from the Animal Health Station flock and 2 from a flock 10 miles south of Townsville. The last two groups were necropsied following positive C.F. reactions on their sera (Laws, in press). The infected sheep killed at the local abattoirs were from three properties. Infected pigs had also been found on these three properties and the property south of Townsville.

Only 3 of the 23 sheep showed clinical signs. One, an adult, was emaciated, while 2 others, both lambs, had abscesses in a metacarpo-phalangeal joint and were lame. One of these also had an increased respiratory rate and a chronic cough. The other isolations were from abscesses in sheep which were clinically normal. From Table I it can be seen that the lung and mediastinal lymph node were most often affected. The abscesses in sheep (Figure 1) were similar to those seen in pigs and had no characteristic features. In two melioidosis abscesses there was some calcification.

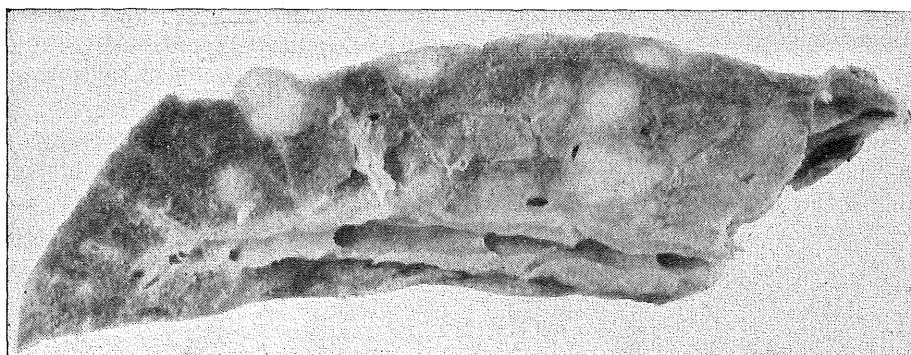


Fig. 1.—*Ps. pseudomallei* abscesses in the lungs of a sheep. Natural infection.

Microscopic examination showed that the changes in tissue infected with *Ps. pseudomallei* varied from acute to chronic.

*Acute.*—In these abscesses there was a central mass of polymorphs surrounded by a zone of macrophages and polymorphs, then a reticulum of loose fibrous tissue containing macrophages, lymphocytes and plasma cells with no well-defined capsule. In lesions of acute purulent bronchopneumonia there was no evidence of alveolar structure in the consolidated lung. In some areas the alveoli were disrupted with red cells and a serous exudate in which were polymorphs and macrophages. Generally the damaged lung showed diffuse infiltration with polymorphs, macrophages, lymphocytes and plasma cells. In some areas there were foci of polymorphs and in others accumulations of lymphocytes and plasma cells. The bronchiolar epithelium was swollen, vacuolated and desquamated and the bronchioles plugged with polymorphs and desquamated cells. There was an increase in lymphocytes in the peribronchiolar tissue. In the less active lung lesions the consolidated lung showed marked epithelialization and a diffuse infiltration with polymorphs and lymphocytes. There were also numerous foci of polymorphs and the bronchioles were plugged with masses of them. Polymorphs and macrophages infiltrated the interlobular connective tissue.

*Chronic.*—Microscopic examination of chronic lesions showed well-defined abscesses. Around the purulent centre there were epithelioid cells and polymorphs surrounded by a layer of epithelioid cells, lymphocytes, polymorphs and plasma cells all enclosed in a wide fibrous tissue capsule.

From the other abscesses which were similar to those infected with *Ps. pseudomallei* the following bacteria were isolated: *Corynebacterium ovis* 188, other species of *Corynebacterium* 9, *Pasteurella septica* 2 and *Streptococcus* sp. 1. No pathogenic bacteria were recovered from 153 abscesses.

