Clinical and pathological observations on goats experimentally infected with *Pseudomonas pseudomallei*

A D THOMAS*, J C FORBES-FAULKNER*, J H NORTON* and K F TRUEMAN†

SUMMARY: The effects in goats of the subcutaneous injection of varying doses of *Pseudomonas pseudomallei* (90 to 500,000 bacilli) suspended in normal saline are described. High doses (⩾ 500 bacilli) caused acute, fatal infections. Lower doses (90 to 225 bacilli) caused acute or chronic disease when infection became established. However, 11 of 18 goats injected with the lower doses of bacilli showed no sign of infection on clinical or bacteriological examination. Response to antibiotic therapy with long acting tetracycline and chloramphenicol was minimal. Goats surviving the initial phase of infection tended to overcome the disease with a corresponding increase in the number of abscesses that were sterile at necropsy. In infected goats, clinical signs included undulating fever, wasting, anorexia, paresis of the hind legs, severe mastitis and abortion. At necropsy, abscesses were found predominantly in the spleen, lungs, subcutaneous injection site and its draining lymph node.

Aust Vet J 65: 43-46

Introduction

Pseudomonas pseudomallei has been isolated from both normal and mastitic goats' milk (Lewis and Olds 1952). An increase in the human consumption of raw goats' milk in northern Queensland has highlighted a potential public health risk and accreditation of goat herds for freedom from melioidosis has been introduced. This has indicated the need for a better understanding of the disease and its clinical and serological responses in goats, so that accurate and sensitive tests for diagnosis can be introduced.

This paper records the clinical and pathological effects in goats subcutaneously injected with different doses of *P. pseudomallei*.

* Oonoonba Veterinary Laboratory
P.O. Box 1085
Townsville Queensland 4810
† Animal Research Institute
Fairfield Road
Yeerongpilly, Queensland 4105

Material and Methods

Goats

The experiment used 8 non-pregnant, 5 pregnant and 24 wether adult Saanen goats obtained from an area recognised as being free from melioidosis. None of their serums reacted at positive titre in complement fixation and indirect haemagglutination tests for melioidosis (Alexander *et al* 1970).

The goats, numbered 1 to 37, were housed in appropriate groups in an isolation facility. Care was taken to prevent cross-infection.

Inocula

The inocula were prepared from either a local sheep, bird or goat isolate of *P. pseudomallei* grown in trypticase soya broth for 24 h at 37°C. Suspensions in sterile saline were standardised optically and a final count determined using a Neubauer cytometer. The inocula were used within 2 h of preparation. One ml of the appropriate suspension was injected subcutaneously just above the coronary band of the

right foreleg of each trial goat. The goat isolate only was used at a range of dose rates comprising >500, 500, 225 and 90 bacilli (Table 1). The goat, sheep and bird isolates were compared at a dose rate of 500 bacilli (Table 2); 0.5 ml of each inoculum was also injected intraperitoneally into guinea pigs to test for virulence.

Eleven goats (nos. 27 to 37) in the control group were injected with 1 ml of sterile saline; of these, 2 were non-pregnant, 3 were pregnant and 6 were wether goats.

Clinical Observations and Samples

All goats were observed daily for abnormalities. Rectal temperatures were recorded twice daily for 2 weeks after inoculation and then each morning until necropsy. Injection sites were carefully examined for abscesses, and any exudate was sampled. Discharge from the udders of mastitic goats was also sampled. Blood samples were collected for culture twice weekly for the first 3 weeks after inoculation and weekly thereafter. Faecal and urine samples were collected weekly for cultural examination.

Therapy

Goats showing signs of high fever, persistent lameness or both were treated with antibiotics. These goats were given 300 to 800 mg doses of long-acting oxytetracycline followed by, or in conjunction with, 400 to 800 mg doses of chloramphenicol. The dose and the number of treatments used varied with the clinical signs of individual goats.

