Bulletin of the World Health Organization, 61 (2): 339-344 (1983)

Ecology of plague in Africa: response of indigenous wild rodents to experimental plague infection

MARGARETHA ISAÄCSON,[1] PAUL TAYLOR,[2] & LORRAINE ARNTZEN[3]

The Mastomys natalensis *species complex, subdivided into genetically distinct species having diploid chromosome numbers 2n = 32 and 2n = 36, is a reservoir for several zoonoses including Lassa fever and plague. This report describes a study to determine whether these sibling species and three other rodent species have different potential as reservoirs for plague. It was found that* M. natalensis *(2n = 32) was significantly more resistant to experimental plague infection (50% survived inoculation with 120 000* Yersinia pseudotuberculosis *subsp.* pestis*) than was* M. coucha *(2n = 36) (none of which survived doses of 190* Y. pseudotuberculosis *subsp.* pestis*). In descending order of resistance were* M. natalensis, Aethomys chrysophilus, M. coucha, Tatera leucogaster *and* A. namaquensis. *No* A. namaquensis *survived inoculation of 10 or more plague bacilli.*

Previous reports on susceptibility to plague or other infections, which were based exclusively on findings in the universally distributed laboratory-bred Mastomys, *are thus not necessarily applicable to the* M. natalensis *species as a whole but probably only to* M. coucha. *The* Y. pseudotuberculosis *subsp.* pestis *fraction-1 passive haemagglutination test appeared to be relatively insensitive in that only 5 out of 47 animals surviving experimental plague infection showed specific antibodies 6 weeks after challenge.*

The geographic distribution of human plague in southern Africa corresponds closely with that of the plague-susceptible species, M. coucha, *while the resistant species,* M. natalensis, *predominates in areas where human plague has not been recorded. The role of* A. namaquensis *in the ecology of plague needs to be carefully studied and its possible importance in plague research should be investigated further.*

The *Mastomys natalensis* species complex, one of Africa's most prevalent wild rodents, plays an important role in the natural cycle of several zoonoses, including Lassa fever and plague.

Although Lassa fever is known to occur only in West Africa, the rodent host is widely distributed over most of the continent. Monath (*1*) suggested several possible biological explanations for this discrepancy, including differences between subpopulations of *Mastomys* in their susceptibility to the virus. With regard to plague, much of the experimental work on *Mastomys* was done before it was appreciated that different responses by sibling species to *Yersinia pseudotuberculosis* subsp. *pestis* and other pathogenic agents might be significant. The colonies of this rodent, maintained in many laboratories around the world, are descended from the original colony still maintained at the South African Institute for Medical Research, i.e., the 2n = 36 species (*2*). In South Africa, this animal became the preferred routine laboratory animal for the study of plague because of its consistently high susceptibility to very low doses of *Y. pseudotuberculosis* subsp. *pestis*. In 1968, Davis et al. (*3*) stated that "in South Africa *Mastomys* is highly susceptible to plague and resistance to *P. pestis* has never been demonstrated". In contrast, other workers (*4*) concluded, on the basis of epidemiological as well as laboratory susceptibility studies, that "*Arvicanthis* and *Mastomys* from Rongai in Kenya were mostly highly resistant to *P. pestis*".

Hallett (*5*), in 1970, found high *Y. pseudotuberculosis* subsp. *pestis* fraction 1 antibody titres in *Mastomys* sera and stated that this was unexpected but that similar results reported from Kenya might indicate that *Mastomys* had acquired genetic resistance in certain hyperenzootic areas. However, in 1953, Davis (*6*) had remarked on the occurrence of a limited plague focus in Morocco, where, in contrast to other African plague foci, *Mastomys* appeared not to be involved in the plague cycle.

The suggestion by Matthey (*7*) in 1966 that *M. natalensis* consisted of at least two genetically different species was unfortunately ignored until 1977

[1] Head, Department of Tropical Pathology, South African Institute for Medical Research, P.O. Box 1038, Johannesburg, South Africa 2000.

[2] Acting Director, Blair Research Laboratory, Harare, Zimbabwe.

