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Identification of Plague Hosts and Vectors in Lushoto District of Tanzania.

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Abstract

Plague is a zoonotic disease, endemic throughout the world except Australia and Antarctica. In Africa the disease has been reported in countries such as Democratic Republic of Congo, Madagascar, Mozambique, Uganda and the United Republic of Tanzania. Surveillance work done in Mbulu and Karatu plague foci revealed the evidence of *Yersinia pestis* in rodents despite lack of disease outbreaks. The information obtained prompted a study to be done in another plague focus of Lushoto. In this study live trapping of wild and commensal rodents was done after which fleas were collected. Nine rodent species of captured and these includes *Mastomys* (33%), *Rattus rattus* (25.9%), *Praomys* (14.3%), *Lophuromys* (14.3%), *Grammomys* (8%), *Beamys* (1.8%), *Arvicanths* (0.9%), *Croccidura* (1.8%) and *Mus* (0.9%). Of the rodents identified *Mastomys* (33%), *Rattus rattus* (25.9%), *Arvicanths* (0.9%), and *Mus* (0.9%) have been implicated to be important hosts *Y. pestis.* Five flea species were also identified and these were *Xenopsylla* spp (39.5%), *Dinopsyllus* spp (22.5%), *Ctenocephalides* spp (26.1%), *Leptopsylla* spp (6.3%) and *Echidnophaga* spp (5.5%) of which *Xenopsylla* spp (39.5%) and *Dinopsyllus* spp (22.5%) have been implicated as efficient plague vectors.

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DNA extracted from fleas and rodents tissues was found negative for *Yersinia pestis* DNA using PCR amplification of the conserved and specific *Y. pestis* plasminogen activator gene. The results of this study have identified possible reservoirs and vectors of plague in Lushoto District.

Key words: Plague; Hosts; Vectors; Lushoto; Tanzania.

1. Introduction

Plague is a zoonotic disease, endemic throughout the world except Australia and Antarctica [1]. The disease is caused by *Yersinia pestis*, which primarily infects a wide range of rodents and is transmitted via flea vectors [2]. The disease persists in many parts of the world with 90% of the plague cases being reported to the World Health Organization each year, come from Africa where public health and living conditions are poor [3]. The natural foci of plague are spread worldwide, mainly in the rodent and flea vector reservoirs. The disease is a public health threat in many parts of the world, especially in sub-Saharan Africa where many countries harbours endemic foci [4].

The disease can cause case fatality rates of 50 to 60% if left untreated. The mode of transmission is mainly through a bite by an infective flea that is harboured by rodent reservoirs or from person to-person through aerosols in case of pneumonic form and by contact with infective fluids of infected animal or human [5]. The oriental rat flea *Xenopsylla cheopis* and the human flea *Pulex irritans* are thought to be important arthropod vectors in transmitting plague to humans [6]. The rodents that are susceptible to *Y. pestis* infection include the roof rat (*Rattus rattus*), and the multimammate mouse (*Mastomys natalensis*). These rodents are often infested with flea species that are capable of transmitting the plague bacteria. The fleas implicated in transmission of plague include the, *Xenopsylla cheopis*, *X. brasiliensis, Dinopsyllus lypusus, Ctenophthalmus cabirus* and occasionally *Ctenocephalides* felis [7].

In Tanzania, *Xenopsylla brasiliensis, Xenopsylla cheopis* and *Dinopsyllus lypusus* are considered to be the most important vectors [8]. Surveillance studies of *Y. pestis* evidence of plague in rodents revealed the presence of *Y. pestis* in Karatu and Mbulu Districts despite of no outbreaks [9]. This work prompted Lushoto district which is considered as a plague foci to be investigated as it used to be an active plague area. Furthermore the need to determine the flea vector efficiency in plague transmission is very important to understand how plague is maintained in natural ecosystems. This study was therefore initiated to identify possible plague hosts and vectors in Lushoto District through Molecular screening of *Y. pestis* DNA. It was envisaged that the data collected would provide the necessary information in combating future disease outbreaks through the identified possible reservoirs and vectors.