### (3) Goats

The organism was isolated from 31 of the 61 abscesses examined in goats and from each nostril of a goat with bilateral nasal discharge. Of the 38 goats in the herd there were no abscesses in 8. *Ps. pseudomallei* was recovered from abscesses in 14 of the remaining 30 goats. A species of *Corynebacterium* was isolated from 1 abscess but no pathogenic bacteria were recovered from the other 30 abscesses examined.

Only two goats showed clinical evidence of disease. One had an elevated temperature, increased respiratory rate and bilateral watery ocular and nasal discharge. The other, D212, showed symptoms of C.N.S. involvement. In the former, there was extensive pneumonia (the consolidated lung contained multiple abscesses a few millimetres in diameter), abscesses in the spleen (up to 1 cm in diameter), abscesses (4 cm in length) in the bronchial and mediastinal lymph nodes, and multiple abscesses a few millimetres in diameter in the liver

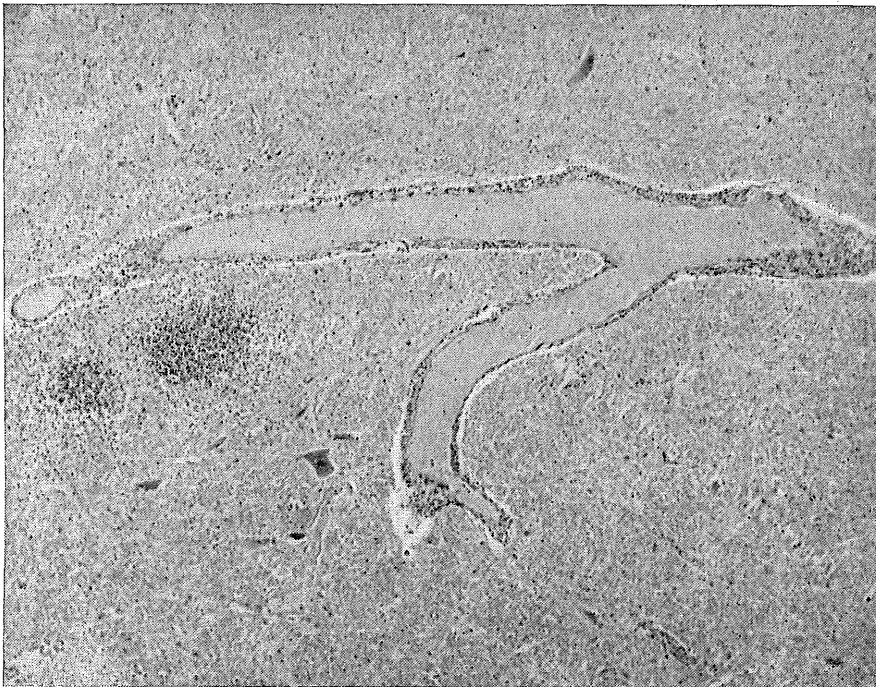


Fig. 2.—Section of the pons of goat D212 showing accumulations of polymorphs and perivascular cuffing. Natural infection. (H. and E. X70).

and kidney. When presented for examination, D212, a young male, was showing ventral and slight lateral flexion of the neck, circling movements and falling onto the right side. While it was recumbent, uncontrolled struggling took place.

At necropsy, the only abnormal macroscopic finding was turbid cerebrospinal fluid. This contained polymorphs. Histological examination showed round cell infiltration of the cerebellar leptomeninges and both round cells and polymorphs were infiltrating the leptomeninges of the pons. In the pons there were numerous micro abscesses, and widespread vasculitis and perivascular cuffing with round cells, polymorphs and mononuclear macrophages (Figure 2). *Ps. pseudomallei* was recovered from the cerebellum but not from cerebrospinal fluid, medulla oblongata, cerebrum or spleen.

The other 12 goats showed no symptoms but the organism was recovered from abscesses in the viscera, most often the lungs, bronchial and mediastinal lymph nodes and from the spleen (Table 1). The abscesses were similar to those seen in sheep and pigs.

