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The role of rodents and small carnivores in plague endemicity in Tanzania

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ABSTRACT. Between 1974 and 2003, blood samples were collected from wild and commensal rodents, and wild and domestic small carnivores in selected villages of seven districts in Tanzania that have experienced human plague outbreaks and seven districts that have not experienced any outbreak of the disease. The samples were tested for antibodies against *Yersinia pestis* Fraction I antigen, using passive haemagglutination (PHA) or ELISA tests. Of the 3354 rodents and 558 small carnivores from the plague infected districts, 122 (3.6%) rodents (captured in Mbulu and Lushoto districts) were plague positive; 29 (5.2%) small carnivores from Mbulu, Arumeru, Hai and Lushoto districts were plague positive, 28 of these were domestic dogs (*Canis familiaris).* PCR tests showed that 17.5% of 211 rodents tested from Lushoto contained *Y. pestis* DNA. In the non-infected districts, 1545 rodents and 171 domestic dogs were tested. 11 (0.7%) of the rodents (captured in Monduli, Chunya and Masasi districts) were plague-positive. In Masasi district, 10.4% (7/67) of the rodents and 43.6% (17/39) of the dogs were positive for anti-*Y. pestis* IgG. It was concluded that wild and commensal rodents as well as wild and domestic small carnivores play a potential role as reservoirs and/or carriers of sylvatic plague in Tanzania, and that the disease exists in areas where human plague outbreaks have not occurred before. In order to update the distribution of the disease it is proposed that further epidemiological surveillance activities are established.

KEY WORDS : Rodents, small carnivores, plague, passive haemagglutination, ELISA. PCR.

INTRODUCTION

Plague has been endemic in Tanzania for more than a century. The first authentically recorded epidemic occurred at Image, Iringa in 1886. At the time of this outbreak, however, it was noted that the local people were quite familiar with the disease which was locally known as *"Chambafu/Shaambafu"* and that they knew it was associated with rodents. It was also noted that communities, under the guidance of their leaders, were burning houses as a means of controlling rodents and fleas and consequently controlling the disease (ROBERTS, 1935; MSANGI, 1968). The second authentically recorded epidemic occurred at Kiziba, Bukoba in 1897 (DAVIS et al., 1968). Likewise, the local people were already familiar with the disease that was referred to as **"***Rubunga***",** and were isolating plague patients as a means of controlling its spread. Based on available information, *Yersinia pestis* was isolated for the first time in Tanzania during this epidemic (DAVIS et al., 1968). The Kiziba focus is probably the oldest in the country as plague was introduced to this area from Uganda as far back as 1883 (CLYDE, 1962). Since then, the disease spread and established itself in many parts of the country especially the Central, North-eastern, Northern and South-western regions (Fig. 1). The spread was facilitated by slave and ivory caravans that mostly moved across the hinterland to the coast and through the Kilimanjaro region to Mombasa in Kenya. Indeed, most established plague foci today are found along the ancient slave and ivory trade routes (MSANGI, 1968; KILONZO, 1981).

Over the years, outbreaks of the disease have occurred in various parts of the country and involved large numbers of human cases and substantial case-fatality rates. During the past half century (1953 – 2003), a total of 8956 plague cases of whom 731 (8.2%) were fatal, were reported from ten districts in the country. Since 1980, however, only three districts (Lushoto, Singida and Karatu) have experienced outbreaks of the disease, and involved 8298 and 646 (7.8%) reported cases and deaths, respectively (KILONZO, 2003).

Prior to the studies reported in this paper, limited investigations were made to understand the species and ecology of rodents involved in the epidemiology of plague in the country. HUBBARD (1973) incriminated many rodent species in Tanzania as suitable reservoirs of plague in view of their hosting of flea species known to be efficient vectors of the disease elsewhere. Some observations made in plague – endemic areas in the country revealed that *Mastomys natalensis*, was the most frequent natural reservoir of plague and that it played an important role in maintaining the disease as it is partly refractory to the infection, and hence, it is not eradicated during plague epizootics (GUGGISBERG, 1966; HUBBARD, 1973). MSANGI (1968) demonstrated the presence of haemagglutination plague antibodies in 0.8% and 1.5% of clinically healthy *M. natalensis* and *Arvicanthis abyssinicus*, respectively. Many other wild rodent species including *Tatera robusta, Grammomys dolichurus; Rhabdomys pumilio* and *Otomys angoniensis* have been suggested as suitable reservoirs of the disease. This has been argued on the basis of seropositive assessments in Kenya where the ecological

and climatic features are similar to those in Tanzania, and the fact that these rodent species are abundant in areas where outbreaks of plague occur frequently and host similar flea species which are found on known rodent reservoirs (DAVIS et al., 1968; HUBBARD, 1973; SIONGOK et al., 1977).

