

## Isolation of *Pasteurella pneumotropica* from rodents in South Africa

BY A. J. SHEPHERD, P. A. LEMAN AND R. J. BARNETT  
*National Institute for Virology, Private Bag X4, Sandringham 2131,  
Transvaal, South Africa*

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### SUMMARY

Four thousand, five hundred and sixteen rodents of 27 species were captured in widely separated localities in South Africa over a period of ten years. Samples of spleen, lung, heart, liver and rectal tissue with faeces were tested for the presence of zoonotic bacteria and 109 isolations of *Pasteurella pneumotropica* were made from 11 species. Latent infection with the organism was found to be widespread although there were temporal fluctuations in prevalence. Field and laboratory evidence suggest that *P. pneumotropica* may be associated with, but not the primary cause of, rodent epizootics in the wild.

### INTRODUCTION

The literature on *Pasteurella pneumotropica* was reviewed by Rogers, Anderson, Palmer & Henderson (1973), and Biberstein (1975). First described and studied by Jawetz (1948), the organism was found to be latent in laboratory rats, mice, guinea pigs and hamsters and produced a necrotizing pneumonia in mice on serial passage (Jawetz, 1950). Subsequently, latent infection was found to be widespread in a number of mammal species, including horses, calves, dogs, cats and man (Henriksen & Jyssum, 1961; Hoag, Wetmore, Rogers & Meier, 1962; van Dorssen, de Smidt & Stam, 1964; Flynn, Brennan & Fritz, 1965; Blackmore & Casillo, 1972; Moore, Allen & Ganaway, 1973). Naturally occurring disease which has been recorded in laboratory mice includes pneumonia, conjunctivitis, abortion and abscesses (Brennan, Fritz & Flynn, 1965; Weisbroth, Scher & Boman, 1969; Needham & Cooper, 1975; Wilson, 1976; Ward, Moffat & Olfert, 1978). Pneumonia, mastitis and other conditions have been reported in rats (Brennan *et al.* 1965; Schulz, Pohl & Mannheim, 1977; Hong & Ediger, 1978). Various conditions have also been recorded in the hamster, dog and kangaroo (Brennan *et al.* 1965).

With the exception of Crowngold (1973), who isolated *P. pneumotropica* from wild rodents in Johannesburg, the organism does not appear to have been studied in wild rodent species. This paper reports isolation of *P. pneumotropica* from South African rodents captured in a plague survey in the period 1971–1981. Field observations are also reported.

