Mammalia 72 (2008): 253–257 © 2008 by Walter de Gruyter • Berlin • New York. DOI 10.1515/MAMM.2008.038

# Potential mammalian reservoirs in a bubonic plague outbreak focus in Mbulu District, northern Tanzania, in 2007

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## Abstract

This study investigated mammalian involvement in an outbreak of bubonic plague in Mbulu District, northern Tanzania, in March 2007. Plague is a rodent-borne zoonotic disease that spreads to humans through fleas infected with *Yersinia pestis*. Live trapping of rodents and shrews was conducted in fallow and crop fields, peridomestic areas, houses and the neighboring forest reserve. Serum was separated from blood of captured animals. A rapid diagnostic test (RDT) was used for diagnosis of plague infection. An ELISA technique was used to detect antibodies against *Yersinia pestis* fraction 1 antigen. Wild and commensal rodents tested positive by RDT, indicating current infection in clinically healthy animals. The ELISA showed that wild rodents (*Lophuromys flavopunctatus*, *Praomys delectorum*, *Graphiurus murinus*, *Lemniscomys striatus*) and commensal rats (*Rattus rattus*, *Mastomys natalensis*, *Mus minutoides*) were *Y. pestis*-positive. Two potential vectors, *Xenopsylla brasiliensis* and *Dinopsyllus lypusus*, were found on wild and commensal rodents with a flea index of 1.8. We conclude that diverse potential mammalian reservoirs and efficient vectors of *Y. pestis* are present in abundance in Dongobesh and could lead to persistence and future plague outbreaks.

**Keywords:** bubonic plague; ELISA; rodents; Tanzania.

## Introduction

Plague is a zoonotic disease for which human infection is usually acquired through the bites of infected rodent fleas. The natural reservoirs of plague are wild rodents and the causative agent is a bacteria, *Yersinia pestis* Lehmann and Neumann 1896. It persists as a chronic disease among many species of rodents around the world and outbreaks often occur in unpredictable patterns. Over 200 mammalian species in 73 genera have been reported to be naturally infected with *Y. pestis*, but rodents are the most important hosts (Perry and Fetherston 1997).

The plague cycle can be urban, involving rats, fleas and humans, or sylvatic, involving wild rodents. Sylvatic plague is widely distributed in western North America, South America (Peru, Ecuador and Brazil), southern, eastern and central Africa, the Middle East and central Asia (WHO 2006). In Madagascar for example, the reservoirs of *Y. pestis* are two species of rats, *Rattus norvegicus* Berkenhout 1769 and *R. rattus* Linnaeus 1758. However, the sylvatic reservoir of *Y. pestis* is not well documented (Chanteau et al. 1998). Studies in Brazil revealed the presence of significant levels of specific anti-F1 antibodies among rodents and wild or domestic carnivores (dogs and cats), indicating that these plague foci were active in spite of the absence of human cases in some of them (de Almeida et al. 1995). In the Lushoto plague outbreak focus in north-east Tanzania, *Mastomys natalensis* Smith 1834, *Lophuromys flavopunctatus* Thomas 1888, *Arvicanthis nairobae* Allen 1909 and *R. rattus* were found to carry anti-plague immunoglobulin antibodies, suggesting that they were natural reservoirs involved in the plague cycle (Kilonzo et al. 2005). Wild and commensal rodents continually interact with each other in the fallow and crop fields created after deforestation (Makundi et al. 2003).

Plague remains a disease of major public health importance globally, with several countries in the world reporting cases (WHO 2004, 2005). Outbreaks in Algeria have shown that plague may re-emerge in the same areas after a long period of silence (WHO 2004). The African countries most affected are Madagascar, Democratic Republic of Congo (DRC), Mozambique, Uganda and Tanzania. DRC reported 1174 plague cases with 50 deaths in 2006 (http://www.who.int/csr/don/archive/disease/plague/en/).