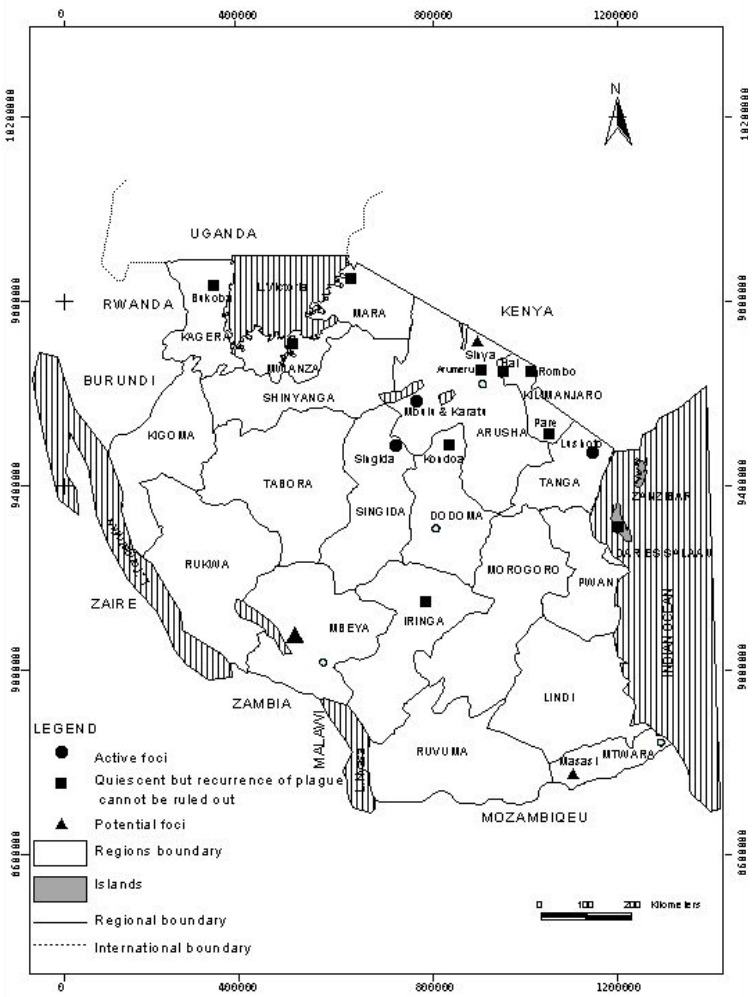

Fig. 1. – Distribution of plague in Tanzania (1953-2003).

Many outbreaks of human plague in the country have been associated with or preceded by large increases and/or mortalities of rodents in the infected area. The Wahehe people in Iringa, for example, reportedly observed that remarkable rat mortalities were associated with *"Chambafu"* (plague) outbreaks long before the arrival of German administrators in 1884 (MSANGI, 1968). LURZ (1913) similarly reported large numbers and deaths of rodent populations prior to the 1912 outbreak of plague in Rombo district. Likewise, the 1948 outbreak of the disease in Iramba district which involved 312 and 178 (57.1%) recorded cases and deaths, respectively, was preceded by large mortalities of rodents in late 1947 (ANONYMOUS, 1948). Similar population build-ups and plague epizootics were reported prior to plague outbreaks in Hanang (then Mbulu) and Same districts in 1951 and 1964, respectively (ANONYMOUS, 1951 and 1964). The first outbreak of human plague in Lushoto district in April, 1980 was preceded by large increases of rodent populations during the years 1978 – 1979 which prompted the use of zinc phosphide for their control in view of the severe damage caused to agricultural crops (MKAMI, 1980; KILONZO & MHINA, 1982).