Necropsy

Those goats that despite treatment with antibiotics, showed anorexia, wasting, persistent paresis of the hind legs or a continuing high fever, were killed when it was evident they would not recover. Control goats and inoculated goats showing no clinical signs of disease were killed at intervals throughout the study to investigate their disease status. A thorough necrospy was performed on each goat. Samples of heart blood, lung, liver, spleen, brain, cerebrospinal fluid, right prescapular lymph node, retropharyngeal lymph nodes, urine, faeces, injection site, uterus, udder supramammary lymph nodes and lesions at any other sites were aseptically collected for bacteriological examination.

Culture

Samples were cultured on sheep blood agar, MacConkey agar, and 3% glycerol agar with added antibiotics and crystal violet (Thomas *et al* 1979). The plates were incubated for 4 d at 37°C. Isolates were identified as *P. pseudomallei* by conventional biochemical tests, methylene blue reduction disc tests (Thomas *et al* 1982) and the Microbact 24E system* (Thomas 1983). Identity of isolates was confirmed by agglutination with rabbit antiserum to *P. pseudomallei*.†

Results

Clinical Signs

All guinea pigs injected with the *P. pseudomallei* inocula died from melioidosis within 1 to 2 d. The 11 control goats were healthy throughout the trial. The 3 pregnant goats kidded normally.

Goats inoculated with a goat strain of P. pseudomallei

The 2 goats (1 and 2) given high doses (6,000 and 500,000 bacilli) had temperature rises up to 41°C within 24 h of inoculation. An undulating fever continued until they were killed, approximately 2 months after inoculation. Signs observed included anorexia, wasting, lameness in the foreleg and paresis of the hind legs. Abscesses developed at the site of injection. The abscess on goat no. 1 burst 9 d after inoculation. The wound was cleaned and it healed in 5 d.

The temperatures of the 2 goats (5 and 6) given doses of 500 bacilli rose to 41.5°C within 48 h after inoculation. An undulating fever continued until they were killed, approximately one month after inoculation. Goat no. 5, which was pregnant at the time of inoculation, gave birth to full term twins 11 d after inoculation. One of the twins was healthy but the other was stillborn. Goat no. 6 developed severe mastitis. The udder was swollen and there was pus in the milk. Both goats had anorexia and lameness of the foreleg.

* Disposable Products, Adelaide, South Australia

† For the purposes of this paper, the isolation of *P. pseudomallei* from infected tissues will be described as "culture positive", and failure to isolate the organism as "culture negative".

TABLE 1

Observations on 22 experimentally infected goats, subcutaneously injected with varying doses of a goat strain of *Pseudomonas pseudomallei

Goat Numbers	1	2	5	6	9	11	13	14	17	18	19
Description†	F	F	pF	F	W	W	W	W	W	W	W
Dose rate	$5x10^5$	$6x10^3$	500	500	225	225	225	225	225	90	90
Therapy (number of injections)	8	6	14	25	1	5	4	4	3	0	0
Necropsy (days after inoculation)	60	51	34	34	31	156	31	31	31	156	71
Number of tissues with culture positive lesions	3	4	5	7	14	3	9	5	13	0	3
Number of tissues with culture negative lesions	0	0	0	0	0	2	0	1	0	2	2

* — 11 inoculated goats did not show clinical signs or pathology

† — F = female; pF = pregnant female; W = wether

TABLE 2

Observations on 6 experimentally infected goats, subcutaneously injected with 500 bacilli of a sheep, goat or bird strain of *Pseudomonas pseudomallei*

Goat Numbers	3	4	5	6	7	8
Description*	F	pF	pF	F	F	F
Dose rate†	S	S	G	G	B	B
Therapy (number of injections)	8	21	14	25	1	6
Necropsy (days after inoculation)	104	34	34	34	96	28
Number of tissues with culture positive lesions	3	12	5	7	0	4
Number of tissues with culture negative lesions	4	0	0	0	2	0

* — F = female; pF = pregnant female

† — Strain of *P. pseudomallei* used: S sheep, G goat, B bird

Four of the 9 wethers given doses of 225 bacilli remained clinically normal. The temperatures of the 5 affected goats (9, 11, 13, 14 and 17) rose to 41.4°C within 3 to 6 d after inoculation. Four of these goats (9, 13, 14 and 17) had undulating fevers until they were killed 31 d after inoculation. Goat no. 11 also had an undulating fever until 31 d after inoculation when the temperature returned to normal, and the goat appeared healthy until the end of the experiment. All 5 goats had lameness of the inoculated foreleg. The lameness in goat no. 11 disappeared one month after inoculation.