Tanzania has a well-documented plague outbreak history, dating back over 100 years (Msangi 1969). Outbreaks of plague in the last 20–30 years (until 2003) were reported in Lushoto, Karatu and Mbulu Districts (Kilonzo and Mtoi 1983, Kilonzo et al. 2005). No human plague cases were reported in Tanzania during 2003–2006. Before 2003, Lushoto District had been reporting human plague cases for 23 years (Davis et al. 2006). Plague had remained quiescent in Mbulu District for 30 years until the recent outbreak in February–March 2007 (Makundi 2007). The outbreak occurred in the villages of Tumati and Arri in the Division of Dongobesh (4°04′ S, 35°22′ E). This was the first recorded human plague outbreak in this area, which is far from the earlier known focus in Mbulu District. A total of 35 suspect plague cases were initially reported, with six deaths. The victims had clinical symptoms of plague, including buboes, high fever, chills, headache, weakness, vomiting, nausea and prostration. A retrospective confirmation of plague by rapid diagnostic test (RDT) using a dipstick assay was made (Makundi et al. 2007). Patient records at the local health center

showed that all the cases were bubonic, indicating transmission by flea bites and therefore involving a mammalian reservoir. Furthermore, interviews with local people in the area indicated that dead rats were seen in peridomestic areas and surrounding crop fields prior to the plague outbreak. The District Agricultural Office also confirmed a rodent outbreak in Mbulu District including the division of Dongobesh during the 2 months preceding the plague outbreak.

Following these field observations, we conducted a study to identify and establish potential mammalian reservoirs of the disease in the outbreak focus.

## Materials and methods

### Location of the outbreak

The study was carried out in the villages of Arri and Tumati, which are in the Division of Dongobesh (4°04′ S, 35°22′ E) at altitudes ranging from 1930 to 2250 m above sea level. The location of Mbulu District in relation to other active plague and historical plague outbreak foci in Tanzania is shown in Figure 1. The locality of the current outbreak in Dongobesh Division (Arri and Tumati villages) in Mbulu District is shown in Figure 2. The outbreak focus borders the Mongahay Forest Reserve.

### Animal trapping, handling and laboratory analysis of rodent sera

Three distinct habitats were identified in the outbreak area: natural forest, fallow land, and crop fields. Sherman live traps were used in the forest, fallow and crop fields. Locally made box traps and Sherman live traps were used for trapping in houses. Trapping was carried out for 3 consecutive nights in any given locality, habitat or house, after which the traps were moved to a different area within the village. In total, there were 861 trap nights with a trap success of 8.1%. Captured small mammals