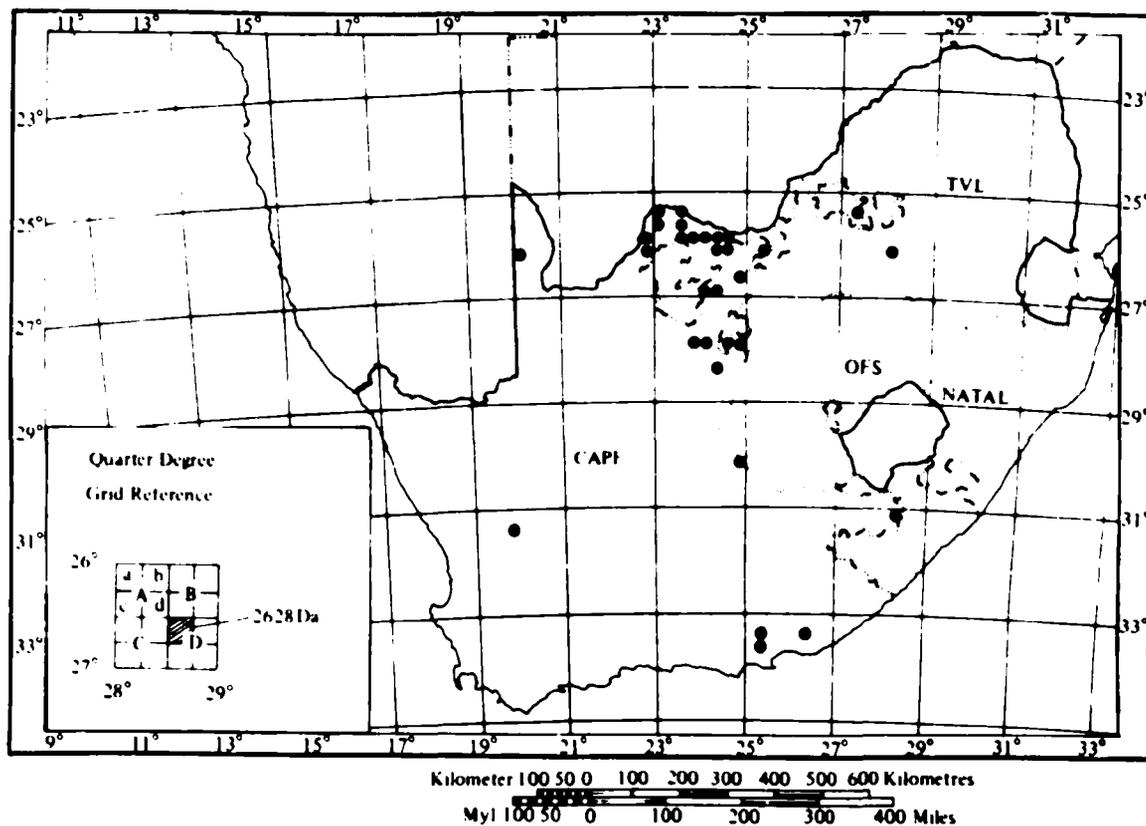


Fig. 1. The distribution of *Pasteurella pneumotropica* isolations from rodents in South Africa. Points of collection indicated by ●. Insert bottom left explains the means of locality reference. (Davis, 1948.)

## MATERIALS AND METHODS

### *Rodents*

Wild and peri-domestic rodents were captured and sent to the laboratory as part of a plague surveillance programme. The collecting localities included the north western, north eastern and south eastern Cape Province, the Orange Free State and the western Transvaal (Fig. 1). Specimens were also examined from coastal ports in Natal. Collections were supplemented by two field trips in the Transvaal to collect springhares and other rodents. The survey began in August 1971, and data presented refer to investigations made from then until August 1981.

### *Bacteriological investigations*

Rodents were anaesthetized with ether and bled by cardiac puncture. The animals were dissected aseptically and pieces of liver, spleen, lung and heart blood were inoculated onto blood agar (5% horse blood with Oxoid Columbia agar base) and MacConkey agar (Mast Ready Pac, Mast Laboratories). Pieces of rectum with formed faeces were inoculated into Selenite F broth (Difco Laboratories). All media were incubated aerobically at 37 °C for 24–48 h. After 24 h the Selenite F broth was subcultured onto blood agar and MacConkey agar plates. During the last five years of the survey only MacConkey agar was used for Selenite F subculture.

Gram negative bacilli were identified by the fermentation of standard 0.75% peptone water sugars with phenyl red indicator. The urease reaction was determined

using a Christensen's urea agar slope and indole production tested for by Kovak's reagent on cultures grown in peptone water. The oxidase reaction was tested for using an aqueous solution of tetramethyl phenylene diamine dihydrochloride prepared just before use. Tests for the decarboxylase (Falkow) and dihydrolase reactions are described by Cowan & Steel (1965).

The first isolations of *P. pneumotropica* from wild rodents in South Africa were made by Crowngold (1973) from *Rattus rattus* and *Tatera brantsii* at Johannesburg. Positive identification of the organism as *P. pneumotropica* was confirmed by the National Communicable Disease Centre, Atlanta, Georgia, U.S.A., and biochemical and cultural characteristics of these isolates were used as a basis for identification of our own isolates (Crowngold pers. comm.).