[3] Senior Research Technologist, MRC Research Unit for Emergent Pathogens, South African Institute for Medical Research, Johannesburg, South Africa.

when Lyons et al. (*8*) reinvestigated the subject. Taylor et al. (*9*) drew attention to the apparent discrepancy between the occurrence of human plague in Zimbabwe and the distribution of plague antibodies in dog sera, and they related this to the rodent distribution. They noted the close relationship between the areas of human plague and the distribution of *Mastomys* (2n = 36) (Fig. 1), as mapped by Green et al. (*10*). The latter also suggested the renaming of members of the *M. natalensis* species complex as *M. natalensis* (2n = 32) and *M. coucha* (2n = 36). Other species may be added as the taxonomy becomes clearer (*9*).

In view of these and other apparently contradictory observations on an animal that has become established as a common laboratory model for a wide range of studies, it seemed appropriate to compare the susceptibility to a major natural pathogen, *Y. pseudotuberculosis* subsp. *pestis*, of the two sibling species of *Mastomys* and of other major rodent species in southern Africa.

To avoid confusion, reference to reports of work carried out on *Mastomys* species before the publication by Green et al. (*10*) will not include the species name. *Mastomys* used in this study are identified by their diploid chromosome numbers. *Aethomys chrysophilus* is also known to be a species complex in Zimbabwe with chromosome diploid numbers of 44 and 50 (*11*), but only the latter (2n = 50) has been recorded in animals from the capture site used in this study (D. H. Gordon, personal communication, 1982).