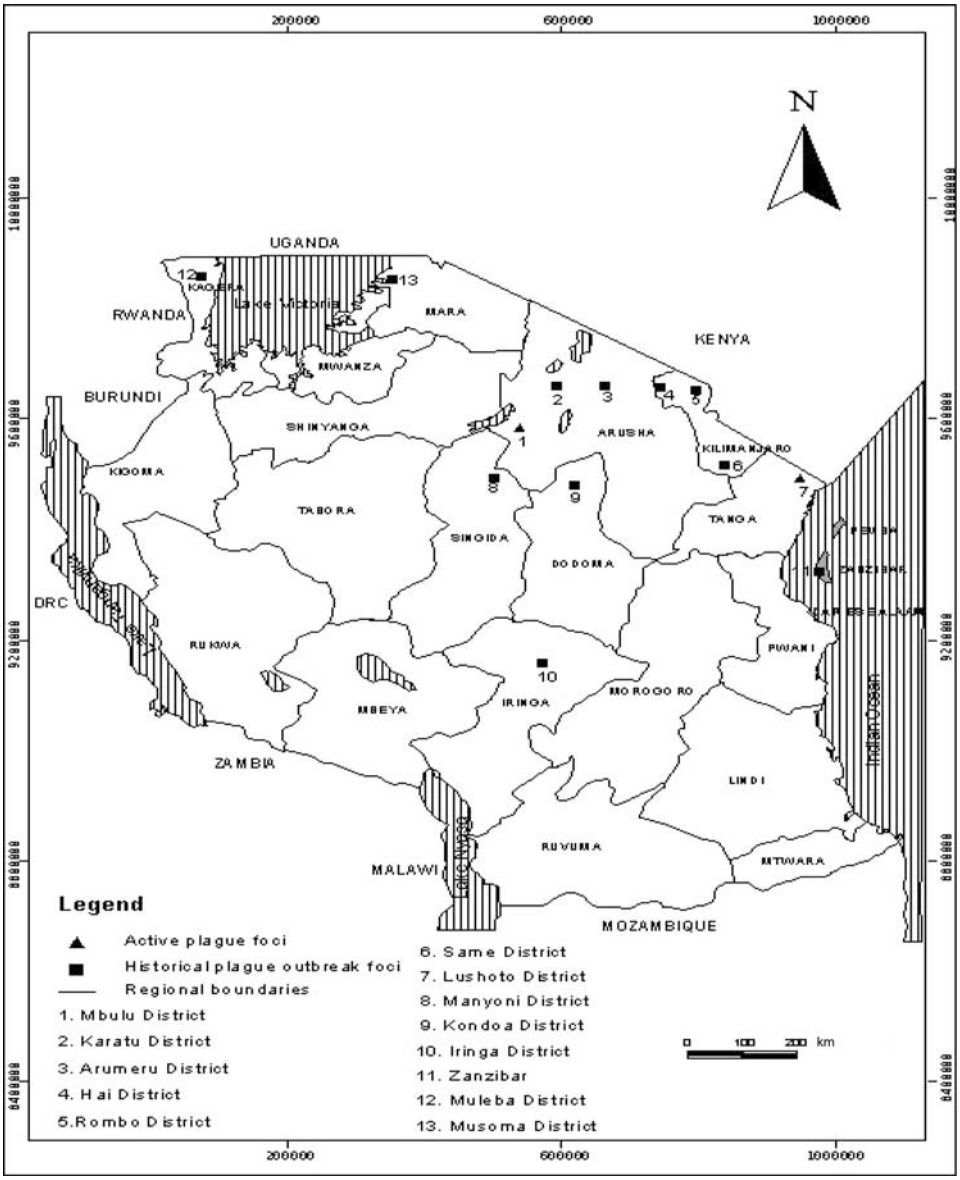


**Figure 1** Active and historical plague outbreak localities in Tanzania.

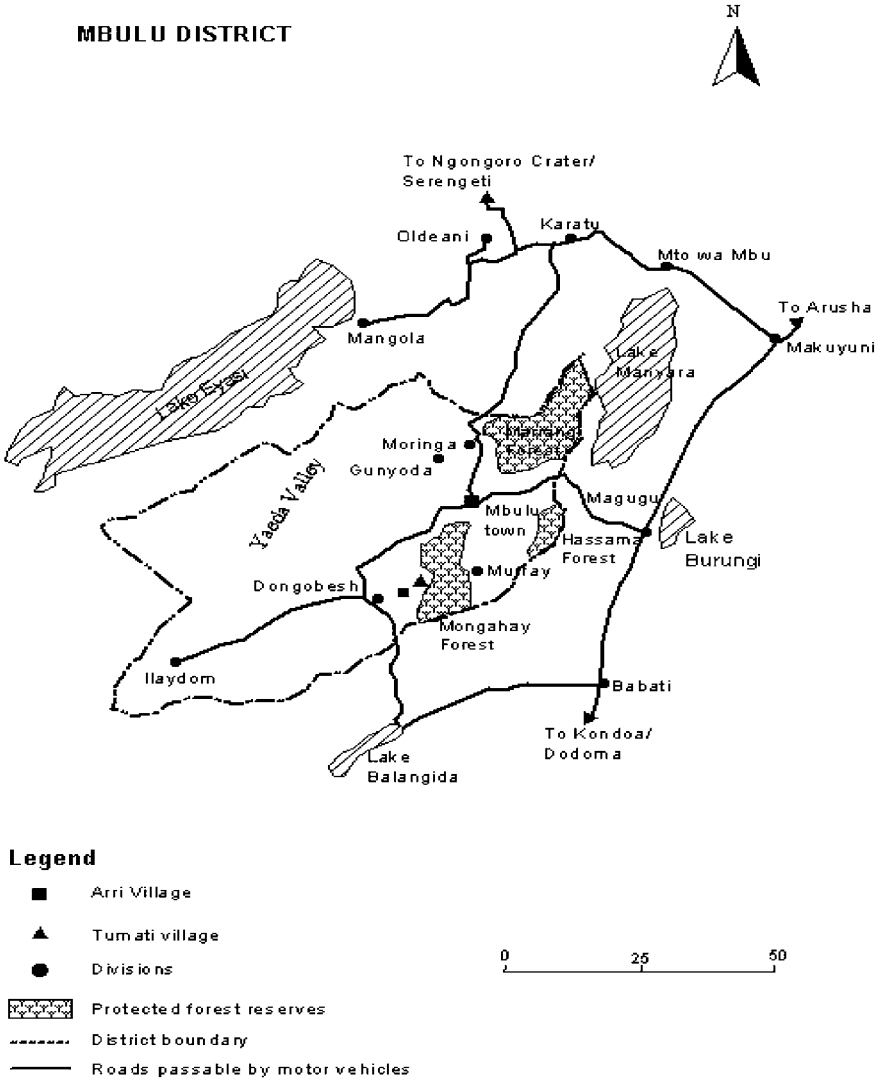


**Figure 2** Map of the Mbulu District showing the plague outbreak villages and location of Mongahay Forest.

were identified, anesthetized by ether, and blood was collected from the orbital vein or heart using micro-capillary tubes or a syringe and needle, respectively. The diagnosis of plague was made by detection of antibodies against specific antigen (fraction 1 or F1 antigen) of the plague bacillus, *Y. pestis*, in rodent blood by an ELISA technique (Williams et al. 1984). We used both anti-F1 positive and negative control sera from South Africa for comparison. The ELISA titers ranged from 1:4 to 1:128.