## RESULTS

The 4516 rodents of 27 species captured and tested are listed in Table 1. One hundred and nine isolations of *P. pneumotropica* were made from widespread localities in South Africa and from 11 species of rodent. The greatest numbers of isolations were made from the north eastern Cape Province (Fig. 1) in a large area stretching from the Botswana border down to the Kimberley district. In this area *P. pneumotropica* was isolated from *Desmodillus auricularis* (21 isolations), *Mastomys coucha* (19), *Tatera brantsii* (7), *Rhodomys pumilio* (5), *Tatera leucogaster* (3), *Mus musculus* (2) and *Mus minutoides* (1). In the south eastern Cape isolations were made from *R. pumilo* (7), *M. coucha* (6), *Rattus rattus* (3) and *D. auricularis* (2). Elsewhere in the Cape isolations were made from *Parotomys brantsii* (1) in the Karoo (map reference 31 19 Bd) and *Gerbillurus paeba* (2) in the Kalahari (26 20 Aa). In the Transvaal isolations were made in Johannesburg (26 28 Aa) from *R. rattus* (19) and *Otomys irroratus* (2). and further north in the Brits district (25 27 Ad) from *M. coucha* (7) and *T. leucogaster* (2).

The biochemical and cultural characteristics for the 109 *P. pneumotropica* isolates are summarized in Table 2. Identification as *P. pneumotropica* was confirmed on a combination of the following characteristics: colonies were small, convex, rounded, greyish, non-motile, and non-haemolytic (after 48 hours a faint zone of  $\beta$  haemolysis became visible around colonies); odour was similar to that of *Haemophilus influenzae*; growth was generally poor in peptone water but the urease reaction was always strongly positive. Most isolates gave typical *P. pneumotropica* profiles although oxidase, indole, ornithine and sucrose negative and mannitol positive variants were isolated. Fifty of the 109 isolates were assessed for ability to grow on MacConkey agar. Of the 21 isolates which were MacConkey positive 17 were originally isolated on MacConkey agar from faeces and 4 were isolated on blood agar and subsequently tested for growth on MacConkey. Ten of the 23 faecal isolates (Table 3) were isolated before Selenite F subculture onto blood agar was discontinued in 1976. Six of these isolates were MacConkey negative and four were positive. After 1976 13 more strains were isolated from faeces on MacConkey agar. All seven strains isolated in 1981, irrespective of source, were found to be able to grow on MacConkey agar.

