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DIEGO LEANDRO REIS DA SILVA FERNANDES

PESTE: DINÂMICA DA DISTRIBUIÇÃO E CIRCULAÇÃO DA INFECÇÃO NO ESTADO DE PERNAMBUCO, BRASIL

Recife
2022

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de doutor em Ciências Biológicas.

Área de concentração: Biotecnologia

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Aprovada em: 25/02/2022

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Agora vai e foi...

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RESUMO

A peste é uma zoonose de roedores silvestres, transmitida por pulgas, que pode infectar outros mamíferos, inclusive humanos. Pela gravidade da doença e sua rápida evolução a peste pode gerar emergências de saúde pública de interesse internacional. Os avanços tecnológicos e científicos ainda não permitem eliminar a infecção dos focos naturais que persistem em numerosos países alternando períodos de atividade e quiescência. No estado de Pernambuco, a zoonose introduzida em 1902, apresenta-se silente desde a década de 1980, mas a qualquer momento, encontrando condições favoráveis, pode voltar a se manifestar. Compreender a dinâmica da infecção para construção dos cenários futuros de risco para sua transmissão e determinar os períodos e locais que exijam maior atenção dos órgãos de saúde pública afim de direcionar as atividades de vigilância nessas áreas. Foram construídos bancos de dados contendo informações sobre os casos humanos, reservatórios, vetores e dados ambientais utilizando dados primários e secundários no período de 1902 a 2005 disponíveis no Serviço de Referência Nacional de Peste da FIOCRUZ PE que foram analisados utilizando ferramentas de geoprocessamento. As análises aplicadas aos casos humanos usando o município de Exu, localizado no foco da Chapada do Araripe (Pernambuco), como área de estudo de caso no período de 1945-1976 permitiu evidenciar a transição da infecção da área urbana para a silvestre e o ressurgimento dos casos após um período de quiescência, independente da reintrodução a partir de outros focos ativos. Os resultados das análises sobre a ocorrência e distribuição geográfica de roedores hospedeiros e de pulgas vetores nas áreas de peste brasileiras revelaram diferenças na composição das faunas rodentia e sifonapteriana no foco da Serra dos Órgãos (RJ) região sudeste, e no nordeste do Brasil que são atribuídas aos diferentes biomas nas duas regiões. Importante flutuação nas populações de roedores foi observada após um período epidêmico no município de Exu, com redução da fauna silvestre representada principalmente por *Necromys lasiurus* e aumento da espécie comensal *Rattus rattus*. Os resultados confirmam a espécie *N. lasiurus* como responsável por epidemias de peste na área focal de transmissão no nordeste do Brasil, uma vez que a redução nas populações desta espécie ao longo do tempo coincide com a quiescência da doença. Além disso, o aumento nas populações de *R. rattus* diretamente relacionado ao processo de urbanização de pequenas localidades rurais não produziu epidemias de peste como se poderia esperar, especialmente considerando sua proximidade com os humanos favorecendo a propagação da peste e outras doenças transmitidas por esses roedores. A análise contribuiu para confirmar o papel das espécies silvestres como hospedeiros amplificadores da peste e dos ratos comensais (*R. rattus*) como possíveis hospedeiros preservadores no período quiescente naquela área de transmissão da infecção. A ocorrência de reservatórios naturais e vetores competentes evidencia o potencial de manutenção e transmissão da bactéria e, consequentemente, a necessidade de manutenção e aprimoramento do programa de vigilância da peste a fim de evitar o acometimento futuro das populações humanas.

Palavras-chave: Peste; Roedores; Pulgas; Epidemiologia; Análise espacial.

ABSTRACT

Plague is a flea transmitted zoonosis of wild rodents, which can attain other mammals, including humans. Due to the severity of the disease and its rapid evolution, the plague can generate public health emergencies of international interest. Technological and scientific advances still do not allow to eliminate the infection from natural foci that persist in many countries alternating periods of activity and quiescence. In the state of Pernambuco, the zoonosis introduced in 1902 has been silent since the 1980s, but at any time, when conditions are favorable, it can reappear. To understand the dynamics of the infection to build future risk scenarios for its transmission and determine the periods and places requiring higher attention from public health agencies in order to direct surveillance activities in these areas. Databases were built containing information on human cases, reservoirs, vectors and environmental data using primary and secondary data available at the National Plague Reference Service FIOCRUZ PE that were analyzed using geoprocessing tools. The analysis of the human cases using the municipality of Exu, located in the Chapada do Araripe (Pernambuco) focus, as a case study area in the period 1945-1976, allowed to highlight the transition of the infection from the urban to the rural area and the resurgence of cases after a period of quiescence, regardless reintroduction from other active focus . The results of the analyzes on the occurrence and geographic distribution of rodent hosts and flea vectors in Brazilian plague areas revealed differences in the composition of the rodent and Siphonaptera faunas from the Serra dos Órgãos (RJ) southeastern region, and from northeastern Brazil foci, which are attributed to the different biomes in the two regions. An important fluctuation in rodent populations was observed after an epidemic period in the municipality of Exu, with a reduction of the wild fauna represented mainly by *Necromys lasiurus* and an increasing of the commensal species *Rattus rattus* populations. The results confirm the species *N. lasiurus* as responsible for plague epidemics in the focal transmission area in northeastern Brazil, since the reduction of their populations over time concurs with the quiescence of the disease. Furthermore, the increasing of the *R. rattus* populations directly related to the urbanization process of small rural locations did not produce plague epidemics as might be expected, especially considering its proximity to humans favoring the spread of plague and other rat transmitted diseases. Our analysis contributed to confirm the role of wild species as amplifying hosts of plague and of the commensal rats (*R. rattus*) as possible preserving hosts in the quiescent period in that infection transmission area. The occurrence of natural reservoirs and competent vectors evidences the potential for maintenance and transmission of the bacterium and, consequently, the need to maintain and improve the plague surveillance program in order to avoid the future involvement of human populations.

Keywords: Plague; Rodents; Fleas; Epidemiology; Spatial analysis

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SIGLAS

BHC	Beta-hexaclorocicloexano
CDC	Centers for Disease Control and Prevention
DDT	Dicloro-difenil-tricloetano
DNC	Doença de Notificação Compulsória
DNS	Departamento Nacional de Saúde
EUA	Estados Unidos da América
HA	Hemaglutinação
LACEN	Laboratório Estadual de Saúde Pública
LNC	Lista de Notificação Compulsória
MS	Ministério da Saúde
OMS	Organização Mundial de Saúde
PCP	Programa de Controle em Peste
RDC	República Democrática do Congo
SINAN	Sistema de Informação de Agravos de Notificação
SISPESTE	Sistema de Informação Nacional de Peste
SRP	Serviço de Referência Nacional em Peste
SVS	Secretaria de Vigilância em Saúde

SUMÁRIO

1 INTRODUÇÃO

A peste, causada pela bactéria *Yersinia pestis,* é uma doença negligenciada, reemergente, de risco ocupacional, e uma grande desconhecida pela sociedade e a quase totalidade dos profissionais de saúde e governos (OLIVEIRA et al., 2011; TAVARES et al., 2019). Entretanto, pode determinar significativos impactos nos sistemas de saúde, pondo em risco a estabilidade social, e, por sua rápida disseminação e transcendência, como nos casos de pneumonia pestosa, pode gerar emergências de saúde pública de interesse internacional, o que exige notificação à Organização Mundial de Saúde (OMS), de acordo com o Regulamento Sanitário Internacional vigente (STENSETH et al., 2008; WORLD HEALTH ORGANIZATION, 2008).

A peste persiste em focos naturais em todos os continentes (exceto a Antártica) à custa de complexo entrelaçamento de fatores bióticos, envolvendo as populações de reservatórios e vetores e a capacidade de persistência da *Y. pestis* na natureza e fatores abióticos, tais como os geológicos, destacando-se os orográficos, e os paisagísticos. Os períodos de silêncio, caracterizados por ausência ou ocorrência de raros casos humanos, podem gerar a falsa impressão da sua eliminação ou erradicação, mas ela pode reaparecer após décadas de um aparente controle (DUPLANTIER et al., 2005; GAGE e KOSOY, 2004; JONES et al., 2019).

Os avanços tecnológicos e os conhecimentos científicos acumulados, *per se*, não foram suficientes para erradicação da peste que continua afligindo países desenvolvidos como os Estados Unidos da América (EUA) e a Federação Russa (JONES et al., 2019). Assim, atualmente, se deve considerar o potencial de disseminação internacional pelo intercâmbio determinado pela globalização da economia e dos mercados, exigindo que todos os países, mesmo os não endêmicos, avaliem sistematicamente a possível emergência de novos focos naturais e o aumento do risco de ocorrência de peste urbana (STENSETH et al., 2008).

Anualmente centenas de notificações de casos humanos são reportados à OMS em países da África, Ásia Central, sudeste da Europa e nas Américas. Esses dados são considerados uma subestimativa da real situação, em decorrência de uma vigilância inadequada e da subnotificação pelo temor do impacto que a peste teria sobre as economias locais e nacionais (STENSETH et al., 2008; WORLD HEALTH ORGANIZATION, 2019). No Brasil existem duas áreas focais independentes: o chamado “foco do Nordeste”, com áreas dispersas pelos estados do Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Bahia, Piauí e norte de Minas Gerais e o “foco da Serra dos Órgãos”, nos limites dos municípios de Nova Friburgo, Sumidouro e Teresópolis, no Estado do Rio de Janeiro. Estes focos são remanescentes da introdução da infecção em 1899 por via marítima através do porto de Santos em São Paulo (GILES, PETERSON e ALMEIDA, 2011; TAVARES et al., 2012). No foco do Nordeste, as áreas mais ativas eram as do Ceará, Pernambuco e Bahia, seguidas das de Alagoas e Paraíba. A partir da década de 1980 todos os focos tenderam à quiescência (ALMEIDA et al., 2020; SAAVEDRA, 2011; SOUZA et al., 2017; TAVARES et al., 2012; 2019).

Muito embora o controle da peste tenha sido desencadeado desde sua entrada no Brasil só a partir de 1930 os dados passaram a ser regularmente documentados e arquivados estabelecendo um amplo acervo (TAVARES et al., 2012). O estudo retrospectivo desses registros pode ajudar a identificar as áreas de risco de exposição humana e, consequentemente, direcionar as atividades de vigilância nessas áreas considerando a possibilidade de reemergência desse agravo no futuro. Nossas análises realizadas utilizando ferramentas de geoprocessamento aplicadas aos dados sobre a infecção nas populações humanas e nos hospedeiros/reservatórios contribuiram para melhor compreensão da dinâmica da zoonose na área estudada.

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2 OBJETIVOS

2.1 GERAL

Definir a dinâmica da infecção pestosa no estado de Pernambuco e caracterizar as áreas de risco para sua transmissão a fim de determinar os períodos e locais que exijam maior atenção dos órgãos de saúde pública para direcionar as atividades de vigilância nessas áreas.

2.2 ESPECÍFICOS

- Analisar a ocorrência espacial e distribuição de casos de peste humana no período de 1902 a 1976 no estado de Pernambuco e utilizando o município de Exu como área de estudo de caso;
- Descrever as características bióticas sobre as populações de roedores/hospedeiros e pulgas/vetores nos focos de peste do Brasil no período de 1952 a 2019;
- Determinar a ocorrência e a distribuição geográfica dos roedores que participam do ciclo da peste no município de Exu no período de 1966 a 2005;

3 REFERENCIAL TEÓRICO

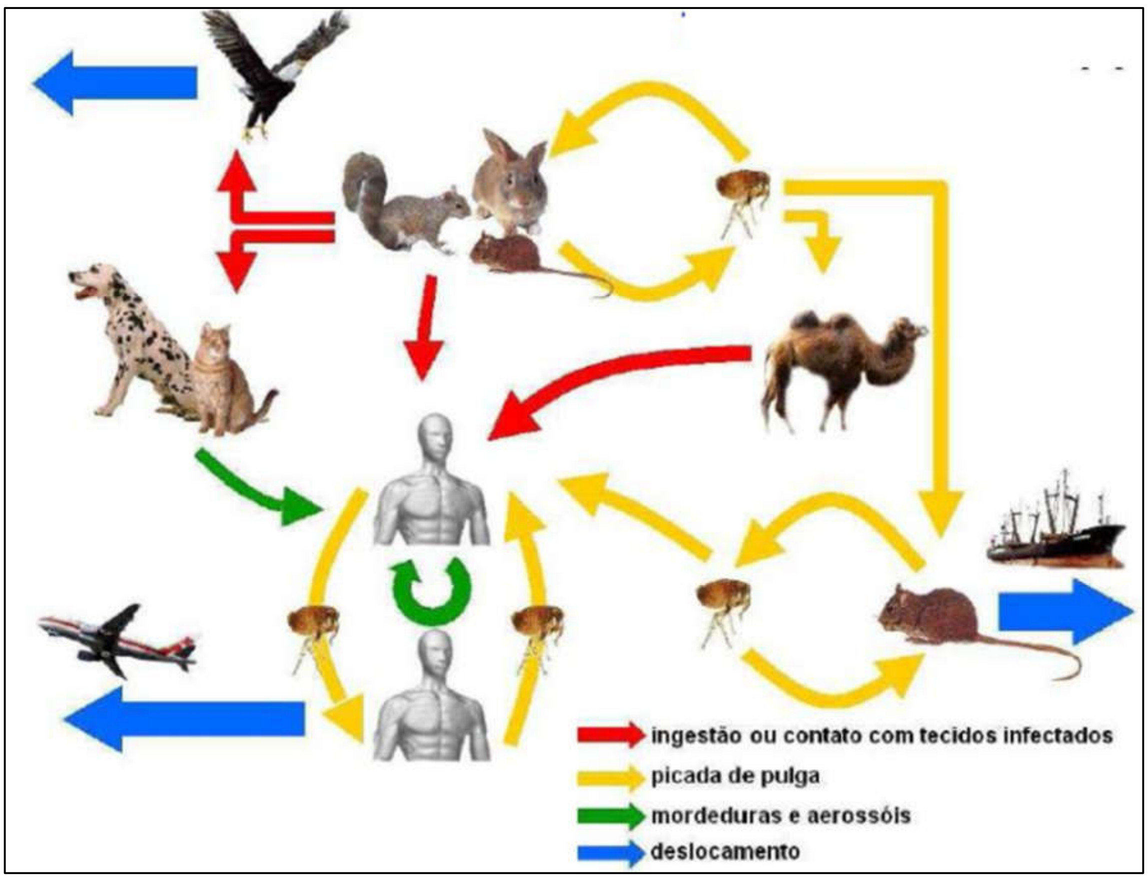
3.1 A PESTE NO MUNDO, BRASIL E PERNAMBUCO

O termo Peste popularmente sugere praga, desgraça, calamidade, maldade e todo um cortejo de perversidades, já o termo Bubônica sugere algo desagradável, doloroso e ambos são muito utilizados como xingamento ou palavrão. Estas são as imagens da doença, a Peste, que aflige a humanidade desde sempre, exercendo infinita influência no imaginário popular (BRAMANTI et al., 2016; TAVARES et al., 2019).

A bactéria causadora da peste, a *Yersinia pestis*, surgiu através de uma diferenciação da *Y. pseudotuberculosis* provavelmente há cerca de 1.500-20.000 anos, originada no Planalto Central da Ásia de onde se expandiu (ACHTMAN et al., 1999; DEMEURE et al., 2019; MORELLI et al., 2010; SPYROU et al., 2018). Os primeiros registros da ocorrência da peste constam no 1º Livro de Samuel, em 1320 a.C e na era Cristã três grandes pandemias estão bem documentadas (RAOULT et al., 2013): a primeira pandemia que ocorreu no século VI ao VIII, denominada “Peste de Justiniano”, que contribuiu para a queda do império romano devido ao alto grau de mortalidade; a segunda que ficou conhecida como “Peste Negra” caracterizada pela forma pneumônica, a mais grave da doença, que se estendeu por toda Europa e norte da África e a terceira pandemia também chamada “Pandemia Contemporânea”, partiu de Yunnan em 1894 na China que vinha registrando surtos desde 1772, atingindo em seguida Hong Kong e se espalhou para diversos países e continentes ao redor do mundo e alcançou as Américas (POLLITZER, 1954). Durante a terceira pandemia em 1894, em Hong Kong, Alexander Yersin identificou pela primeira vez o bacilo da peste, o qual possui atualmente a denominação de *Yersinia pestis*, em sua homenagem. Em 1896, Yersin desenvolveu o primeiro antissoro contra a bactéria, utilizando-o para o tratamento da doença e em 1898 Paul-Louis Simond descobriu o papel da pulga na transmissão da doença (BUTLER, 2014; POLLITZER, 1954).

A peste nas duas primeiras pandemias se espalhou lentamente entre os países já que as rotas terrestres eram o principal meio de transporte da época (MORELLI et al., 2010). Com o desenvolvimento da navegação a vapor a velocidade de disseminação aumentou consideravelmente aliado as condições precárias de higiene das embarcações infestadas de ratos e pulgas que encontravam excelente meio de subsistência entre as cargas dos navios e a infecção pode facilmente ser transportada por longas distâncias gerando a terceira pandemia. Com as medidas de antiratização das embarcações (ratproofing) e a forte atuação da vigilância sanitária dos portos mundiais essa rota de propagação da peste foi controlada (LINK, 1951). Contudo com a facilidade de deslocamento dos atuais meios de transporte e o mundo globalizado que dispõem de aviões que em menos de 24h é possível se estar em qualquer continente do planeta, a peste pode encontrar nesses meios uma maneira de se propagar, naturalmente através da contaminação em suas áreas de focos e criminalmente por ações de bioterrorismo que atualmente se tornou uma preocupação mundial (Figura 1) (STENSETH et al., 2008).

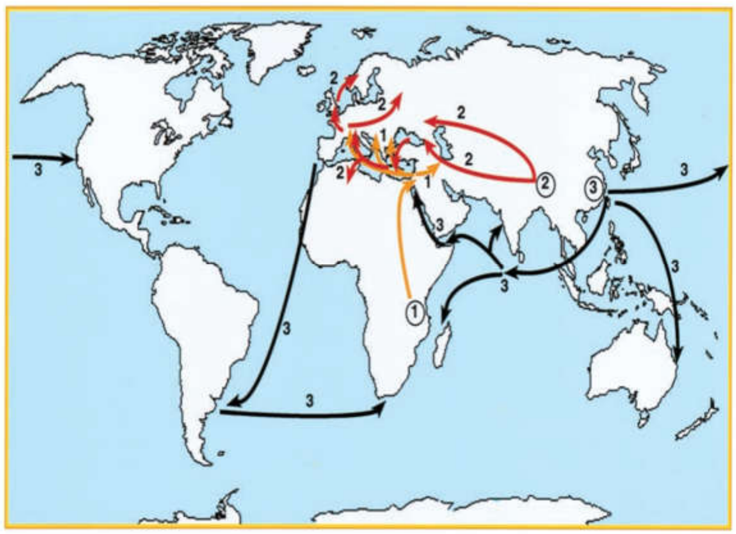
Figura 1 – Transmissão e disseminação da peste



Fonte: modificado de STENSETH et al. (2008).

Estudos combinando dados históricos, arqueológicos e genômicos têm contribuído para melhor conhecer a dinâmica da peste, da *Y. pestis* e das pandemias globalmente ao longo dos séculos (Figura 2) (ACHTMAN et al., 1999; DEMEURE et al., 2019; MORELLI et al., 2010; SPYROU et al., 2018).