In the current study it was possible to establish an accurate diagnosis of plague infection in rodents using RDT by dipstick assay (Chanteau et al. 2003, Thullier et al. 2003). The dipsticks used for the current study originated from the Pasteur Institute, Madagascar, and were used to detect antibodies against the F1 antigen of *Y. pestis* in rodent sera to provide some indication of current infection.

Flea ectoparasites were removed from animals by brushing the fur; these were collected in a large aluminum pan, counted and recorded, and later identified to species level in the laboratory. The fleas were not tested for evidence of infection with *Y. pestis* during the current study, but they have been preserved for future tests to detect *Y. pestis* DNA using PCR.

## Results

Table 1 provides the results of ELISA tests of animal sera. Of 63 animal sera examined, 37 (58%) were positive for antibodies against *Y. pestis* F1 antigen by ELISA. The results demonstrate a high rate of exposure to *Y. pestis* among the rodent species. The titer range (1:4–1:128)

**Table 1** Species composition and presence of specific antibodies against *Yersinia pestis* F1 antigen in rodents and shrews.

| Species | Sera | | |
|---|---|---|---|
| | Total (n) | Positive for *Y. pestis* antibodies | |
| | | (n) | (%) |
| *Mastomys natalensis* | 21 | 13 | 61.9 |
| *Lophuromys flavopunctatus* | 11 | 9 | 81.8 |
| *Rattus rattus* | 15 | 7 | 46.7 |
| *Lemniscomys striatus* | 3 | 1 | 33.3 |
| *Praomys delectorum* | 4 | 1 | 25.0 |
| *Graphiurus murinus* | 2 | 1 | 50.0 |
| *Crocidura* sp. | 5 | 3 | 60.0 |
| *Mus minutoides* | 1 | 1 | 100.0 |

used in these tests provides a measure of either current infection (1:64–1:128) or developing/receding infection (1:4–1:64). The forest biotope was dominated by *L. flavopunctatus* and *Praomys delectorum* Thomas 1910, for which 54.5% tested positive for antibodies against *Y. pestis* F1 antigen. Two *Graphiurus murinus* Smuts 1832 were captured in the forest, but only one tested positive for antibodies against the *Y. pestis* F1 antigen (Table 1). *Mastomys natalensis* was the most abundant rodent species in the fallow /crop field mosaic and in peri-domestic areas. Other species captured in fallow and crop fields included *L. flavopunctatus*, *Lemniscomys striatus* Pagenstecher 1885 and *Mus minutoides* Smith 1834. *Rattus rattus* was only captured indoors and 46.7% were positive for antibodies against the *Y. pestis* F1 antigen. Even rodents with F1 antibodies detected at the high titer (1:128) appeared to be healthy animals and did not show any clinical signs of plague. *Crocidura* sp. Wagler 1832 also tested positive for antibodies against *Y. pestis* F1. Table 2 shows the plague diagnosis results for rodents using the RDT. Out of 30 rodent sera tested, 15 (50.0%) were both RDT- and ELISA-positive for antibodies against *Y. pestis* F1.

A total of 65 fleas, of which 48 (72.3%) were *Dinopsyllus lypusus* Jordan *et* Rothschild 1913 and 18 (27.7%) were *Xenopsylla brasiliensis* Baker 1904, were collected from wild and commensal rodents, with an overall flea index of 1.8 (Table 3).

**Table 3** Flea species and their numbers collected from different rodent hosts.

| Rodent species | No. of animals | Flea ectoparasite species* | |
|---|---|---|---|
| | | *Dinopsyllus lypusus* | *Xenopsylla brasiliensis* |
| *Mastomys natalensis* | 16 | 22 | 8 |
| *Rattus rattus* | 9 | 5 | 10 |
| *Praomys delectorum* | 1 | 1 | 0 |
| *Lophuromys flavopunctatus* | 9 | 18 | 0 |
| *Lemniscomys striatus* | 1 | 1 | 0 |
| Total | 36 | 47 | 18 |

*Flea index=(Total no. of fleas/Total no. of rodent hosts)=1.8.

