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Brief Original Article

Rodents and other small mammal reservoirs in plague foci in northeastern Brazil

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Abstract

Introduction: Plague is an acute, infectious zoonotic disease, primarily of wild rodents and their fleas, that affects humans and other mammals. In Brazil, several plague foci are located in the northeast region. Plague surveillance based on monitoring of rodents was discontinued in 2007, and the current information on rodent populations is unsatisfactory. Our purpose was to update the information on rodents and other small mammals in plague foci in northeastern Brazil.

Methodology: Nine surveys in the historically most important northeastern plague areas were conducted in 2013-2015.

Results: In this study, 393 animals (13 rodent and four marsupial species) were entrapped. The plague bacterium *Yersinia pestis* was not detected in tissue sample cultures from the 225 animals that were analyzed. Eighty sera samples were analyzed for anti-F1 antibodies by hemagglutination (HA) and protein A ELISA tests, and all were negative, except for one marsupial, *Monodelphis domestica*, which was HA positive.

Conclusions: Qualitative and quantitative differences in the animal populations were observed in the areas surveyed, and the antibody positive marsupial indicated that plague continues to circulate in the wild.

Key words: Plague; *Yersinia pestis;* antibodies; rodents; marsupials.

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Introduction

Plague is an acute, infectious zoonotic disease, primarily of wild rodents and their fleas, that is caused by the bacterium *Yersinia pestis*, which can affect both humans and other mammals. More than 200 rodent species are hosts/reservoirs of the infection [1]. Plague has a global distribution, with natural foci in Africa, Asia, and North and South America. According to the World Health Organization, 1000-3000 cases of the disease occur in humans annually [2-3].

Brazil has several plague foci that are within various ecological complexes: Ibiapaba, Baturité, Araripe, Triunfo, Borborema, Diamantina, Planalto Oriental, and Espinhaço, which are in the states of Ceara (CE), Pernambuco (PE), Piauí (PI), Rio Grande do Norte (RN), Paraiba (PB), Alagoas (AL), Bahia (BA) and Minas Gerais (MG), respectively, constituting the so-called "Northeast Focus," and Serra dos Orgãos in the state of Rio de Janeiro (RJ) (Figure 1). These foci are dated to when plague was first introduced in 1899 by sea at the beginning of the third Pandemic [4].

For several decades, the methodology of the plague surveillance and control program in Brazil consisted of collecting rodents and fleas and testing for the plague bacillus [5-6] and serological surveys to detect anti-plague antibodies in sentinel animals (*e.g.,* rodents and domestic carnivores: dogs and cats) [7-11]. Based on an analysis of the results, the detection of antibodies was at least tenfold more sensitive among dogs than that in cats and rodents.

Domestic or wild carnivores (*e.g.,* dogs, cats, foxes, and skunks, among others) that prey on rodents can acquire *Y. pestis* infection from the bites of infected fleas and by eating infected prey; therefore, the sampling of a few carnivores is estimated as equivalent to sampling hundreds of rodents [12].

Therefore, plague surveillance in Brazil was restricted to serological surveillance of domestic dogs, and beginning in 2007, the monitoring of rodents and fleas was discontinued. Whereas studies on rodent populations in plague areas date back almost two decades, the current state of knowledge has faded over the years and further studies are required. Additionally, important changes have occurred in the focal areas, and the effects of climate change and anthropogenic changes in recent decades remain to be properly evaluated [13]. Furthermore, marsupials have not been fully investigated, with these small carnivores disregarded because they are not considered sufficiently abundant to play any role in plague epizootiology or epidemiology in Brazil [14].

The aim of this paper was to update the information on rodents and other small mammals from the plague areas in northeast Brazil to determine the potential plague hosts in the different foci and to learn whether changes have occurred in the composition of the rodent populations compared with previous collections.