Figura 2 – Rotas da peste nas três pandemias



Inferred route	Historical association	
East China–Kurdistan	Silk Road (200 BC–1400)	1*
China–Africa	Zheng He expeditions (1409–1433)	2*
Within China	Evolution of 1.ORI in Yunnan	
China–Calcutta/Hawaii–United States	Plague ships (1895 and 1899)	3*
Hong Kong–Vietnam	China to Vietnam (1906)	
China–Bombay	Plague ship from Hong Kong (1896)	
Morocco–Senegal	Plague outbreaks (1909 and 1914)	
India–Europe–South America	Indian rice ship from Rotterdam to Argentina/ Uruguay (1899)	
China–Vietnam–Burma	Burma to China (1905)	
India–Suez/Madagascar–Turkey	Plague ships from India	

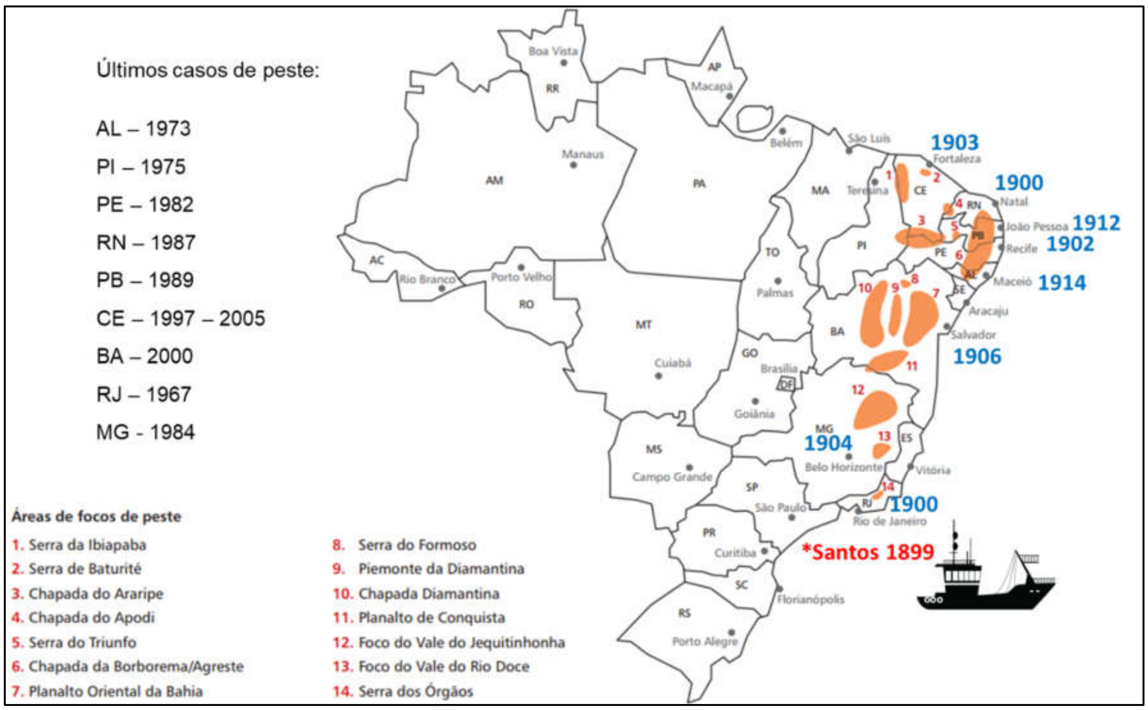
*Origem e dispersão da peste respectivamente na primeira (1), segunda (2) e terceira (3) pandemias. Diferentes linhages genéticas da *Y. pestis* foram responsáveis pelas diversas pandemias.

Fonte: adaptado de ACHTMAN et al. (1999), MORELLI et al. (2010).

A peste chegou ao Brasil no ano de 1899 pelo porto de Santos – São Paulo, se disseminou inicialmente nas cidades litorâneas dando início a Fase Portuária da doença, atingindo os portos do sul ao norte do país, até 1912: Rio de Janeiro e Ceará em 1900; Rio Grande do Sul e Pernambuco em 1902; Maranhão e Pará em 1903; Bahia em 1904, Espírito Santo, Paraná e Sergipe em 1906 e Paraíba em 1912 (POLLITZER e MEYER, 1965).

A peste foi imediatamente combatida nos portos do Brasil pelos Estados envolvidos e em seguida, a partir de 1935, pelo Departamento Nacional de Saúde (DNS). Entretanto a infecção seguiu seu curso natural e a partir de 1907 através das vias terrestres (rodovias e ferrovias) foi atingindo cidades e municípios interioranos, caracterizando assim a Fase Urbana. Com os avanços da saúde pública e o controle sanitário viabilizados pelo governo federal, a infecção foi eliminada das áreas portuárias e urbanas, o que não impediu que a partir de 1930 alcançasse fazendas e sítios infectando os roedores autóctones, dando início a chamada Fase Rural ou Silvestre e assumindo então a condição de enzootia e estabelecendo focos naturais (Figura 3) que permanecem até o momento atual nos estados de Pernambuco, Paraíba, Piauí, Ceará, Rio Grande do Norte, Alagoas, Bahia e norte de Minas Gerais os quais constituem o “foco do Nordeste”, e também na Serra dos Órgãos (Rio de Janeiro) (POLLITZER, 1954; POLLITZER e MEYER, 1965; BALTAZARD, 1968).

Figura 3 – Origem, disseminação e focalização da peste no Brasil



Fonte: acervo do Serviço Nacional de Referêcia em Peste (2022)

3.2 EPIDEMIOLOGIA DA PESTE

A peste embora seja frequentemente considerada uma doença do passado continua produzindo surtos e epidemias em diversos países na África, Ásia e Américas. Os períodos de silêncio, caracterizados por ausência ou ocorrência de raros casos humanos, podem gerar a falsa impressão da sua eliminação ou erradicação, mas ela pode reaparecer após décadas de um aparente controle (BERTHERAT, 2019; DUPLANTIER et al., 2005; GAGE e KOSOY, 2005; JONES et al., 2019).

Quadro 1 – Reemergência da peste no mundo

REEMERGÊNCIA DA PESTE APÓS PERÍODOS DE QUIESCÊNCIA		
UGANDA	1982	22 anos
AFRICA DO SUL	1982	10 anos
BOTSWANA	1989	Mais de 45 anos
QUÊNIA	1990	10 anos
ÍNDIA	1994	27 anos
MALAWI	1994	31 anos
INDONÉSIA	1997	27 anos
ZÂMBIA	1997	Mais de 33 anos
ARGÊLIA	2003	Mais de 50 anos
YOLONG (Yunnan)	2005	Mais de 100 anos
LÍBIA	2009	Mais de 20 anos
PERU	2010	13 anos

Fonte: BERTHERAT (2010)

Apesar de circular predominantemente entre os roedores silvestres em zonas rurais e afastadas de centros urbanos há registro de casos ou surtos nas populações urbanas como a epidemia de peste pneumônica em Madagascar em 2017 (RANDREMANANA et al., 2019; STENSETH et al., 2008; WORLD HEALTH ORGANIZATION, 2019).

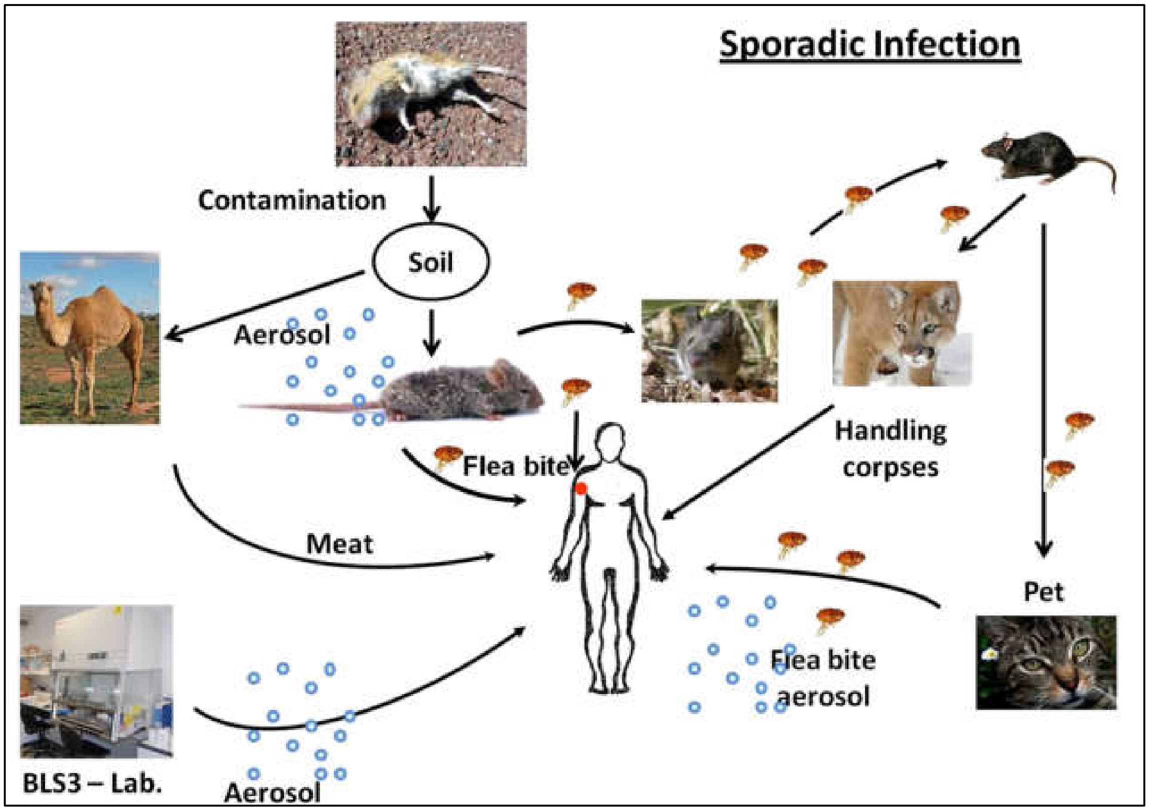
Nos últimos anos (2013 a 2018) os casos de peste no mundo vêm diminuindo, exceto em Madagascar e na República Democrática do Congo (RDC) que mantiveram taxas elevadas e foram responsáveis por mais de 94% dos casos de peste que ocorreram no mundo nesse período. Além desses, o Peru, Estados Unidos da América (EUA), China e Mongólia têm notificado casos continuamente (BERTHERAT, 2019).

No Brasil a peste só começou a ser notificada e registrada regularmente a partir de 1935 com a criação do Departamento Nacional de Saúde (DNS). Antes desse período os dados são incompletos e esparsos porque o controle da doença estava a cargo de cada Estado atingido. Até a década de 1970 as ocorrências variaram de 20 a 100 casos anuais, eventualmente ocorrendo surtos ou epidemias como as dos estados da Bahia em 1974-1975, em 1975 na Chapada do Araripe (Ceará - Pernambuco) e a última na Paraíba (1986-1987) no Planalto da Borborema. A partir de 1990 os casos foram rareando até 2005 quando ocorreu o último caso no Ceará (ALMEIDA et al., 1989; ARAGÃO et al., 2007; 2009; TAVARES et al., 2012). Eventualmente ainda ocorrem notificações de casos suspeitos, mas sem confirmação laboratorial. Entretanto, inquéritos sorológicos das atividades de vigilância, ainda vêm detectando anticorpos antipestosos em animais sentinelas (cães domésticos), confirmando que apesar da regressão da doença entre as populações humanas a bactéria ainda circula nas áreas focais (COSTA et al 2017; SOUZA et al 2017; TAVARES et al., 2012).

A cadeia epidemiológica da peste é complexa envolvendo os reservatórios/hospedeiros (roedores sinantrópicos comensais e silvestres), outros mamíferos (carnívoros domésticos: cães e gatos e silvestres: pequenos marsupiais), vetores (pulgas) e o homem além de fatores abióticos nas áreas de foco e nichos específicos (ZEPPELINI, ALMEIDA e CORDEIRO-ESTRELA, 2016).

O complexo ciclo epidemiológico da peste pode ser descrito em quatro ciclos bioecológicos: (1) o ciclo enzoótico, que ocorre entre os hospedeiros naturais, como entre algumas espécies de roedores moderadamente resistentes; (2) o ciclo epizoótico ou silvestre, que implica a transmissão entre os roedores mais sensíveis à infecção, que ao morrerem deixam livres suas pulgas infectadas e que na busca por alimento infectam outros hospedeiros (3) o ciclo domiciliar, resultante do intercâmbio entre animais domésticos e humanos com roedores silvestres infectados e/ou suas pulgas; (4) o ciclo pneumônico da doença, que ocorre por transmissão direta pessoa a pessoa, podendo ocasionar grandes epidemias, denominado peste dêmica (Figura 4) (BRASIL, 2008; RAOULT et al. 2013).

Figura 4 – Vias de transmissão da peste



Fonte: RAOULT et al. (2013)

Embora os roedores sejam os principais hospedeiros/reservatórios diversos outros mamíferos são susceptíveis à infecção pela *Y. pestis* e podem servir de fonte para a transmissão ao homem. Os próprios *pets* convencionais (cães e gatos), mas também insetívoros (porcos-espinhos), pequenos marsupiais (timbu e gambá), lagomorfos (coelhos e lebres), além de ovinos, camelos e macacos (OLIVEIRA et al., 2011; PERRY e FETHERSTON, 1997). Eventualmente ocorrem surtos de peste gástrica pelo consumo da carne crua ou mal-cozida de camelos e marmotas (DAI et al., 2018; RAOUL et al 2013; BIN-SAEED, AL-HAMDAN e FONTAINE, 2005; KEHRMANN et al., 2020).

As aves são refratárias à infecção pela *Y. pestis* mas podem ser responsáveis pelo transporte de pulgas, principalmente as aves de rapina atuando como um vetor mecânico da peste levando carcaças de animais mortos ou pulgas infectadas de um local a outro até por grandes distâncias (MAHMOUDI et al, 2021; STENSETH et al., 2008).

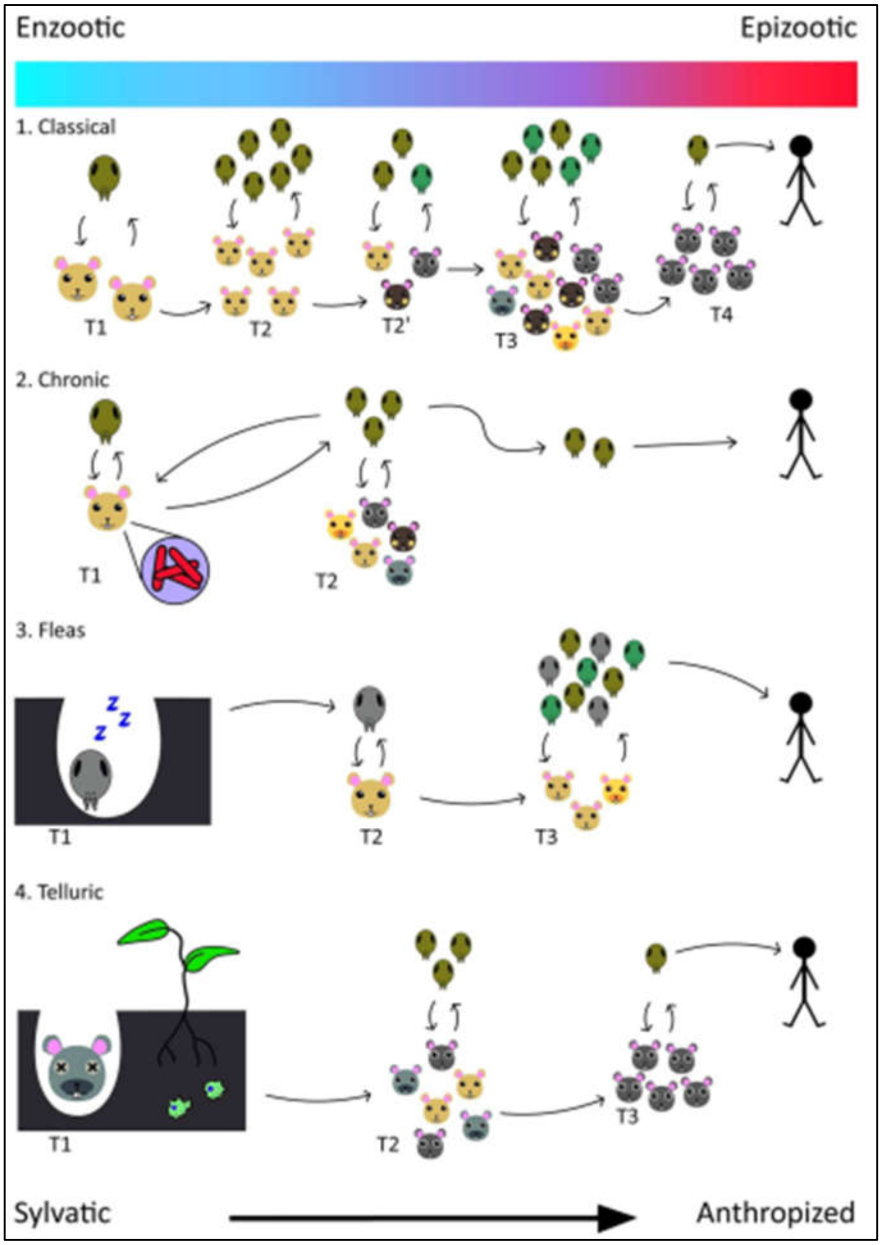
Diversos fatores contribuem para as epizootias entre os roedores e consequente extravasamento (*spill over*) da doença para humanos, como a influência de alguns eventos climáticos. Existe uma correlação positiva entre a ocorrência de epizootias e a cascata trófica, decorrente de maior precipitação em uma determinada área e com isso um maior crescimento de vegetais que servirão de aporte nutricional para o aumento da população de roedores hospedeiros e consequentemente uma maior probabilidade de epizootias e casos humanos (Figura 5) (DUBYANSKY, DUBYANSKA e BOGATYREV, 1992; ENSCORE et al., 2002; PARMENTER et al., 1999).

Diante da complexidade do ciclo epidemiológico da peste e da imensa disponibilidade de hospedeiros e vetores, apesar de todos os conhecimentos científicos e tecnologias atualmente disponíveis, a peste tem apresentado grande resiliência à erradicação (JONES et al., 2019; ZEPPELINI, ALMEIDA e CORDEIRO-ESTRELA, 2016).

3.3 A DOENÇA

A peste é uma infecção bacteriana causada pela *Y. pestis*, um cocobacilo, gram-negativo da Família Enterobacteriaceae. A *Y. pestis* é classificada pelo Centers for Disease Control and Prevention (CDC) no Grupo A de agentes biológicos de bioterrorismo e na classe 3 de risco biológico pela gravidade da doença que causa e o potencial de risco para a segurança das nações, por se tratar de um agente biológico com aplicações de bioterrorismo (INGLESBY et al., 2000; PERRY e FETHERSTON, 1997). O acesso da *Y. pestis* ao organismo humano pode ocorrer através da pele pela picada de pulgas infectadas ou por abrasões da pele pelo contato direto com materiais infectados; através da conjuntiva ocular; mucosa do aparelho respiratório por aspiração de partículas expelidas por pacientes de peste pneumônica ou animais (*pets*, principalmente gatos); ou por deglutição através da mucosa do aparelho digestivo pelo consumo de carnes cruas de animais infectados (BIN-SAEED, AL-HAMDAN e FONTAINE, 2005; EIDSON et al., 1988; GAGE, et al., 2000; KEHRMANN et al., 2020).

Figura 5 – Ciclos enzoóticos e epizoóticos da peste



De cima para baixo: 1. modelo clássico, 2. modelo de infecção crônica, 3. pulgas como reservatório, 4. Peste telúrica. O eixo horizontal informa a ocorrência espacial dos fenômenos, partindo de uma paisagem silvestre para uma situação urbana / periurbana, indicando risco à saúde pública. A barra de gradiente indica a possibilidade de transição de um ciclo enzoótico a um epizoótico. Espécies de roedores diferenciadas por cor e padrão, espécies de pulgas apenas por cor. As setas indicam a interação entre os componentes do ciclo em determinado tempo T (setas duplas), ou a progressão de cada etapa do modelo. Setas apontando para humanos indicam infecção humana em epizootias.

Fonte: ZEPPELINI, ALMEIDA E CORDEIRO-ESTRELA (2016)

As contaminações podem ocorrer nas situações mais diversas tais como atividades em laboratórios, assistência à saúde, na agricultura, lazer (turismo ecológico, caça, pesca) ou por ações de bioterrorismo (ALMEIDA, LEAL E TAVARES, 2019).

