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Experimental plague infection in South African wild rodents

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SUMMARY

Susceptibility studies were undertaken to determine the response of some South African wild rodent species to experimental plague (*Yersinia pestis*) infection.

A degree of plague resistance was found in three gerbil species captured in the plague enzootic region of the northern Cape Province, these being the Namaqua gerbil, *Desmodillus auricularis*, (LD50 1×10^6 organisms), the bushveld gerbil, *Tatera leucogaster*, (LD50 $9{\cdot}1 \times 10^5$) and the highveld gerbil, *T. brantsii* (LD50 4×10^2). Animals from a population of the four-striped mouse, *Rhabdomys pumilio*, captured in the plague area of Port Elizabeth, proved moderately resistant to experimental plague infection (LD50 $1{\cdot}3 \times 10^4$) while those from another population of the same species captured in a plague-free area of the Orange Free State were extremely susceptible (LD50, 5 organisms). The response of both populations however was a heterogeneous one. Marked differences in susceptibility were also found between two populations of multimammate mice, *Mastomys natalensis* ($2n = 32$) although both originated from areas outwith the known distribution of plague in southern Africa.

The 50% infectious dose was relatively high in *T. leucogaster* ($3{\cdot}2 \times 10^2$) and *D. auricularis* ($1{\cdot}7 \times 10^3$), but was low (2–16 organisms) in the other rodent species tested.

The plague antibody response, determined by enzyme-linked immunosorbent assay (ELISA), was extremely short-lived in *T. leucogaster*, only 10% of inoculated animals remaining seropositive at low titres after 11 weeks. Antibodies persisted for only slightly longer in the sera of *T. brantsii* which were reinoculated with 2×10^3 plague organisms 6 weeks after initial challenge.

The demonstration of the existence of both susceptible and resistant populations of *R. pumilio* and *M. natalensis* indicates that these species must be considered as potential plague reservoir hosts in parts of South Africa.

The results suggest that resistance to plague infection in previously epizootic hosts in the northern Cape Province such as *Tatera* sp. and *D. auricularis* has arisen through continual selective pressure of the organism. If the findings are applicable to gerbil populations in other plague enzootic regions of South Africa it is probable that acquired plague resistance has been responsible for the absence of gerbil epizootics and consequently for the dramatic decline in human plague outbreaks in South Africa since 1950.

INTRODUCTION

Bubonic plague (*Yersinia pestis*) was introduced into South Africa via the major seaports in the closing years of the 19th century (Mitchell, 1927). Initially confined to urban settlements and transmitted by domestic rats and their fleas, infection soon spread to wild rodents causing an increasing number of human outbreaks in rural areas from 1914 onwards. Once introduced into the susceptible wild rodent populations, spread was rapid and a series of epizootics, most conspicuous in gerbils (*Tatera* sp. and *Desmodillus auricularis*) and multimammate mice, *Mastomys coucha*, swept throughout South Africa and eventually to neighbouring countries on the subcontinent (Davis, 1948). Subsequent epizootics initially followed a periodicity of 5–6 years but gradually became more dispersed in time and place (Davis, 1964). Despite continuing surveillance no plague epizootics in gerbils or other species have been reported in South Africa in recent years, although serological surveys have shown that plague infection persists in wild rodent populations (Hallett, McNeil & Meyer, 1970; Shepherd & Leman, 1983).