Methodology

Fieldwork

Fieldwork was performed following standard guidelines [12,15]. Nine one-week expeditions were conducted during 2013-2015 in the historically more important plague areas in the states of PE, BA, and CE. Rodent live traps (Sherman and Tomahawk) were placed in corn, bean, cotton, cassava, and sugarcane fields and in grasslands and forests. Traps were set in late afternoon and recovered early in the morning on the following day. Trapped animals were handled under anesthesia for collection of ectoparasites, sexing, and identification to species or genus, followed by euthanasia, weighing and measuring. Samples of blood and tissues (spleen or liver) were collected for analyses; skin and carcasses were preserved for taxonomic studies and deposited in zoological collections. In this study, the updated nomenclature of rodents according to Bonvicino *et al* [16] was adopted.

Laboratory analyses of samples

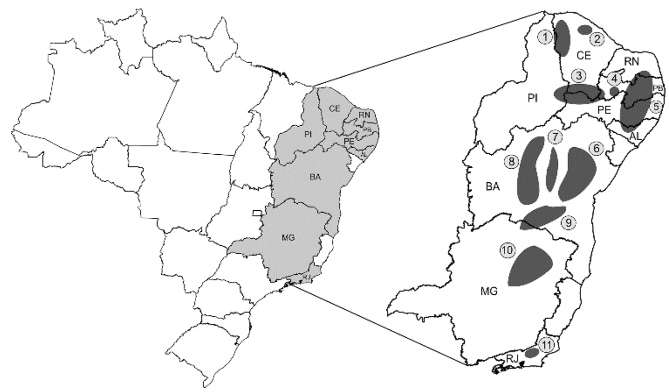
Serological analysis

A hemagglutination (HA) test [17] and a protein A (Protein A-Peroxidase *Staphylococcus aureus*/horseradish. Sigma-Aldrich, St. Louis, USA) ELISA [17,18] were used to detect *Y. pestis* anti-F1 antibodies in sera samples from selected animals.

Bacteriological analysis

Tissue samples (spleen or liver) were preserved on Cary-Blair shipment and transport medium (Zhejiang Gongdong Medical Technology CO. LTD, Taizho, China) and from selected animals, were ground and plated directly on brain-heart infusion agar (BHI) medium (HiMedia Laboratories Pvt Ltd 23, Vadhani Industrial Estate, LBS Marg, Ghatkopar West, Mumbai, India) for identification and isolation of *Y. pestis*. Plates were incubated at 28°C for 48-72 h and inspected daily for the growth of characteristic colonies, which were further tested for lysis by anti-plague bacteriophage [17].

Figure 1. Plague foci in Brazil and location of study areas: 1. Serra da Ibiapaba; 2. Serra de Baturité; 3. Chapada do Araripe; 4. Serra Triunfo; 5. Planalto da Borborema; 6. Planalto Oriental da Bahia; 7. Piemonte da Diamantina; 8. Chapada Diamantina; 9. Planalto da Conquista; 10. Serra do Espinhaço; 11. Serra dos Órgãos.



Results

A total of 393 animals were entrapped during the nine surveys, which included 13 species of rodents, *i.e., Necromys lasiurus (Zygodontomys lasiurus pixuna, Bolomys lasiurus), Calomys expulsus (Calomys callosus), Cerradomys langguthi (Oryzomys subflavus), Pseudoryzomys* spp.*, Euryoryzomys* spp.*, Oecomys* spp.*, Oligoryzomys stramineus (Oryzomys eliurus, Oligoryzomys eliurus), Holochilus sciureus, Oxymycterus* spp.*, Wiedomys pyrrhorhinos, Rattus rattus, Thrichomys laurentius (Cercomys cunicularis inermis, Thrichomys apereoides),* and *Galea spixii,* and four marsupials, *i.e., Monodelphis domestica, Gracilinanus* spp., *Didelphis albiventris,* and *Marmosa* spp. (Table 1).

All 80 sera samples were negative for anti-F1 antibodies by hemagglutination (HA) and protein A ELISA tests (Table 2), with the exception of the serum from one marsupial, *M. domestica*, which was HA positive, although with low titer (1/16).

The plague bacillus was not found in any culture of the 225 animal tissue samples analyzed (Table 2). All cultures were multi-contaminated.