#### (4) Cattle

Melioidosis was diagnosed in 3 cows. Two were bred within a few miles of Townsville and the third 50 miles to the south of that city.

Cow A234 was a well-fleshed, paraplegic animal found recumbent in the bed of a river, downstream from infected piggeries. It was killed for necropsy. An abscess 3 cm in diameter was present in the perirenal fat and several abscesses up to 3 cm in diameter were seen in the spleen. No macroscopic lesions could be seen in the spinal cord. However, on histological examination of the lumbar and thoracic spinal cord, minute abscesses and considerable perivascular round cell infiltrations of the grey and white matter were seen as well as leptomeningitis. *Ps. pseudomallei* was recovered from a splenic abscess and the abscess in the perirenal fat. The spinal cord was not examined bacteriologically.

Cow A341 has been described by Laws and Mahoney (in press). This case occurred on a property where melioidosis had been diagnosed in pigs and sheep. When first examined it was recumbent and had a temperature of 105.4°F. Death occurred 18 hr later. At post-mortem examination there was a lesion of the medulla oblongata and the adjacent leptomeninges. Histological examination showed a purulent and non-purulent encephalitis, myelitis and leptomeningitis. *Ps. pseudomallei* was isolated from the medulla oblongata.

Cow B297 was examined by a field officer, who submitted specimens of liver, kidney and suprarenal gland. The C.N.S. was not examined. *Ps. pseudomallei* was isolated from abscesses in the suprarenal gland.

#### (5) Horses

Horse C119 was a thoroughbred gelding depastured with several others on river flats in the vicinity of Townsville. The first indication of illness was when the owner saw the animal standing alone. He noticed that it had an unusual



head posture and walked with a stiff gait. When examined by us it was in lateral recumbency, with tetanic muscular spasm, abnormal curvature of the neck and dorsal rotation of the eyes. The animal died 24 hr after being first noticed sick. No lesions were seen at post-mortem examination. Histological examination showed both round cells and polymorphs infiltrating the leptomeninges of the medulla oblongata and pons. In both the medulla oblongata and pons there were numerous abscesses varying in size from a few polymorphs to 0.5 mm in diameter. Also there was vasculitis, and slight perivascular cuffing with round cells and polymorphs (Figure 3). The cerebrospinal fluid, medulla oblongata, pons, cerebrum and hippocampus were examined bacteriologically. *Ps. pseudomallei* was recovered from the medulla oblongata only.