## Discussion

This is the first documented epizootic of plague in Mongahay Forest and surrounding villages in Dongobesh division. The presence of antibodies against *Y. pestis* F1 antigen in rodent sera is of considerable importance in determining potential reservoirs of plague in the Dongobesh plague focus. Based on ELISA tests of small mammal sera from various localities, Kilonzo et al. (2005) demonstrated that plague reservoirs were widely distributed in Tanzania. The RDT was most useful in demonstrating that animals that appeared clinically healthy had a current plague infection. The study revealed that several species of rodents are naturally involved in the epidemiology of plague in the area. Apart from rodents, infections in other animals are unimportant in the long-term survival of *Y. pestis* (Perry and Fetherston 1997). Animals in fallow peri-domestic areas and in houses tested positive for antibodies against the F1 antigen, which further suggests that *Y. pestis* was circulating in these populations. *Lophuromys flavopunctatus* and *P. delectorum* were predominant in Mongahay Forest, but were also captured at the edge of the forest, and *L. flavopunctatus* was also captured in fallow fields surrounding the village. This indicates co-existence and interaction with species occurring in the fallow areas, particularly *M. natalensis*. *Xenopsylla brasiliensis* and *D. lypusus* were found on both wild and commensal rodent species. These flea species are considered to be good plague vectors (Schwan 1986, Kilonzo et al. 2006). It has been reported that flea indices were correlated with plague incidences in the Lushoto outbreak villages in north-eastern Tanzania (Laudisoit et al. 2007). In this focus, plague outbreaks are seasonal (Davis et al. 2006); flea indices $<1.0$ were reported during the non-outbreak season and indices $>1.0$ were observed during plague outbreaks (Kilonzo et al. 1992). The rodent flea index of 1.8 observed in Dongobesh is quite high and could be one of the factors contributing to efficient transmission of the disease between rodent populations and humans. In a study of plague prevalence in the Karatu District, which neighbors Mbulu District, Kilonzo et al. (2006) also observed predominance of *X. brasiliensis* (45.8%) and *D. lypusus* (54.2%).

It would appear that in the current outbreak, an enzootic cycle of the disease was present in the area before the disease surfaced in the human population. This suggests that the Dongobesh area was an unknown natural focus of plague, since there were no reported incidences of the disease elsewhere in Tanzania from where it could have been introduced. One important factor that supports the hypothesis of the presence of enzootic sylvatic plague in the area is the high proportion of rodents that did not show any easily observable clinical manifestation, but were positive for antibodies against *Y. pestis* F1 antigen by the dipstick (RDT) and ELISA techniques. The fact that local people had seen dead animals in the fields further suggests that some animals were highly susceptible to plague infection whereas others were not. The classic epidemiologic model for plague considers it an enzootic infection of mostly resistant wild rodents. The findings of the current study therefore led to the hypothesis that the outbreak of human plague in Dongobesh began with an

**Table 2** Rapid diagnostic test by dipstick assay of rodent sera from clinically healthy animals.

| Rodent species | Locality of capture | No. of sera tested | No. positive (%) |
|---|---|---|---|
| *Mastomys natalensis* | Crop fields/fallow/peri-domestic | 10 | 3 (30) |
| *Praomys delectorum* | Mongahay Forest | 4 | 4 (100) |
| *Lophuromys flavopunctatus* | Mongahay Forest | 9 | 6 (66.7) |
| *Rattus rattus* | In-house | 7 | 2 (28.6) |

epizootic in wild rodents that resulted in infection of commensal rodents. In particular, a rodent outbreak had occurred prior to the disease incidence and, owing to deforestation, crop fields and residences are in close proximity to the currently protected Mongahay Forest Reserve. This elevates the risk of infection in humans. Exposure to plague could have occurred in the peridomestic environment. The findings of the current study suggest that the forest reserve and its rodent fauna could have an important role in the epidemiology of plague in Dongobesh and surrounding areas. Thus, an epidemiological surveillance of plague on a wider geographical scale is warranted to identify other natural foci and potential outbreak areas in the whole of Mbulu District in northern Tanzania.

## Acknowledgements

This study was financially supported by the ECORAT Project and the Pest Management Center (SPMC), Sokoine University of Agriculture. We wish to thank the Ministry of Health and Social Welfare, the staff of Mbulu District Hospital, in particular Mr. Lekei, and Manyara Regional Health Officer Ms. Kombe for logistical support. Staff of the SPMC, in particular Mr. G. Mhamphi and Halid Kibwana, are thanked for their contribution to field and laboratory work.

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