Seven of the 9 wethers given the lowest dose of 90 bacilli showed no clinical signs. The temperatures of the other 2 goats (18 and 19) rose to 41.1°C within 4 to 8 d after inoculation. Goat no. 18 had an undulating fever until killed 5 months after inoculation. Goat no. 19 had an undulating fever until killed 2½ months after inoculation because it was in poor condition. No antibiotic treatment was given to either of these goats (Table 1).

Goats Inoculated with 500 Bacilli of a Bird or a Sheep Strain of P. pseudomallei

The temperatures of the 2 goats receiving the sheep strain (3 and 4) and the 2 goats receiving the bird strain (7 and 8) rose to 41.5°C within 48h after inoculation. Two of the goats (4 and 8) had undulating fevers until they were killed approximately one month after inoculation. Goat no. 4 was pregnant at the time of inoculation and aborted 9 days later. This goat developed severe mastitis. The temperatures of goats nos. 3 and 7 returned to normal 15 days after inoculation. Because it was in poor condition, goat no. 7 was killed 3 months after inoculation. Goat no. 3, after initial lameness of the inoculated foreleg, remained healthy until it was killed 3½ months after inoculation.

Pathology and Bacteriology

All 11 control goats and 11 inoculated but clinically normal goats had no gross lesions at necropsy, and were culture negative.

Lesions were seen at necropsy in all 15 goats which showed clinical signs (Tables 1 and 2). Fibrous adhesions were present in the thoracic and abdominal cavities in 6 of these goats.

Abscesses varied in size from 1 mm up to 3 cm in diameter with the majority being in the range 2 to 5 mm. The number of abscesses in organs varied from single lesions to multiple abscesses which tended to coalesce in the lungs. The abscesses were discrete and firm becoming well encapsulated with age. The content was white to cream and occasionally had a greenish tinge, and varied in consistency from viscous, through caseous to dry and crumbling. The latter type was associated with older lesions which were often sterile.

Isolation of *P. pseudomallei* was mainly from abscesses, except in the brain, where 2 of the 3 culture positive samples were not recovered from lesions, and in a few of the infected lymph nodes which were 2 to 3 times normal size with hyperaemia of the cut surface.

P. pseudomallei was isolated from 13 of the 15 goats showing clinical signs; the remaining 2 goats (7 and 18) had sterile abscesses in the spleen and swollen right prescapular lymph nodes.

P. pseudomallei was recovered from the spleen (11 goats), prescapular lymph node of the inoculated leg (10), lung (9), injection site (9), retropharyngeal lymph node (7), kidney (5), mediastinal lymph node (5), liver (4), mesenteric lymph node (3), rumen wall (3), brain (3), cerebro-spinal fluid (2), sternum (2), mandibular lymph node (2), nasal turbinates (2), uterus (2), supramammary lymph nodes (2), udder (2) and milk (2). There were single isolations from faeces, urine, intercostal lymph node, bronchial lymph node, superficial inguinal lymph node, tonsils, shoulder tendon and the skin of the prepuce.

P. pseudomallei was cultured from the abomasal contents, heart, lung, liver and kidney of the aborted foetus. The stillborn full term foetus was culture negative but *P. pseudomallei* was isolated from the lungs of its twin, when killed at 25 d after birth.

Faecal and urine samples taken throughout the trial were culture negative, although faeces covered with contaminated placental fluids were culture positive in one case (goat no. 4).