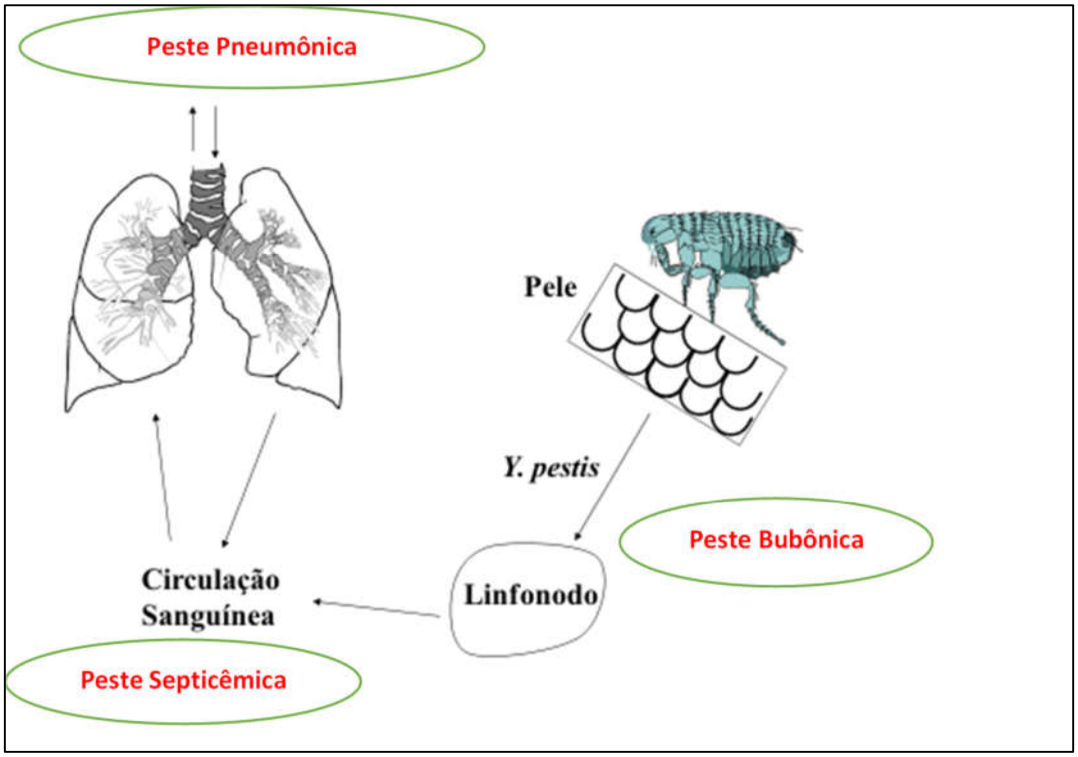
O espectro da infecção varia dependendo do organismo do paciente, virulência e carga do inóculo, desde formas clínicas de altíssima gravidade a aquelas assintomáticas e oligossintomáticas, somente detectáveis em inquéritos sorológicos (ALMEIDA, LEAL E TAVARES, 2019). As principais formas clínicas da doença são: Peste bubônica; Peste pneumônica e Peste septicêmica. Outras formas mais raras podem ocorrer tais como a faríngea, gástrica, meníngea, cutânea primária e endoftálmica (BRASIL, 2008; BUTLER, 2014; PERRY e FETHERSTON, 1997).

A forma predominante da doença é a bubônica (80 a 95% dos casos) com mortalidade de 10 - 20%, transmitida pelas picadas de pulgas infectadas. Os bacilos introduzidos através da pele são levados pela corrente sanguínea/linfática até o linfonodo mais próximo ao local da picada, onde se multiplicam e ocorre a liberação de fatores de inflamação responsáveis pela dor, rubor e edema no local. Esse processo inflamatório do linfonodo é denominado de bubão que caracteriza a forma clínica da peste bubônica. Além do bubão o paciente apresenta calafrios, cefaleia, febre alta, mialgias, anorexia, náuseas, vômitos e dores generalizadas (Figura 6). O período de incubação da peste bubônica varia de dois a seis dias. O bubão é extremamente doloroso, e sua localização varia de acordo com a porta de entrada dos bacilos, sendo localizados principalmente na região ínguino-crural (70%) e menos frequente nas regiões axilar (20%) e cervical (10%) (BRASIL, 2008).

A forma septicêmica primária ocorre pela penetração da bactéria diretamente na corrente sanguínea através abrasões da pele ou conjuntiva ocular e tem como sintomas: início súbito, febre elevada, hipotensão arterial, dispneia, hemorragia cutânea as vezes serosas e mucosas atingindo os órgãos internos, fácies de estupor e grande prostração. Se não tratada pode levar ao coma e ao óbito em dois a três dias. A forma septicêmica também ocorre de modo secundário, na fase terminal de outras formas não tratadas da doença (BRASIL, 2008).

A forma pneumônica pode ser primária ou secundária à outras formas da doença por disseminação hematogênica dos bacilos. É uma forma grave da doença e altamente perigosa pela alta taxa de contágio, podendo provocar rapidamente epidemias. O quadro clínico inicia com uma infecção grave, evoluindo rapidamente com febre muito alta, calafrios, arritmia, náuseas, vômitos, hipotensão, obnubilação e astenia intensas dores no tórax, dificuldade de respiração (dispneia) e taquipneia, cianose, expectoração sanguinolenta, fluida e com bactérias presentes, levando a morte caso não haja intervenção médica com tratamento precoce (BRASIL, 2008).

Figura 6 – Fisiopatologia da peste



Fonte: arquivo SRP

3.4 PULGAS VETORES DE PESTE

As pulgas são artrópodes pertencentes à Classe *Insecta*, Ordem Siphonaptera caracterizada pelo aparelho bucal especializado do tipo picador/sugador sendo ambos os sexos, machos e fêmeas, hematófagos; são ectoparasitas principalmente de mamíferos (94%) entre os quais os roedores são os mais afetados (74%) e em menor proporção (6%) também parasitam as aves. As pulgas são transmissoras da peste e outros agravos como a tularemia e o tifo murino.

Além de vetores da *Y. pestis* as pulgas também podem atuar como reservatórios dessa bactéria desempenhando um papel importante no ciclo da zoonose. Pulgas infectadas ou não podem sobreviver durante vários meses no interior das tocas dos roedores onde o microclima favorece a sobrevivência dos bacilos no próprio organismo dos insetos ou no solo contaminado pelas fezes infectadas eliminadas pelas pulgas. Diversos fatores influenciam a capacidade vetora e a transmissão pelas pulgas: tempo de digestão e excreção do sangue absorvido, quantidade de bactérias ingeridas, capacidade de bloqueio parcial ou total do proventrículo, tempo de repasto sanguíneo além de suas predileções por hospedeiros específicos garantindo a manutenção da enzootia assim como o desencadeamento de epizootias (EISEN e GAGE, 2009; HINNEBUSH, JARRETT e BLAND, 2017).

De cerca de 3 mil espécies de pulgas já identificadas mundialmente pelo menos 280 espécies e subespécies de 62 gêneros se infectam pela *Y. pestis* e pelo menos 60 espécies ocorrem no Brasil (LINARDI e GUIMARÃES, 2000).

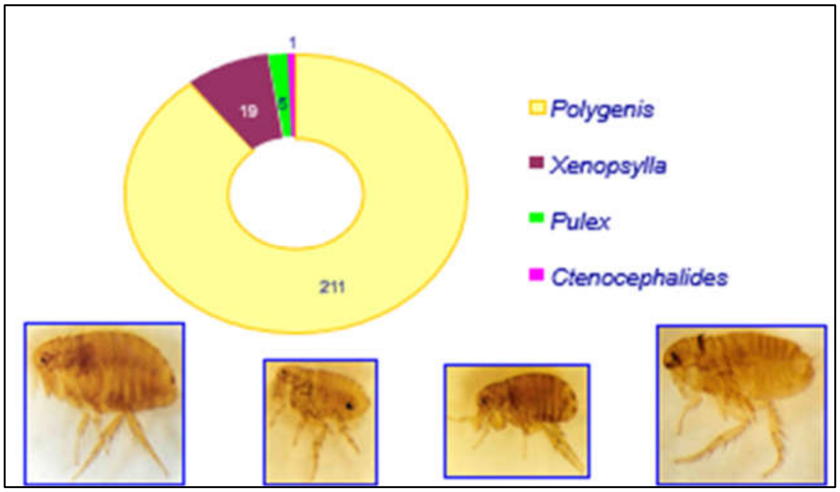
Nas áreas focais de peste do Nordeste do Brasil foi registrada a ocorrência de pulgas ectoparasitas do gênero *Polygenis* (*Polygenis bohlsi jordani, P. tripus, P. bohlsi bohlsi e P. roberti roberti)* predominantes nos roedores silvestres; *Xenopsylla cheopis* (pulga do rato), *Pulex irritans* (conhecida como pulga do homem, ubiquitária e também parasita animais domésticos, *Ctenocephalides felis (*ectoparasita de cães e gatos) e *Adoratopsylla antiquorum (*parasita de pequenos marsupiais, carnívoros, predadores de roedores) (BRASIL et al., 1989; OLIVEIRA et al., 2009; KARIMI, EFTEKHARI e ALMEIDA, 1974b).

Polygenis spp e as espécies *X. cheopis*, *P. irritans e C. felis* foram encontradas naturalmente infectadas pela *Y. pestis nas* áreas focais de peste do Nordeste (BRASIL et al., 1989; KARIMI, EFTEKHARI e ALMEIDA, 1974b), Figura 7.

A capacidade vetorial de *P. bohlsi jordani e P. tripus* foi comprovada por estudos experimentais em laboratório e foi considerado que essas espécies desempenham um papel importante na propagação da peste entre os roedores durante as epizootias nos focos do Nordeste responsáveis pelos casos humanos (KARIMI et al., 1974b; BALTAZARD, 2004). A capacidade vetorial de *P. bohlsi bohlsi e P. roberti roberti* ainda não foi estudada e permanece desconhecida.

No foco da Serra dos Órgãos, as espécies *P. rimatus e P. pradoi* são predominantes nos roedores silvestres (CARVALHO et al., 2001; GUIMARÃES, 1972). A habilidade vetorial dessas espécies permanece desconhecida, bem como o papel, se houver, na epidemiologia da peste neste foco.

Figura 7 – Frequência por espécie das pulgas infectadas pela *Y. pestis* nos focos do nordeste do Brasil (1966-1986)



Fonte: acervo do SRP

Apesar das controvérsias (BLANC, 1956; HINNEBUSH, JARRETT e BLAND, 2017) sobre os mecanismos de transmissão e o papel vetor de algumas espécies de pulgas, tem sido evidenciado que as espécies *P. irritans e C. felis* estão envolvidas na transmissão da peste em focos da Tanzânia, Uganda e Madagascar (LAUDISOIT et al., 2007; EISEN et al., 2008; RATOVONJATO et al., 2014). Considerando a proximidade entre os *pets* e os seres humanos a possibilidade de transmissão da peste por estas espécies nos focos brasileiros não deve ser desconsiderada (OLIVEIRA et al., 2009; 2011).

Uma análise genética (VNTR - múltiplos locos do número variável de repetições em tandem) em quatro pares de cepas de *Y. pestis* originadas de *P. b. jordani* (pulgas/vetores) e seus respectivos hospedeiros/roedores (*Necromys lasiurus)* revelou estreita relação genética entre elas comprovando a relação epidemiológica desses isolados (OLIVEIRA et al., 2012).

3.5 ROEDORES E A PESTE

A ordem *Rodentia* é a mais numerosa entre a classe dos *Mammalia* que possui cerca de 40 famílias, 389 gêneros e 2 mil espécies, correspondendo a cerca de 40% das espécies de mamíferos existentes na Terra. Os roedores são extremamente eficientes na adaptação a qualquer ambiente terrestre que lhe forneça condições de sobrevivência, suportando diferentes climas, temperaturas e altitudes, podendo mostrar uma imensa gama de adaptações fisiológicas. Os roedores podem abrigar vários agentes biológicos que podem causar agravos de alta letalidade, como ricketsioses, hantaviroses e a peste (BRASIL, 2002; MAHMOUDI et al., 2021).

Das duas mil espécies de roedores existentes no mundo cerca de 230 espécies já foram encontradas naturalmente infectadas pela *Y. pestis* (BONVICINO et al., 2015). Em determinadas situações os roedores silvestres podem desencadear uma epizootia de peste podendo infectar outros mamíferos, inclusive o homem (PERRY e FETHERSTON, 1997).

A susceptibilidade dos roedores à infecção pela *Y. pestis* varia entre as espécies e consequentemente o papel de cada espécie no ciclo epidemiológico da infecção. Nos focos de peste do nordeste do Brasil ocorrem diversas espécies com diferentes níveis de susceptibilidade (Quadro 2). Algumas espécies como o *Necromys lasiurus*, são mais sensíveis, sofrendo grande mortandade nas epizootias, servindo de amplificador e difusor da infecção. Outras espécies como o *Galea spixii e o Rattus rattus* são relativamente resistentes e podem desempenhar um papel na manutençãoda infecção nas áreas focais (BALTAZARD, 2004).

Quadro 2 – Principais roedores dos focos de peste do nordeste do Brasil

FAMÍLIA	**SUBFAMÍLIA**	**GÊNEROS, ESPÉCIES**
Muridae	*Murinae* (sinantrópicos comensais)	*Rattus rattus,*
	Sigmodontinae (sinantrópicos silvestres)	*Akodon cursor, Necromys lasiurus, Calomys expulsus, Cerradomys langguthi, Holochilus sciureus, Nectomys squamipes, Oligoryzomys stramineus, Oxymycterus dasytrichus, Rhipidomys sp, Wiedomys pyrrhorinos*

Caviidae		*Galea spixii, Kerodon rupestris*
Echimydae		*Thrichomys laurentius, Proechimys sp.*

Fonte: adaptado de BRASIL (2008).

3.6 VIGILÂNCIA EM SAÚDE: PREVENÇÃO E CONTROLE, TRATAMENTO E DIAGNÓSTICO

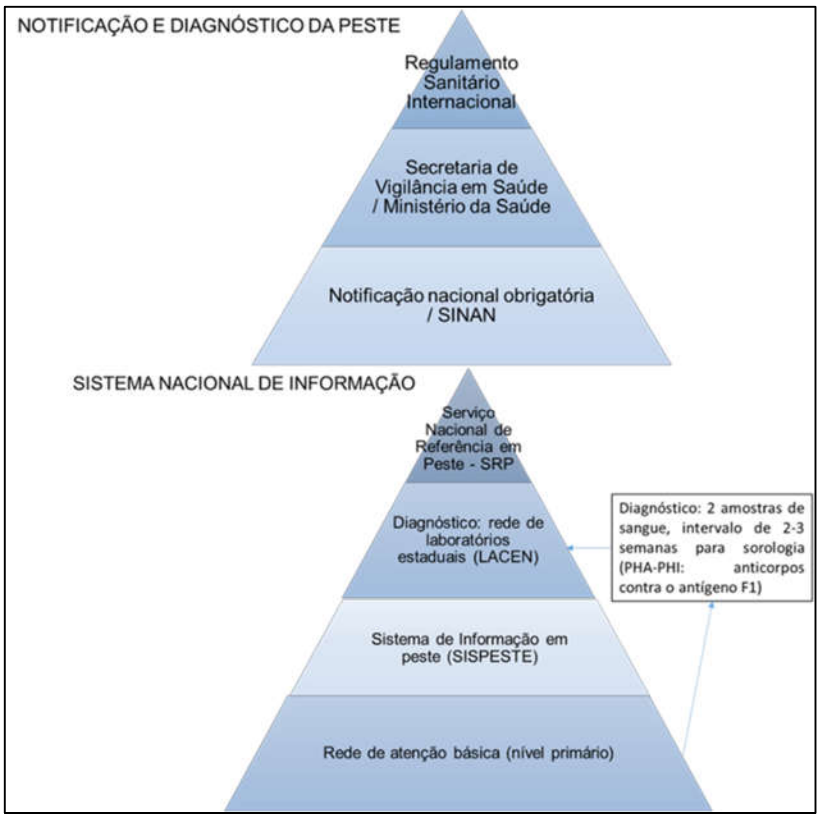
3.6.1 Prevenção e controle

A peste consta na Lista de Notificação Compulsória (LNC) do Brasil, referente às doenças, agravos e eventos de importância para a saúde pública de abrangência nacional em toda a rede de saúde, pública e privada. Sendo doença de notificação compulsória (DNC) é obrigação dos serviços de saúde a comunicação imediata de casos suspeitos. Todo caso de peste deve ser investigado em até 48 horas após a notificação, avaliando a necessidade de adoção de medidas de controle pertinentes (Figura 8) (BRASIL, 2011).

O procedimento padronizado pela Secretaria de Vigilância em Saúde (SVS) do Ministério da Saúde (MS) é o seguinte: a) todos os casos notificados devem ser investigados e os dados registrados no Sistema de Informação de Agravos de Notificação (SINAN); b) a Assistência Básica responde pelo atendimento dos pacientes; c) confirmada a suspeita, coletam-se duas amostras de sangue para exames sorológicos (a primeira na fase aguda e a segunda após duas a três semanas); d) as amostras devem obrigatoriamente ser acompanhadas pela Ficha de Investigação de Caso Humano de Peste do Sistema de Informação Nacional de Peste (SISPESTE) (Figura 8).

O SINAN foi criado para operacionalizar os processos de notificação compulsória, pelos profissionais de saúde e responsáveis pelos estabelecimentos de saúde, sejam eles públicos ou privados nas ocorrências ou suspeitas de doenças, agravos ou eventos de saúde pública relacionados em portaria (BRASIL, 2016) e atualizado pela portaria Nº 264, de 17 de fevereiro de 2020, a qual inclui a Peste como doença de notificação imediata ao Ministério da Saúde (até 24h). A criação do SINAN de maneira prática permitiu que a gestão operacionalizasse os processos de notificação aproximando-a das unidades notificadoras (BRASIL, 2007). O fluxo de notificação da atenção básica para peste inclui desde a identificação do paciente, atendimento e coleta de amostras para exames laboratoriais ao preenchimento da Ficha de Investigação de Caso Humano de Peste do SISPESTE.

Figura 8 – Esquema hierárquico de notificação dos casos de peste



Fonte: AUTOR (2020)

Desde a entrada da peste no Brasil foram desencadeadas medidas profiláticas nas áreas focais envolvendo profilaxia ofensiva (despulização e desratização) e profilaxia defensiva (antiratização - tornando o ambiente impróprio para os roedores). O trabalho de profilaxia era executado casa-por-casa, dentro de uma área de 6 km de raio, tendo como centro o local do caso humano. A desratização era realizada por meio de limpeza do terreno em torno das casas e eliminação dos ratos empregando desde a captura por ratoeiras ao lança chamas e iscas raticidas (FREITAS, 1957;

POLLITZER e MEYER, 1965). A despulização era realizada por pulverizações de Dicloro-Difenil-Tricloroetano (DDT) (15% - 75%) ou Beta-hexaclorocicloexano (BHC) (30 %) em suspensão aquosa, continuamente até três vezes por ano por muitos anos, o que levou à seleção de resistência, por pressão contínua, nas pulgas das casas *X. cheopis* e *P. irritans* e provavelmente determinou o recrudescimento da peste no Brasil na década de 1960 (KARIMI, EFTEKHARI e ALMEIDA, 1974b; BALTAZARD, 2004).

A vigilância da peste durante décadas consistiu na pesquisa da *Y. pestis* nos roedores e pulgas e detecção de anticorpos específicos antipestosos (anti-F1) por hemaglutinação (HA) entre animais sentinelas nas áreas focais estabelecendo um amplo acervo de dados sobre as populações dos hospedeiros e vetores. Uma análise dos resultados revelou maior sensibilidade dos testes sorológicos entre os cães domésticos. Por conseguinte, o monitoramento de roedores e pulgas foi descontinuado e a vigilância foi restrita à sorologia de cães a partir de 2007 (BRASIL, 2008; COSTA et al., 2017; TAVARES et al., 2012).