Table 1. Isolation of *Pasteurella pneumotropica* from captured rodents

Species	Year of isolation												Total	
	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981			
<i>Pedetes capensis</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	0/2
<i>Petromus typicus</i>	0/4	—	—	—	0/4	0/2	—	—	—	—	—	—	—	0/10
<i>Cryptomys hottentotus</i>	—	—	0/1	0/7	—	—	—	—	—	—	—	—	—	0/9
<i>Mystromys albicaudatus</i>	0/3	—	—	—	0/2	—	—	—	—	—	—	—	—	0/5
<i>Saccostomus campestris</i>	0/10	0/5	—	0/1	—	—	—	—	—	—	—	—	—	0/16
<i>Malacothrix typica</i>	0/2	—	—	—	—	0/2	—	—	—	—	—	—	—	0/4
<i>Desmodillus auricularis</i>	0/6	22/171	1/36	0/1	0/1	0/2	0/4	0/1	0/3	—	—	—	—	23/225
<i>Gerbillurus paeba</i>	—	2/6	—	—	—	0/1	—	—	—	—	—	—	—	2/7
<i>Tatera brantsii</i>	0/30	3/76	3/49	0/29	0/150	0/103	1/129	0/31	0/20	0/14	0/2	—	—	7/633
<i>Tatera leucogaster</i>	0/10	2/28	0/7	0/15	0/23	0/80	1/18	0/11	—	2/25	0/1	—	—	5/218
<i>Aethomys chrysophilus</i>	0/103	—	—	0/1	—	0/17	0/24	0/27	—	0/3	0/2	—	—	0/177
<i>Aethomys namaquensis</i>	0/13	0/15	0/9	0/21	0/19	0/48	0/61	0/24	—	0/1	0/1	—	—	0/212
<i>Lemniscomys griselda</i>	0/7	—	—	—	—	—	—	—	—	—	—	—	—	0/7
<i>Mastomys coucha</i>	0/188	12/98	3/82	0/102	0/94	0/238	0/100	0/98	0/47	12/143	5/55	—	—	32/1245
<i>Mus minutoides</i>	0/3	1/25	0/5	0/3	—	0/2	0/1	0/1	—	0/2	—	—	—	1/42
<i>Mus musculus</i>	0/5	—	0/3	—	—	0/35	0/28	0/18	0/7	1/12	1/9	—	—	2/117
<i>Praomys verreauxi</i>	—	—	—	—	—	—	—	—	0/1	—	—	—	—	0/1
<i>Rattus norvegicus</i>	0/5	0/24	—	—	—	—	—	—	0/2	—	—	—	—	0/31
<i>Rattus rattus</i>	0/8	0/40	22/123	0/3	0/1	0/29	0/1	0/20	0/5	0/1	—	—	—	22/231
<i>Rhabdomys pumilio</i>	0/39	7/189	0/111	0/37	0/14	0/84	0/83	0/36	0/265	4/89	1/23	—	—	12/970
<i>Thallomys paedulus</i>	—	0/3	—	—	—	—	—	0/1	—	—	—	—	—	0/4
<i>Zelotomys woosnami</i>	—	—	—	—	—	0/1	—	—	—	—	—	—	—	0/1
<i>Otomys angoniensis</i>	—	—	0/15	—	—	0/3	0/2	0/7	0/2	0/1	—	—	—	0/30
<i>Otomys irroratus</i>	0/2	0/14	2/42	0/3	0/2	0/1	0/1	0/8	0/3	0/24	—	—	—	2/100
<i>Otomys unisulcatus</i>	0/1	0/7	0/79	0/2	0/24	0/30	0/31	0/23	—	0/1	—	—	—	0/198
<i>Parotomys brantsii</i>	0/3	0/2	1/11	—	0/1	—	—	—	—	—	—	—	—	1/17
<i>Parotomys littledalei</i>	—	—	—	—	0/3	—	—	0/1	—	—	—	—	—	0/4
Totals/year	0/442	49/703	32/573	0/225	0/338	0/678	2/483	0/307	0/355	19/319	7/93	—	—	109/4516

\* Isolations/animals tested.

Table 2. *Biochemical and cultural characteristics of 109 Pasteurella pneumotropica isolates from wild rodents*

Test	Variants*							
	1	2	3	4	5	6	7	8
Number of isolates	54	21	21	6	2	3	1	1
Indole	+	+	+	-	+	+	+	+
Oxidase	+	+	+	+	-	+	+	+
Urease	+	+	+	+	+	+	+	+
Motility	-	-	-	-	-	-	-	-
Glucose (acid)	+	+	+	+	+	+	+	+
Glucose (gas)	-	-	-	-	-	-	-	-
Sucrose (acid)	+	+	+	+	+	+	-	+
Lactose (acid)	+/-	+/-	-	-	-	-	-	-
Maltose (acid)	+	+	+	+	+	+	+	+
Dulcitol (acid)	-	-	-	-	-	-	-	-
Mannitol (acid)	-	-	-	-	-	+	-	-
Sorbitol (acid)	.	.	-	.	.	.	.	.
Inositol (acid)	.	.	-	.	.	.	.	.
H <sub>2</sub> S	-	-	-	-	-	-	-	-
KCN	+/-	+/-	.	-	+/-	+/-	.	.
Arginine dihydrolase	.	.	-	.	.	.	.	.
Ornithine decarboxylase	+	+	+	+	+	+	+	-
Lysine decarboxylase	+/-	+/-	-	+/-	+/-	+/-	-	-
MacConkey agar	.	-	+	-	-	.	.	.