P. pseudomallei was isolated from only one of the blood samples (goat no. 5).

Discussion

P. pseudomallei is a soil saprophyte (Thomas *et al* 1979), and has been isolated in concentrations up to 40,000 bacilli/g of soil during the summer months in northern Australia (Thomas and Forbes-Faulkner 1981). Wound infection in sheep and goats is a common route of entry for *P. pseudomallei* (Thomas 1981; Fatimah *et al* 1984). Hence, subcutaneous inoculation of the foreleg of the experimental goats was used to simulate field conditions of infection.

The severity of the disease produced was dependent upon the dose of *P. pseudomallei* given. When the goat strain was used, there was a 100% infection rate when 500 or more bacilli were injected. The course of the disease was acute and sufficiently severe to warrant euthanasia in all 4 goats despite antibiotic treatment. Five of the 9 goats (55%) given a dose of 225 bacilli became infected. Four of these goats developed an acute, severe form of melioidosis while the fifth goat developed a chronic form of the disease. When 90 bacilli were injected into 9 goats, only 2 goats (22%) became infected, developing a chronic form of the disease. One of these goats was able to overcome the infection (no positive cultures at necropsy, 5 months after inoculation).

These results differed from those of Narita *et al* (1982) who inoculated 8 goats subcutaneously with $\geqslant 6.5 \times 10^7$ bacilli of a goat strain of *P. pseudomallei*. They observed a temperature rise of 1.5 to 3.0°C and swollen femoral lymph nodes in all 8 goats but no paresis of the hind legs or undulating fevers. Seven of the goats recovered from illness 5 days after inoculation although all were culture positive when killed between 7 and 23 days after inoculation. It is possible that the goat strain of *P. pseudomallei* used by Narita *et al* (1982) was a relatively avirulent strains. Avirulent strains of *P. pseudomallei* can produce local and systemic disease but only if given in very large doses (Howe *et al* 1971).

Three *P. pseudomallei* strains isolated from different hosts (goat, sheep and bird) were compared at a dose rate of 500 bacilli in this study. All gave a similar disease pattern (Table 2) with 4 goats (2 inoculated with the goat strain and 1 each with a sheep and a bird strain) developing acute melioidosis and 2 goats (1 inoculated with a sheep strain and the other with a bird strain) developing chronic melioidosis. It could be assumed that the virulence of all 3 isolates was equivalent.

Sterility of older lesions with possible regression has been observed in pigs (Omar *et al* 1962) and goats and sheep (Olds and Lewis 1954). Of the 15 goats in this study showing clinical signs of melioidosis, 5 goats survived for longer than 2½ months. When comparing the findings in these goats with the 10 goats that were killed within 1 to 2 months after inoculation, it can be shown that the number of tissues with culture positive lesions, on average, decreased from 7.1 to 1.8 and the number of tissues with sterile lesions increased from 0.1 to 2.4 in the longer surviving goats. The regression of lesions could have resulted from antibiotic treatment. However, the 2 goats which received 90 bacilli were given no antibiotic treament and thus appeared to be an example of natural regression as may be seen in field cases.

The posterior paresis seen in some of the experimental cases also occurs in natural infections (Lewis and Olds 1952; Laws and Hall 1963; Omar 1963; Fatimah *et al* 1984). *P. pseudomallei* has a predilection for the central nervous system, possibly gaining access through the cranial nerves (Omar *et al* 1962; Laws and Mahoney 1964; Ladds *et al* 1981).

The spleen, lungs and prescapular lymph nodes of the experimental goats were the major sites of isolation of *P. pseudomallei*, as has been found in field cases by Olds and Lewis (1954), Omar (1963) and Laws and Hall (1963); and in experimental cases by Narita *et al* (1982). The wide distribution

of lesions in the lungs of some of the experimental goats indicated a bacteraemic spread from the injection site, even though only one blood culture was positive for *P. pseudomallei.*

Two goats developed mastitis, and the milk was culture positive. *P. pseudomallei* has been isolated from normal goats' milk (Lewis and Olds 1952; Thomas unpublished data) and from mastitic goats' milk (Lewis and Olds 1952; Omar 1963). There have been no reports recording human cases of melioidosis from the drinking of infected milk. However, the results indicate a potential health risk.