The establishment of plague foci and distribution patterns in Tanzania has been based on outbreaks of the disease among human populations, rather than on substantiation of the disease among natural reservoirs in the particular area. In the past, very limited investigations were carried out to substantiate natural reservoirs, secondary reservoirs and/or carriers of the disease. In order to know its actual distribution, and hence be able to forecast outbreaks, adequate information on the reservoirs and carriers as well as its endemicity level and the population densities of its efficient vectors is desirable. The purpose of the present study was to partly fulfill this objective.

MATERIALS AND METHODS

Time and areas of study :

These studies were conducted at different times of the year, between 1974 and 2003. Seven districts that have experienced at least one recorded outbreak of human plague, and the same number of districts which have never recorded any outbreak of the disease, were selected for the study. The first category of districts (infected) comprised Singida, Mbulu, Arumeru, Hai, Rombo, Same and Lushoto. The second (un-infected) category of districts comprised Masasi, Chunya, Igunga, Monduli, Muheza, Kilombero and Morogoro-Rural (Fig. 2). At least two villages in each district were selected for the surveys. In plague-infected districts, the selected villages included the ones where the most recent outbreaks occurred.

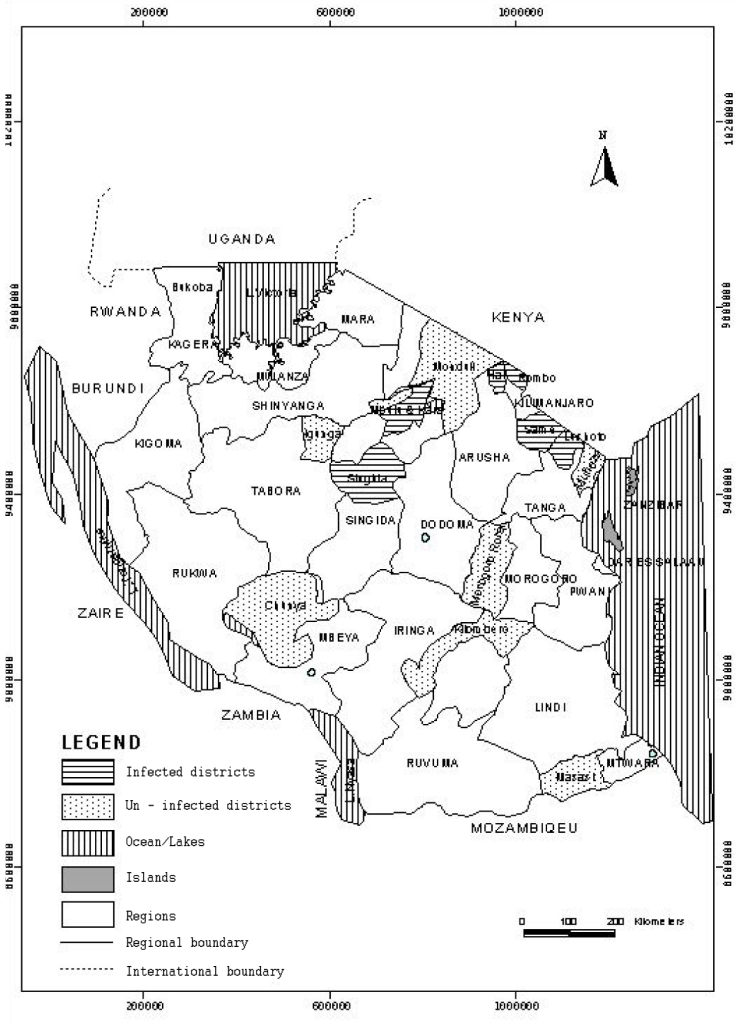

Fig. 2. – Areas surveyed for plague in Tanzania.

Trapping, collection and processing of specimens :

Sylvatic and commensal rodents were live-trapped using Sherman, Chauvancy or Box traps baited with peanut butter or roasted sardines. Traps were inspected in the morning with captures removed and taken to a central processing location. Live-captured animals were anaesthetized with ether and brushed with ether-soaked cotton wool to kill its arthropod ectoparasites which were then removed by scrubbing the fur of the animal with a small brush. Each animal was bled from the heart using a disposable syringe and needle or from the orbital vein using capillary tubes and capped microtubes. Flea ectoparasites were sorted, counted and preserved in 70% ethanol for their subsequent identification. Blood samples were left at room temperature overnight for spontaneous separation of serum with a minority of samples separated by centrifugation. Sera were preserved at 0-4ºC while in the field and at –20ºC after returning to the laboratory. In a few occasions, sera were preserved in liquid nitrogen.