It is generally accepted that rodent species which are partially or moderately resistant to plague infection are more likely to be maintenance hosts than species which are extremely susceptible (Poland & Barnes, 1979). Thus in parts of the world plague-resistant rodents such as *Microtus* spp. and *Peromyscus* spp. in the United States of America (Quan & Kartman, 1956), *Arvicanthis* spp. and *Mastomys* spp. in Kenya (Heisch, Grainger & D'Souza, 1953) and *Meriones* spp. in Iran (Baltazard *et al.* 1960) are held to be primarily responsible for the persistance of the organism. Davis (1953*a*, 1964), however, believed South Africa to be an exception. Limited experimental work indicated that with the exception of the Namaqua gerbil, *D. auricularis*, which was resistant, all South African rodent species were uniformly susceptible to plague infection (Pirie, 1927*a*; Davis, 1963). Together with field observations of gerbil epizootics, this led to the conclusion that gerbils alone were responsible for the perpetuation of plague in South Africa; all other rodent species being secondarily infected (Fourie, 1938; Davis, 1953*a*, 1964). Carry-over of infection between epizootics was attributed to fleas which could remain infected in deserted gerbil burrows for up to 3 months (Pirie, 1927*b*; Davis, 1953*a*). Recently, however, evidence has accumulated which suggets that wild rodent species other than gerbils may be more important in plague ecology than was previously suspected. Hallett, McNeil & Meyer (1970) reported high titre antibodies in the bush Karoo rat, *Otomys unisulcatus*, and the springhare, *Pedetes capensis*, and traced infection in a human outbreak directly to *O. unisulcatus* in an area from which gerbils had been eradicted. More recently Shepherd & Leman (1983) detected plague antibodies in the four-striped mouse, *Rhabdomys pumilio*, in the eastern Cape Province and field studies during a human outbreak suggested that *R. pumilio* was the principal plague reservoir host in that area (Shepherd *et al.* 1983). In addition, Isaacson, Taylor & Arntzen (1983) reported that of five Zimbabwean wild rodent species tested, the red veld rat, *Aethomys chrysophilus*, and the multimammate mouse, *Mastomys natalensis* ($2n = 32$) were moderately resistant to experimental plague infection.

In view of the disparity between Davis' (1953*a*, 1964) theory that plague is perpetuated solely by gerbils and the recent evidence implicating other species, it

was decided to reinvestigate the susceptibility to plague of some South African wild rodent species.

A second aspect of the investigation involved serological testing of susceptibility experiment survivors for plague antibodies in order to relate the findings to results obtained in surveys.

MATERIALS AND METHODS

Plague strains and antigen

Virulent *Yersinia pestis* strain SAIMR A33243, isolated from a human case in Ovamboland, South West Africa/Namibia, was used in susceptibility studies. Reference avirulent strain A1122 was used to prepare Fraction 1 (F1) antigen according to methods supplied by Dr T. Quan of the Centers for Disease Control (C.D.C.), Fort Collins, Colorado, USA.

Serology and bacteriology

The enzyme-linked immunosorbent assay (ELISA) and ELISA-inhibition (EI) tests were used to detect antibody to the F1 plague antigen as previously described (Shepherd *et al.* 1984). Commercial (Cappel Laboratories, Cochranville, Pa. 19330, USA) anti-mouse (IgA, G and M) horseradish peroxidase-conjugated immunoglobulin was used at optimal dilution. O-phenylene diamine (OPD) was used as indicator reagent and absorbance read at 492 nm on a Titertek Multiscan.

The fluorescent antibody (FA) test (Moody & Winter, 1959) was used to detect plague bacilli in impression smears of organs of rodents which died after inoculation.

The organs of animals which were sacrificed 21 days post inoculation (p.i.) were plated onto blood agar for isolation of *Y. pestis*. Tests for virulence factors (F1 antigen, V and W antigens, pigmentation and pesticin) of plague isolates were performed as described by Surgalla, Beesley & Albizo (1970).

Animals

Three gerbil species were trapped at several sites in the Vryburg and Kimberley districts of the northern Cape Province where plague appears to be enzootic (Shepherd & Leman, 1983; Special Pathogens Unit, unpublished). These were the bushveld gerbil, *Tatera leucogaster*, the highveld gerbil, *T. brantsii*, and the Namaqua gerbil, *D. auricularis*.

The four-striped mouse, *R. pumilio*, was obtained from two sites. These were Coega, near Port Elizabeth in the eastern Cape Province where a human plague outbreak occurred in 1982 (Shepherd *et al.* 1983) and from several sites in the Philippolis and surrounding districts of the southern Orange Free State, where regular plague surveillance has detected no plague activity in recent years (Special Pathogens Unit, unpublished).

The multimammate mouse, *Mastomys* sp., in southern Africa comprises two sibling species with different karyotypes (Green, Gordon & Lyons, 1978). The $2n = 32$ chromosome species, provisionally named *M. natalensis*, is distributed in the eastern part of southern Africa while the $2n = 36$ chromosome species named *M. coucha* occurs in the western region (Green *et al.* 1980). Specimens

of *M. natalensis* were obtained from Richards Bay, Natal, and from a laboratory colony maintained at the South African Institute for Medical Research, Johannesburg, derived from animals captured at Tzaneen, eastern Transvaal. Both localities lie outside the southern African plague enzootic region (see Davis, 1964).