Intrauterine spread of melioidosis has been recorded in pigs (Rogers and Anderson 1970) and in a goat (Retnasabapathy 1955). Both of the inoculated pregnant goats in this trial had kids that were culture positive. Also *P. pseudomallei* was isolated from the uterus of the goat that aborted and another non-pregnant animal. The potential for vertical transmission of melioidosis in goat herds should not be underestimated.

In this study, *P. pseudomallei* was isolated from the urine and faeces of only one of the 15 infected goats, and then only at necropsy. This goat had a septicaemia with culture positive lesions in 12 other sites throughout the body. This supports the view that the spread of melioidosis by contaminated urine and faeces of animals is rare (Omar *et al* 1962; Nouvel *et al* 1976) although cases of faecal spread of the disease in horses in France (Galimand and Dodin 1982) have recently been reported.

P. pseudomallei is resistant *in vitro* to a wide range of antibiotics (Thomas *et al* 1981), but is usually susceptible *in vivo* to tetracycline and chloramphenicol (Hezebicks and Nigg 1958). Well encapsulated abscesses would protect *P. pseudomallei* from the action of antibiotics. In this study, antibiotic treatment was given when required and not on the prolonged basis recommended by Howe *et al* (1971) and Lee and Chua (1986). The 3 goats that were pregnant and/or had mastitis were given the greatest number of antibiotic doses (14, 21 or 25 injections) but this did not stop progression of the disease in the 34 days after inoculation. Treatment is not recommended, except for valuable animals, due to the unpredictable results and the expense of long term antibiotic therapy.

Acknowledgments

The authors thank H Long, R Jack and G Bowden for their assistance.

References

Alexander A D, Huxsoll D L, Warner Jr A R, Shepler V and Dorsey A (1970) — *Appl Microbiol* **20:** 825

Fatimah I, Ikede B O and Mutalib R A (1984) — *Vet Rec* **114:** 67

Galimand M and Dodin A (1982) — *Bull Soc Vet Prat de France* **66:** 51

Hezebicks M M and Nigg C (1958) — *Antbiotics and Chemotherapy* **8:** 543

Ladds P W, Thomas A D and Pott B (1981) — *Aust Vet J* **57:** 36

Laws L and Hall W T K (1963) — *Qld J Agric Sci* **20:** 499

Laws L and Mahoney D F (1964) — *Aust Vet J* **40:** 202

Lee M K and Chua C T (1986) — *Aust NZ J Med* **16:** 75

Lewis F A and Olds R J (1952) — *Aust Vet J* **28:** 145

Narita M, Longanathan P, Hussein A, Jamaluddin A and Joseph P G (1982) — *Natl Inst Anim Health Q (Jpn)* **22:** 170

Nouvel J, Dodin A, Dementoy M-C and Chove M-A (1976) — *Bull Acad Vet de France* **49:** 223

Olds R J and Lewis F A (1954) — *Aust Vet J* **30:** 253

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

Omar A R, Cheah Kok Kheong and Mahendranathan T (1962) — *Brit Vet J* **118:** 421

Retnasabapathy A (1955) — as cited by Omar *et al* (1962)

Rogers R J and Anderson D J (1970) — *Aust Vet J* **46:** 292

Thomas A D (1981) — *Aust Vet J* **57:** 146

Thomas A D (1983) — *Vet Microbiol* **8:** 611

Thomas A D and Forbes-Faulkner J C (1981) — *Aust Vet J* **57:** 535

Thomas A D, Forbes-Faulkner J C and Duffield B J (1981) — *Vet Microbiol* **6:** 367

Thomas A D, Duffield B J and Forbes-Faulkner J C (1982) — *Qld J Agric Anim Sci* **39:** 35

Thomas A D, Forbes-Faulkner J C and Parker M (1979) — *Am J Epidemiol* **110:** 515

(Accepted for publication 15 October 1987)