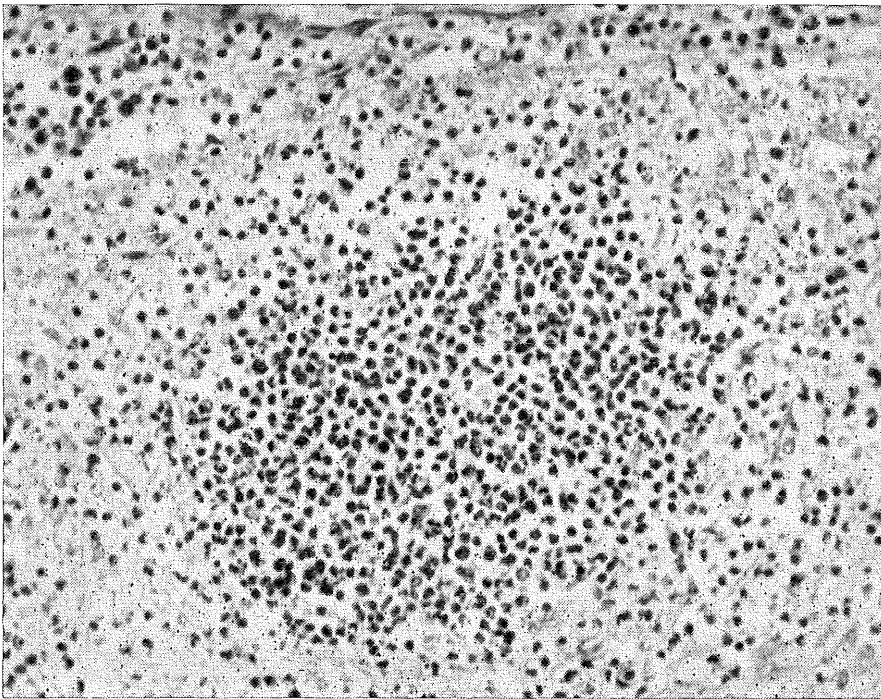


Fig 3.—Micro-abscess in the medulla oblongata of horse C119. Natural infection. (H. and E. X300).

### (c) Manifestations and Pathology of Experimental Infections

#### (1) Cattle

Calf 901, five months of age, had a mild pyrexia for six days after inoculation. By this time an abscess approximately 5 cm in diameter had developed at the site of inoculation. *Ps. pseudomallei* was recovered from pus aspirated from this abscess six weeks after inoculation. The abscess had regressed to approximately 3 cm in diameter by the 14th week and there was no palpable lesion after a further three weeks. When necropsied 15 months after inoculation there was no evidence of infection in the calf.

## (2) Sheep

All 10 sheep experimentally infected developed a pyrexia following inoculation. In each, an abscess developed at the site of inoculation. *Ps. pseudomallei* was recovered from these abscesses by aspiration, six weeks after inoculation, in Sheep Nos. 1 and 2 and at necropsy of the remaining 8 sheep.

At necropsy of these 10 sheep *Ps. pseudomallei* was recovered from abscesses in the following tissues of the number of animals indicated: spleen 4; nose, turbinates and/or septum 3; prepectoral lymph node 2; mediastinal lymph node 1; liver 1; kidney 1; suprathyaryngeal lymph node 1; prescapular lymph node 1; suprarenal gland 1. Abscesses from which no *Ps. pseudomallei* was recovered were also present in: lung 1; prescapular lymph node 1; nose, turbinates and/or septum 1. *Ps. pseudomallei* was recovered from the C.N.S. of 2 sheep only, although 4 other animals had microscopic lesions of this tissue.

Of these experimentally infected sheep, Sheep No. 1 survived for 22 months and was then killed. At this time, it showed no evidence of infection. Sheep No. 2 survived for 12 months. It died from misadventure. *Ps. pseudomallei* was recovered from abscesses in the mediastinal lymph node, liver and spleen. Sheep No. 3 died 25 days after inoculation despite treatment with chloramphenicol, sodium sulphadiazine, and tetracycline.

In the nasal cavity, spleen, right suprarenal gland and at the site of inoculation there were abscesses from which *Ps. pseudomallei* was recovered. Sheep No. 9 was killed on the 9th day after inoculation as part of the epidemiological studies. It was treated on the 5th day with tetracycline. At necropsy, abscesses, from which *Ps. pseudomallei* was recovered, were present at the site of inoculation and in the adjacent prepectoral lymph node.

The remaining 6 sheep—Nos. 4, 5, 6, 7, 8 and 10—showed symptoms of damage to the C.N.S. None had gross lesions of the C.N.S. but all had microscopic lesions.

Sheep No. 4 on the 3rd day after inoculation had a rectal temperature of 106°F. Treatment with chloramphenicol and sodium sulphadiazine was not successful. After treatment the temperature fell, but later rose again. On the 17th day ataxia of the hind-limbs was evident. This became gradually worse and by the 23rd day the hindquarters were paralysed. Death occurred on the 29th day. At necropsy, the hind-limbs were oedematous. Abscesses were present at the site of inoculation, in the nasal turbinates and nasal septum. Some of the abscesses in the latter sites had ulcerated. There were several abscesses in the spleen (Figure 4) and multiple abscesses a few millimetres in diameter in the lungs. Also, there was an abscess in the right kidney and suprathyaryngeal lymph node. No macroscopic lesions were seen in the C.N.S. However, histological examination showed a focus of round cells in the spinal cord at the level of the 12th thoracic vertebra. Both the grey and the white matter of the spinal cord at the 2nd lumbar vertebra had a bilateral purulent and non-purulent inflammation. The leptomeninges of the lumbar and sacral spinal cord were