Specimens of the white-tailed rat, *Mystromys albicaudatus*, and *M. coucha*, which were used as controls on the virulence of the plague strain used, were obtained from laboratory colonies maintained at the Special Pathogens Unit. The extreme susceptibility of the laboratory colonies of both these species to plague had previously been determined (Davis, 1963).

The populations of *T. brantsii* and *T. leucogaster* tested consisted almost entirely of mature adult animals, the majority having been in captivity for up to 2 years prior to the experiments. The other species tested consisted of animals ranging from subadult to adult.

Animals received from the field were bled from the heart under ether anaesthesia and the sera tested for plague antibodies by ELISA and EI tests. The animals were allowed at least 4 weeks to recover before being challenged.

Susceptibility tests

Virulent *Y. pestis* strain SAIMR A33243 was seeded into brain heart infusion broth and incubated for 18 h at 28 °C. Serial tenfold dilutions were made in physiological saline and 0·5 ml of the required dilution inoculated subcutaneously into the hind leg. The titre of the original culture and thus of the dose administered to the rodents was calculated by viable plate counts on blood agar at terminal dilutions. As a control on the virulence of the culture used, *M. coucha* with an LD50 of < 10 plague bacilli were inoculated with the highest two dilutions of the culture on each test.

Plague deaths were confirmed by FA test. Surviving animals were killed 21 days p.i., bled from the heart, autopsied, and their tissues cultured for the presence of viable plague bacilli. The sera were assayed for antibodies to plague FI antigen by ELISA and EI tests.

After 21 days surviving *T. brantsii* and *T. leucogaster* were bled from the heart under ether anasthaesia and kept alive for antibody studies. *T. leucogaster* were re-bled at weeks 7 and 11 p.i. *T. brantsii* were re-inoculated with 2×10^3 plague bacilli at 6 weeks p.i. and the antibody status of survivors determined at 10, 14, 18 and 22 weeks p.i.

The 50% lethal dose (LD50) and the 50% infectious dose (ID50), proof of infection being based on death or seroconversion, were calculated according to the method of Reed & Meunch (1938).

RESULTS

The results of the susceptibility tests, showing the numbers of animals which survived experimental plague infection, those which seroconverted and the mean ELISA titre of seropositive rodents, are presented in Table 1. The calculated ID50 and LD50 values and the mean number of days to death in each species are presented in Table 2.

Table 1. *Results of subcutaneous inoculation of South African wild rodents with* Yersinia pestis, *and serological response of survivors*

Species and source of origin	Approximate no. of bacilli inoculated						
	5	10	10^2	10^3	10^4	10^5	10^6
Mastomys natalensis (Transvaal-nonenzootic)	5/3/2 (8)*	5/1/1 (16)	5/5/3 (19)	5/0/– (—)	5/4/3 (277)	5/1/1 (1024)	—
Mastomys natalensis (Natal-nonenzootic)	5/4/0 (—)	5/1/1 (1024)	5/0/– (—)	5/0/– (—)	5/0/– (—)	5/0/– (—)	5/0/– (—)
Mystromys albicaudatus (Laboratory)	5/4/1 (1024)	8/0/– (—)	5/0/–(—)	5/1/1 (8192)	5/0/– (—)	5/0/– (—)	5/0/– (—)
Desmodillus auricularis (northern Cape-enzootic)	—	4/4/3 (373)	4/4/0 (—)	4/4/3 (11)	4/4/3 (75)	4/3/2 (8)	—
Tatera brantsii (northern Cape-enzootic)	5/3/0 (—)	5/5/3 (179)	5/1/1 (2048)	5/4/4 (76)	5/0/– (—)	5/0/– (—)	—
Tatera leucogaster (northern Cape-enzootic)	10/10/0 (—)	10/9/0 (—)	10/8/6 (16)	10/10/6 (64)	10/10/10 (134)	10/8/8 (182)	5/1/1 (128)
Rhabdomys pumilio (Orange Free State-nonenzootic)	5/1/0 (—)	5/0/– (—)	5/0/– (—)	5/1/1 (32)	5/1/1 (512)	5/1/1 (64)	—
Rhabdomys pumilio (eastern Cape-enzootic)	8/7/6 (284)	8/6/6 (135)	8/5/5 (307)	8/6/5 (336)	8/5/5 (473)	8/1/1 (2048)	8/0/– (—)

* Number inoculated/number survived/no. of seroconversions (mean ELISA titre).