Discussion

Although plague remains active in numerous natural foci occupying large areas of different countries, the disease is largely neglected. Furthermore, surveys of plague reservoirs are scarce. Although Brazil has investigated plague more than any other South American country [19], the most extensive and complete research was developed until the 1980s [13].

Table 1. Distribution of the trapped animals by geographical origin.

Animals		Geografical origin (State and focus)							Total	%
		CE = 143		PE = 60			BA = 189			
		Baturité	Ibiapaba	Borborema	Triunfo	Araripe	Piemonte	Diamantina		
Rodents	*Necromys lasiurus*	13	34	5	15	1	9	18	95	24.2
	Calomys expulsus	0	0	0	0	0	42	8	50	12.8
	Cerradomys langutthi	7	16	5	5	1	26	21	81	20.6
	Pseudoryzomys spp	0	0	0	0	0	0	1	1	0.3
	Euryoryzomys spp	0	9	0	0	0	0	0	9	2.3
	Oecomys spp	0	2	0	0	0	0	0	2	0.5
	Oligoryzomys stramieus	0	0	0	1	0	33	5	39	9.9
	Holochillus sciureus	0	3	0	1	0	0	0	4	1.0
	Oxymicterus spp	0	10	0	0	0	0	0	10	2.6
	Wiedomys pirhorrohinus	1	0	0	0	0	0	0	1	0.3
	Rattus rattus	5	2	0	0	1	1	13	22	5.6
	Thrichomys laurentius	0	7	0	3	14	0	6	30	7.7
	Galea spixii	0	0	0	0	6	0	0	6	1.5
	Without information	0	1	0	0	0	1	0	2	0.5
Marsupials	*Monodelphis domestica*	4	11	2	1	0	0	4	22	5.4
	Gracilinanus spp	0	15	0	0	0	0	0	15	3.8
	Didelphis albiventris	0	1	0	0	0	0	1	2	0.5
	Marmosa	0	2	0	0	0	0	0	2	0.5
	Total	**30**	**113**	**12**	**26**	**23**	**112**	**77**	**393**	**100**
	%	**7.6**	**28.8**	**3.0**	**6.6**	**5.9**	**28.5**	**19.6**	**100**	

Table 2. Bacteriological culturing and hemagglutination (HA) and protein A ELISA tests for analyzed animals.

Species	***Bacteriological analyses***					***Serological analyses***				
	Distribution by State			Total	%	Distribution by State			Total	%
	CE	PE	BA			CE	PE	BA		
Necromys lasiurus	27	06	09	42	19	0	0	6	6	7.5
Calomys expulsus	0	0	40	40	18	0	0	15	15	18.7
Cerradomys langguthi	21	05	26	52	23	0	0	25	25	31.3
Oligoryzomys stramieus	0	0	31	31	14	0	0	7	7	8.7
Holochilus sciureus	01	0	0	01	0.5	0	0	0	0	0
Oxymicterus spp	10	0	0	10	4	0	0	0	0	0
Rattus rattus	01	01	01	03	1	4	1	12	17	21.3
Thrichomys laurentius	0	09	0	09	4	0	0	0	0	0
Galea spixii	0	06	0	06	3	0	5	0	5	6.3
Monodelphis domestica	12	02	0	14	6	1	0	3*	4	5
Gracilinanus spp	15	0	0	15	7	0	0	0	0	0
Didelphis albiventris	01	0	0	01	0	0	0	0	0	0
Marmosa spp.	0	0	0	0	0	1	0	0	1	1.2
Without information	01	0	0	01	0.5	0	0	0	0	0
Total	**89**	**29**	**107**	**225**	**100**	**6**	**6**	**68**	**80**	**100**
%	**39.5**	**13.0**	**47.5**	**100**	**-**	**7.5**	**7.5**	**85.0**	**100**	**-**

*01 Positive HA – negative protein A ELISA tests.

This study demonstrated that the mammal species formerly involved in the epizootiological cycle of plague in Brazil remained in the historically most important plague areas. In these areas, thirteen rodent and four marsupial species were recorded, with most of these species previously reported in the plague areas of northeast Brazil [5, 9-10,14,20]. The taxonomy of small mammal species is under constant revision, and therefore, some rodents were assigned to other genera or species, and in the literature, some species are referred to with different names.