extensively infiltrated with polymorphs and round cells. *Ps. pseudomallei* was recovered from the abscess at the site of inoculation and abscesses in the kidney, spleen, nasal cavity, supratharyngeal lymph node and the lumbar and sacral spinal cord.

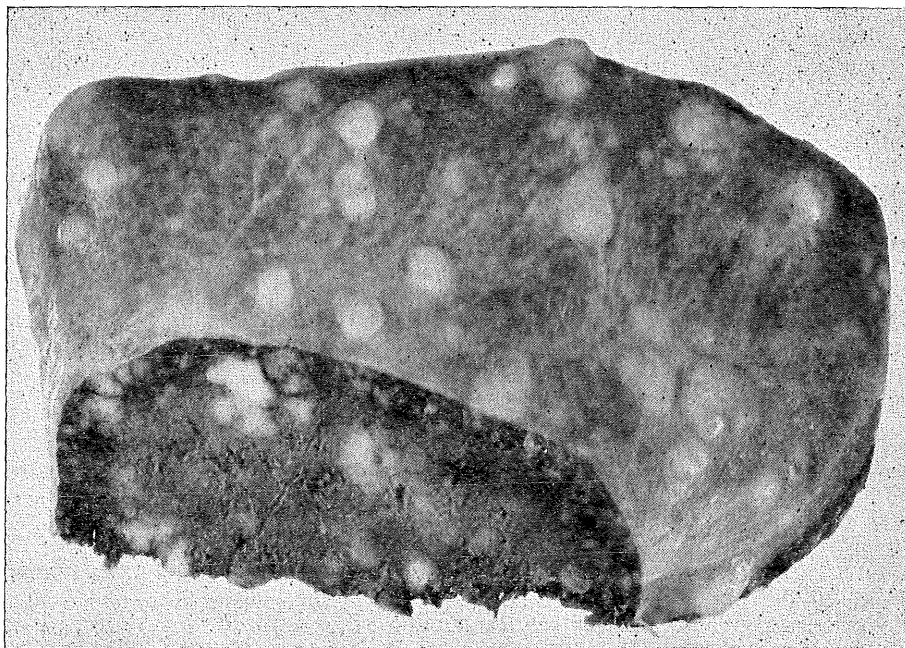


Fig. 4.—*Ps. pseudomallei* abscesses in the spleen of a sheep. Experimental infection.

Sheep No. 5 had a high temperature on the 3rd day after inoculation and remained febrile, except after treatment with sodium sulphadiazine on the 19th day, when the temperature dropped to 102.8°F. On the 20th day tremors of the muscles of the head and neck were noticed. The head was extended and the nose elevated. The animal was unable to eat. On the 25th day it was killed by exsanguination. At necropsy abscesses were found at the site of inoculation and in the nasal septum. Erosion of the right humero-radial articulation was seen. Histological examination demonstrated a mild perivascular cuffing of vessels in the midbrain, a purulent and non-purulent inflammation of the cerebellum, medulla oblongata and leptomeninges, also perivascular cuffing and leptomeningitis in the spinal cord at the level of the 7th cervical vertebra. *Ps. pseudomallei* was recovered from the subcutaneous abscess, nasal cavity, cerebrospinal fluid and cerebellum.