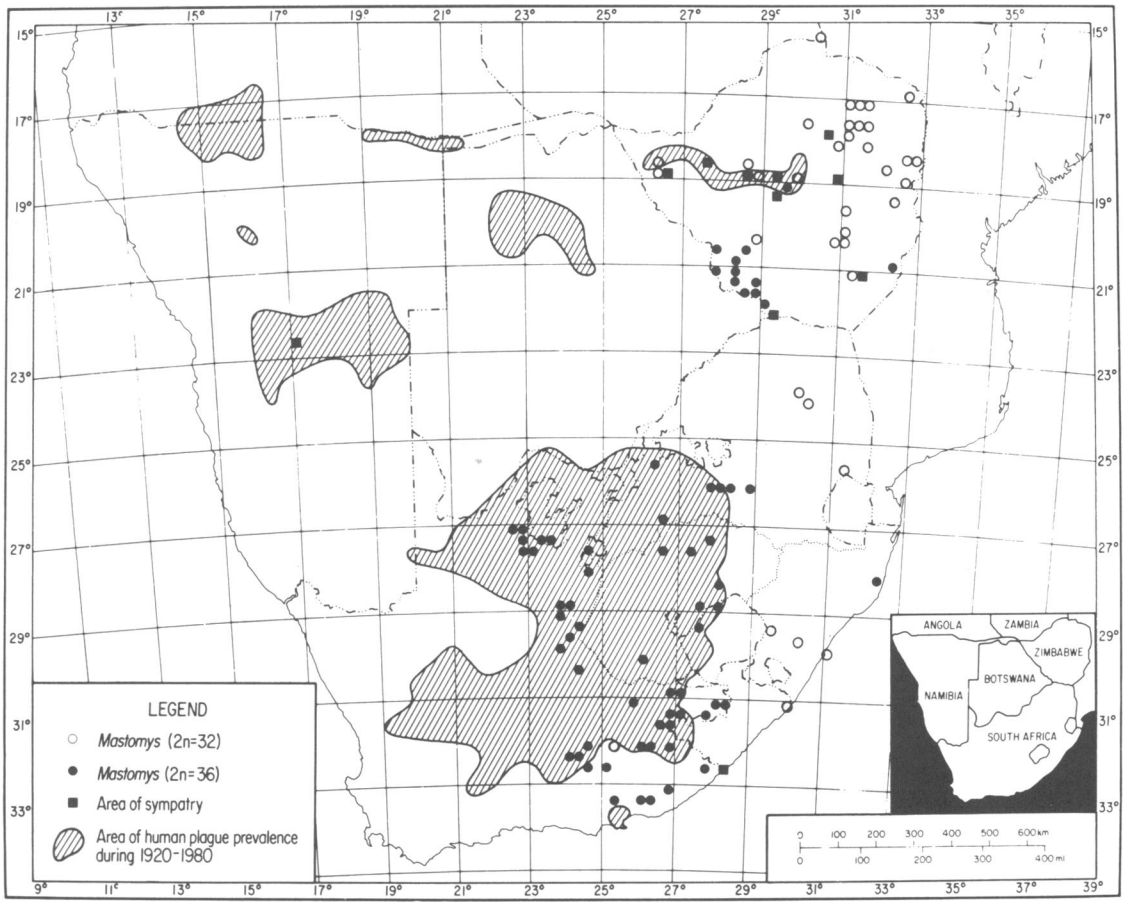

Fig. 1. Relationship between the distribution of the two chromosomal species of *Mastomys* and the prevalence of human plague in southern Africa from 1920 to 1980 (data partly from Green et al. (*10*) and Taylor et al. (*9*)). Note that karyotyping of *Mastomys* has been carried out on those from South Africa and Zimbabwe, but not yet on those from Botswana and Namibia.

MATERIALS AND METHODS

Animals

All rodents were trapped live in Zimbabwe in an area where plague has not been known to occur (17°55′ S, 30°45′ E) and *Mastomys* were typed according to their electrophoretic haemoglobin pattern, which is quite distinct for each of the chromosomal types (*12*). Serum was obtained from each animal to establish its plague antibody status prior to challenge. The animals were dusted with insecticide, kept in quarantine for some time, and then forwarded to South Africa for the challenge studies. Animals in the first batch were held in individual cages, but those in subsequent batches were kept in pairs, not necessarily of different sex. A further quarantine period was observed during which the animals were again dusted with insecticide. They were fed with standard mouse cubes and given water *ad libitum*. After inoculation with *Y. pseudotuberculosis* subsp. *pestis*, the animals were held in a Vickers' flexible film, negative-pressure animal containment isolator, fitted with air inlet and exhaust through HEPA filters.

The species used were: *Mastomys* (2n = 32) (the multimammate mouse), *Mastomys* (2n = 36), *Tatera leucogaster* (the bushveld gerbil), *A. chrysophilus* (the African or red veld rat), and *A. namaquensis* (the golden or Namaqua rock rat). Ten animals of each species were used per challenge dose. They were observed twice daily and autopsies were performed on the animals that died, when tissue was taken from heart, lung, spleen, and liver for culture. Survivors were sacrificed and exsanguinated 6 weeks after challenge for determination of plague antibody.

Challenge strain

Y. pseudotuberculosis subsp. *pestis* strain SAIMR/F329/68, isolated in 1968 from a flea during a bubopneumonic plague epidemic in Lesotho, was used. It is characterized by the presence of a relatively stable pigment factor. Prior to challenge, the isolate was grown in brain-heart infusion broth and passaged through white mice and through laboratory-bred *Mastomys* (2n = 36). Dilutions were made in peptone water and challenge was by subcutaneous inoculation into a hind leg of 0.2 ml of the designated dilution. Control animals were inoculated with diluent only.

Viable plate counts were done immediately before and after each batch of inoculations. For the counts, 1-ml aliquots of the 10^{-5} and 10^{-6} dilutions were divided among five blood agar plates per dilution. These were incubated at 28 °C for 48 h, after which the colonies were counted and the mean doses received by the animals were calculated.

Serology

The presence of antibodies against the *Y. pseudotuberculosis* subsp. *pestis* fraction 1B antigen was determined by means of the passive haemagglutination test using a microtitration method (*13*). Pre- and post-challenge sera of survivors were tested in parallel.

RESULTS

It was noted that *Mastomys* (2n = 32) was considerably more aggressive and excitable than *Mastomys* (2n = 36).

The results of the study are summarized in Table 1, from which it may be concluded that *Mastomys* (2n = 32) is highly resistant to experimental plague infection, in that 50% of animals survived challenge with 120 000 *Y. pseudotuberculosis* subsp. *pestis*. On the other hand, *Mastomys* (2n = 36) was found to be highly susceptible, no animals having survived a dose as low as 190 bacilli. With increasing dose, the interval between inoculation and death progressively decreased.