O conhecimento dos focos é essencial para a metodologia das atividades de vigilância. O monitoramento das áreas focais deve ser continuo para prevenir as contaminações das populações humanas que de alguma maneira, seja em atividades de trabalho ou de lazer, penetrem no ecossistema da infecção. As comunidades das áreas de risco devem ser informadas sobre os sinais de epizootia que atingem primeiro os roedores e precedem os casos humanos, e orientadas a evitar contato com os animais silvestres ou carcaças de animais mortos (BRASIL, 2008).

3.6.2 Tratamento

O tratamento para peste deve ser imediato devido à gravidade e rapidez da evolução da doença, a fim de deter uma bacteremia e evitar uma toxemia, sendo indicado, diante de uma suspeita, o início do tratamento antes mesmo da confirmação dos exames (BUTLER, 2014).

Historicamente foi utilizada a soroterapia para o tratamento da peste no Brasil graças aos trabalhos desenvolvidos por Oswaldo Cruz (CRUZ, 1906). A mortalidade pela peste decresceu partir de 1943 com o emprego da sulfa-antibioterapia: com o advento das Sulfanamidas (1937-1938) e sua utilização em larga escala após 1940-1941 e da estreptomicina (1944) com utilização em larga escala após 1948 os óbitos declinaram sensivelmente (FREITAS, 1957).

Os quimioterápicos atualmente recomendados são os aminoglicosídeos (gentamicina), tetraciclinas (doxiciclina), cloranfenicol, sulfonamidas (sulfadiazina) e fluoroquinolonas (ciprofloxacina). Não devem ser utilizados os betalactâmicos, macrolídeos (eritromicina) e azalídeos (azitromicina) no tratamento por serem ineficazes contra a *Y. pestis* (BRASIL, 2008).

A *Y. pestis* tem se comportado uniformemente suscetível aos antimicrobianos, entretanto em 1995 foram isoladas de pacientes em Madagascar duas cepas multirresistentes: uma resistente a todos os antimicrobianos recomendados para o tratamento e profilaxia da peste e a outra resistente a menor variedade de produtos. Foi demonstrado que a resistência pode ocorrer pela transferência horizontal de genes no intestino das pulgas. A possibilidade de que outras cepas resistentes podem emergir por esse mesmo mecanismo é preocupante do ponto de vista clínico ou pela possibilidade do emprego de tais cepas em ações de bioterrorismo. Considerando a possibilidade do uso de cepas natural ou artificialmente resistentes, a vigilância da resistência aos antibióticos em *Y. pestis* deve se tornar sistemática em todo o mundo (GALIMAND, CARNIEL e COURVALIN, 2006).

Apesar dos esforços ainda não foi desenvolvida uma vacina eficaz para as diferentes formas de peste (bubônica e pneumônica) que confira imunidade duradoura e sem efeitos adversos (BUTLER, 2014; SUN e SINGH, 2019).

3.6.3 Diagnóstico

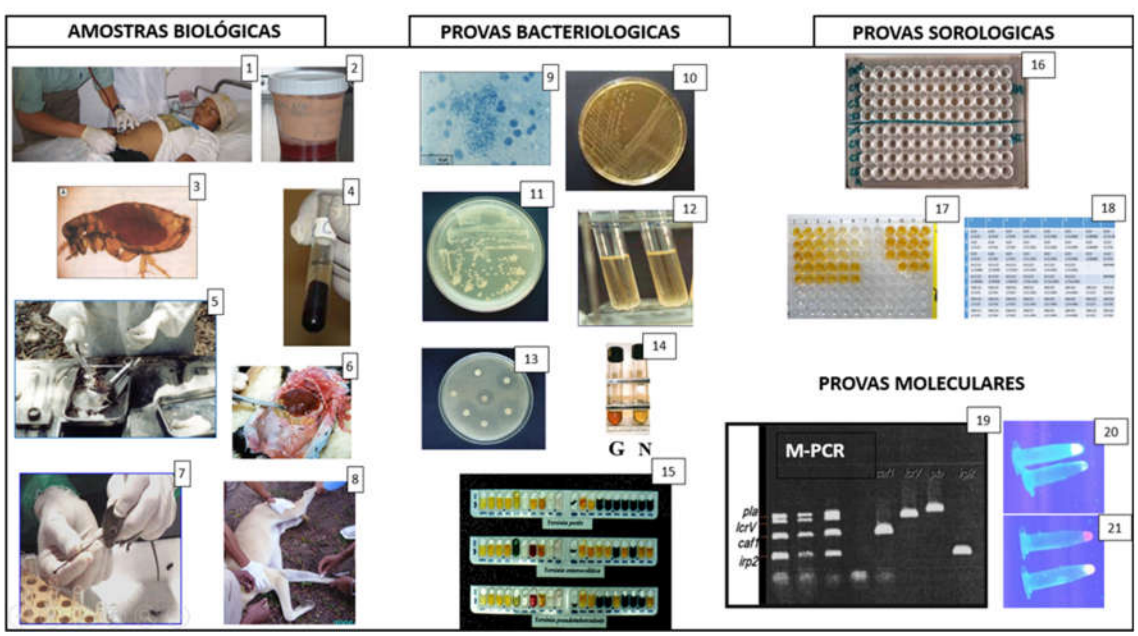
São considerados casos suspeitos os pacientes com quadro clínico de início súbito, febre alta, com ou sem linfadenite regional dolorosa e manifestações gerais graves com ou sem sintomas respiratórios. Os casos com quadro clínico de peste e confirmação laboratorial são classificados como Positivo Classe I (critério clínico-laboratorial) e os casos com quadro clínico sugestivo em área com ocorrência confirmada laboratorialmente de peste humana ou animal são classificados Positivo Classe II (critério clínico-epidemiológico). Os casos suspeitos com diagnóstico laboratorial negativo e sem história epidemiológica compatível são descartados como negativos (ARAGÃO et al., 2007).

O diagnóstico laboratorial de peste no Brasil foi inicialmente exclusivamente bacteriológico. A partir de 1966 com a implantação em Exu (PE) de um laboratório para estudos sobre a peste todas as amostras coletadas dos pacientes foram sistematicamente analisadas. A metodologia do diagnóstico foi aperfeiçoada pela introdução de novas técnicas e os resultados agilizados (BALTAZARD, 2004). As amostras obtidas dos pacientes eram analisadas por cultura, sensibilidade ao fago antipestoso e/ou inoculação subcutânea de animais de laboratório (camundongos albinos Swiss Webster) (KARIMI, ALMEIDA e ALMEIDA, 1974a).

O diagnóstico sorológico foi implantado a partir da década de 1980 (ALMEIDA et al., 2020; TAVARES et al., 2012) e acompanhando o desenvolvimento tecnológico no final da década de 1990 foram introduzidas técnicas imunoenzimáticas (ALMEIDA e FERREIRA, 1992) e de Biologia Molecular para diagnóstico da peste em amostras clínicas, nos hospedeiros vertebrados e pulgas vetores (LEAL e ALMEIDA 1999; LIRA et al., 2014; SILVA et al., 2012; TAVARES et al., 2020).

Atualmente são empregadas técnicas bacteriológicas, sorológicas e moleculares no diagnóstico da peste no Brasil (Figura 9). A automação não é recomendada devido a possibilidade de resultados falso-positivos (ALMEIDA et al., 2020; TAVARES et al., 2019).

Figura 9 – Metodologia do diagnóstico da peste no SRP



Amostras biológicas. 1: Punção de linfonodo; 2: escarro sanguinolento; 3: pulga; 4: sangue/soro; 5-6: necropsia de roedor; 7-8: coleta de sangue. Provas bacteriológicas. 9: esfregaço de baço de roedor corado Azul de Loeffler; 10: cultura em gelose peptonada; 11: bacteriófago positivo; 12: cultura em caldo peptonado; 13: antibiograma; 14: provas bioquímicas; 15: galeria 20 E. Provas sorológicas. 16: Hemaglutinação; 17-18: ELISA. Provas moleculares. 19: Multiplex-PCR; 20-21: LAMP.

Fonte: AUTOR/Acervo do SRP (2020)

3.7 ANÁLISE ESPACIAL EM SAÚDE E A PESTE

O geoprocessamento pode ser definido como um conjunto de ferramentas adequadas para manipular informações espacialmente referidas, e quando aplicado a questões de saúde pública permite o mapeamento de doenças e a avaliação de riscos epidemiológicos de interesse para a saúde. Existem diversas maneiras de se conceituar, identificar e quantificar riscos ambientais e de vulnerabilidade social (ELLIOTT et al., 2009). Dentro do universo do geoprocessamento está a análise espacial, uma ferramenta que auxilia de maneira visual e prática a tomada de decisões por parte das equipes de saúde. O uso das análises espaciais na vigilância em saúde tem se tornado de suma importância para a localização e o monitoramento de áreas de risco de agravos de importância epidemiológica para a saúde. Sua contribuição no campo da saúde sistematiza as medidas de controle de maneira prática quando se trata de grandes áreas territoriais, alocando recursos cada vez mais escassos nos ambientes e espaços críticos. Essa inovação na vigilância epidemiológica traz benefícios na redução e focalização de coletas amostrais de campo, diminuindo dessa maneira os custos de vigilância e o impacto ambiental na fauna, além de possibilitar uma resposta mais rápida das autoridades competentes no caso de um alerta de possível epizootia em andamento.

O geoprocessamento se apresenta como um moderno instrumento de divulgação de resultados das investigações epidemiológicas, permitindo que epidemiologistas entendam a dinâmica das doenças e suas variações no espaço e no tempo. Além disso, as informações geradas podem ser facilmente compreendidas e interpretadas pelos profissionais e usuários dos serviços de saúde. Já se utilizava de rotina o espaço gráfico na saúde com o uso de croquis de localidades pela vigilância e serviço de controle de doenças que eram produzidos e utilizados pela extinta Superintendência de Campanhas de Saúde Pública (SUCAM) (ROJAS, BARCELLOS e PEITER, 1999). Atualmente o uso do geoprocessamento facilita a compreensão de informações originadas de investigações epidemiológicas gerando dados que podem ser compreendidos e interpretados pelos profissionais e usuários dos serviços de saúde de maneira visual e interativa a exemplo de como uma determinada doença se comporta no espaço, no tempo e quais variáveis interferem nessa relação. O que torna atrativo o uso dessa ferramenta para o estudo da peste é a diversidade de análises e perspectivas inéditas aplicadas a cada foco em atividade ou não, englobando em diferentes camadas informações sobre espécies de hospedeiros mamíferos, pulgas vetores, clima, relevo e cobertura vegetal elucidando dessa maneira diferentes vieses bióticos e abióticos que apenas a análise estatística não alberga. Pode ser aplicada para comparar diferentes focos de peste esclarecendo por exemplo como diferentes tipos de vegetação, altitude e fauna podem influir na atividade de uma área ou outra de peste em determinado tempo (LIU et al., 2018; GILES, PETERSON e ALMEIDA, 2011).

Atualmente alguns grupos de pesquisa já utilizam dessas ferramentas afim de prever situações de risco para o surgimento da peste através de modelos preditivos, inferindo o risco de uma área sem informação prévia de dados ecológicos a partir de uma área de foco de peste conhecida. Para isto aplica-se a terceira lei da geografia segundo a qual quanto mais semelhante o ambiente geográfico, mais semelhante é o processo geográfico e os atributos associados (ZHU et al., 2018). No entanto é necessário que se tenha conhecimento de diferentes conjuntos de variáveis e covariáveis ambientais ao longo do tempo para garantir a significância do método. Sendo as principais influenciadoras das populações de roedores e pulgas: temperatura média, precipitação e umidade relativa anuais, além das covariáveis como quantidade de roedores e pulgas (DU, ZHU e WANG, 2020). Outra variável ambiental de importância é a cobertura vegetal que provem alimento, moradia, condições de sobrevivência e reprodução dos roedores, consequentemente favorecendo o crescimento da população de pulgas ectoparasitas (XU et al., 2011; GAGE et al., 2008; STENSETH et al., 2008).

O uso dessa ferramenta de análise espacial já está em prática no combate e prevenção da peste, sendo usada para inferir o índice de pulgas de uma área não conhecida (DU, ZHU e WANG, 2020). Uma das contribuições dessas formas de utilização é o fortalecimento do conhecimento histórico de como a peste se comportou nas distintas pandemias que assolaram a humanidade. O estudo realizado por Yue et al., (2017) através mapeamento das rotas de comércio e os casos de peste nas cidades circunvizinhas revelou que durante a pandemia de peste que assolou a Europa Medieval existiu uma forte relação negativa entre a proximidade dos surtos de peste e a proximidade das principais rotas de comercio entre as cidades, ou seja, quanto mais próxima (menor distância) a cidade das rotas de comércio maiores eram as reincidências de surtos de peste (maior o número de surtos de peste). Essa conclusão só foi possível graças a forte documentação e registro dos casos e localidades dos eventos somado ao uso do geoprocessamento, ferramentas de análise espaciais e epidemiologia descritiva dos dados históricos.

Diversos trabalhos produzidos utilizando essas ferramentas no estudo da peste trouxeram novos conhecimentos e fortaleceram consensos apenas pressupostos por levantamentos pontuais, como por exemplo a introdução e dispersão da peste nos Estados Unidos com estimativas da velocidade de disseminação da peste na direção oeste-leste do País (ADJEMIAN et al., 2007).

Enfim, o uso dessa ferramenta de análise espacial já está em prática e vem sendo usada por diversos pesquisadores para elucidação de aspectos ainda ignorados na epidemiologia da peste e poderá contribuir com novos subsidios para aplicação nas atividades da metodologia de prevenção e controle da peste.

4 CONSIDERAÇÕES FINAIS

No nosso estudo o emprego de ferramentas de análise espacial e geoestatística aplicado aos casos humanos de peste no estado de Pernambuco contribuiu para o entendimento da dinâmica da zoonose: sua expansão geográfica desde sua chegada pelo porto do Recife em 1902 até sua focalização na área rural. Do litoral, a infecção se espalhou por via terrestre afetando inicialmente algumas cidades do interior do estado e atingiu as mesorregiões do Agreste e o Sertão e encontrando condições favoráveis entre a fauna rodentia autóctone estabeleceu focos naturais permanentes na Chapada da Borborema, Maciço de Triunfo e Chapada do Araripe.

Usando o municipio de Exu no foco da Chapada do Araripe como estudo de caso foi possível observar no período de 1945 a 1976 a trajetória da infecção humana da área urbana para a área rural no município, e a reemergência dos casos após um período de quiescência sem reintrodução de outros focos. Em 1961, após seis anos de quiescência, a peste reapareceu na zona rural, em um sítio distante 16 km da cidade de Exu. Este caso serviria de alerta e as epidemias a partir de 1964 poderiam ter sido evitadas ou contidas. No entanto, não havia um sistema de vigilância ou um modelo preditivo na época. Portanto, o caso não foi devidamente considerado e, consequentemente, nos 15 anos seguintes até 1975, a peste se espalhou por todo o território do município e atingiu locais que não haviam sido acometidos nos períodos epidêmicos anteriores. Subitamente, depois de um longo período de atividade contínua e em um momento de maior expansão quando atingiu vários municípios da Chapada do Araripe, a infecçao desapareceu repentinamente neste foco desde 1975.

Os resultados das análises sobre a ocorrência e distribuição geográfica dos roedores hospedeiros e de pulgas vetores nas áreas de peste brasileiras revelaram aspectos particulares nas diferentes áreas de foco relacionadas aos diferentes biomas, além de diferenças nas populações dos roedores nos períodos epidêmicos e no período de quiescência da doença.

Nossas análises também contribuíram para confirmar o papel da espécie silvestre *Necromys lasiurus* como hospedeiros amplificadores da peste responsáveis por *spillover* para as populações humanas. Observou-se alta prevalencia das populaçoes de *N. lasiurus* durante os períodos epidêmicos enquanto que a redução dessas populações ao longo do tempo resultou na quiescência da doença.

Os estudos revelaram o impacto do processo de urbanização de pequenas localidades rurais nas populações dos ratos comensais (*Rattus rattus)* que apresentaram importante crescimento populacional. Algumas localidades rurais que no inicio dos estudos eram pouco povoadas e as habitações esparsas atualmente formam vilas com uma importante densidade de habitantes e de roedores comensais. Apesar de sua abundância, os *R. rattus* não causaram epidemias de peste como seria de esperar, especialmente considerando sua proximidade com os humanos favorecendo a propagação da peste e outras doenças transmitidas por esses roedores.

Em conjunto, pelas análises dos dados referentes a peste humana e nos roedores foi possível observar a expansão geográfica da infecção desde sua chegada por via maritima, sua focalização entre os roedores silvestres e o declínio da epidemia na área de transmissão da Chapada do Araripe que permanece em quiescência.

No entanto, este silêncio não deve ser interpretado como extinção do foco, pois a qualquer momento, por um mecanismo desconhecido a infecção pode reativar e para evitar o acometimento futuro das populações humanas é de extrema importância manutenção e aprimoramento do programa de vigilância da peste.

5 SÚMULA CURRICULAR

RESUMOS PUBLICADOS EM ANAIS DE CONGRESSOS E EVENTOS CIENTÍFICOS

- Strategies of plague surveillance and control in Brazil. SOBREIRA, M.; **FERNANDES, D.L.R.S**; LEAL, N. C.; ALMEIDA, A. M.P. In: 13th International *Yersinia* Symposium, 2019, Antananarivo, Madagascar.
- Rodent reservoirs and flea vectors in Brazilian plague foci. **FERNANDES, D.L.R.S**; SOBREIRA, M.; LEAL, N. C.; GOMES, E. C. S.; ALMEIDA, A. M. P. .In: 13tn International *Yersinia* Symposium, 2019, Antananarivo, Madagascar.
- Geolocalização das cepas de *Yersinia pestis* da coleção de culturas FIOCRUZ-CYP. **FERNANDES, D. L. R. S**.; SOBREIRA, M.; GOMES, E. C. S.; LEAL, N. C.; ALMEIDA, A. M. P. In: 55º Congresso da Sociedade Brasileira de Medicina Tropical e XXVI Congresso Brasileiro de Parasitologia, 2019, Belo Horizonte, MG.
- Flutuação populacional de roedores reservatórios em área focal da peste no Estado de Pernambuco. **FERNANDES, D. L. R. S.**; DUARTE, B. M.; SILVA, M. P. F.; GOMES, E. C. S.; ALMEIDA, A. M. P. In: 55º Congresso da Sociedade Brasileira de Medicina Tropical e XXVI Congresso Brasileiro de Parasitologia, 2019, Belo Horizonte, MG.
- Pulgas dos focos de peste do Estado de Pernambuco. SILVA, M. P. F.; **FERNANDES, D. L. R. S**.; GOMES, E. C. S.; DUARTE, B. M.; ALMEIDA, A. M. P. In: 55º Congresso da Sociedade Brasileira de Medicina Tropical e XXVI Congresso Brasileiro de Parasitologia, 2019, Belo Horizonte, MG.
- Estudo retrospectivo da ocorrência de sifonápteros envolvidos na transmissão da peste no Estado de Pernambuco. SILVA, M. P. F. ; **FERNANDES, D. L. R. S**. ; DUARTE, B. M.; GOMES, E. C. S.; ALMEIDA, A. M. P. In: 55º Congresso da Sociedade Brasileira de Medicina Tropical e XXVI Congresso Brasileiro de Parasitologia, 2019, Belo Horizonte, MG.
- Análise retrospectiva da ocorrência de roedores envolvidos no ciclo epidemiológico da peste no município de Exu, sertão pernambucano nos anos de 2007 e 2015. **FERNANDES, D. L. R. S.**; GOMES, E. C. S.; ALMEIDA, A. M. P. In: 54º Congresso da Sociedade Brasileira de Medicina Tropical, 2018, Recife, PE.
- An overview of the plague in Brazil: historical and present status. ALMEIDA, A. M. P.; SOBREIRA, M.; LEAL, NILMA, C.; **FERNANDES, D. L. R. S.**; PEREIRA, S. V.

C.; TAVARES, C. In: The International Conference on Plague Prevention and Control, 2018, Harbin, China. Collection of Abstracts, 2018.