Table 2. *Mean number of days to death, and calculated 50% infectious dose and 50% lethal dose of South African rodents experimentally infected with* Yersinia pestis

	ID50	LD50	Mean no. of days to death	Range
Mystromys albicaudatus	10	14	4·5	2–8
Desmodillus auricularis	$1{\cdot}7 \times 10^3$	$1{\cdot}1 \times 10^6$	12	—
Tatera brantsii	16	4×10^2	5·0	2–14
Tatera leucogaster	$3{\cdot}2 \times 10^2$	$9{\cdot}1 \times 10^5$	6.3	4–10
Mastomys natalensis (Natal-nonenzootic)	12	14	4.4	2–9
Mastomys natalensis (Transvaal-nonenzootic)	5	$1{\cdot}4 \times 10^3$	6.4	3–16
Rhabdomys pumilio (Orange Free State non-enzootic	2	5	4·0	2–9
Rhabdomys pumilio: (eastern Cape-enzootic)	2	$1{\cdot}3 \times 10^4$	3·9	2–11

The virulence of the strain used for challenge was confirmed by the response of the two laboratory colonized species. *M. coucha* and *M. albicaudatus*, of known susceptibility. With the exception of a single *M. albicaudatus* which survived an inoculum of 5×10^3 plague bacilli, all the control rodents failed to survive a challenge of greater than five organisms. The LD50 of *M. coucha* approximated seven organisms (data not shown).

All three of the gerbil species captured in the northern Cape Province showed some degree of resistance to plague infection. This was most strikingly observed in *D. auricularis* and *T. leucogaster* and to a lesser extent in *T. brantsii*.

The two populations of *R. pumilio* tested showed different responses to experimental inoculation with *Y. pestis*. The population from the plague-endemic Port Elizabeth area was relatively resistant while that from the Orange Free State was extremely susceptible. Similarly the two populations of *M. natalensis* differed in their plague response. The laboratory colony originating in the eastern Transvaal proved moderately resistant while the population from Natal was almost as susceptible as *M. coucha*.

The response of both populations of *R. pumilio* to experimental plague infection was markedly heterogeneous. In the resistant population a number of animals died at all levels of challenge while in the susceptible population three animals survived even at relatively high levels of challenge. Despite the difference in susceptibility between the two populations, the mean number of days to death in each group was similar.

In addition to the variation in the plague LD50 values, differences between species were evident in the 50% infectious dose (Table 2). *T. leucogaster* and *D. auricularis* had a relatively high plague infection threshold level while the ID50 for all the other species tested was relatively low. Both the susceptible and resistant population of *R. pumilio* had an extremely low ID50 of two organisms.

In *T. brantsii*, *T. leucogaster* and *R. pumilio* the frequency of seroconversion increased relative to the dose administered and with inocula greater than the ID50