In Hai, Rombo, Arumeru and Mbulu districts, small wild carnivores were live-captured with steel cage traps or killed by handgun. Venous blood was aseptically collected from the captured/shot animals and similarly processed. Collection and processing of blood from domestic dogs and cats was effected after obtaining informed consent from their owners.

Testing for plague infection :

A total of 4899 sera were tested against *Yersinia pestis* Fraction I (FI) antigen, using the Passive haemagglutination (PHA) test and controlled by the Passive haemagglutination inhibition (PHAI) test. Furthermore, 289 serum samples from Lushoto and Masasi districts were also tested by the Enzyme Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) techniques for detection of current and past infections.

RESULTS

A total of 3354 rodents were tested for anti-plague antibodies in the seven districts with established plague endemic foci. Of these, 122 (3.6%) were positive for specific haemagglutination antibodies against *Y. pestis* (Table 1a). Of the rodents tested in Lushoto district, 222 were also tested with the ELISA technique. Of these, 7.7% and 11.3% were positive for anti-plague IgG and IgM respectively (Table 1b). A total of 211 rodents from Lushoto were also subjected to PCR tests and 17.5% of them contained *Y. pestis* DNA (Table 1c). The seropositive rodents were captured in Mbulu and Lushoto districts, where recent and active human plague cases, respectively, have been recorded. A total of 1545 rodents were evaluated in the districts where outbreaks of the disease have not been reported. Of these animals, 15 (1.5%) were plague positive and had been captured in the Monduli, Chunya and Masasi districts (Table 2a).

A total of 729 small carnivores were examined from six plague-infected and two un-infected districts (Table 3). Of these, 47 (6.4%) were positive for plague antibodies. The majority (95.7%) of the positive carnivores were domestic dogs from the Arumeru, Mbulu, and Lushoto districts (plague-infected), and the Masasi district (uninfected). Other plague positive carnivores identified were one wild cat (*Felis lybica*) from the Hai district and one domestic cat (*Felis catus*) from the Masasi district (Table 3)..

TABLE 1a

Species and infection rates of rodents in districts with previous records of plague outbreaks :
(1a) Results of PHA tests

Rodent species tested	Nos. tested and (%) positive in each district							
	Singida	Mbulu	Arumeru	Hai	Rombo	Same	Lushoto	Total
Rattus rattus	96 (0)	37(8.1)	12 (0)	118 (0)	37 (0))	131 (0)	804 (3.4)	1235
Mastomys natalensis	355 (0)	43 (9.3)	78 (0)	19 (0)	84 (0)	35 (0)	552 (4.2)	1166
Arvicanthis nairobae	7 (0)	3 (0)	20 (0)	2 (0)	39 (0)	-	292 (2.2)	363
Lophuromys sp.	-	3 (0)	-	3 (0)	-	-	133 (6.8)	139
Pelomys fallax	-	-	-	-	-	-	60 (20)	60
Grammomys dolichurus	-	1 (0)	-	-	-	-	55 (3.7)	56
Otomys spp.	-	-	-	-	-	8 (0)	124 (11.3)	132
Heliosciurus sp.	-	-	-	-	-	-	17 (0)	17
Praomys spp.	-	-	-	-	-	-	3 (0)	3
Tatera robusta	21 (0)	-	3 (0)	-	8 (0)	16 (0)	3 (0)	51
Lemniscomys striatus	-	26 (11.5)	-	12 (0)	1 (0)	-	-	39
Rattus norvegicus	-	1 (0)	-	-	-	-	-	1
Cricetomys gambianus	-	-	-	9 (0)	28 (0)	-	1 (0)	38
Rhabdomys pumilio	-	-	35 (0)	-	4 (0)	-	-	39
Tachyoryctes daemon	-	-	1 (0)	-	2 (0)	-	-	3
Acomys spinossismus	-	-	-	-	-	8 (0)	-	8
Aethomys spp	4 (0)	-	-	-	-	-	-	4
Total	483 (0)	114 (8.3)	149 (0)	163 (0)	203 (0)	198 (0)	2044 (5.5)	3354 (3.6)