PARTICIPAÇÃO DE CURSOS DE ATUALIZAÇÃO (ouvinte)

- Participação no minicurso "Fórum Social Brasileiro Para Enfrentamento De Doenças Infecciosas E Negligenciadas - 2018 / 3º Encontro Brasileiro De Movimentos Sociais De Luta Contra Doenças Negligenciadas" no 54º Congresso da Sociedade Brasileira de Medicina Tropical de 02 a 05 de setembro de 2018, Recife, PE.
- Curso de "Esquistossomose: manejo clínico e epidemiológico na Atenção Básica" – UNA-SUS, FIOCRUZ-PE 22/10/2018.
- Participação no I Simpósio de Coleções Biológicas do Nordeste SCBio, UNICAP, 13/12/2018;
- Participação no curso "Modelagem de nicho ecológico com base em dados de coleções científicas" I Simpósio de Coleções Biológicas do Nordeste SCBio, UNICAP realizado em 10/12/2018;
- Curso Triatomíneos e Vigilância Entomológica (MEDTROP 2019) 55º Congresso da Sociedade Brasileira de Medicina Tropical e XXVI Congresso Brasileiro de Parasitologia, 2019, Belo Horizonte, MG

PARTICIPAÇÃO DE COMISSÕES

- Participação na condição de parecerista para concessão de bolsas e apoio financeiro aos projetos de extensão, de pesquisa-ação e/ou inovação, na seleção do Programa Institucional de Bolsas de Extensão e Cultura - Edital PIBExC2018 – UFPE.

ORIENTAÇÕES CONCLUIDAS

- Orientação de trabalho de conclusão de curso (2019) de aluna graduanda de Biomedicina da UNINASSAU sobre "Ocorrência de ectoparasitos envolvidos no ciclo da peste no estado de Pernambuco de 1966 a 1979";
- Orientação de trabalho de conclusão de curso (2019) de aluna graduanda de Enfermagem da UNIVISA sobre "Levantamento de ocorrência de ectoparasitos envolvidos na transmissão de *Yersinia pestis* no estado de Pernambuco";

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APÊNDICE A - SPATIOTEMPORAL ANALYSIS OF BUBONIC PLAGUE IN PERNAMBUCO, NORTHEAST OF BRAZIL: CASE STUDY IN THE MUNICIPALITY OF EXU

Publicado no periódico PLoS ONE: Fernandes DLRdS, Gomes ECdS, Bezerra MF, e Guimarães RJdPS, de Almeida AMP (2021) Spatiotemporal analysis of bubonic plague in Pernambuco, northeast of Brazil: Case study in the municipality of Exu. PLoS ONE 16(4): e0249464. https://doi.org/10.1371/journal.pone.0249464.

No estado de Pernambuco a peste introduzida em 1902 apresenta-se silente desde a década de 1980. Para entender melhor a dinâmica da doença nas áreas focais de transmissão foram utilizadas ferramentas de geoprocessamento para analisar a ocorrência espacial e distribuição de casos de peste humana no período de 1945 a 1976 utilizando o município de Exu como área de estudo de caso. Os resultados permitiram evidenciar a transição da infecção de áreas urbanas para áreas silvestres e o ressurgimento de casos após um período de quiescência, independentemente da reintrodução de outros focos ativos.

RESEARCH ARTICLE

Spatiotemporal analysis of bubonic plague in Pernambuco, northeast of Brazil: Case study in the municipality of Exu

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Data Availability Statement: In this reviewed version we added to the supplemental files our data bank on patients clinical and epidemiological features, as well as a case-by-case sheet with all

Abstract

Along with other countries in America, plague reached Brazil through the sea routes during the third pandemic. A brief ports phase was followed by an urban phase that took place in smaller inland cities and finally, it attained the rural area and established several foci where the ecological conditions were suitable for its continued existence. However, the geographic dispersion of plague in Brazil is still poorly studied. To better understand the disease dynamics, we accessed satellite-based data to trace the spatial occurrence and distribution of human plague cases in Pernambuco, Northeastern Brazil and using the municipality of Exu as study case area. Along with the satellite data, a historical survey using the Plague Control Program files was applied to characterize the spatial and temporal dispersion of cases in the period of 1945–1976. Kernel density estimation, spatial and temporal clusters with statistical significance and maximum entropy modeling were used for spatial data analysis, by means of the spatial analysis software packages. The use of geostatistical tools allowed evidencing the shift of the infection from the urban to the wild-sylvatic areas and the reemergence of cases after a period of quiescence, independent of the reintroduction from other plague areas.

Introduction

Plague is a focal zoonosis, affecting primarily rodents and eventually, humans and other mammals. The infection spectrum is wide and the main presentations are the bubonic, pneumonic and septicemic forms; the contamination occurs mainly through flea bites, inhalation of aerosols or contact with infected secretions or tissues [1]. It is caused by *Yersinia pestis*, a Gram-negative bacillus that belong to the Enterobacteriaceae family [2] and is categorized in the Biohazard Class 3 and Bioterrorism Agents Group A [3]. This zoonosis is one of the oldest and confirmed plague cases in the State of Pernambuco, including dates, locations and the coordinates for each municipality affected.

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most feared diseases of mankind and remains a threat still nowadays. Over the centuries, this infection wiped out millions of lives, impacted the people way of life, having a huge influence in science and arts over the ages and it still represents the iconic perception of a pandemics until nowadays [4].

Currently, the global incidence of human plague is the lowest reported by the WHO in 30 years. During the 2013–2018 period, the areas that have reported human cases were limited to sub-Saharan Africa, Asia, and North and South America, and most cases are reported by Madagascar, followed by the Democratic Republic of the Congo [5].

However, it is not uncommon the sudden reappearance of cases after several decades of epidemiological silence in natural plague foci causing fear, panic and loss of life and in the economics. In 2003 the plague reappeared in Algeria after >50 years of quiescence [6]. In 1994, the city of Surat, India, experienced a pneumonic plague outbreak that caused panic, population evasion and a severe impact on local economy [7]. Other episodes occurred in 2009 in the city of Ziketan, China, and in 2019 in the Mongolian border with China and Russia which resulted in the closure of the borders between these countries [5].

Along with other countries in America, plague only reached Brazil during the third pandemic, in the year of 1899. Transported by steamships, the disease caused its first outbreaks in port cities. However, due to inland transportation of goods, plague quickly reached Brazilian countryside, establishing several natural foci where the ecological conditions were congenial for its persistence [8]. The Brazilian plague foci are scattered throughout a large area ranging from the Northeastern State of Ceará to the Southeastern State of Minas Gerais and another separated area at the State of Rio de Janeiro [9–11].

The activities of the infection in these foci are independent in time and space [9]; after several successive outbreaks of varied sizes until the decade of 1980, cases in Brazil decreased and the last confirmed human case was in 2005 [12, 13]. Despite its current quiescent state, we must remain vigilant and maintain rigorous epidemiological surveillance. Of note, attempts to eradicate plague by some countries (USA, USSR) were unsuccessful and therefore this was discontinued and disapproved [14, 15].

The availability of new technological resources allowed the development of new studies on the plague activities, which is crucial for improving the understanding of the disease dynamics and to establish effective monitoring and surveillance strategies, capable of recognizing eventual issues that may precede spillovers to human populations. This study aimed to better understand the plague dynamics in Pernambuco, Northeastern Brazil by analyzing the spatial occurrence and distribution of cases and using the municipality of Exu as study case area by performing a historical survey of human cases and characterizing the spatial and temporal dispersion of cases.

Methods

Study area

The study was carried out in the state of Pernambuco, Northeast Brazil, and the municipality of Exu was used for the case study of plague. This municipality lies in the mesoregion *Sertão*, has an area of 1,336,788 km^2, an estimated population in 2019 of 31,825 inhabitants, Municipal Human Development Index (2010) of 0.576 and a warm and dry climate with scarcity and irregular rainfall (Biome *Caatinga*). Situated in the ecological complex of Chapada do Araripe, 600–700 m in altitude, about 200 km long and 30 km wide, is limited to the municipalities of Bodocó to the west, Granito to the south, Moreilândia to the east and to the north with Crato in the state of Ceará [16, 17].

Data collection

Data on the occurrence of human plague cases in the state of Pernambuco were obtained from the forms named *Comunicado Sobre Ocorrência de Peste Humana* (Notification on the Occurrence of Human Plague) from the Plague Control Program; CONCEPAS system (http://cyp.fiocruz.br/index?services); and activity records from the Plague Laboratory, available in the National Reference Service in Plague of the Aggeu Magalhães Institute, Fiocruz PE.

Information on the early occurrences of plague in the state is sparse and incomplete, therefore for the 1902 to 1944 period only data relating the occurrence of the first case per municipality were obtained. For the 1945 to 1976 period, collected data included the date and place of the occurrence, patient's gender, age, clinical features, and classification of the case (suspected, positive or negative). All data were compiled and organized into a database (DB) using Excel software.

For geospatial analyses, the vector data obtained were: municipal limits of 1970 and 2010 from the Brazilian Institute of Geography and Statistics (IBGE) (https://www.ibge.gov.br/geociencias/organizacao-do-territorio/15774-malhas.html?=&t=downloads). The drainage (hydrography) from the Mineral Resources Research Company (CPRM) (https://www.cprm.gov.br/en/Hydrology-83). The Digital Elevation Model (DEM) data was obtained from the Shuttle Radar Topography Mission (SRTM) refined for the Brazilian territory from the original resolution of 3 arc seconds to 1 arc second using a geostatistical approach (http://www.dsr.inpe.br/topodata/) using the script (https://code.earthengine.google.com/ccf3b9ff46eb845e1b88f68550e9a22a) on the Google Earth Engine (GEE) platform. All geospatial data were obtained from free access and use platforms.

Data analysis

The DB was separated into two groups: (1) DBM (Database by Municipality), containing both the years of the first plague case by municipality during 1902–1966, and all cases recorded by municipality for the 1945–1976 period; (2) DBE (Exu Database), which was separated into two sub-groups corresponding to the epidemic periods of 1945–1954 and 1961–1976 and a quiescence period of 1955–1960. The localities of the DBE cases (suspected, positive or negative) in the study period (1945–1976) were georeferenced in loco, with a GPS (Global Positioning System), model eTrex Vista Cx, Garmin (Kansas City, USA), configured in the UTM (Universal Transverse Mercator) projection system, Datum WGS-84. To georeference, a landmark (house, church or gate) was standardized for each of the localities. The GPS data was transferred to GPS TrackMaker Pro 4.9.603 (Geo Studio Technology, Belo Horizonte, Brazil) and the geographic coordinates were organized and stored in the shape file format, that was used with the DB to create a spatial database (SDB).

Descriptive epidemiology was used to analyze the distribution of cases by gender, clinical features, age, location (urban or rural area) and period of the occurrence. Unfortunately, due to the lack of standardization of the records over time, some of the clinical variables were not available for all patients.

The spatial analyzes performed in these groups were: (1) map of spatial and temporal distribution to spatially visualize the location of the disease and the number of cases in the municipalities of Pernambuco and in the localities (*sitio*, *fazenda*, *povoado*) of Exu (choropleth maps); (2) Kernel density estimation (KDE) to identify the location of clusters for case occurrences. For KDE, the following parameters were used: quadratic function, density calculation and adaptive radius on both banks; (3) spatial scanning map (Scan) to identify spatial and temporal clusters with statistical significance. The Scan used the Poisson model (Retrospective Space-Time analysis scanning for clusters with high rates using the Discrete Poisson model) based on

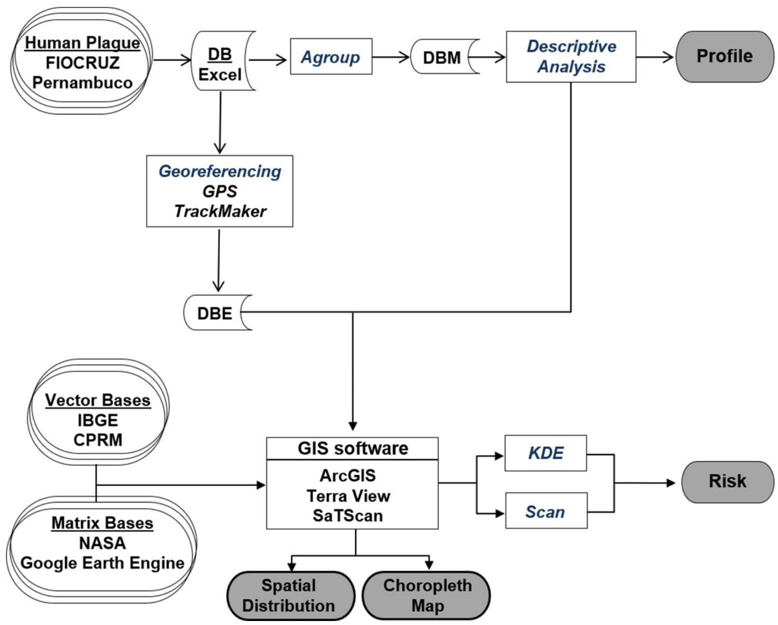

Fig 1. Workflow for collection, storage and analysis of spatiotemporal data.

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the resident population in Pernambuco for the analysis in the DBM and the Exponential model using the DEM attribute obtained by Google Earth Engine (GEE) to search for spatial and/or temporal clusters of exceptionally short or long survival in DBE (Retrospective Space-Time analysis scanning for clusters with short or long survival using the Exponential model).

Data processing, interpretation, visualization and analysis were performed using ArcGIS (http://www.arcgis.com/), SatScan (https://www.satscan.org/) and TerraView (http://www.dpi.inpe.br/terralib5/wiki/doku.php). Fig 1 illustrates the methodology for collecting, storing and analyzing spatiotemporal data. The surface and boundaries of the municipality of Exu during the study period are different from the present, because some small rural communes called *Povoados* (Tabocas, Viração, Timorante) were emancipated and became new urban areas named *Vilas* (Villages).

Results and discussion

Entry and dissemination of the human plague in Pernambuco, Northeast Brazil

In 1902, just three years after the plague entered Brazil, the state of Pernambuco was affected by the disease [11]. According to the data collected in this study, from the introduction until the last case (1982), 56 municipalities (30.4%) of the extant 184, registered plague cases (Fig 2). Since its arrival, strict sanitary control measures undertaken eliminated the infection from the port city (Fig 2A), however, they failed to prevent its spread to the countryside [8, 11]. During the period of 1913–1918, the disease spread to the *Agreste* mesoregion, reaching eight clustered municipalities (Fig 2B). In 1919, the plague spread further throughout the state, reaching two new mesoregions, one municipality in the *Zona da Mata* or Forest Zone and four in the *Sertão* (Fig 2C). From 1920–1936, 16 new municipalities were attaint for the first time including an only one municipality in São Francisco mesoregion (Fig 2D).

The Fig 2E and 2F presents the two largest epidemic periods for the state. In the first period, 1945–1954, only one new municipality was affected, in the year 1948 (Fig 2E), in the second,

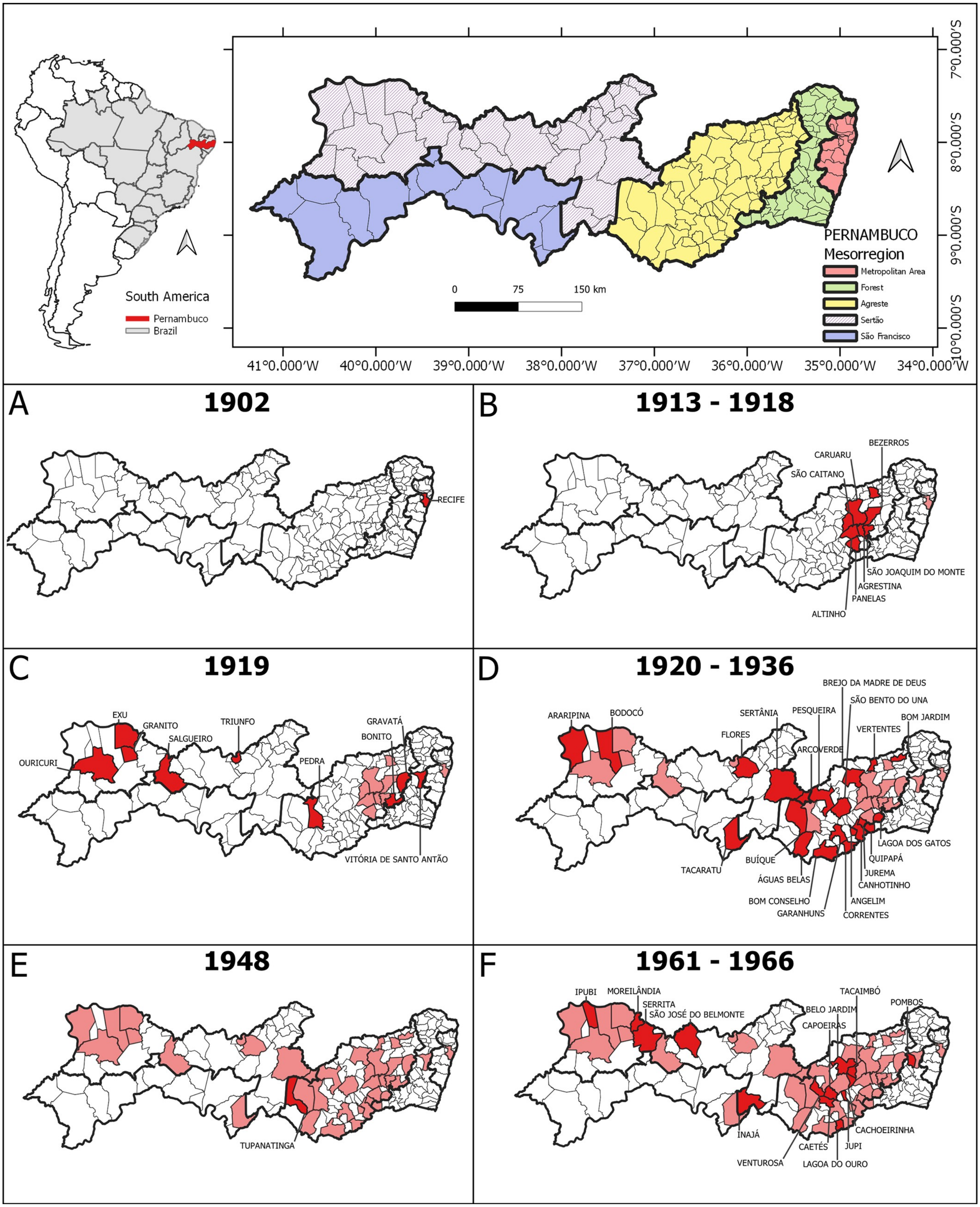
South America
Pernambuco
Brazil
PERNAMBUCO
Mesorregion
Metropolitan Area
Forest
Agreste
Sertão
São Francisco
0
75
150 km
41°0.000′W
40°0.000′W
39°0.000′W
38°0.000′W
37°0.000′W
36°0.000′W
35°0.000′W
34°0.000′W
7°0.000′S
8°0.000′S
9°0.000′S
10°0.000′S
A
1902
RECIFE
B
1913 - 1918
BEZERROS
CARUARU
SÃO CAITANO
SÃO JOAQUIM DO MONTE
AGRESTINA
PANELAS
ALTINHO
C
1919
EXU
GRANITO
TRIUNFO
SALGUEIRO
OURICURI
GRAVATÁ
BONITO
PEDRA
VITÓRIA DE SANTO ANTÃO
D
1920 - 1936
BREJO DA MADRE DE DEUS
SÃO BENTO DO UNA
ARARIPINA
BODOCÓ
SERTÂNIA
PESQUEIRA
BOM JARDIM
VERTENTES
FLORES
ARCOVERDE
LAGOA DOS GATOS
QUIPAPÁ
JUREMA
CANHOTINHO
TACARATU
BUÍQUE
ÁGUAS BELAS
BOM CONSELHO
GARANHUNS
ANGELIM
CORRENTES
E
1948
TUPANATINGA
F
1961 - 1966
IPUBI
MOREILÂNDIA
SERRITA
SÃO JOSÉ DO BELMONTE
TACAIMBÓ
POMBOS
BELO JARDIM
CAPOEIRAS
INAJÁ
CACHOEIRINHA
CAETÉS
JUPI
VENTUROSA
LAGOA DO OURO

Fig 2. Emergence and dissemination of human plague cases by municipality in the mesoregions of the state of Pernambuco, Northeast Brazil, 1902–1966. On top: Localization of Pernambuco and Brazil in South America and the mesoregions. A-F: Spatial and temporal distribution of the human plague cases, showing the municipalities affected for the first time (dark red) and those previously affected (light red). Shapefiles of Pernambuco and counties limits were obtained from IBGE (public-domain access).