\* Variants: 1-3, Typical *P. pneumotropica*. 1, Ability to grow on MacConkey not determined; 2, MacConkey negative; 3, MacConkey positive; 4, indole negative; 5 oxidase negative; 6, mannitol positive; 7 sucrose negative; 8, ornithine negative.

Table 3 lists the tissues from which *P. pneumotropica* was isolated in each species of rodent. Isolations were most frequent from the lung (63), heart (34) and faeces (23). The liver (10), and spleen (9) were less frequently infected.

The results show temporal fluctuations in the prevalence of the organism in rodents. After numerous and widespread isolations in 1972-73 a quiescent phase, broken only by two isolations in 1977, persisted until widespread prevalence was again noted in 1980-81.

*Field Observations*

In May and August 1980 two field trips were made to Vaalkop Dam in the Brits district of the Transvaal (25 27 Ad) to collect springhares and other rodents. In May it was evident that on the study farm and others nearby there had been an epizootic among gerbils (*Tatera leucogaster*). Few were trapped and most gerbil warrens were deserted. Three gerbil carcasses, in an advanced state of decay, were recovered from excavated warrens. *P. pneumotropica* was not isolated from the carcasses but was isolated from one captured gerbil. Large numbers of *M. coucha* were caught living and breeding in peanut stacks and seven isolations of *P. pneumotropica* were made from this species. No *M. coucha* were trapped or observed anywhere on the farm in August after peanut harvesting.

Table 3. *Rodent tissues from which Pasteurella pneumotropica was isolated*

Species	Tissue					No. of animals
	Lung	Heart	Spleen	Liver	Faeces	
<i>D. auricularis</i>	16	4	—	1	3	23
<i>G. paeba</i>	—	2	—	—	—	2
<i>T. brantsii</i>	3	3	—	1	2	7
<i>T. leucogaster</i>	4	—	2	3	—	5
<i>M. coucha</i>	17	2	3	3	13	32
<i>M. minutoides</i>	—	1	—	—	—	1
<i>M. musculus</i>	2	—	1	1	1	2
<i>R. rattus</i>	15	19	—	—	—	22
<i>R. pumilio</i>	3	1	3	1	4	12
<i>O. irroratus</i>	2	2	—	—	—	2
<i>P. brantsii</i>	1	—	—	—	—	1
Totals	63	34	9	10	23	109

One apparently healthy female gerbil which was brought back to the laboratory in August with several males, and caged with them, was found moribund several days later and died the same day. *P. pneumotropica* was isolated from the lung, liver, spleen, kidney, heart, uterine horn, trachea and naso-pharynx of this rodent. It seemed certain that *P. pneumotropica* septicaemia was the ultimate cause of death. The female was in a potentially stressful situation in being caged with several males and much fighting had occurred.

#### DISCUSSION

The results of this survey suggest that *P. pneumotropica* is as common in South African wild rodents as it is in laboratory colonies. The affinity for the lung which induced Jawetz (1950) to propose the name *P. pneumotropica* was well marked. Faecal isolations reported previously by Wheeler (1967), Moore *et al.* (1973) and Hong & Ediger (1978) also occurred frequently.

It is probable that transmission of the organism takes place via several pathways. Hong & Ediger (1978), reporting on cases of mastitis in rats due to *P. pneumotropica*, found that the organism became established in the oral cavity of young rats up to six weeks old before moving into the gut. Coprophagy is often observed in young captive wild rodents kept at this laboratory, and it is possible that this may be an important source of infection in the wild. Inhalation of bacteria from the contaminated environment, faecal suspension (Wheeler, 1967) and naso-genital contact when the vagina and uterus are infected (Blackmore & Casillo, 1972), may lead to respiratory tract and lung involvement. Colonization of the gut may occur secondarily to lung infection due to regurgitation and swallowing of organisms, similar to the transport from lung to gut of larvae of some nematode species. Ward *et al.* (1978), reporting on the presence of the organism in male rat genitalia, postulated transmission by sexual intercourse.