2. Methods

2.1. Study area

Lushoto District is situated in Tanga region, in the West Usambara Mountains, a part of the Eastern Arc Mountains. With an elevation ranging from 900 to 2,250 m above sea level, Lushoto District (04°22′–05°08′S,

038°05′–038°38′E) covers a surface area of 3,500 km^2, of which 2,000 km^2 are arable land and 340 km^2 are forest reserve [10].

Fleas were collected from rodents trapped in the forests, forests edge, farms and peridomestic areas by using the Sherman Trap. The fleas were collected from the captured rodents using an animal grooming comb after anaesthetizing them with diethyl ether. The fleas were brushed on a white cloth for visibility and easy collection. The collected fleas were stored in 70% ethanol before analysis after which they were identified using field manual as previously described by Stuart [11]. The fleas were placed in pools of one-10 individuals (corresponding to the same animal host and flea species) and then tested for the presence of *Y. pestis* DNA.

For further analysis, the ethanol preserved fleas were subsequently dried on sterile filter paper in a laminar biosafety hood. The fleas were placed in the Eppendorf tubes with 100 µl of brain-heart infusion broth (Oxoid, Hampshire, England) and then triturated with a sterile pipette as previously described by Hang'ombe [12]. The triturated samples were then boiled at 95°C for 10 min and then followed by centrifugation for 10 sec at 10,000 *xg*, where 1 µl was used as a template for PCR testing. Negative control template employed brain-heart infusion broth only, and fleas collected from a non-endemic plague area. Briefly, PCR amplification was performed for the detection of the *Y. pestis* plasminogen activator gene using primers *Yp pla1* (5'TGC TTT ATG ACG CAG AAA CAG G3') and *Yp pla2* (5'CTG TAG CTG TCC AAC TGA AAC G3') as previously described by Hang'ombe et al [12]. The primers amplify a 344 bp region of the *Y. pestis* plasminogen gene. PCR was done using the Phusion™ flash high fidelity PCR master mix (Finnzymes Oy, Finland). Specific *Y. pestis* detection was identified by the presence of a specific 344 bp DNA band on 1.5% agarose gel, stained with ethidium bromide and evaluated under UV transilluminator. The estimation of the sizes of PCR products was done according to the migration pattern of a 100-bp DNA ladder.

3. Results

The study was initiated by gathering records regarding plague in Lushoto district where the outbreaks started in 1980 and were yearly being reported up to 2003 (Table 1).

3.1. Tables

Table 1: Reported Plague cases in Lushoto from 1980 to 2003

Year	Recorded/affected villages	Suspected/reported cases	Number of reported deaths	Percentage Death
1980	2	49	11	22.4
1981	1	9	6	66.7
1982	9	76	18	23.7
1983	2	569	49	8.6
1984	11	603	41	6.8
1985	18	129	22	17.1

1986	23	360	57	15.8
1987	32	470	57	12.1
1988	34	452	13	2.9
1989	9	29	5	17.2
1990	28	459	58	12.6
1991	39	1203	68	5.7
1992	2	16	2	12.5
1993	3	18	0	0.0
1994	19	444	50	11.3
1995	14	831	74	8.9
1996	33	826	59	7.1
1997	30	499	30	6.0
1998	13	286	3	1.0
1999	15	364	11	3.0
2000	11	76	2	2.6
2001	1	4	0	0.0
2002	9	97	3	3.1
2003	5	38	1	2.6
TOTAL	48	7907	640	8.1

A total of 112 rodents (Table 2) representing nine species were trapped from five different sites which included forest, forest edge, in farms along water stream and per domestic areas.