Based on bacteriological or serological testing, the following species of rodent are naturally infected: *R. rattus, Z. lasiurus pixuna (N. lasiurus), G. spixii, O. subflavus (C. langguthi), O. eliurus (O. stramineus), C. callosus (C. expulsus), Akodon arviculoides, C. cunicularius (T. apereoides)*, *Nectomys squamipes,* and *Oxymicterus quaestor*. The naturally infected marsupials are *M. domestica* and *D. albiventris* [5,8-10,21].

The role of most of these species in the epidemiology of plague remains unknown. Karimi *et al* [21] determined the susceptibility to *Y. pestis* of the most common species from the plague focus of Chapada do Araripe (PE). Moreover, these authors detected differences in the susceptibility among specimens of the identical species with different geographical origins [21].

The species *C. langguthi* was previously identified as *O. subflavus*. The genus *Oryzomys* includes numerous species, and the *subflavus* species is recognized as a complex group that contains many species and genera. *Cerradomys* was coined to accommodate the *O. subflavus* group [22].

Specimens of *Pseudoryzomys* spp., *Euryoryzomys* spp., and *Oecomys* spp. were formerly misidentified as *O. subflavus* during the trapping conducted by previous plague workers. During this study, these specimens were distinguished from *C. langguthi*. Therefore, among the specimens referred to as *O. subflavus* [5,10,14], some individuals might be from any one of three genera.

The roles of marsupials, wild carnivores, and other predators of rodents (i.e., *M. domestica, D. albiventris, Marmosa* spp., and *Gracilinanus* spp.) in plague epidemiology in the Brazilian foci are not known and are perhaps underestimated. However, natural plague infection was detected by isolation of *Y. pestis* from *M. domestica* and its flea parasites and serologically in *M. domestica* and *D. albiventris* [5, 8-9, 14]. Experimentally, antibodies persisted for at least 3 years in ferrets (black-footed ferrets, carnivores) after receiving vaccinations containing the *Y. pestis* F1 and V antigens [23]. The alleged scarcity of the marsupials could be due to the fact that the former trappings did not target their habitats [14]. In this study, trapping in the forests provided more specimens, including the only HA positive animal.

Although immune enzymatic assays are more sensitive than HA analysis, all of the sera samples analyzed by the Protein A ELISA were negative. Protein A has universal affinity for immunoglobulins (IgG) from almost all species of domestic and wild mammals and for IgA and IgM in some species [18]. Thus, the protein A conjugate can detect infection early, when infection would not be detectable by methods based on the use of anti-IgG conjugates. In the present study, all bacteriological analyses were negative, with all cultures multi-contaminated, which might have masked the growth of *Y. pestis*.

Necromys lasiurus, incriminated for plague amplification in nature [24], was more abundant in Serra da Ibiapaba and *C. expulsus, O. stramineus,* and *C. langguthi* were more abundant in Piemonte da Diamantina (BA). In addition to differences in susceptibility among specimens of the identical species but from different geographic origins, this difference in the composition of the rodent communities raises questions about the role of the identical species at different sites. To improve the understanding of the role of different rodent and other small mammal species in the epidemiology of plague in Brazilian foci areas, further studies are required.

Plague has declined in Brazil since the 1990s. However, plague is a cyclical disease in time and space; it can remain dormant for decades and then reappear suddenly, and the factors involved in the emergence or re-emergence are not fully understood [25]. Therefore, at any moment, unknown environmental, social, or other changes may trigger reemergence of plague in the Brazilian foci.

Conclusions

The results of the sampling conducted during this work revealed qualitative and quantitative differences in the rodent populations from the six areas that were studied, with higher abundance in Serra da Ibiapaba (CE) and Piemonte da Diamantina (BA); thus, these areas are more vulnerable to rodent-transmitted diseases. The antibody positive marsupial indicated that plague continues to circulate in the wild.

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