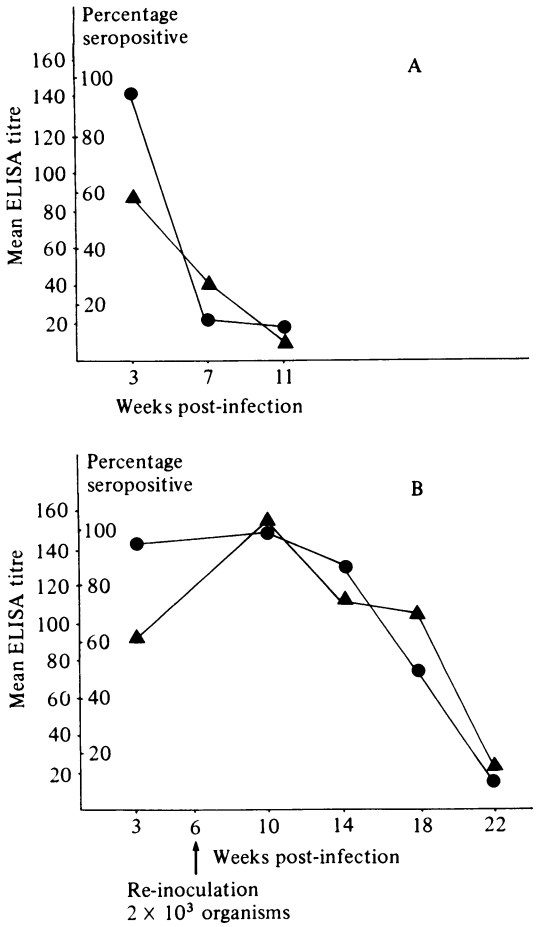

Fig. 1. Persistence of enzyme-linked immunosorbent assay (ELISA) antibodies to *Yersinia pestis* in experimentally inoculated *Tatera leucogaster* (A) and *T. brantsii* (B). ●, Mean ELISA titre of seropositive animals. ▲, Percentage of inoculated animals remaining seropositive.

almost all surviving animals seroconverted. In contrast, *D. auricularis* and the resistant *M. natalensis* population had a heterogeneous antibody response with a percentage of surviving animals remaining seronegative even at high levels of challenge.

The mean ELISA antibody titre in *T. leucogaster* and *R. pumilio* appeared to be directly related to the challenge dose while antibody levels in *D. auricularis* were generally low and showed no relationship to the inoculum size.

The mean ELISA titre of *T. leucogaster* which seroconverted and the percentage of animals remaining seropositive declined rapidly with time (Fig. 1) so that only 10% of the animals originally surviving infection remained seropositive by 11 weeks p.i. Thirteen *T. brantsii* which survived experimental infection were reinoculated with 2×10^3 plague bacilli. One of the five which had remained seronegative after the first inoculation died and four seroconverted. The mean antibody titre

of reinoculated gerbils increased only marginally but antibodies persisted for longer than in *T. leucogaster* which survived a single dose.

Y. pestis was isolated after 21 days from lesions at the inoculation sites on the hind leg of two *R. pumilio* from Port Elizabeth and one *R. pumilio* from the Orange Free State. All three animals had ELISA antibody titres $\geqslant 128$. Plague was also reisolated from a liver lesion in a *M. albicaudatus* which had an ELISA plague antibody titre of 8192. All four of the plague strains isolated proved to be typically virulent and identical to the parent strain.

DISCUSSION

Many rodent species throughout the world have been implicated in the plague cycle but, outwith the United States of America, few studies have distinguished between reservoir hosts and amplifying or epizootic hosts (Poland & Barnes, 1979). Reservoir rodent hosts are relatively resistant to plague infection with the result that mortality in them is seldom evident. Plague transmission in such species tends to occur in relatively stable foci in which human cases are usually infrequent. In contrast, amplifying hosts are generally extremely susceptible to plague infection and suffer explosive, severe epizootics, often traversing great distances. The risk of human plague cases occurring is greatest during or after such epizootics owing to hostless infective fleas at large in the environment.

In southern Africa it is historically evident that gerbils of the genera *Tatera* and *Desmodillus* were the primary amplifying hosts and most human cases generally followed gerbil epizootics (Fourie, 1938; Davis, 1948; 1953*a*, *b*). Human outbreaks were most often associated with concurrent mortality among *Rattus rattus*, infection being transmitted from gerbils to the domestic environment by the semidomestic *M. coucha*. Judging from previous reports of heavy mortality in gerbils during epizootics in the northern Cape Province (Pirie, 1927*b*; Davis, 1953*b*) it appears that gerbils in that region were no less susceptible than those tested from other areas by Pirie (1927*a*) and Davis (1963) or those from present day plague-free areas in Zimbabwe (Isaacson, Taylor & Arntzen, 1983). As shown by this study, however, present day gerbil populations in the northern Cape Province are resistant to plague infection. It seems probable that the plague resistance evident in all three gerbil species has been acquired in response to continual selective pressure of the organism by removal of susceptible individuals. Acquired resistance to plague in rodent populations has been widely observed in *Rattus* sp. throughout the world (Pollitzer, 1954) and also in epizootic hosts such as California ground squirrels, *Citellus beecheyi*, in western USA (Barnes, 1982).

The rate at which plague resistance is acquired by individual species must vary since *D. auricularis* from the Kimberley district of the northern Cape Province were already resistant when Pirie (1927*a*) conducted his pioneering susceptibility studies. He suspected that this resistance was acquired since epizootics in this species had occured in the same area 3 years prior to his tests. A second *D. auricularis* population captured from a plague-free area proved more susceptible to experimental infection (Pirie, 1927*a*). *D. auricularis* was subsequently observed to suffer almost as heavily as *Tatera* sp. during epizootics in other parts of South Africa (Fourie, 1938; Davis, 1953*b*).