Sheep No. 6 had a fever from the 3rd to 16th day following inoculation, when it became comatose. No treatment was attempted. On the 10th day the right hind leg was paralysed and the animal was unable to rise. Its condition deteriorated and death followed. Necropsy showed a subcutaneous abscess at

the site of inoculation and an abscess in the adjacent prescapular lymph node. The nasal septum and turbinates were ulcerated. Histological examination showed C.N.S. involvement. In the medulla oblongata there was a non-purulent inflammation of the grey matter and a mild round cell infiltration of the leptomeninges. Multiple abscesses were present in the cerebellum, pons and spinal cord at the level of the first cervical vertebra in addition to lesions similar to those seen in the medulla oblongata. The spinal cord at the level of the 7th cervical vertebra was normal. *Ps. pseudomallei* was recovered from the subcutaneous abscess and prescapular lymph node. No bacteriological examination of the C.N.S. was done.

Sheep No. 7 had a febrile reaction for one week following inoculation, after which the temperature gradually decreased. On the afternoon of the 21st day paresis of the hind limbs was observed. If helped to its feet, this sheep would stand, with trembling muscles, and slowly flex its hocks until it again became recumbent. As the condition had not improved on the next day, it was killed for necropsy. There was a subcutaneous abscess 4 cm in diameter at the site of inoculation but no other gross lesions. Microscopically, the spinal cord at the 2nd thoracic vertebra had a perivascular infiltration of round cells in the white matter and to a lesser extent in the grey matter. There was a slight accumulation of round cells on the pia mater. Similar lesions were seen at the level of the 12th thoracic vertebra. A more extensive non-purulent myelitis and leptomeningitis was evident in the spinal cord in the lumbar and sacral regions. *Ps. pseudomallei* was isolated from the site of inoculation only, although samples of the thoracic, lumbar and sacral spinal cord were examined.

Sheep No. 8, after inoculation, showed a rise of rectal temperature to 106.4°F in the morning and 107.2°F in the afternoon of the next day. During the next week it fell to normal. On the 27th day, the sheep was seen to bite at its left side and fall onto its right side in lateral recumbency. After struggling, it regained its feet, but subsequently fell again. The next day this animal could not stand without assistance and all limbs were paralysed. It was killed for necropsy. The only macroscopic lesion was an abscess at the site of inoculation. Histological examination did not show any abnormality of the cerebellum, cerebrum or pons. The leptomeninges at the 7th cervical vertebra had an accumulation of round cells, polymorphs and cellular debris adjacent to the ventral fissure of the spinal cord. The lesion extended into the grey and the white matter of the cord as a round cell infiltration. In the lumbar spinal cord, there was also non-purulent inflammation of both grey and white matter and a leptomeningitis. *Ps. pseudomallei* was recovered from the site of inoculation only. The prepectoral lymph node, liver, spleen, kidney, cerebellum, cervical, thoracic, lumbar and sacral spinal cord were cultured with negative results.

Sheep No. 10 had a normal pre-inoculation rectal temperature but 18 hr afterwards it was 108°F. The animal remained febrile and was treated with 0.5 g of tetracycline on the 4th day when it was showing slight lateral rotation of the head. When it was killed on the 9th day, abscesses at the site of

inoculation and in the prepectoral and prescapular lymph nodes were seen. On histological examination of the brain a small perivascular round cell accumulation was seen in the medulla oblongata. *Ps. pseudomallei* was recovered from the site of inoculation, the spleen and the prepectoral lymph node.

#### IV. DISCUSSION

The number of isolations of *Ps. pseudomallei* (194 strains from 116 animals) showed that it was not a rare infection of animals in the vicinity of Townsville.

Approximately 30 per cent. of the abscesses examined from pigs yielded *Ps. pseudomallei* and it was the most frequently recovered organism from abscesses in pigs. In sheep, *Ps. pseudomallei* was recovered from only 11 per cent. of the abscesses which were examined, while *Corynebacterium ovis* was recovered from 47 per cent. Only one goat herd was examined and half of the 61 abscesses examined yielded *Ps. pseudomallei*.

