Melioidosisis in intensive piggeries in south eastern Queensland

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SUMMARY: The epidemiology of melioidosis was investigated in 8 intensive piggery units which used water from the same river in south eastern Queensland. In 3 consecutive years cases of disease followed heavy rainfall and flooding. Although Pseudomonas pseudomallei was not isolated from water or soil samples the water supply was suspected as the source of infection. Affected pigs were detected at slaughter by the presence of abscesses most commonly in the bronchial lymph nodes (40%) and spleen (34%). One hundred and fifty nine cases were observed at slaughter from a total of 17,397 animals at risk. Infection by inhalation of water aerosols derived from nipple drinkers, hose sprays and a water misting cooler was considered to be responsible for the bronchial lymph node lesions. These outbreaks occurred outside the area in which melioidosis is generally regarded as being endemic.

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Introduction

Animals contract infection with melioidosis from the environment in those parts of the world where the causative agent Pseudomonas pseudomallei exists as a saprophyte in soil and water. In northern Queensland melioidosis occurs sporadically in pigs raised on soil (Laws and Hall 1963, Thomas 1981). On one occasion pigs raised in an intensive piggery were infected via the water supply (Thomas et al 1981). More recently in southern Queensland, clinically normal baconer pigs from a group of intensive piggeries were condemned at slaughter due to melioidosis. Because of this unusual occurrence an epidemiological study was undertaken to determine the extent and cause of the outbreak.

Materials and Methods

Field Studies

Eight intensive piggeries in the Mundubbera and Gayndah areas from which cases of melioidosis had originated were visited and water supply, housing, feed source, and pig movements were investigated. Managers of all other piggeries in the area were canvassed by telephone for the same information and 5 of the larger piggeries that used river water but were unaffected were visited. The units were mapped to show their relation to the river system which was then investigated for features relevant to the disease. Records of monthly rainfall and river flooding were plotted for the 3 years of the study.

Water and soil samples from 2 adjacent units (M1 and M2) with a common water intake and a high prevalence of melioidosis were collected for cultural examination. Water samples of 100 ml and later 1 litre were taken from the river near the common intake each fortnight from November 1981 to June 1982 (14 collections). Samples of water and sediment from the storage tanks of M1 and M2 were collected at the same time. From June to December 1982 a different sampling procedure was used. Moore swabs were left in place for 1 week in the river water at the intake and at a nearby point of leakage from the pipe and soil sampled weekly by the method of Thomas et al (1979) (25 collections). The sites for soil collection were the river bank near the intake, the overflow area of tank M1 and also the bank of a small earthen dam near units M1 and M2. A method was devised to fix a Moore swab within the intake pipes of units M1/M2 and G3. From December 1982 water was sampled by this means and soil sampling continued as for the previous period. Rats for laboratory examination were collected on 2 occasions from units M1 and M2.

Laboratory Studies

In the early stages of the investigation all abscesses of lymph node and body organs, as well as infected skin wounds, were collected at slaughter from pigs from the affected piggeries. In later studies no samples were taken from skin lesions because they were invariably negative for P. pseudomallei. The sites of lesions in affected pigs were recorded. A range of normal tissues—bronchial, cervical and gastrohepatic lymph nodes, spleen, tonsil and faeces—were cultured from 15 pigs with culture positive lesions. Specimens were examined in a biohazard hood and a description recorded. With experience, a decision to culture was made on the basis of site of the lesion and its appearance. Sterile instruments were used to excise a small portion (3 to 4 g) of the abscess which included the capsule. This was dipped in alcohol, flame and then
homogenised in 5 ml of broth using a stomacher. Blood agar and MacConkey agar plates were streaked with a loopful of homogenate and incubated at 37°C in an aerobic atmosphere. Culture plates were examined daily for 5 days and *P. pseudomallei* was identified by its characteristic colonial and microscopic morphology, and biochemical activity.

Water and soil samples (100 g) were examined using a selective enrichment culture method (Thomas et al 1979). Larger water samples (1 litre) were filtered after overnight settling using a membrane with 0.45μm pore size. The membrane was placed in enrichment broth. Moore swim water samples were examined by the method used for soil samples.

**Prevalence Study**

Infected swine were detected by examination of abscases at abattoir inspection. Confirmation was obtained by laboratory examination of samples of all lesions. A small number of typical lesions with respect to site and appearance but negative on culture were considered due to *

*P. pseudomallei* infection. The prevalence of disease in the boar population at slaughter was estimated for each unit from abattoir records. The temporal distribution of cases was plotted.