TABLE 1b

(1b) Results of ELISA tests on rodent sera from Lushoto district
(Range of titres :IgG : 1 :4 - 1 :128; IgM : 1 :8 - 1 :512; Minimum specific titre = 1 :4)

Rodent species	No. tested for antibodies	No. & % positive for IgG	No. & % positive for IgM
Mastomys natalensis	142	15 (10.6)	13 (9.2)
Arvicanthis nairobae	25	6 (23.1)	7 (26.9)
Lophuromys sp	14	1 (7.7)	2 (15.4)
Rattus rattus	19	2 (10.5)	2 (10.5)
Grammomys dolichurus	14	-	-
Praomys spp.	6	-	-
Mus (L) minutoides	1	-	-
Petrodromus sp.	1	1 (100)	1 (100)
Total	222	17 (7.7)	25 (11.3)

TABLE 1c

(1c) : *Yersinia pestis* DNA in rodents captured in Lushoto district :
results of Polymerase Chain Reaction (PCR) tests (Minimum specific titre =Lowest dilution of the test serum that produces positive reaction with specific Fraction I plague antigen).

Rodent species	Number tested	Number positive	% positive
Mastomys natalensis	131	25	19.1
Arvicanthis nairobae	25	7	28.0
Lophuromys sp	14	1	7.1
Rattus rattus	19	3	15.7
Grammomys dolichurus	14	0	0
Praomys sp.	6	0	0
Mus (Leggada) minutoides	1	0	0
Petrodromus sp. (Elephant shrew)	1	1	100
Total	211	37	17.5

TABLE 2a

(2a) Species, numbers and infection rates of rodents and insectivores tested in districts with no records of plague outbreaks. Figures in brackets refer to percentage of animals positive for plague. In Masasi, sera were tested by ELISA technique for detection of antibodies, in other districts PHA tests were used.

Animal species tested	Numbers tested and % infection in each district							
	Masasi	Chunya	Igunga	Monduli	Muheza	Kilombero	Morogoro Rural	Total
Rattus rattus	7 (0)	105 (2.9)	22 (0)	5 (0)	14 (0)	15 (0)	133 (0)	301
Mastomys natalensis	56 (12.5)	379 (0.8)	105 (0)	104 (1.9)	35 (0)	109 (0)	262 (0)	1050
Arvicanthis nairobae	-	6 (0	-	4 (0)	-	-	-	10
Aethomys sp.	2 (0)	-	-	-	-	-	-	2
Tatera robusta	2 (0)	17 (0)	-	7 (0)	6 (0)	-	1 (0)	33
Saccostomus campestris	-	2 (0)	-	-	-	-	-	2
Crocidura hirta	-	-	5 (0)	-	3 (0)	1 (0)	35 (0)	44
Lemniscomys griselda	-	-	-	-	-	-	3 (0)	3
Mus sp.	-	-	-	-	-	-	100 (0)	100
Total	67 (10.4)	509 (1.2)	132 (0)	120 (1.7)	58 (0)	125 (0)	534 (0)	1545 (1.5)

TABLE 2b

(2b) : Observation of Y. *pestis* F1 by ELISA tests of rodent sera collected in Masasi district

Species	No. tested for F1	No. & % positive for F1
Mastomys natalensis	45	14 (55.5)
Rattus rattus	7	1 (14.3)
Tatera sp.	2	0 (0)
Aethomys sp.	2	0 (0)
Total	56	15 (26.8)

DISCUSSION AND CONCLUSIONS

The present observations broadly indicated that many species of rodents are suitable and serve as natural reservoirs of plague in Tanzania. All these species could play an important role in the epidemiology of the disease. These observations are consistent with those reported from Kenya (DAVIS et al., 1968). As the examined rodents were clinically healthy when live-trapped, these animals are at least partly refractory, and hence, potentially capable of maintaining the disease enzootically for long periods. GUGGISBERG (1966) and HUBBARD (1973) suggested that *M. natalensis* was the major reservoir of the disease in Kenya and Tanzania and concluded that it was responsible for maintaining and passing the infection to the house rat, *R. rattus*, and to humans. Our observations are consistent with these reports; however, our data also indicated that other field rodent species including *Arvicanthis nairobae, Lemniscomys striatus, Lophuromys spp., Pelomys fallax, Grammomys dolichurus*, *Otomys* spp. and *Rattus rattus* could play similar roles in disease maintenance. Current studies in the Lushoto plague focus suggest close interaction and exchange of flea ectoparasites between sylvatic and commensal rodents, thus facilitating