The majority of animals with melioidosis were symptomless carriers. Of the 116 naturally infected animals, 104 were clinically normal and most of them came to slaughter for human consumption. Further, 2 of the 10 experimental sheep survived inoculation with massive doses of the organism and, after an initial response, showed no clinical signs of infection. One had infected visceral abscesses when it was killed 12 months after inoculation.

In Queensland, Cottew, Sutherland, and Meehan (1952) reported acute melioidosis in both natural and experimental infections in sheep. Also in Queensland, Lewis and Olds (1952) described two acute and four chronic cases in naturally infected sheep, while Olds and Lewis (1954) reported that in goats, although acute infections occurred, the disease was more often chronic. Olds and Lewis (1955) described an acute case of melioidosis in a pig. Suttmoller, Kraneveld, and Van der Schaaf (1957) described an acute outbreak of melioidosis in sheep, but stated that, in Aruba, melioidosis in sheep, goats and pigs was more often a chronic disease. Harries *et al.* (1948) stated that in man melioidosis varied in severity from a fulminating disease often rapidly fatal to one of slow suppuration in one or two sites. However, the description by these authors, Whitmore (1913), Stanton and Fletcher (1932), Mirick *et al.* (1946), and Rimington (1962) are mostly of the acute fatal disease of man. The infections reported here indicate that in domestic animals melioidosis is more often a chronic disease except when the central nervous system is infected.

No outbreaks similar to the one reported by Cottew, Sutherland, and Meehan (1952), in which an estimated 80 of 4,000 sheep on a property died, were reported in this 5-year period.

Melioidosis produces acute to chronic suppurative lesions which Runnels (1954) pointed out may be caused by any of several organisms. In the various species, examination of abscesses which were indistinguishable from those from which *Ps. pseudomallei* was recovered yielded one or more of a variety of pathogenic bacteria.

*Ps. pseudomallei* resembles *M. mallei*, and nasal lesions similar to those seen in glanders occurred in our experimentally infected sheep and also in those described by Cottew, Sutherland, and Meehan (1952). They did not occur in any of the natural infections.

The most common site of natural infection in sheep was the lungs and associated lymph nodes. Both Cottew, Sutherland, and Meehan (1952) and Lewis and Olds (1952) reported abscesses most commonly in the lungs. However, the former described abscesses in the lungs of only 3 of 10 sheep experimentally infected. In the experimental infections of 10 sheep reported in this paper, only 1 sheep had abscesses in the mediastinal lymph node from which *Ps. pseudomallei* was recovered. Another sheep had pneumonia with abscess formation, but *Ps. pseudomallei* was not recovered. There were no abscesses in the bronchial lymph nodes.

Invasion of the C.N.S. by *Ps. pseudomallei* is not common, so the animals affected in this way have been described in some detail. The lesions were not often visible macroscopically. They consisted of micro abscesses and/or round cell infiltrations of the meninges and around the vessels as shown in Figures 2 and 3. These lesions were seen in experimentally infected sheep and in naturally infected cattle, a horse and a goat.

Fournier (1953) recorded a meningitis and *Ps. pseudomallei* infection in a man after the removal of a shrapnel splinter from his cranium. Cottew, Sutherland, and Meehan (1952) and Lewis and Olds (1952) referred to lesions and symptoms of the C.N.S. in sheep. Laws and Mahoney (in press) noticed a fibrinous plaque in the leptomeninges of a cow. In the C.N.S. infections reported in this paper, the only macroscopic abnormality was slightly turbid cerebrospinal fluid in a goat. This lack of macroscopic lesions made it difficult to confirm infection of the C.N.S. A cultural examination of the C.N.S. for bacteria was done on 8 of 9 animals with similar microscopic lesions and which were known to be infected. *Ps. pseudomallei* was recovered from this tissue in only 5 of them. Apparently the small foci of infection were missed in the sampling.

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