**Results**

**Field Studies**

During the 3-year observation period 159 cases of melioidosis were detected from 8 intensive piggeries. All 8 piggeries used untreated river water pumped to holding tanks and then reticulated to the piggery. All were located within 50 km on the Burnett River system at a latitude of 25.5°S. Five units (M1-M5) with the same owner at Mundubbera and 3 units (G1-G3) with different owners at Gayndah were affected. M1 and M2, 2 large breeding and growing units with a total of 700 sows and 3500 progeny were 30 m apart. Their water supply was pumped from an intake pipe just upstream of the Mundubbera town weir, on the Burnett River, and held in 2 concrete tanks adjacent to the units. A muddy sediment was present on the floor of the weir and also on the bottom of the concrete tanks. Unit M3, with 3500 growers, received half of the weaners bred at units M1 and M2. The water intake for M3 was from a water hole with a gravelly floor in the Boyne River near its junction with the Burnett.

The remaining affected farms were breeding and growing units with progeny numbers approximately 10 times the number of sows. Units M4 and M5 contained 200 and 130 sows respectively. Their water intakes were in sandy and rocky water holes in the Burnett River. Units G1, G2 and G3 had 180, 200 and 58 sows, respectively. Units G1 and G2 had the same water supply. The water was pumped from a water hole with muddy floor at the Gayndah township to a concrete storage tank which supplied on-farm tanks. The G3 unit drew its water from a deep hole with muddy floor in the Burnett River.

Pressure nipple drinkers were used in all affected units and passageways between boar pens were high pressure sprayed every 1 to 2 days in M1 and M2. A water misting cooling system was used in hot weather above grower pens in unit G3. Apart from occasional stub boars, there were no introductions to the affected piggeries.

Three of the 5 unaffected large piggeries in the same vicinity pumped water from gravelly waterholes in the river to large earthen dams before use. One of these was a growing unit of 600 pigs and the other 2 had 70 and 250 sows with progeny. Another of the unaffected units with 120 sows used the same water supply as units G1 and G2. A single sterile splenic abscess typical of melioidosis was seen in a boar from this unit in 1982. The fifth unaffected piggery with 40 sows pumped

| TABLE 1 |
|------------------|------------------|------------------|------------------|------------------|------------------|
| **Prevalence of cases of melioidosis found at slaughter in pigs from 8 units in southern Queensland** |
| **Mundubbera units** |
| Year | M1 | M2 | M3 | M4 | M5 |
| 1981 | 11/674*(1.6) | 5/1052(0.5) | 1/1431(0.1) | 0/3200(0) | 0/3200(0) |
| 1982 | 20/1055(1.9) | 57/1448(3.9) | 13/2856(0.5) | 8/1161(0.7) | 9/994(1.0) |
| 1983 | 1/577(0.2) | 12/772(1.6) | 1/1010(0.1) | 0/517(0) | 0/429(0) |
| **Gayndah units** |
| Year | G1 | G2 | G3 |
| 1982 | 2/532(0.4) | 9/628(1.4) | 7/282(2.5) |
| 1983 | 0/457(0) | 3/747(0.4) | 0/243(0) |

* Pigs affected/pigs marketed during the period of each outbreak (1981, 12 weeks; 1982, 27 weeks for M units, 12 weeks for G1, G2, 16 weeks for G3, 1983, 12 weeks).† Percentage of affected pigs are shown in parenthesis

| TABLE 2 |
|------------------|------------------|------------------|------------------|------------------|------------------|
| **Sites of melioidosis abscesses found in pigs from Mundubbera and Gayndah units 1981-1983** |
| Unit | Site | Bron LN§ | Sp† | G-H LN§ | Cerv LN§ | Lung | Liver | Kidney | Other† | TOTAL |
| M1 | 20 | 21 | 3 | 2 | 3 | 3 | 2 | 2 | 2 | 56 |
| M2 | 35 | 36 | 5 | 4 | 10 | 5 | 3 | 2 | 100 |
| M3 | 8 | 5 | 3 | 4 | 0 | 0 | 1 | 0 | 17 |
| M4 | 6 | 4 | 1 | 1 | 0 | 2 | 0 | 0 | 11 |
| M5 | 7 | 4 | 0 | 0 | 0 | 0 | 1 | 0 | 15 |
| G1 | 1 | 1 | 1 | 1 | 0 | 2 | 0 | 0 | 7 |
| G2 | 6 | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 220 |
| G3 | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 7 |

* Bronchial lymph node
† Spleen
§ Gastrohepatic lymph node
§ Superficial inguinal lymph node 1 Seminal vesicle 1 Uterus 1 Heart 1


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water directly from a gravelly waterhole in the river to a holding tank at the piggery. Nine other smaller piggeries which were unaffected also used water directly from the river.