A. namaquensis was extremely plague-sensitive, a dose of 10 bacilli having killed all inoculated animals. It also showed the shortest interval between inoculation and death, the median value of 3.0 days with a dose of 1900 bacilli being equalled only by *Mastomys* (2n = 36) when exposed to a dose of 120 000 bacilli.

The *Y. pseudotuberculosis* subsp. *pestis* fraction 1 haemagglutination (PHA) tests, which were done on 155 adult animals used in the study, yielded negative results prior to plague infection. Of 47 survivors, only 5, i.e., 3 *Mastomys* (2n = 32) and 2 *A. chrysophilus*, had specific antibodies six weeks after infection. Titres ranged from 1:4 to 1:16 in four animals with one, *A. chrysophilus*, having a titre of 1:128; the animals had been given between 1900 and 120 000 bacilli. Ten suckling infants born to three *A. chrysophilus* females prior to inoculation were left with their parents after the latter were infected. In each family one parent died (two females and one male). The infants, who learned to fend for themselves, all survived and did not develop clinically apparent illness or demonstrable plague antibodies.

DISCUSSION

The role of *Mastomys* as a natural disease host has probably been most intensively investigated in relation to the epidemiology of bubonic plague. The simple technique used to distinguish *Mastomys* with chromosome numbers 2n = 32 and 2n = 36 made it

Table 1. Results of subcutaneous inoculation of virulent *Y. pseudotuberculosis* subsp. *pestis* isolate SAIMR/F329/68 in 155 African wild-trapped rodents[a]

	No. of bacilli inoculated									
	10		1.9×10^2		1.9×10^3		1.9×10^4		1.2×10^5	
Mastomys (2n = 32)					2/10	(8.5)	0/10		5/10	(4)
Mastomys (2n = 36)	1/10	(6)	10/10	(7.5)	9/9	(4)			10/10	(3)
T. leucogaster	7/10	(7)	10/10	(6)	8/8	(4.5)				
A. chrysophilus					4/9	(6)	5/10	(7)	8/10	(4)
A. namaquensis	10/10	(4.5)	10/10	(4)	9/9	(3)				

[a] Five animals in the 1.9×10^3 dose group were killed by their cage-mates shortly after inoculation and are therefore excluded from the study. Results are given as number of deaths from plague/number of animals inoculated. Figures in parentheses give the median survival time (in days) of the animals that died.

possible to capture and type the relatively large number of animals needed to carry out a plague susceptibility study. Although colonies of the two species are being successfully bred in our laboratory, wild-trapped rodents were used in preference to laboratory-bred animals for this preliminary study, to ensure that host factors would match closely those found in their natural environment.

In our study, we have shown that *Mastomys* (2n = 32) and *Mastomys* (2n = 36) differ significantly in their susceptibility to *Y. pseudotuberculosis* subsp. *pestis* in that the former is highly resistant whereas the latter is very sensitive (Table 1). *T. leucogaster*, the bushveld gerbil, which has long been believed to play an important role in southern African plague epidemiology, seemed rather more sensitive than *Mastomys* (2n = 36). *A. namaquensis* was the most sensitive of all while the response of *A. chrysophilus* was similar to that of *Mastomys* (2n = 32).

Both *Aethomys* and *Mastomys* are essentially arboreal rats, with tendencies to domesticity, and their role in plague is believed to be that of bridging the gap between the sylvatic reservoir (gerbils) and man (*14*). Our results, however, indicate that *A. chrysophilus* and *A. namaquensis* differ markedly in their susceptibility to plague and therefore may play different roles in the plague cycle.

It was also shown that an inverse correlation exists in the animals between degree of exposure and duration of survival after exposure. This probably reflects a dose-dependent incubation period.