Table 2*:* Rodents and shrews observed according to villages and species trapped

Species	Villages					
	Gologolo	Mavumo	Viti	Manolo	Total	Percentage
Mastomys	2	10	12	13	37	33.0
Rattus	4	13	5	7	29	25.9
Praomys	7	2	0	7	16	14.3
Lophuromys	9	6	0	1	16	14.3
Grammomys	2	2	0	5	9	8.0
Beamys	0	1	0	0	1	0.9
Arvicanthis	0	1	0	0	1	0.9
Croccidura	0	0	2	0	2	1.8
Mus	0	1	0	0	1	0.9
TOTAL	24	36	19	33	112	100.0

Most of the rodents trapped were in normal condition except five of them which had splenomegaly and these were from *Mastomys* and *Rattus* species. The species involving, *Mastomys* (33%) and *Rattus rattus* (25.9%) were trapped in all sampled areas followed by *Lophuromys* and *Praomys* in three areas except Viti village (14.3%), while *Grammomys* (8.0%) and *Beamys* (1.8%) were captured at Mavumo, only *Croccidura* (1.8%) at Viti and *Mus* (0.9%) at Mavumo. This study also observed that there was random mixing of rodent's species in the different sites whereby the forest rodents were trapped in farms as well as the house rats *Rattus rattus* which was trapped in the farms. According to (Table 5) it was observed that *Mastomys* was highly infested by fleas followed by *Rattus* species.

A total of 253 fleas (Table 3) which represents five species were collected from various rodents' species, the most observed flea species were the *Xenopsylla species* (39.5%), followed by *Ctenocephalides species* (26%) and the *Dinopsyllus species* (22.5%). All the flea species were collected in all villages while *Leptopsylla spp* (6.3%) and *Echidnophaga spp* (5.5%) were collected in only one village Manolo. It was observed that (Table 4) the flea –rat indices of each category of rodents were above 0.5, *Mastomys* 2.7, *Rattus* 1.7, *Praomys* 4.1, *Lophuromys* 1, and *Grammomys* 1.6.The results showed that there is no evidence of *Yersinia pestis* bacterium currently in the rodents and fleas because there was no detection of *Y. pestis* DNA after PCR analysis.

Table 3: Fleas species observed in the Study area

Species	Villages					
	Gologolo	Mavumo	Viti	Manolo	Total	Percentage
Xenopsylla spp	10	42	22	26	100	39.5
Ceratopylla spp	25	15	1	16	57	22.5
Ctenocephalides spp	43	17	4	2	66	26.1
Leptopsylla spp	0	0	0	16	16	6.3
Echidnophaga spp	0	0	0	14	14	5.5
TOTAL	78	74	27	74	253	100.0

4. Discussion

Plague has been the scourge of mankind for many years. In Lushoto district the first outbreak was recorded in 1980. The disease persisted for 23 years. During this period 7907 peoples were suspected to have been infected by the disease. About 640 deaths equivalent to 8.1% mortality rate were reported. After 2003 the disease regressed almost 10 years due to employment of different control measure and enforcement of environmental sanitation laws. This study was initiated to monitor the identified hosts and vectors for the evidence of plague. The results showed that the *Yersinia pestis* bacterium is not present currently in the rodents and fleas because there was no evidence of *Y. pestis* DNA after PCR assay. Although it was observed that all potential hosts and effective vectors for the transmission of the disease were available.

Rodent hosts suspected to be involved in the past outbreaks namely, *Mastomys natalensis* (33.0%), *Rattus rattus* (25.9%), *Mus musculus* (0.9%) and *Arvicanthis* (0.9%) were trapped and these results agree with other works (Table 4) [8, 9]. The same specie of rodent including *Rattus spp* was also observed to have evidence of *Yersinia pestis* DNA in Zambia [12]. This indicates that they are the most important hosts for maintaining the bacterium and they may serve as the transmission vessel for the bacterium into human being population.