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1961–1966, which was the period with the highest number of cases, 14 new municipalities registered plague cases (Fig 2F). It is important to note that after 1966, no new municipality registered occurrence of plague case until 1982, the date of the last notification in the state.

Establishment of the plague in Pernambuco, Northeast Brazil

According to the epidemiological records, there were 954 plague-suspected cases notified in Pernambuco during the period from 1945 to 1976. Out of these, 525 (55.0%) were considered positive based on the epidemiological-clinical criterion for the classification of cases at the time [9, 11].

The 954 plague-suspected and the 525 positive cases originated from 57 (31%) and 37 (20%), respectively, of the extant 184 municipalities in the state of Pernambuco (Fig 3A). Exu (red) concentrates the largest number of positive cases (267), followed by Bodocó (45 cases),

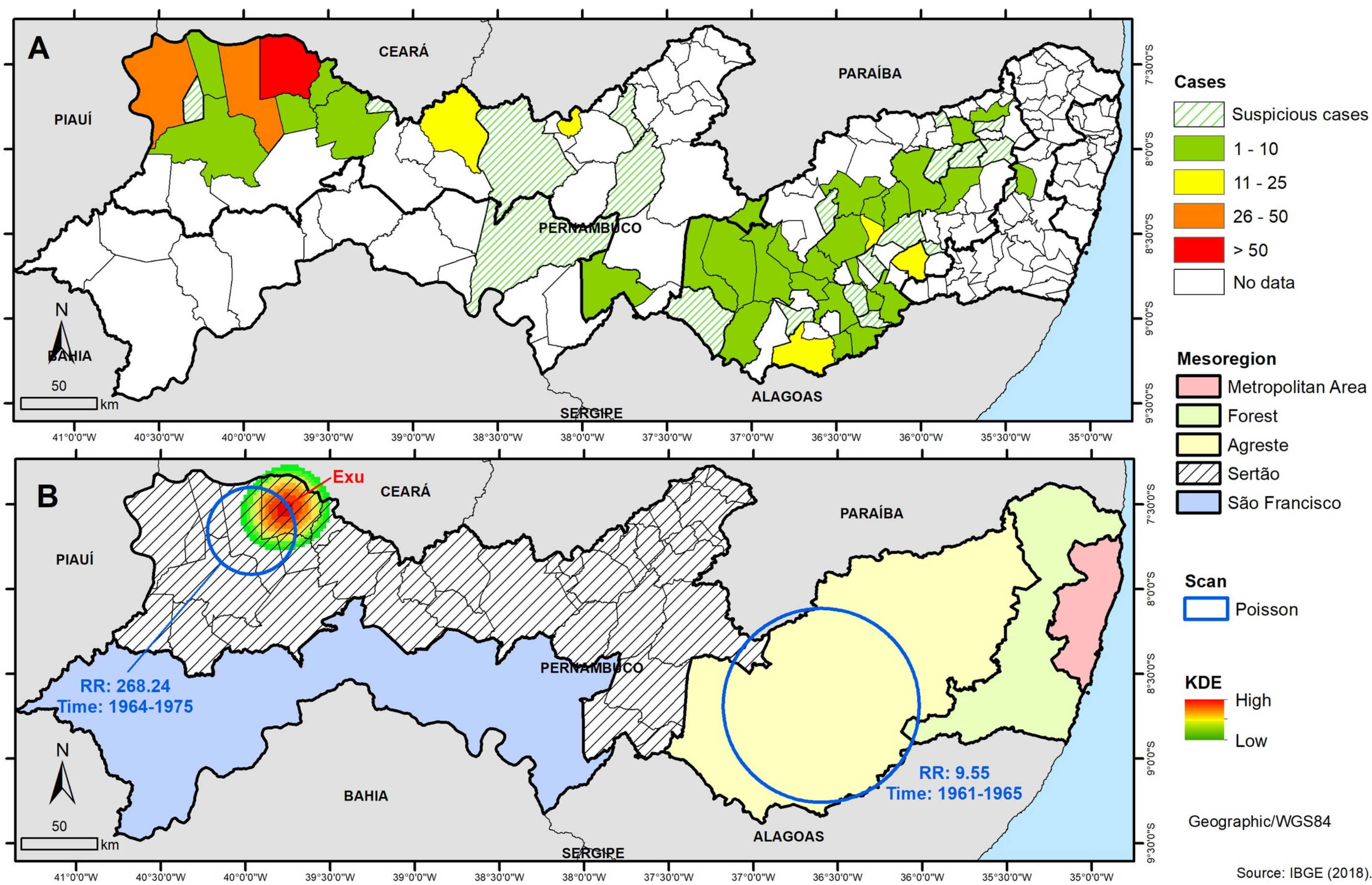

Fig 3. Spatial distribution and risk analysis of human plague cases in Pernambuco, Northeast Brazil between the years 1945–1976 with the identification of the mesoregions. (A) Spatial distribution of human plague by number of occurrences per municipality; (B) Identification of risk areas for the occurrence of the disease by application of KDE and Scan in cases of human plague in Pernambuco. Shapefiles of Pernambuco and counties limits were obtained from IBGE (public-domain access).

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Araripina (37 cases) and other five municipalities, all located in the ecological complex of Chapada do Araripe, in the *Sertão* mesoregion. In this same mesoregion, the ecological complex of Triunfo—São José do Belmonte registered 17 and 12 positive cases (yellow), respectively. This mesoregion, despite not concentrating the largest number of plague afflicted municipalities, is doubtless of great epidemiological importance, since it concentrates the largest number of plague cases in Pernambuco during the studied period (398 cases—75,8% of the total positive cases).

In the *Agreste* mesoregion, the highest number of positive cases was registered in the municipalities of Bom Conselho (20), Cachoeirinha (13) and Panelas (11) and other 31 municipalities registered one to 10 plague-positive cases. These municipalities are located in the Planalto da Borborema, another focal plague area involved in the 1961–1976 epidemics in the state of Pernambuco.

It is noteworthy that in the period of the study, when the plague was already established in Pernambuco, no new cases were reported in the metropolitan region or in the mesoregion of *São Francisco*, although in this later at least one case had been registered in the 1920–1936 period as seen in Fig 2D. In the *Forest* mesoregion, only one municipality registered three positive cases (green). The absence of cases in these areas could be related to their ecological and geographical conditions, such as altitude, vegetation and the temperature unsuitable for the persistence of the infection. In contrast, in the *Agreste* and *Sertão* mesoregions, the plague encountered the congenial ecotopes to maintain the wild cycle and establish permanent foci in the ecological complexes of Chapada do Araripe and Planalto da Borborema [9, 10].

The Kernel density analysis (KDE) of the number of cases reported in Pernambuco revealed that the municipality of Exu from the Chapada do Araripe focus is at higher risk for the occurrence of plague (Fig 3B). Exu appeared at the epicenter of the Kernel patch that radiates in decreasing intensity as it moves away from the Chapada slope towards the plains and the neighboring municipalities of Moreilândia, Bodocó, Granito and Crato. The Fig 3B shows as well the presence of two statistically significant spatiotemporal clusters obtained by the Scan analysis, one that encompasses the municipality of Exu with a relative risk (RR) 268.24 times higher for the occurrence of plague in comparison with the other municipalities of Pernambuco in the period 1964–1975 and another cluster located in the *Agreste* mesoregion with RR = 9.55 in the period 1961–1965.

These preliminary findings led us to perform a more detailed analysis using the Municipality of Exu as a model to better understand the dynamics of plague's transmission and maintenance in Brazil.

Plague in Exu

From the 525 positive cases in the state of Pernambuco, 267 (50.9%) originated in the municipality of Exu. All cases reported in Exu in the analyzed period were the primary bubonic form, characterized by the presence of the bubo, which was generally single, extremely painful, accompanied by high fever and torpor. The temperature (recorded in 43 patients) ranged from 37° to 41°C (mean 38.7°C) and the buboes (recorded in 42 patients) located in the following anatomical sites: inguinal-crural (36), axillary (4), cervical (2). There was no record of pneumonic plague, but 14 patients had septicemia confirmed by a positive *Y*. *pestis* blood culture and this septicemia was probably evolution from the primary bubonic infection [18, 19].

Although data on response to treatment and patient follow-up were unavailable for several cases, those that could be accessed had satisfactory response to the standard treatment by Sulfadiazine and Streptomycin and most cases recovered; the few deaths were attributed to lack or late treatment as stated by Karimi et al. [18]. According to Freitas [19] with the use of sulfaantibiotics therapy replacing the use of serum in treatment from 1943 on, deaths declined significantly.

Regarding the age at diagnosis, patients were grouped in: early childhood (0 to 6 years = 44), childhood (7 to 12 years = 39), adolescents (13 to 18 = 20), adults (19 to 59 = 38) and elderly (≥60 = 3). Among the patients, 220 were men (61.1%) and 140 women (38.9%). Regarding seasonality, from 1961 to 1976, although cases were recorded in all months of the year but February and April, most cases occurred from July to November, with 8 to 17 cases per month. A positive association was observed between the plague cases and increased rodent reservoir and flea vector populations and higher number of naturally infected rodent and fleas in the fields in the municipality of Exu [9, 18, 20].

Analysis of the spatial distribution of the cases reveals that Exu city (urban area) concentrates the largest number of cases and is surrounded by smaller spots in neighbor rural sites. The spatial contiguity between them favors the exchange between the commensal and wild rodent hosts (Fig 4). Of note, once the infection disseminated to the rural zone, most affected areas (localities x cases) were concentrated close to the Chapada slope, while rather dispersed in the plateau (brown). Indeed, according to Baltazard [9], all the plague cases from the municipalities of Exu and Bodocó occurred along the green and fertile slopes with numerous islets of dense brushwood, permeated by springs issues from the Chapada. The few cases in the contiguous plains were limited to a 30–50 km zone neighboring the Chapada slopes. This is a congenial ecosystem for the coexistence of the wild rodent reservoirs of the bacteria, the flea vectors and the humans [21].

In order to study the dissemination of plague cases in the municipality of Exu over time, three distinct periods were stratified: (1) ten years of activity (1945–1954); (2) quiescence for six years (1955–1960) and (3) 15 years of activity (1961–1975). Based on this stratification, it was possible to visualize the dissemination of the plague from the urban to the rural areas (Fig 5A and 5B).

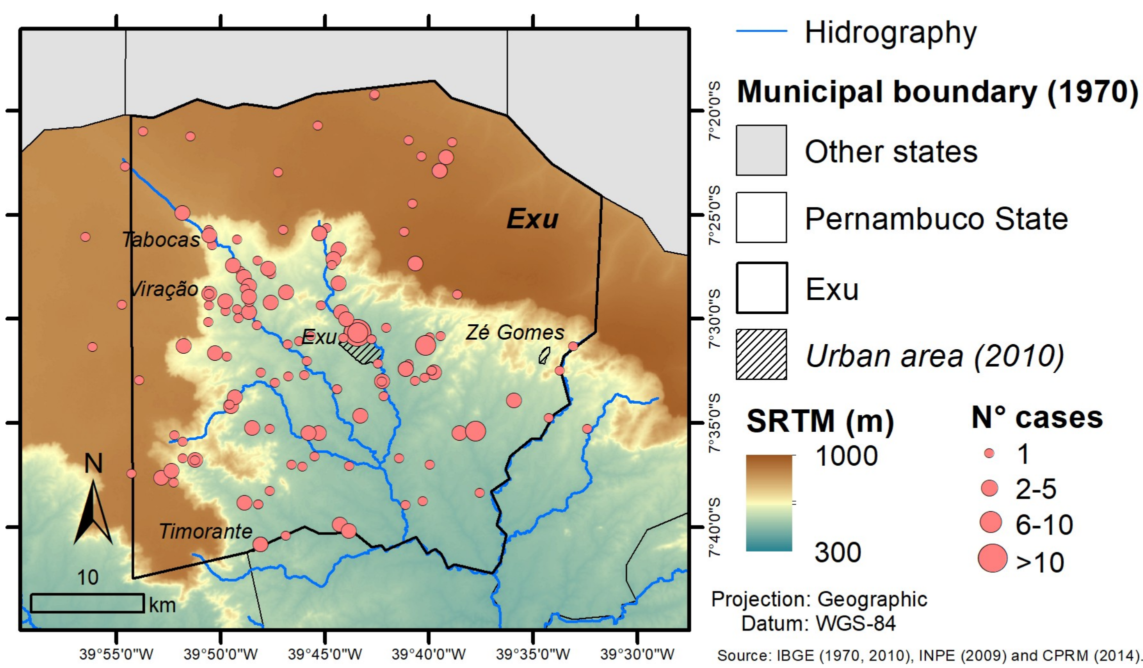

Fig 4. Spatial distribution of human plague cases in Exu, Pernambuco, Northeast Brazil, in the period 1945–1976 considering the number of cases per locality and the relationship with altimetry. Shapefile of Exu was obtained from IBGE; DEM from SRTM (http://www.dsr.inpe.br/topodata/); and Hydrography from CPRM. Images were used for illustrative purposes only.

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In the period of 1945–1954, from the 74 cases in the municipality, 32 (43%) were urban and 42 (57%) were rural, spread in 33 localities. In Exu city the most numerous cases were recorded in 1945 (9) and 1946 (19), followed by 1948 (2), 1951 (1) and 1952 (1). This period can be considered the end of the urban phase (Fig 5A) when the infection raged into the city houses through the population of the commensal rats (*Rattus rattus*) and was likely transmitted by the rat fleas (*Xenopsylla cheopis*) or by *Pulex irritans*, the so called "human flea" [22] and even by *Ctenocephalides felis*, the cat fleas. Karimi et al. [20] reported the occurrence of infected free-living *X*. *cheopis* and *P*. *irritans* in the soil of the houses or in the bedding of the deceased patients from Exu. Further evidence of the potential role of *C*. *felis* and *P*. *irritans* in plague transmission was provided elsewhere [23–25]. No cases were registered in the city during the following 11 years (1952–1963), reappearing only in 1964, three years after the re-emergence and the outburst of plague cases in the rural areas.

In contrast to the first epidemic period (1945–1954), there were only 9 cases in the city (4.7%) in the second period (1961–1975), while in the rural area there were 184 cases (95.3%) (Fig 5B). This change in the occurrences pattern could be due to the control measures employed to eliminate rats and fleas from the city houses based on the use of poisons and insecticidal (DDT and BHC) spraying [11, 19]. On the other hand, the continued pressure of spraying insecticides up to three times a year for many years, led to the insecticidal resistance among the *Xenopsylla* and *Pulex* flea populations, which may have contributed to the upsurge of the plague in the 1960s [9, 20].

Fig 5C shows that at the beginning of the first period (1945–1954), the cases were concentrated in the city of Exu and surroundings as well as in the small rural communes and surroundings such as the *povoados* Tabocas, Viração and Timorante (green) and at the end of the period (red) the cases occurred further away from the city. In the beginning of the second period (Fig 5D), the cases were concentrated in the rural area around the *povoados* Viração and Tabocas (green), and at the end of the period (red) they spread across the plain towards the *povoado* Timorante and to the south, to the neighboring municipality of Bodocó.

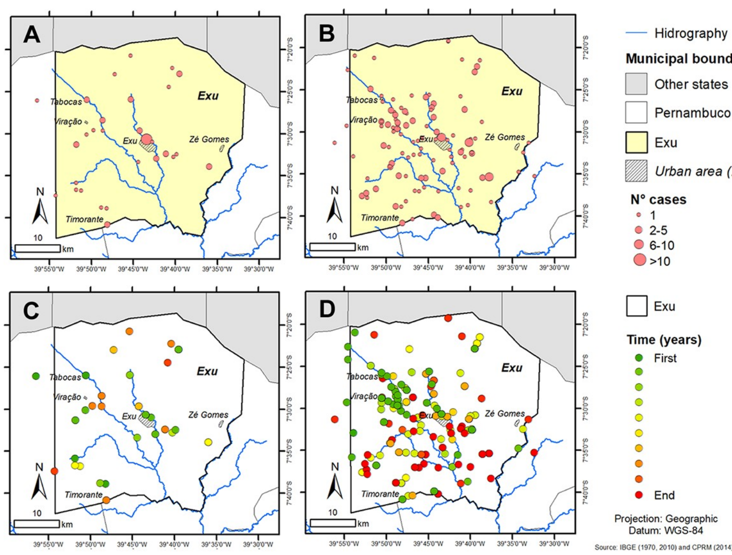

Fig 5. Spatiotemporal distribution of human plague cases in Exu, Pernambuco, Northeast Brazil. (A) Showing the concentration of human plague cases in the urban area of Exu in the period of 1945–1955; (B) Dispersion of cases to the rural areas during the second epidemic period 1961–1975; and the timeline shift within each period stratified by color scale: dark green, cases at the beginning of each period and dark red, those at the end of each period (C) distribution of cases by locality 1945–1954 and (D) 1961–1975. Shapefile of Exu was obtained from IBGE; and Hydrography from CPRM. Image was used for illustrative purposes only.

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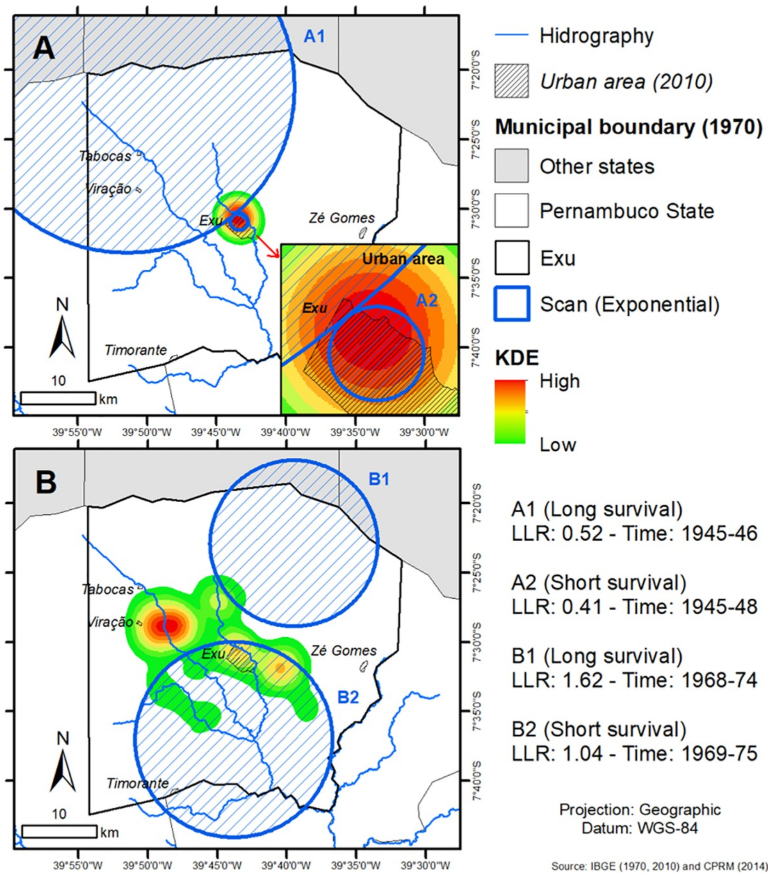

Fig 6. Application of KDE and Scan in the human plague cases in Exu, Pernambuco, Northeast Brazil in the period 1945–1955 (A) and 1961–1975 (B). Retrospective Space-Time analysis scanning for clusters with short or long survival using the Exponential model. Log Likelihood Ratio (LLR). Shapefile of Exu was obtained from IBGE; and Hydrography from CPRM. Image was used for illustrative purposes only.