Suckling infants of females with fatal plague infections did not become clinically ill, neither did a group of healthy control animals kept in the isolator throughout the study. In the absence of ectoparasites therefore, even intimate contact, as occurs between female animals and their suckling offspring, did not result in plague transmission. None of the animals had demonstrable haemagglutinating plague antibodies prior to challenge and only 5 out of 47 survivors had demonstrable antibodies six weeks after challenge. In our experience, field studies of rodent serology appear to be rather fruitless as only a small percentage of animals can be shown to have antibodies (*9*). Our current results raise two possibilities: either the *Y. pseudotuberculosis* subsp. *pestis* fraction 1 passive haemagglutination test is not highly sensitive, or surviving animals eliminate bacilli by local defence mechanisms at the site of inoculation and a humoral antibody response does not occur.

An important result of this study is the demonstration of entirely different responses by *Mastomys* (2n = 32) and *Mastomys* (2n = 36) to a natural pathogen. These findings have important implications with regard to *Mastomys* as an experimental laboratory animal in various research fields (*15*). Especially in the African context, these results apply to *Mastomys* in its role as an ecological link in many zoonoses, such as plague (Fig. 1), Lassa fever, salmonelloses, African tickbite fever, and arbovirus infections. In this respect, a great deal of work needs to be done to redefine the relative roles of the two *Mastomys* species, not only with regard to their susceptibility to pathogenic microorganisms but also in relation to their behaviour, ectoparasite infestation, etc. The role of *Mastomys* as an experimental laboratory animal needs to be very carefully defined, as it is *M. coucha* (2n = 36) and not necessarily *M. natalensis* (2n = 32) which spontaneously develops adenocarcinoma of the stomach, and which has been found to be an ideal experimental host for organisms causing schistosomiasis and filariasis, and for many other pathogens.

Although most *Mastomys* colonies in laboratories throughout the world are probably derived from the original South African colony, which had the 2n = 36 chromosome number, a strong plea is made that laboratories should determine the diploid chromo-

some number of their colonies and identify the animals accordingly when publishing research findings. Finally, the epidemiological implications of these findings are probably applicable to medically important animal reservoir hosts in other parts of the world, indicating a need for reevaluation of old and current data and methodology.

RÉSUMÉ

L'ÉCOLOGIE DE LA PESTE EN AFRIQUE : RÉACTION DE RONGEURS SAUVAGES INDIGÈNES À UNE INFECTION PESTEUSE EXPÉRIMENTALE

Une étude a été entreprise en vue de déterminer la sensibilité à l'infection pesteuse de plusieurs espèces indigènes de rongeurs sauvages dont on pense qu'elles jouent un rôle dans l'écologie de la peste en Afrique. Il s'agissait de *Tatera leucogaster*, d'*Aethomys chrysophilus*, d'*Aethomys namaquensis* ainsi que de membres du complexe d'espèces *Mastomys natalensis*. Les espèces du genre *Aethomys* sont essentiellement constituées de rats arboricoles à tendance domestique qui peuvent jouer un rôle analogue à celui des espèces du genre *Mastomys* en assurant la jonction entre les réservoirs de peste «sylvatique»[a] (gerbilles et autres rongeurs sauvages) et l'homme. Les précédentes études ne prenaient pas en considération l'existence d'espèces jumelles de *Mastomys* morphologiquement analogues mais génétiquement distinctes, avec un nombre diploïde égal à 32 et à 36, espèces qui ont été respectivement rabaptisées *Mastomys natalensis* et *Mastomys coucha*. Il est établi que la colonie parente sud-africaine, utilisée dans le passé et dont des descendants ont été distribués à de nombreux laboratoires du monde entier, est constituée de *M. coucha*. Cette espèce s'est révélée si fortement et si régulièrement sensible à l'infection par *Yersinia pseudotuberculosis* subsp. *pestis* qu'elle est devenue l'animal de laboratoire classique tant pour le diagnostic de la peste que pour la recherche.