In 7 of the 8 affected units and in all 5 unaffected units investigated, all pigs were housed in pens with an impervious concrete floor with an area of slats. In one of the affected units (G1) the non-lactating sows were kept in unsurfaced yards open to the weather. Six of the 8 affected units used the same commercial rations and the other 2 used home mixed rations. The commercial ration was also widely used in unaffected piggeries. Rats were present on all units visited in moderate to large numbers.

Investigations revealed that the only significant earth work on the river system was the construction, between 1980 and 1983, of a major dam with a rock and concrete wall on the Boyne River 86 km from its junction with the Burnett.

Laboratory Studies

Lesions of melioidosis in baconers at the abattoir consisted of 0.2 to 2 cm abscesses with a homogeneous moist or dry caseous exudate contained in a thin fibrous capsule. The exudate was usually light green, occasionally off white, and the capsule was white. The appearance was sufficiently characteristic to differentiate abscesses from those caused by other agents. Abscesses surrounded by an area of pneumonia consolidation were seen in the lungs. Histologically, the abscess exudate was a caseated mass of inflammatory cells with neutrophils discernable only in the periphery. A narrow zone of macrophages surrounded by fibrous tissue comprised the capsule. Club formations were rarely seen in the periphery of the exudate.

Cultures of faeces and normal tissues from 15 pigs which had typical culture positive abscesses resulted in one isolate of P. pseudomallei. The isolate was from the faeces of a pig with extensive lung and kidney lesions. P. pseudomallei was not isolated from the soil or water samples.

A sample of 10 rats collected from units M1 and M2 during the 1981 outbreak were examined for gross lesions with negative results. A second sample of 20 rats collected in 1983 also had no gross lesions. Pooled liver, kidneys, lungs and heart and a separate faecal sample from each rat in the second sample were cultured but P. pseudomallei was not isolated.

Prevalence Study

The prevalence of melioidosis cases in baconers from each unit during the period of each outbreak is shown in Table 1. The combined monthly frequency of cases from all units is shown in Figure 1. In addition Figure 1 shows the monthly rainfall and Burnett River flooding at Mundubbera for the years 1981-83. Cases of melioidosis occurred soon after river flooding in February 1982 and May 1983. However in 1981 the first cases were detected in June some months after the river flooding of that year. The sites of melioidosis lesions in bacon pigs from the 8 units over the 3 year period is shown in Table 2. Lesions most commonly affected the bronchial lymph nodes (40%) and spleen (34%). In 30% of cases lesions were seen only in the bronchial lymph nodes and in 21% lesions only occurred in the spleen. In unit G3 6 of 7 pigs affected in 1982 had lesions only in the bronchial lymph node.

Discussion

The seasonal occurrence of cases following heavy rainfall and river flooding (Figure 1) suggests that surface water runoff is the source of infection of the pigs although this was not confirmed by the apparently insensitive methods used. It is also possible that the disturbance of soil caused by the building of the dam on the Boyne River contributed to the bacterial contamination of the rivers. P. pseudomallei is held by clay particles in soil (Thomas et al 1979). A previous report (Thomas et al 1981) of melioidosis in an intensive piggery demonstrated clay contamination of the drinking water as the source of infection.

The failure to culture P. pseudomallei from the fortnightly water and tank sediment samples taken throughout the 1982 outbreak may have been due to insufficient frequency of
sampling or inability of the laboratory technique to detect small numbers of organisms. The Moore swab sampling also failed to demonstrate *P. pseudomallei*, but the period of sampling did not coincide with an outbreak of disease. The "inline" Moore swab sampling of water, in operation during the 1983 outbreak, also failed to demonstrate *P. pseudomallei*. This technique may be insensitive and culture of large volumes of water may be necessary.