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Such findings demonstrate that the first epidemics started in the city urban area and disseminated to the rural areas mainly where the human detector was more numerous. In the second epidemics, which counted the largest number of cases (193–72.3% of cases), the plague was already disseminated practically throughout all the municipality territory. In this period the plague was well established among the wild fauna [21]. The rodent *Necromys lasiurus* is the most common wild rodent, the most frequently infected by plague and carrying infected fleas (*Polygenis bolhsi jordani* and *P. tripus*) which played likely the more important role in the spread of plague and the human cases [9, 18, 20].

The analysis of risk areas by KDE in the municipality of Exu confirmed the dynamics of the plague dispersion. In the first epidemic period (1945–1954) cases predominated in the city—urban phase, represented by the risk spot (Fig 6A). It is also possible to note the presence of two significant spatiotemporal clusters obtained by the Scan analysis, one with a long survival rate located in the urban center of Exu with an LLR of 0.41 in the period from 1945–1948 and another with a short survival rate located in the northwest part of the municipality (rural area) with LLR = 0.52 in the period from 1945–1946. Such findings highlight the higher risk and duration for the occurrence of cases in the urban zone in that period, certainly associated with commensal rats *R. rattus* and the rat fleas *X. cheopis* [20].

In the second epidemic period (1961–1975), the risk spot for the occurrence of cases (KDE) is concentrated in the rural area, mainly in the *povoados* Viração, Tabocas and Zé Gomes (Fig 6B), where the number of human detectors was higher. There was also a marked presence of two significant spatiotemporal clusters obtained by the Scan analysis, one with a long survival rate located in the northern part of the municipality (rural area) with LLR = 1.62 in the period 1968–1974 and the other with short survival rate located in the part south of the municipality (enrolling the urban center of Exu) with LLR = 1.04 in the period 1969–1975. These results demonstrate the higher prevalence of the plague in the rural areas during this second epidemic period in Exu.

These results enlighten the dynamics of the epidemization and epizootization of the plague in Exu. However, the absence of cases in the quiescence period (1955–1960) could be misleading considering that there was no investigation of the circulation of the bacillus in the nature and the only indicator of the infection activity was the human cases and it can be assumed that the occurrences could be under-reported [9].

Globally, the mechanisms of persistence of the infection in the plague foci in different regions of the world during the interepizootic periods are not yet fully understood [4, 14]. It was hypothesized that during the quiescence period the infection could remain in the nature in an enzootic form as a low-level circulation of the rodent-flea-rodent cycle with more time in the fleas' organism or in the rodents as a chronic form of the plague. It is also postulated that the bacteria would endure inside the burrows of certain rodent species, where the microclimate would allow its survival in debris from dead animals, in the soil contaminated with flea feces and in soil parasites [9, 14, 21, 26].

Conclusion

During the studied period it was demonstrated the transition of the infection from the urban to the rural areas in Exu, Pernambuco state, Northeast of Brazil and the reemergence of cases after a quiescence period without reintroduction from other foci. After six years of quiescence, the plague reappeared in 1961 in the rural area, in a *sitio* 16 km far from the city of Exu. This case would be served as an alert and the epidemics from 1964 onward could had been avoided or constrained. However, there was not a surveillance system or a predictive model at that time. Therefore, the case was not properly considered and consequently, in the following 15 years until 1975, the plague spread throughout the municipality territory and reached places that had not been affected in the previous epidemic periods.

At the beginning of the first epidemic period analyzed (1945–1954), the infection was still raging mainly in the urban area maintained by the commensal rats and their fleas and then moved to the rural area from which it reemerged after 6 years of silence, reappearing in the city only three years later while it was already largely active on the rural areas. Unexpectedly after a long period of continuous activity and at a time of larger expansion when it reached several municipalities in the Chapada do Araripe, the plague suddenly disappeared in this focus since 1975. This disappearance was confirmed not only by the absence of human cases but also by the absence of the plague bacillus among the rodent hosts and flea vectors and a decreasing in positive sentinel/indicator animals through the bacteriological and serological surveillance activities [12, 27]. The causes for this disappearance are not known. Purportedly it could be attributed to the rarefaction of rodent populations decimated by successive epizootics and which were unable to recover due to climatic changes [9].

This change of pattern was surprising and contrary to what was expected, as predicted by Baltazard [9], which would be a gradual reduction of the cases and the retreat of the infected area with persistence in Exu until the total halt of the infection activities in the focus.

However, this silence must not be interpreted as extinction of the focus, because at any time, by an unknown mechanism the infection can reactivate. Due to its cyclical characteristics—alternating periods of activity and quiescence, depending on a series of complex factors —the plague can reemerge, causing new epizootics and reaching the human populations [28, 29].

Since it is so widespread in wildlife rodent reservoirs and considering the particularities of the focal areas, the eradication of the plague is a momentarily unattainable objective and not even recommended in the face of the failure of the eradication attempts carried out by some countries [15, 30]. It is therefore essential to maintain the monitoring and control of this zoonosis in order to avoid future spillovers for the human populations.

Supporting information

S1 Data.
(XLSX)

Author Contributions

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APÊNDICE B - RODENT HOSTS AND FLEA VECTORS IN BRAZILIAN PLAGUE FOCI: A REVIEW

Publicado no periódico *Integrative Zoology*: D. L. Reis da Silva Fernandez et al. Rodent hosts and flea vectors in Brazilian plague foci: a review. Integrative Zoology 2020; 0: 1–10. doi: 10.1111/1749-4877.12480

O presente artigo revisa e discute a ocorrência e distribuição geográfica de roedores hospedeiros e de pulgas vetores nas áreas de peste brasileiras durante o período de 1952 a 2019 baseado em dados históricos dos programas de controle e vigilância de peste disponíveis no Servivo de Referencia Nacional em Peste (SRP) da FIOCRUZ PE e pesquisas em campo pelo SRP. Os estudos revelaram diferenças na composição das faunas rodentia e sifonapteriana no foco da Serra dos Órgãos (RJ) região sudeste, e no nordeste do Brasil que são atribuídas aos diferentes biomas nas duas regiões. O roedor *Necromys lasiurus*, considerado amplificador da peste nos focos do Nordeste do Brasil não foi encontrado na Serra dos Órgãos (RJ). O papel das diversas espécies na epidemiologia da peste no foco da Serra dos Órgãos não está definido.

Integrative Zoology 2020; 0: 1–10 doi: 10.1111/1749-4877.12480

REVIEW

Rodent hosts and flea vectors in Brazilian plague foci: a review

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Abstract

Plague, caused by the *Yersinia pestis* bacterium, has several foci scattered throughout a large area from the Brazilian territory that ranges from the Northeastern State of Ceará to the Southeastern State of Minas Gerais and another separated area at the State of Rio de Janeiro. This review gathers data from plague control and surveillance programs on the occurrence and geographic distribution of rodent hosts and flea vectors in the Brazilian plague areas during the period of from 1952 to 2019. Furthermore, we discuss how the interaction between *Y. pestis* and some rodent host species may play a role in the disease dynamics. The absence of human cases nowadays in Brazil does not mean that it was eradicated. The dynamics of plague in Brazil and in other countries where it was introduced during the 3rd pandemic are quite alike, alternating epidemics with decades of quiescence. Hence, it remains an important epidemic disease of global concern. The existence of a large animal reservoir and competent vectors demonstrate a need for continuous surveillance to prevent new outbreaks of this disease in humans.

Key words: fleas, plague, rodents, transmission, vectors

INTRODUCTION

It has been historically accepted that plague, caused by the bacterium *Yersinia pestis*, arrived to Brazil during the third pandemic through the seaport of Santos, located in the State of São Paulo. The first Brazilian case of plague was reported in October 1899, and from then onward up to 1906, it assailed almost all the great Brazilian harbors. Prompt control measures taken by the health authorities successfully controlled the infection in the ports. However, these measures did not prevent the disease from spreading to inland cities via railways and other means of transportation. During the decades 1920–1930s, the disease began to afflict small towns, farms, and ranches (sítios) in the rural areas (Pollitzer 1954; WHO 1965).

Recently, whole genome sequencing of several Brazilian *Y. pestis* strains isolated from different sources and periods revealed a rather low genetic diversity amongst the strains when compared to strains from other countries. These findings provided evidence that the plague spread throughout the Brazilian territory starting from a single introduction (Vogler *et al*. 2019). Similar patterns can be observed in other locations affected during the 3rd pandemic (Cui *et al*. 2013).

Entering through seaports, the infection afflicted first the brown rat-population of *Rattus norvegicus*, and in the rural zone of the Northeast, the commensal (*Rattus*

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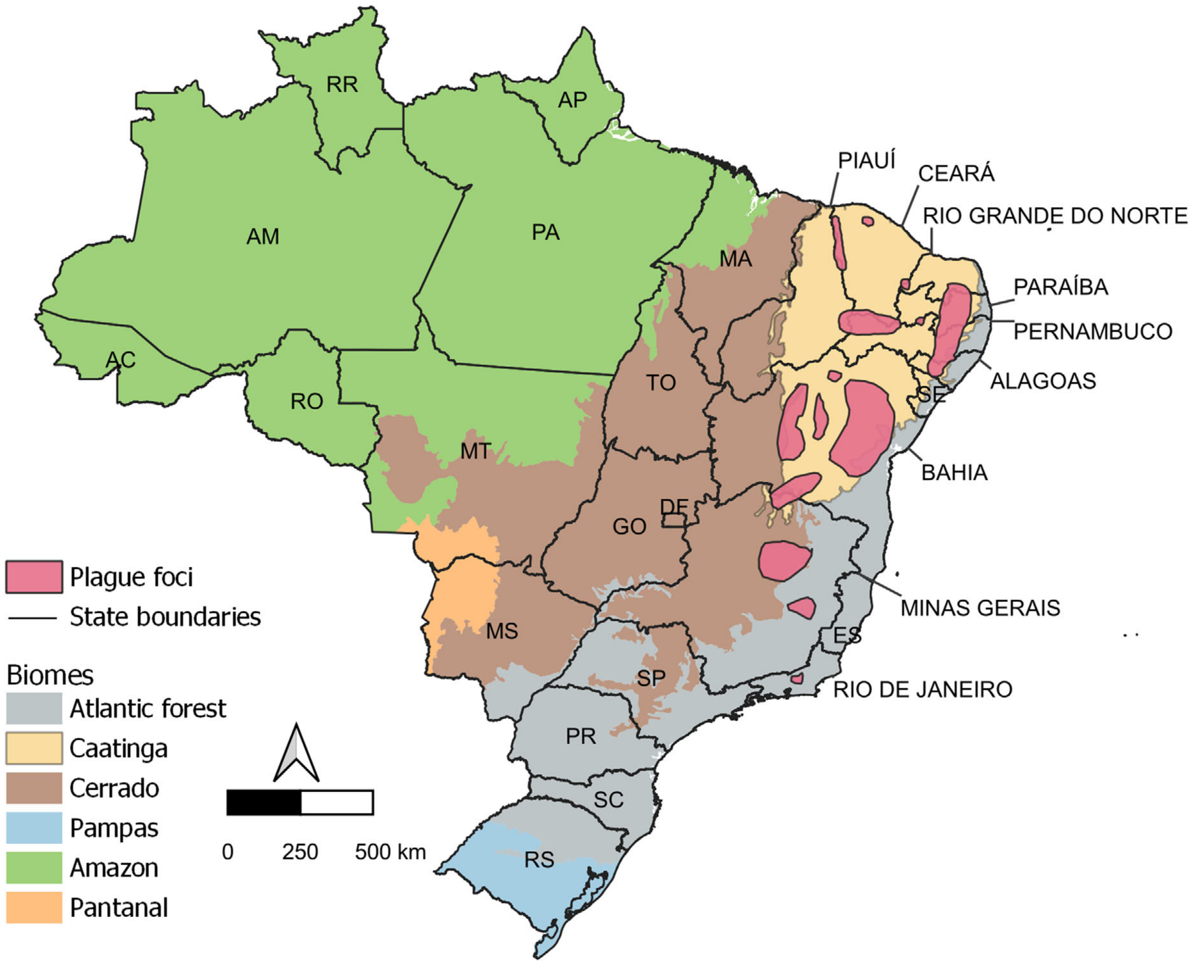

Figure 1 Map of Brazil showing the states, the biomes and the plague areas. The location of the Brazilian plague foci is shown in pink in the States of Piauí, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Bahia, Minas Gerais, and Rio de Janeiro. Other colors represent the Brazilian biomes: Atlantic forest, Caatinga, Cerrado, Pampas, Amazon, and Pantanal.

rattus). Following its natural course, the infection encountered the susceptible autochthonous wild or sylvatic fauna and established several natural foci where the ecological conditions were suitable for its persistence (WHO 1965).

These foci persist until nowadays scattered through the Northeastern States of Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Piauí, Alagoas, Bahia, and north of Minas Gerais, constituting the so called "Northeast focus." Another smaller and more isolated focus is located at the "Serra dos Orgãos" region in the southeastern State of Rio de Janeiro. (Giles *et al*. 2011; Almeida *et al*. 2020). The localization of the Brazilian foci is shown on Fig. 1.

Although no transmission to humans have been recorded in these areas since 2005 (Sousa *et al*. 2017; Zeppelini *et al*. 2018), the plague can still reemerge, due to its cyclical behavior, characterized by alternating periods of activity and quiescence (Stenseth *et al*. 2008). Understanding the determining factors for the occurrence of the infection is crucial to establish effective control measures to prevent spill over into human populations. Therefore, rigorous monitoring of host and vector populations allows an early detection of *Y. pestis* activity in the nature and consequently, a broader window of opportunity for triggering prompt control measures (Gage 2012).

Early records of plague in Brazil are sparse, as disease control was carried out by each State Department of Health. Only after 1935/1936, when the nationwide plague control and health policies were set, the data was properly collated and archived. Since 2002, disease control activities were decentralized to the municipal administrations (Tavares *et al*. 2012).

Over time, several studies have been carried out to assess the occurrence and distribution of the Rodentia and Siphonaptera faunas and to understand the possible role of different rodents and fleas in the maintenance, epizootization, and epidemization of plague in the focal areas. Biological features, susceptibility to infection, and vector ability were studied in laboratory using experimentally developed rodent and flea colonies (Karimi *et al*. 1974a,b; Almeida *et al*. 1981; Baltazard 2004; Tavares *et al*. 2012).

Following improvements in the understanding of geographical distributions, field behaviors, and genomic features, the nomenclature of small mammals has been updated and some of them were assigned to other genera or species (Burgin *et al*. 2018). As the accurate taxonomic identification is of utmost importance to understand the plague dynamics, the nomenclature of the rodent hosts was revised and updated following Bonvicino *et al*. (2015).

Here, we review and discuss information on the rodent hosts and flea vectors from the Brazilian plague foci gathered by the plague control and surveillance programs, between the years 1952 and 2019.

MATERIALS AND METHODS

Data collection

The present paper reviews the main features of plague rodent hosts and flea vectors, as well as their role in the dynamics of *Y. pestis* throughout the Brazilian territory during the last century. This work was carried out by consulting the literature and records from local control and surveillance programs, collected from 1952 to 2019, now available at the Nacional Reference Service of Plague (Serviço de Referencia Nacional de Peste) from the Aggeu Magalhães Institute (IAM, Recife, Brazil).

Rodents and fleas collection

Animal capture and handling methods varied according to the recommendations in each period. Further details can be found at the original publications (Freitas 1957; Bahmanyar & Cavanaugh 1976; Mills *et al*. 1995; Costa *et al*. 2017; Zeppelini *et al*. 2018). In short, rodents collection was carried out overnight using rodent live traps (type Chauvancy, Tomahawk, and Sherman); trapped animals were brought to a field processing site for collection of ectoparasites, sexing, and identification to species or genus; the ectoparasites were collected by brushing the rodents fur over a water container and transferred to small vials containing 2.5% saline for identification at the laboratory. The rodents were either kept in quarantine until death or euthanized.

Laboratory analysis

Dead animals were autopsied and examined for gross lesions, and tissue samples were taken for smear examination and culture for *Y. pestis* identification and isolation. Triturates of flea pools and rodent tissue samples were plated onto plain agar medium; the plates were incubated at 28 °C for 48 to 72 h and checked daily to observe the colony growth and the lysis by the anti-plague bacteriophage (Bahmanyar & Cavanaugh 1976; Karimi 1978). Serological surveillance was performed by the Hemagglutination assay (HA) with hemagglutination Inhibition control (HI) to detect specific antibodies for the *Y. pestis* capsular protein Fraction 1 or F1 (Chu 2000).

RESULTS AND DISCUSSION

Currently, the global incidence of human plague is the lowest reported by the WHO in 30 years (WHO 2019). The tendency of plague in Brazil also decreased since the years 1980, with the last confirmed human case in 2005 (Sousa *et al*. 2017; Almeida *et al*. 2020). However, it is not uncommon to observe a sudden reappearance of human cases after several decades of epidemiological silence in natural plague foci (Stenseth *et al*. 2008; WHO 2019). Therefore, understanding the disease dynamics is essential to establish effective surveillance strategies, capable of recognizing eventual epizootics that may precede spill overs to human populations. Hence, surveillance, monitoring, and control actions must be continued during plague silent periods (Gage 2012).

Due to the dissemination of *Y. pestis* from its original habitat to other regions of the globe, the dynamics of the pathogen–hosts–vectors interaction is unique in each ecosystem (Bramanti *et al*. 2016). To shed some light at the panorama of plague ecological features in Brazil, this review pinpoints the occurrence and distribution of plague-associated rodent hosts and flea vectors. We also discuss the possible roles of the different species in the dynamics of plague through an appraisal of the main studies carried out in different contexts from 1952 to 2019.

Inventory of wild-rodents fauna

In Brazil, the studies on wild rodents and their ectoparasites have become an important part of the activities of the plague control programs as soon as the infection in the wildlife was surmised. The assessment of plague infection in wild rodents revealed a broad range of naturally infected species in the wild. These findings led to the development of experimental studies in the laboratory to determine the susceptibility of the most prevalent species and the vector capacity of the wild flea ectoparasites (WHO 1965). Several projects were carried out ever since: Inventory by the Nacional Plague Service (Serviço Nacional de Peste); Studies on the Chapada do Araripe plague focus; Plague surveillance program; Revaluation of the rodent populations.

From 1952 to 1955, the National Plague Service carried out a large inventory of the fauna of rodents and other small mammals and their ectoparasites in the plague areas of the Northeast to assess the occurrence and distribution of the Rodentia and Siphonaptera faunas. The collections were carried out in the peridomestic perimeter of rural areas (cultivated fields) and in sylvatic areas (remaining fragments of native vegetation) aiming to know the rodents diversity in different environments. The sylvatic ecotopes had higher diversity but lower abundance. During this study, 44 220 small mammals were caught, of which, 40 262 were rodents from 7 families and 22 genus and species (Freitas 1957).

From 1966 to 1974, a large research program took place in the plague focus of Chapada do Araripe plateau in the State of Pernambuco, focusing on understanding the mechanisms of the persistence, focalization, epizootization, and epidemization of plague in Brazil (Karimi *et al*. 1974a,b, 1976; Petter 1999; Baltazard 2004).

Other series of data on rodents occurrence was obtained along the activities of the plague surveillance program. For several decades the rodents were trapped, mainly at the peridomestic environment, for detection of the plague bacillus and/or antiplague antibodies. From 1978–1984, laboratory analysis and data collection were performed by the National Plague Reference Service. The surveys among the rodents were discontinued in 2007 due to new evidences that serological survey of plague antibodies among stray dogs is an efficient and more cost effective tool for plague surveillance (Tavares *et al*. 2012; Souza *et al*. 2017).

The last rodent inventory was carried out between 2013–2019 in the most prominent historical plague areas to assess the effects of both climate and anthropogenic changes on the plague-associated rodents populations (Costa *et al*. 2017; Zeppelini *et al*. 2018).