A wide variation in biochemical reactions was noted in the isolates of this survey,

but according to the taxonomic review of the genus *Pasteurella* by Mannheim, Pohl & Hollander (1980) all isolates are identifiable as *P. pneumotropica*. No other *Pasteurella* species were isolated in this survey. In common with the strains described by Simmons & Simpson (1977), Schulz *et al.* (1977) and Mannheim *et al.* (1980) we isolated variants which were able to grow on MacConkey agar. However, 29 out of 50 isolates tested were unable to grow on MacConkey agar and it is likely that more such strains would have been isolated from faeces had not the use of blood agar for Selenite F subculture been discontinued in 1976. Other variants recorded in this survey have also been reported previously. These include oxidase negative (Henriksen & Jyssum, 1961; Simmons & Simpson, 1977), indole negative (Hooper & Sebesteny, 1974; Simmons & Simpson, 1977; Schulz *et al.* 1977), ornithine negative (Cowan, 1974) and mannitol positive (Henriksen & Jyssum, 1961; Hoag *et al.* 1962; Simmons & Simpson, 1977). However, no other sucrose negative reports were found in the literature although Heyl (1963) reported a delayed sucrose reaction only after 48 h. Variation in colony colour in indole negative strains, noted previously by Hooper & Sebesteny (1974) and Simmons & Simpson (1977), was not observed.

Rodent populations in South Africa undergo periodic fluctuations usually with marked population explosions followed by decline (van der Merwe & Keogh, 1970). Evidence indicates that the temporal fluctuations in isolations of *P. pneumotropica* are associated with high populations and mortality among rodents. In 1972 *P. pneumotropica* was associated with high population levels of the Namaqua gerbil (*D. auricularis*) in the northern Cape Province prior to a dramatic decline in the population. Interestingly, an epizootic among Namaqua gerbils, caused by a *Pasteurella* species unrelated to any known at that time, was recorded previously in South Africa (Pirie, 1929). Rodent populations were high when isolations were made in the Transvaal in 1980 and in the Kimberley district of the Cape Province in 1981. Field evidence suggests that *P. pneumotropica* was almost certainly associated with mortality among gerbils (*T. leucogaster*) on a farm in the Transvaal in 1980 and must be viewed with suspicion regarding the subsequent disappearance of *M. coucha* from the same farm.

The question of how the normally latent *P. pneumotropica* could become involved in an epizootic is not wholly clear. Increased susceptibility of rodents to disease is one of the consequences of high population levels and crowding (Christian, 1963). Kaplan *et al.* (1980) found evidence that latent or inapparent viruses of field rodents in Britain became activated by immunosuppression due to stress caused by population increase.

*P. pneumotropica* can act as a secondary bacterial pathogen in viral infections. Several viruses are known to lower murine host resistance to bacteria, including influenza (Sellers *et al.* 1961) and reovirus (Philips, Stanley & Walters, 1970). Jakab (1974) found that serial inoculations of Sendai virus and *P. pneumotropica* led to *P. pneumotropica* pulmonary infection, haemorrhage and death in mice. Ward *et al.* (1978) suspected mouse leukaemia viruses present in their laboratory mouse colony as being the primary factor in cases of abortion due to *P. pneumotropica*. *Mycoplasma pulmonis* has also been found to act additively with *P. pneumotropica*

to produce pneumonia in mice (Brennan, Fritz & Flynn, 1969). Viruses and mycoplasmas were not tested for in this survey.

In the light of our findings it would seem that *P. pneumotropica* may cause mortality in African wild rodents under conditions of stress or infection by other agents.

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