As a preventive measure automatic "inline" chlorination units were installed in the water intake pipes of units M1, M2 and G3 in late 1982. Subsequently, a reduced incidence occurred in units M1 and M2 in 1983 and none in the smaller unit G3. However the effect of the chlorinators was uncertain since both chlorination units had operating problems in 1983.

Piggeries which drew water from muddy water holes appeared to be predisposed to melioidosis. Five of the 8 affected piggeries and only 1 of the 5 unaffected piggeries used muddy water holes. In addition an intermediate earthen dam for water settling used by 3 of the unaffected units may have reduced bacterial numbers by allowing them to settle with clay particles. The unaffected unit sharing the same water supply as units G1 and G2 probably was challenged in view of the sterile spleenic abscesses found in one pig. The use of water misting for cooling in G2 appears to be the reason for the higher prevalence of disease in this unit. The unaffected 40 sow piggery and the 9 other unaffected piggeries which were not visited were less likely to have cases of melioidosis because pig numbers were small.

Variations in prevalence of melioidosis between the affected units and in different years (Table 1) indicates variation in infective challenge. However, units M1 and M2 with the same water supply, and presumably the same infective challenge, were markedly different in disease prevalence in each of the 3 years. An additional source of infection such as pig to pig transmission within the unit could be responsible for the differences.

The distribution of melioidosis lesions in bacons from affected units (Table 2) gives indications of the pathogenesis and routes of infection. The frequent occurrence of bronchial lymph node lesions (40%) is evidence that inhalation to the lower respiratory tract is common, although lung lesions (7%) were infrequent. Inhalation of infection from aerosols derived from pressure nipple drinkers and from high pressure spraying appears likely. The water misting system used for cooling in unit G3 was suspected to be the cause of the high proportion of bronchial lymph nodes lesions in pigs from this unit.

The high proportion of cases with splenic lesions (34%) indicates that bacteraemia occurs frequently with the disease in pigs. When only splenic lesions were detected (21%) the route of infection is not apparent. Lesions in the liver and gastrointestinal lymph node occurred more frequently than lesions in the kidney but again the route of infection is undetermined.

The above results contrast with those obtained by Laws and Hall (1963). They found the most common site of melioidosis lesions to be the mandibular lymph node and the spleen followed by the bronchial lymph node and the lung. The pigs they examined were raised on soil, and oral infection appeared the most frequent route.

Lesions caused by *P. pseudomallei* were not found in sows and boars culled from the affected units during the 3 years of the investigation. No deaths due to melioidosis occurred in pigs of any age. It therefore appears that pigs of all ages have a high natural resistance to *P. pseudomallei* under conditions of good husbandry and nutrition and that resolution of lesions is the general rule. However, in Malaysia an acute progressive disease was reported in pigs imported to that country from Australia (Omar 1962). Environmental stress at a strain of *P. pseudomallei* of higher virulence may have been responsible for the severity of the disease in that country.

*P. pseudomallei* is a recognised human pathogen, and frequent contact with culture positive lesions by meatworkers and meat inspectors is a cause for concern. With very rare exceptions infections were localised in abscesses in the pigs examined. Therefore, inadvertent contact or aerosol transmission is much less likely to occur than is the case with other human pathogens, for example species of Brucella or *Coxiella burnetti*. Use of gloves and thorough cleansing and disinfection of contaminated knives in hot water are recommended to prevent transmission at the meatworks. Recently *P. pseudomallei* infection of the wrist of a man who worked in a Northern Territory meatworks has been reported (Anon 1983).

All 159 pig carcasses with melioidosis lesions were totally condemned at the abattoir resulting in a loss of about $16,000. A ban on slaughter of all pigs from affected farms, which would have caused much greater financial loss, was contemplated by abattoir staff, but not imposed when the low human risk factor was explained.

There are several reports of melioidosis in animals outside of the zone 23°N and 23°S considered in the past to be the endemic area (Redfearn and Palleroni 1975). An endemic focus of disease in sheep is present in the south west of Western Australia (32°S) (Ketterer and Bannford 1967). *P. pseudomallei* has been established in soil of the Paris Zoological gardens (49°N) and other sites in France and affected horses disseminate the organism in faeces (Galimand and Dodin 1983). In southern Queensland (27.5°S) there is evidence of sporadic infection in cattle (Ketterer et al 1975). The cases in pigs in the central Burnett area of Queensland (25.5°S) represent a new endemic focus, and it appears that contaminated river water is the source.

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