TABLE 3

Species and infection rates of small carnivores in Tanzania. Dog sera from Masasi District were tested by ELISA technique.

Districts	Animal species examined and % infected								
	Canis familiaris	*Felis catus*	*Genetta genetta*	*Civettictis civetta*	*Crocuta crocuta*	*Felis lybica*	*Otocyon megalotis*	*Ichneumia albicauda*	Total
Arumeru	35 (11.4)	-	-	-	-	-	-	2 (0)	37 (10.8)
Hai	39 (0)	-	2 (0)	-	-	2 (50)	-	-	43 (2.3)
Rombo	-	-	-	1 (0)	-	-	-	1 (0)	2 (0)
Mbulu	55 (3.6)	-	-	-	2 (0)	2 (0)	3 (0)	-	62 (3.2)
Singida	19 (0)	-	-	-	-	-	-	-	19 (0)
Lushoto	388 (5.7)	7 (0)	-	-	-	-	-	-	395 (5.6)
Chunya	129 (0)	-	-	-	-	-	-	-	129 (0)
Masasi	39 (43.6)	3 (33.3)	-	-	-	-	-	-	42 (42.9)
Total	704 (6.4)	10 (10)	2 (0)	1 (0)	2 (0)	4 (25)	3 (0)	3 (0)	729 (6.4)

transfer of the disease causative agents (MAKUNDI et al., 2003).

Our observations further indicated that only two of the seven plague-active foci districts (Lushoto and Mbulu) demonstrated detectable haemagglutination antibodies. This was probably attributable to the fact that specimen collection in these districts was carried out during or soon after outbreaks of the disease, whereas in other districts the study was done several years after the occurrence of the last reported outbreak. These observations could suggest that the use of rodents alone is not enough for the detection of plague endemicity over long periods of time. The presence of both IgG and IgM immunoglobulins among rodent populations in Lushoto district was an indication of past and current infections but does not suggest how long ago the animals carrying IgG were infected.

Furthermore, our data showed that areas where outbreaks of human plague have not occurred before can also harbour animal reservoirs of the disease. Surveys in Monduli, Chunya and Masasi districts were carried out at times of epidemics of rodent populations, and where some rodent species were found to have been exposed to infection with *Y. pestis*. Considering the limited home range of the infected rodents, it is expected that the animals contracted the disease locally, thus suggesting the existence of endemic foci in these areas despite the absence of reported human plague outbreaks.

The presence of specific anti-plague antibodies and/or antigens in small carnivores (domestic dogs, domestic and wild cats) in districts where plague has occurred before (Arumeru, Mbulu, Lushoto and Hai) and in a district (Masasi) where the disease has not occurred before, suggests involvement of these animals in the epidemiology of plague in Tanzania as observed elsewhere (POLAND & BARNES, 1979; TAYLOR et al., 1981; ANONYMOUS, 1984). These animals are known to be efficient carriers of the disease and not more than 2% are killed by the pathogen (KARIMI, 1974 – Pers. Comm.). Anti-plague antibodies are also known to persist in small carnivores for long periods and the animals can occasionally serve as sources of human infection (RUST et al., 1971; BARNES, 1990).

Despite the low sampling and infection rates of carnivores, other than dogs, in our survey, the data suggest that such animals should, be involved in epidemiological studies aimed at establishing endemic foci of plague. Epidemiological surveillance services for plague should be established and regularly carried out in many districts; especially those, which experience frequent outbreaks of rodent populations. Such services will facilitate updating the distribution of natural foci of the disease in the country, the forecasting of outbreaks and allowing the prompt application of appropriate preventive measures. This improved information network will also enable researchers, extension personnel, community members and other people handling rodents in various parts of the country, to take the necessary precautions.

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