Increasing plague resistance in populations of gerbil amplifying hosts would result in a steady decrease in the mortality rate with repeated exposure, presumably leading to the decline and disruption of gerbil epizootics witnessed by Davis (1964). The decline in gerbil epizootics has occured throughout South Africa suggesting that the acquired plague resistance among gerbils in the northern Cape Province, demonstrated in this study, has also occured in other plague enzootic areas of the country. Since most human plague outbreaks in South Africa were previously associated with epizootic plague in gerbils it is possible that the dramatic decrease in human plague cases since 1950 (Hallett, McNeil & Meyer, 1970) has resulted from the increase of plague resistance and subsequent decline of epizootics in gerbils. Only one recorded human plague outbreak has occurred in South Africa in the past decade, this being in an area where gerbils are absent (Shepherd *et al.* 1983).

In 1903 the four-striped mouse, *R. pumilio*, was the first South African wild rodent in which plague mortality was observed (Mitchell, 1927). The observation that plague infection spread relatively slowly in this species in the eastern Cape Province (Mitchell, 1927) led Pirie (1927*a*) to suggest that *R. pumilio* was the principal plague reservoir in that area. Unfortunately this suggestion was subsequently ignored until 1982, when a human plague outbreak occurred near Port Elizabeth in the eastern Cape Province. Studies in the vicinity of the outbreak showed that *R. pumilio* numbers remained high, without obvious epizootic disease, despite serological evidence of plague infection in the population (Shepherd *et al.* 1983). Further confirmation that *R. pumilio* acts as a plague maintenance host in the eastern Cape Province was obtained in this study since animals captured from the vicinity of the 1982 human outbreak showed a moderate resistance to plague infection. A second *R. pumilio* population captured in a plague-free area of the Orange Free State was extremely susceptible to experimental plague infection. Interestingly, there were similarities in the plague response of the two populations, with the 50% infectious dose being identical for each, as was the mean number of days to death in animals which succumbed. In addition, the response to plague infection within each population was a heterogeneous (Table 1). The comparative resistance of the Port Elizabeth population may have arisen by genetic selection since susceptible animals would be eliminated by plague thus increasing the frequency of resistant genes in the population (Hubbert & Goldenberg, 1970). The heterogeneous response of *R. pumilio* to plague resembles that of north American maintenance hosts such as the California vole *Microtus californicus* and the deer mouse *Peromyscus maniculatus*, where individual populations vary markedly in their response to plague infection (Quan & Kartman, 1956, 1962; Hubbert & Goldenberg, 1970). Heterogeneously resistant populations of *M. californicus* contain a percentage of susceptible animals which die with bacteraemia of sufficient intensity to reinfect fleas (Goldenberg, Quan & Hudson, 1964). Plague transmission is therefore maintained at low levels in enzootic foci in which rodent mortality is seldom evident. It seems probable that a similar plague maintenance mechanism exists within *R. pumilio* populations in South Africa.

The response of the two populations of *M. natalensis* differed markedly in plague susceptibility tests. The laboratory colony which originated in the eastern Transvaal was moderately resistant to plague infection, similar to the population tested from Zimbabwe by Isaacson, Taylor & Arntzen (1983). In contrast, the

population captured from Natal proved to be extremely susceptible. The discrepancy is surprising in view of the fact that both *M. natalensis* populations tested in this study and the Zimbabwean population tested by Isaacson, Taylor & Arntzen (1983) originated in areas where human or rodent plague have not previously been recorded. It therefore seems unlikely that the resistance of the northern *M. natalensis* populations has been modified by past plague exposure, although the possibility cannot be entirely discounted until adequate plague surveys of these areas have been undertaken. The results of the plague antibody studies in *T. leucogaster* and *T. brantsii* (Fig. 1) demonstrated that the humoral antibody response in the former species was short lived. Antibodies persisted for a slightly longer period in the sera of *T. brantsii* which were reinoculated although the mean antibody titre was not significantly raised. It seems probable that the duration of antibody response in rodents may vary according to species and even in individual populations within a species. Chen & Meyer (1974) reported a variable duration of antibody response in experimentally infected *Rattus* spp. while Williams, Moussa & Cavanaugh (1979) noted that plague antibody titres in experimentally infected *C. beecheyi* remained unchanged from 6–42 weeks post infection.