La présente étude a permis d'obtenir deux types de données nouvelles. En premier lieu, on a constaté que *A. namaquensis* était nettement plus sensible à *Y. pseudotuberculosis* subsp. *pestis* que *M. coucha* et, en second lieu, on a observé que les espèces jumelles du complexe *Mastomys* sont nettement différentes quant à leur réaction vis-à-vis de *Y. pseudotuberculosis* subsp. *pestis*. *M. coucha* comme on pouvait s'y attendre, s'est révélé très sensible, en revanche *M. natalensis* était relativement résistant. Parmi les autres résultats obtenus, figure le fait que cette sensibilité accrue se caractérise par un laps de temps plus court entre l'exposition à une dose l'étale et la mort et par un taux de mortalité plus élevé. *Aethomys chrysophilus* forme également un complexe d'espèces, toutefois seules les espèces ayant un nombre diploïde de chromosomes 2n = 50 figuraient dans cette étude. Les variations aussi observées dans la réponse à un germe pathogène naturel montrent qu'il est nécessaire de revoir l'écologie d'un certain nombre de zoonoses dans les régions du monde où des complexes d'espèces leur servent de réservoirs.

[a] appelée aussi «peste des rongeurs sauvages».

REFERENCES

1. MONATH, T. P. Lassa fever: review of epidemiology and epizootology. *Bulletin of the World Health Organization*, **52**: 577-591 (1975).
2. DAVIS, D. H. S. & OETTLÉ, A. G. The multimammate mouse *Rattus (Mastomys) natalensis* Smith: A laboratory-adapted African wild rodent. *Proceedings of the Zoological Society, London*, **131**: 293-299 (1958).
3. DAVIS, D. H. S. ET AL. Serological survey of plague in rodents and other small mammals in Kenya. *Transactions of the Royal Society for Tropical Medicine and Hygiene*, **62**: 838-861 (1968).
4. HEISCH, R. B. ET AL. Results of a plague investigation in Kenya. *Transactions of the Royal Society for Tropical Medicine and Hygiene*, **47**: 503-521 (1953).
5. HALLETT, A. F. ET AL. A serological survey of the small mammals for plague in southern Africa. *South African medical journal*, **44**: 831-837 (1970).
6. DAVIS, D. H. S. Plague in Africa from 1935-1949. A survey of wild rodents in African Territories. *Bulletin of the World Health Organization*, **9**: 665-700 (1953).
7. MATTHEY, R. Cytogénétique et taxonomie des rats appartenant au sous-genre *Mastomys* Thomas (Rodentia: Muridae). *Mammalia*, **30**: 105-119 (1966).
8. LYONS, N. F. ET AL. G-banding chromosome analysis of *Praomys natalensis* (Smith) (Rodentia: Muridae) from Rhodesia. 1. 36 chromosome population. *Heredity*, **38**: 197-200 (1977).
9. TAYLOR, P. ET AL. The status of plague in Zimbabwe. *Annals of tropical medicine and parasitology*, **75**: 165-173 (1981).
10. GREEN, C. A. ET AL. The distribution, identification, and naming of the *Mastomys natalensis* species complex in southern Africa (Rodentia: Muridae) *Journal of zoology, London*, **192**: 17-23 (1980).
11. GORDON, D. H. & RAUTENBACH, I. L. Species complexes in medically important rodents: chromosome studies of *Aethomys, Tatera* and *Saccostomus* (Rodentia: Muridae, Cricetidae). *South African journal of science*, **76**: 559-561 (1980).

12. GREEN, C. A. ET AL. Biological species in *Praomys (Mastomys) natalensis* (Smith), a rodent carrier of Lassa virus and bubonic plague in Africa. *American journal of tropical medicine and hygiene*, **27**: 627–629 (1978).
13. CHEN, T. H. & MEYER, K. F. An evaluation of *Pasteurella pestis* fraction-1 specific antibody for the confirmation of plague infections. *Bulletin of the World Health Organization*, **34**: 911–918 (1966).
14. DAVIS, D. H. S. A plague survey of Ngamiland, Bechuanaland Protectorate, during the epidemic of 1944–45. *South African medical journal*, **20**: 462–467, 511–515 (1946).
15. ISAÄCSON, M. ET AL. Susceptibility of members of the *Mastomys natalensis* species complex to experimental infection with *Yersinia pestis*. *Journal of infectious diseases*, **144**: 80 (1981).