Table 4: Rodent infested with multiple flea species

Rodent Species	Flea Species	Number of Fleas
Mastomys	*Ceratophylla spp*	21
	Xenopsylla spp	22
	Ctenocephalides spp	56
Rattus rattus	*Ceratophylla spp*	1
	Xenopsylla spp	72
	Ctenocephalides spp	1
Praomys	*Ceratophylla spp*	5
	Xenopsylla spp	1
	Ctenocephalides spp	0
Grammomys	*Ceratophylla spp*	20
	Xenopsylla spp	1
	Ctenocephalides spp	0
Lophuromys	*Ceratophylla spp*	2
	Xenopsylla spp	0
	Ctenocephalides spp	9

In this study *Mastomys* species was observed to be highly infested by various flea species as observed in past studies [13]. There was also co-infestation of flea to all rodents captured and this kind of infestation shows that there is a high rate of interaction between various rodent species and their vectors. The interaction mentioned above is the best indicator of facilitating transmission of the infection in the sylvatic as well as in the human population immediately when the bacterium happens to invade the hosts and vectors. In this study flea vectors suspected to be involved in the past outbreaks were also identified as *Xenopsylla cheopis* (39.5%) which is an efficient vector because of its proventriculus, which creates a location for growth of *Y.* pestis [13]. The flea becomes blocked with *Y. pestis* and then it is unable to swallow a full blood meal [14, 15].

In an attempt by the flea to dislodge the blockage, the flea infects new mammalian hosts. Also *Dinopsyllus spp*

(22.5%) was found and this specie was described to be capable of transmitting the *Y.* pestis [7]. The same hosts and vectors observed in the study area has been observed in other plague endemic areas within Tanzania and in other countries which are plague endemic like Zambia and Madagascar[9,12,16]. It was observed that the Flea-rat index was observed to be 2.26 which would cause the flea to leave the rodents in search of other alternative hosts which may be a mammal and in this case the disease may be transmitted if the fleas are infective (Table 5). There is no outbreak because the fleas were not infected by the bacterium, therefore reducing the chances of the fleas transmitting the disease from one rodent to another and to human being.

Table 5: Calculated Specific flea index and Percentage Incidence Index

Rodent spp	Total number caught	Total number Infested	Total number Of fleas	PII	SF
Mastomys spp	37	22	100	59.5	2.7
Rattus spp	29	24	57	82.8	1.97
Praomys spp	16	10	66	62.5	4.1
Lophuromys spp	16	7	16	43.8	1
Grammomys spp	9	8	14	88.9	1.6
Beamys spp	1	0	0	0	0
Arvicanthis spp	1	0	0	0	0
Croccidura spp	2	0	0	0	0
Mus spp	1	0	0	0	0
The general flea-rat index					2.26

5. Conclusion

The study concluded that at the moment there is no evidence of the bacterium in the rodents and flea population in Lushoto but the absence of the bacterium in the investigated hosts and vectors cannot rule out the absence of the disease, because it has been observed that the disease may be harbored in soil, rodent's burrows, nests and in other small wild mammals. Furthermore it might have been difficult to recover the bacterium because the sampling population might have been small. Along with this information the community awareness about plague is higher as at the same time people are employing environmental sanitation programs such as plastering their houses frequently as previously mentioned by Kilonzo [8]. The other reason of not recovering the bacteria may be that, the bacterium is circulating at very low levels in the reservoirs to the extent that it is not able to infect the vectors and hence infect the human population as observed by Kilonzo [8].

The rodents captured in the study areas which are important plague hosts are *Rattus rattus, Mus musculus, Mastomys natalensis and Arvicanthis nairobae* which agree with the previously work [8]. While the flea vectors belonging to *Xenopsylla spp* and *Dinopsyllus spp* were identified and these fleas are efficient plague vectors.

The flea-rat index of 2.26 was very high which suggests the possibility of an outbreak if the bacterium shall invade the population at any time.

6. Recommendation

For the purpose of prevention and control of future outbreaks or sporadic outbreaks of plague in Tanzania, the following measures that could assist are recommended:

- The study suggests that regardless of the absence of the bacterium DNA in rodents and Fleas at the moment frequent surveillance must be done to ensure preparedness.
- The government should ensure the continuation of environmental sanitation, law enforcement plus rodents and fleas controls in the area so that to cut off the flea-rat cycle to invade human being.
- Furthermore the study insist that research to be done in other hypothesized reservoir sites e.g. soils, rodent burrows, small mammals and wild animals.
- Quick intervention must be done on control of flea as shown the rat-flea index is very high.

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