In addition to the short duration of humoral antibodies in *T. leucogaster*, the high 50 % infectious dose in this species would result in a low seroconversion rate in response to plague challenge. Williams, Moussa & Cavanaugh (1979) reported that the response of *C. beecheyi* to experimental infection with a range of plague organisms up to the maximum ($2{\cdot}4 \times 10^4$) of a single flea bite (Burroughs, 1947) was that 28 % of the squirrels died, 19 % seroconverted and the remaining 53 % survived without seroconversion. Our results for the experimental plague infection of *T. leucogaster* (below $2{\cdot}4 \times 10^4$ bacilli) revealed similar ratios of 10 % mortality, 33 % seroconversion and 57 % survival without seroconversion.

The results on reinoculation of *T. brantsii* also confirmed the results of previous authors (Chen & Meyer, 1974; Williams, Moussa & Cavanaugh, 1979); animals which survive challenge with low doses of *Y. pestis* without seroconversion remain susceptible to the organism on subsequent reinoculation. Of the five *T. brantsii* which remained seronegative after initial exposure, one died and four seroconverted after a second challenge with 2×10^3 organisms.

In *M. natalensis* and *D. auricularis*, two species found to be plague resistant in this study, several individuals failed to seroconvert even when inoculated with numbers of *Y. pestis* substantially above the 50 % infectious dose. Such animals were termed 'naturally resistant' by Chen & Meyer (1974) who observed this phenomenon in experimentally infected *Rattus* sp. Isaacson, Taylor & Arntzen (1983) reported similar findings in experimentally infected *M. natalensis* and *A. chrysophilus* from Zimbabwe.

The results of this study suggest that a combination of factors in plague-resistant South African rodents, including a short-lived antibody response, a relatively high 50 % infectious dose, and a natural resistance precluding seroconversion may be partially responsible for the low plague antibody rates observed in serological surveys in southern African wild rodent populations (Taylor, Gordon & Isaacson, 1981; Shepherd & Leman, 1983). It follows from these observations that the animals used in this study, although seronegative prior to experimental infection, may have been previously exposed to *Y. pestis* in the wild.

It has been suggested that atypical plague strains which have reduced virulence for mammals may be of importance in the persistence of the organism in enzootic foci (Poland & Barnes, 1979). Williams, Harrison & Cavanaugh (1975) demonstrated that $F1^-$ Variants arose in and caused chronic lesions in laboratory rats which have been immunized and challenged with $F1^+$ plague strains. It was therefore of interest that in this study plague was reisolated from three *R. pumilio* and one *M. albicaudatus* 21 days after infection. However, all four strains proved to be identical to the parent strain. The site of isolation near the injection site on the hind leg of the three *R. pumilio* suggested that these were resolving lesions. While it is of interest that such relatively long term plague infections may occur in experimentally infected South African rodents, surveys have failed to find evidence of chronic plague infection by atypical or virulent strains in the wild (Pirie, 1927*b*; Shepherd & Leman, 1985).

The results of this study have demonstrated that, contrary to previous belief, plague-resistant populations exist in several rodent species throughout South Africa. In some species resistance has arisen in enzootic areas by selective pressure of the organism, while some populations of *M. natalensis* exhibit resistance apparently without prior exposure to plague.

Clearly the concept of plague maintenance by a series of epizootic cycles in gerbils (Davis 1964) is no longer tenable. The results of this study and that of Isaacson, Taylor & Arntzen (1983) suggest that small murid species such as *R. pumilio*, *M. natalensis* and *A. chrysophilus* contain resistant populations in which plague mortality may not be readily apparent. Such species may perpetrate plague in southern Africa in scattered foci which are difficult to detect.

The role of gerbils in the plague cycle in southern Africa needs to be reinvestigated. While it seems likely that limited plague epizootics could occur in *T. brantsii* in the northern Cape Province, the role of highly plague-resistant *T. leucogaster* and *D. auricularis* is obscure and it seems possible that in enzootic areas these species have made the transition from epizootic to reservoir hosts.

Further susceptibility studies on suspected reservoir species in other South African plague foci should facilitate a more thorough understanding of the complex ecology of plague in the subcontinent.

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