Rodents occurrence and distribution in the plague areas

The above inventories revealed the occurrence of 30 species of rodents distributed through 7 families: Cricetidae, subfamily Sigmodontinae (15 species), Caviidae (03 species), Echimyidae (05 species), Erethizontidae (01 species), Dasyproctidae (01 species), Sciuridae (02 species), Muridae (03 species). Figure 2 shows the distribution of the rodent species by the Brazilian states.

In the literature, some species can have their names modified through time, for example, *Necromys lasiurus* was previously named as *Bolomys lasiurus* and *Zygodontomys lasiurus pixuna)* (Bonvicino *et al*. 2015). Also the species *Pseudoryzomys* spp., *Euryoryzomys* spp., and *Oecomys* spp. were formerly misidentified as *O. subflavus*. Therefore, among the specimens referred to as *O. subflavus*, some individuals might be from any one of three genera (Costa *et al*. 2017). The genus *Oryzomys* was split into 11 genera and *Cerradomys* was coined to accommodate the *O. subflavus* group (Weksler *et al*. 2006). In this review, we followed the nomenclature revised by Bonvicino *et al*. (2015). The current nomenclature of the 30 species, caught in the peridomestic and sylvatic ecotopes from all the Brazilian foci can be seen in Table 1.

The commensal rat (*R. rattus*) is omnipresent in all plague areas from all the Brazilian states; they are found both inside the houses and in the peridomestic ecotopes and they are relatively resistant to the *Y. pestis* infection (Karimi *et al*. 1974a; Butler *et al*. 1982; Coutinho *et al*. 1982). Regarding the wild-sylvatic species, some were regularly caught in all the inventories while others were found fortuitously through an eventual sampling in specific biotopes directed to that species (Fig. 2).

In the Northeast plague area, *Necromys lasiurus* is by far the most common wild rodent among the Cricetidae/Sigmodontinae in all states (47.2%), followed in frequency by the species of *Cerradomys* (9.9%), *Oligoryzomys* (3.9%), *Wiedomys* (2.8%), *Akodon* (2.6%), *Calomys* (2.4%), and *Nectomys* (1.8%). Other species (*Holochilus, Oxymycterus, Rhipidomys*) occurred in very low numbers (Fig. 2).

In the plague area of the state of Rio de Janeiro, *Akodon* (37.4%) is the most prevalent species, followed by *Oligoryzomys* (18.9%), *Oxymycterus* (13.9%), *Nectomys* (10.4%), *Thaptomys nigrita* (0.8%), and others. Of note, of *T. nigrita* occurred only in this specific plague area while *Necromys* was absent (Fig. 2). Differences in the rodent and flea faunas from the two areas can be imputed to environmental differences between these biomes

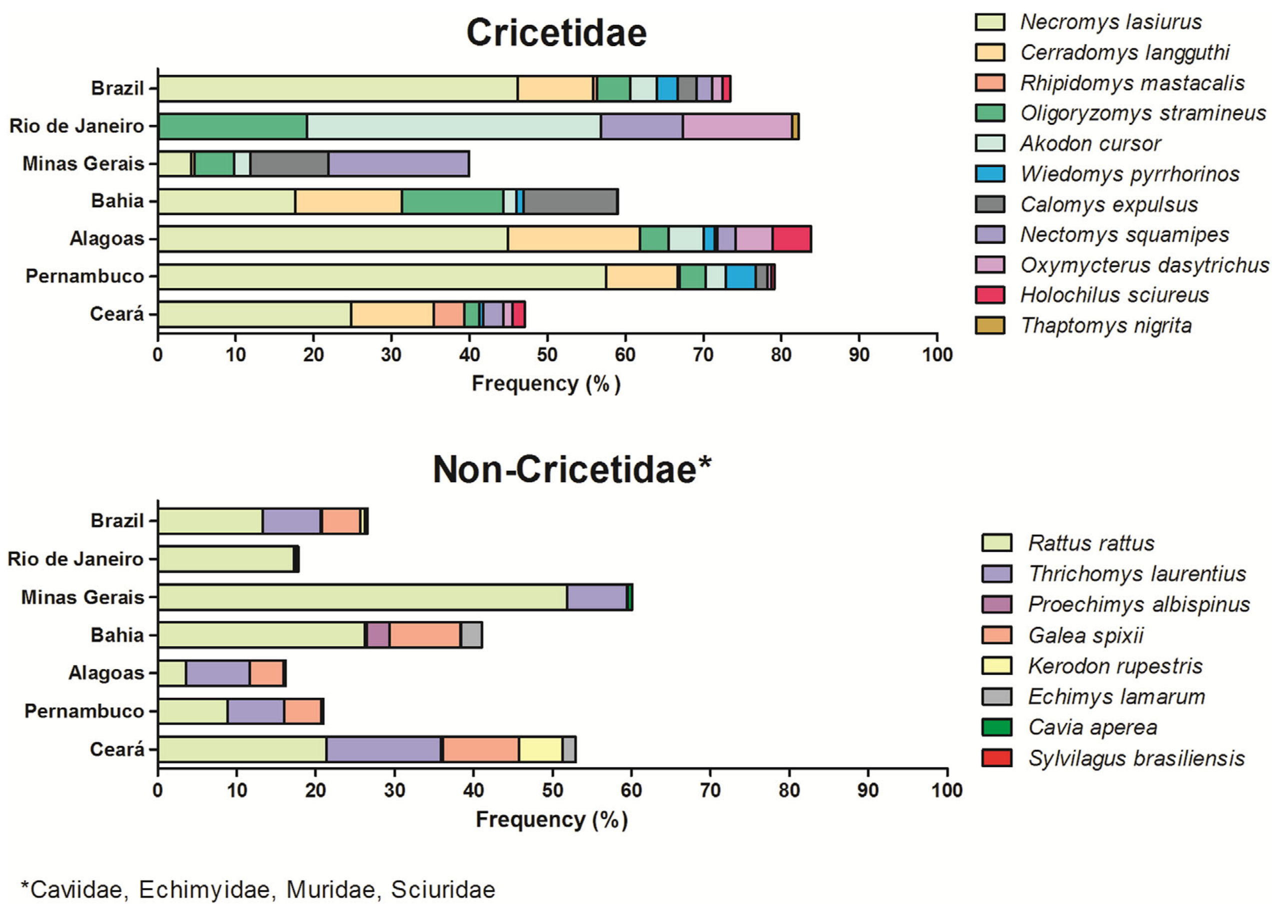

Figure 2 Distribution of the occurrence of the rodent species by Brazilian states. The bar labeled as Brazil represents the sum of the rodents from all the inventories in this review. Distinct species frequencies can be observed among the Rio de Janeiro (Southeastern) and the Northeastern foci; 176 × 130 mm (300 × 300 DPI).

(Fig. 1) and the primary or amplifier host is to be defined for the plague area of Rio de Janeiro.

It is noteworthy that some low prevalence species may eventually exhibit sudden and explosive pullulation or population growth named “ratadas.” These phenomena are correlated with an unusual availability of a specific food (Sobral & Oliveira 2014). Two of these episodes were well documented in the State of Bahia, when two species that usually constitute a small fraction of the rodent population inventory (*Wiedomys pyrrhorinos* and *Calomys callosus*) were the major protagonists of the “ratadas” in 2002 and 2015, respectively.

Plague hosts and associated increasing in human plague risk

From the 30 rodent species described in the plague areas, 13 were found naturally infected, harboring either active infection or antibodies against *Y. pestis* (Table 1). The proportions of each species in positive rodents captured by the surveillance services in Brazil are represented on Fig. 3. Moreover, in addition to the 13 species, 5 others were presumed to be susceptible to have natural infection in the wild (Pollitzer 1954; WHO 1965), but the proof of the infection is yet to be established. Finally, there are no references of natural plague infection among the remaining 12 species (Table 1).

Results from studies performed at the Chapada do Araripe focus (PE) establish the species *Necromys lasiurus* as the epizootic (amplifier) host, spreading the infection to other species, even the less susceptible, as the commensal rat, and eventually causing spill overs to human populations. *N. lasiurus* is the more frequently infected by plague (Fig. 3) and carrying infected fleas (*Polygenis* spp.). They are highly plague susceptible, prolific, and ubiquitous; they shelter usually in sites covered by

Table 1 Rodent species from the Brazilian plague areas, current nomenclature, and status of natural infection

Natural infection with *Yersinia pestis*		
Confirmed†	Presumed‡	Undetected§
Cricetidae	**Cricetidae**	**Cricetidae**
Akodon cursor	*Wiedomys pyrrhorinos*	*Delomys dorsalis*
Calomys expulsus	**Caviidae**	*Euryoryzomys* spp.
Cerradomys langguthi	*Cavia aperea*	*Oecomys* spp.
Holochilus sciureus	*Kerodon rupestres*	*Pseudoryzomys simplex*
Necromys lasiurus	**Echimyidae**	*Rhipidomys mastacalis*
Nectomys squamipes	*Echimys lamarum*	*Thaptomys nigrita*
Oligoryzomys stramineus	**Sciuridae**	**Echimyidae**
Oxymycterus dasytrichus	*Sylvilagus brasiliensis*	*Euryzygomatomys spinosus*
Caviidae	**Muridae**	*Phyllomys* sp.
Galea spixii	*Rattus norvegicus*	*Proechimys albispinus*
Echimyidae	*Mus musculus*	**Sciuridae**
Thrichomys laurentius		*Guerlinguetus* sp.
Muridae		**Erethizontidae**
Rattus rattus		*Coendou prehensilis*
		Dasyproctidae
		Dasyprocta prymnolopha

†The species in which either active infection or plague antibodies were detected. ‡Natural infection was not laboratory proved. §Without register of natural infection.

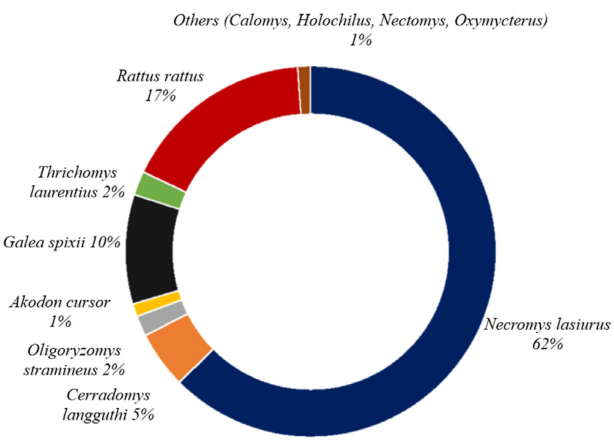

Figure 3 Species of rodents found naturally infected with *Yersinia pestis*. The values represent the proportion of each species in all either active infection or plague antibodies were detected; 158 × 130 mm (150 × 150 DPI).

a low and dense vegetation and in certain climatic conditions; they dig burrows. Their dispersal area overlaps the location of human cases. Therefore, the growth of *Necromys* population and the rise of its flea index (the ratio between fleas—the vector and rodents—the host) is acknowledged as a warning signal of the plague threat. Hence, permanent monitoring of the *Necromys* populations is recommended for the plague surveillance (Karimi *et al*. 1974a,b, 1976; Almeida *et al*. 1981; Baltazard 2004).

Factors of the persistence of the plague during quiescence

Several hypotheses have been proposed to explain the persistence of the plague bacillus during periods of quiescence: the traditional idea of rodent assemblage being the reservoir on the wild; survival of infected fleas in the burrows of the plague dead rodents; species or populations capable of developing a chronic infection (granuloma-like lesions) and infect vectors continuously (Gage 2012; Zeppelini *et al*. 2016).

These hypotheses can account for the conservation of the bacillus during short inter-epizootic periods but they do not explain the decade-long silent periods that sometimes occur between two epidemics.

Other hypotheses can better explain this phenomenon: The maintenance of the bacillus could take place inside the rodent burrows, where the microclimate would allow its long-term survival on the carcasses of rodents, in the corpses of fleas, or in the bedding of dead animals or even in soil protozoa inhabitants. The epizootic would rekindle by reoccupation of the infected burrows by new rodents which would be contaminated by digging in these sites (fossorial and telluric mechanism) (Baltazard 2004; Drancourt *et al*. 2006).

Another possible explanation is that the infection could be maintained at the enzootic state in rodents and their fleas: After the term of the epizootic by depletion of susceptible hosts, the infection would remain in a chronic form in resistant populations. According to Karimi *et al.* (1974a, 1976), susceptible rodents are those whose mortality is almost 100% during an epizootic and resistant species are those in which at least 30% of animals survive the epizootic. Occasionally, the animals' resistance would be put at fault (aging, stress, famines, overcrowding). This failure could lead to a sepsis in a few animals from which fleas become infected. The fleas would be able to keep the bacilli until they meet new hosts. The transmission cycle would thus be kept at a low rate: there would be a long period of conservation of the bacillus in the rodent's organism and in the flea until the density of the populations of susceptible animals promotes transmission to an epizootic frequency (Poland & Barnes 1979; Gage 2012).

An attractive hypothesis for the long-term conservation of the *Y. pestis* in the Northeast foci was the permanence of the plague bacterium among the high populations of *Trichomys apereoides* (Echimyidae) and *Galea spixii* (Caviidae). These species find shelter into supposedly permanent habitats such as chinks and crevices of rocks. *T. apereoides* is highly plague-susceptible and could harbor, carry, and disseminate infected fleas. The *Galea* is plague-resistant and could maintain viable bacteria encapsulated into micro abscesses (Karimi *et al.* 1974a; Coutinho *et al*. 1982).

On the other hand, Petter (1999) assumed that the commensal *R. rattus* might play a role in maintaining the enzootic cycle. In the rural area they live either inside the houses where they make their nests in holes in the ground or walls and dig burrows on the soil, and in the peridomestic areas (Karimi *et al*. 1976). Moreover, they also could maintain viable bacteria encapsulated into micro abscesses (Butler *et al*. 1982; Coutinho *et al*. 1982), allowing periodic flea re-infection and consequently, reactivation of the epizootic cycle. In spite of the studies the enzootic (maintenance) plague hosts in the Brazilian plague foci is not yet defined nor the maintenance mechanism.

Studies about the fossorial and telluric plague

Several studies were performed to assess the hypothesis of conservation of the *Y. pestis* on the soil and the fossorial and telluric plague (Baltazard 2004; Drancourt *et al*. 2006). In our experiments, the plague bacillus was maintained successfully on sterile soil during 12 months into tubes tightly closed and buried into different environments in different conditions and analyzed at different time points. The fossorial ability and contamination with infected soil while digging was studied in experimental terrariums build with *Rattus rattus, Necromys, Thrichomys*, and *Kerodon* (Karimi *et al*. 1976; Almeida *et al*. 1981; Baltazard 2004). After the animals settled into the terrariums, laboratory infected fleas (*Xenopsylla* or *Polygenis*) were added; the terrariums were sealed and inspected afterward by introduction of naïve detectors and examination of samples of the soil and littering from the burrows. Furthermore, samples from soil and littering from the burrows from the fields were collected during the epizootic period and analyzed for *Y. pestis* detection (Karimi *et al*. 1976; Baltazard 2004). These experiments did not support the hypothesis and the plague fossorial and telluric was not demonstrated in Brazil.

A bias in these studies is that only traditional bacteriological techniques (culture and inoculation of laboratory animals) were then employed for the detection of the *Y. pestis* from the burrow or terrarium sample remains. The molecular biology techniques later introduced into the plague program were not available (Leal & Almeida 1999; Melo *et al*. 2003; Chioratto *et al*. 2007).

Rodent flea ectoparasites and role in plague transmission

The rodents collected through the several inventories harbored a total of 14 species from the families *Ctenophthalmidae* (01), *Pulicidae* (03), *Rhopalopsyllidae* (09), and *Stephanocircidae* (01). The same *Ctenophthalmidae* and *Pulicidae* species occurred in both plague areas (Northeast and Southeastern foci), but the *Stephanocircidae* only in the southeastern area. Different *Rhopalopsyllidae* species occurred in each area and one (*Polygenis tripus*) in both (Guimarães 1972; Brasil *et al*. 1989; Carvalho *et al*. 2001). The species of fleas and area of occurrence are given in Table 2.

Xenopsylla cheopis, the so-called rat flea and historically considered the classic plague vector, was prevalent

Table 2 Main flea species found in the plague foci in Northeast and Serra dos Órgãos (RJ), Brazil

Family/species	Northeastern foci	Serra dos Orgãos (RJ) focus
Stephanocircidae		
Craneopsylla minerva		X
Ctenophthalmidae		
Adoratopsylla antiquorum	**X**	X
Rhopalopsyllidae		
Polygenis atopus		**X**
Polygenis pygaerus		**X**
Polygenis pradoi		**X**
Polygenis rimatus		**X**
Polygenis roberti		**X**
Polygenis roberti roberti	**X**	
Polygenis bohlsi bohlsi	**X**	
Polygenis tripus	**X**†	**X**
Polygenis bohlsi jordani	**X**†	
Pulicidae		
Pulex irritans	**X**†	**X**
Xenopsylla cheopis	**X**†	**X**
Ctenocephalides felis	**X**†	**X**

†Naturally infected with *Yersinia pestis*.

among the commensal rats (*R. rattus*). It can also be found among the wild rodents but in very small number.

The vector capacity and the potential role of *Polygenis* spp. in the genesis of human plague was subject of debate (WHO 1965; Baltazard 2004). The species of *Polygenis* were considered inefficient vectors by the current notion that transmission of *Y. pestis* by blocked fleas represents the primary, if not almost exclusive, model by which flea-borne plague transmission occurs. This concept is often referred to as the proventricular blockage model or classical transmission model. Therefore, transmission by unblocked fleas generally was assumed to be minimal and relatively unimportant compared to transmission by blocked fleas (Gage 2012). Currently, other mechanisms of transmission are recognized such as mechanical transmission (mass transmission) by large number of flea bites and early phase transmission (EPT) or transmission by unblocked fleas during the first few days after becoming infected and before a complete blockage can form (Eisen & Gage 2011).

Studies on the plague transmission by *Polygenis bohlsi jordani* and *Polygenis tripus* using flea colonies raised in the laboratory (Baltazard & Eftekhari 1957) proved that they are efficient plague vectors and they might play important roles in the spread of plague during epizootics in the Northeast foci (Karimi *et al*. 1974b; Baltazard 2004).

As for *P. b. jordani*, the studies revealed that they survive for 30 days after a septicemic meal; one single specimen could transmit the infection to susceptible rodents; they are able to bite and to feed on humans (Karimi *et al*. 1974b). Therefore, besides the ability to transmit the bacterium among the rodents they also could transmit the infection from the rodents to the humans. The analysis of the occurrences during the epidemic period suggested that they could answer for numerous human infections. Therefore, they were considered the most effective vector on the northeast foci and their indices an efficient alarm signal of plague activities in the nature (Baltazard 2004).

In the foci of the Northeast, *P. bohlsi jordani* and *P. tripus* are the predominant species regarding the frequency among the wild rodents, while in the Serra dos Orgãos, the predominant species are *P. rimatus* and *P. pradoi* (Guimarães 1972; Karimi *et al*. 1974b; Brasil *et al*. 1989). The vector ability of these species remains unknown, as well as the role if any, of *P. tripus* on this focus.

CONCLUSION

Until the 1970s, Brazil experienced recurrent cycles of plague activity interspersed with 5 to 10 years of quiescent periods and geographic dispersion. However, some of the most relevant plague areas from Brazil had no reports of human cases for more than 50 years and tend to quiescence ever since. The absence of new cases must be accurately assessed, as there are no fair explanations. Considering the analysis of its secular tendency, this could be just a long-term cyclical phenomenon. Since 1986, the presence of *Y. pestis* bacterium has not been detected among the rodents or their fleas and serological surveillance of sentinel animals shows a decreasing trend. Of note, the amount of sentinel animals analyzed by serosurveys reduces every year, and declining rodent populations and flea index was also observed and no rodent die offs reported.

This panorama requires constant attention for eventual fluctuations on the rodent population as well as its ectoparasites that may be a signal of plague activity. Several hypotheses have been put forward to explain the persistence of the plague bacillus during periods of quiescence and the causes for re-emergence. An eventual reappearance of plague activities could derive from social degradation, climate changes, expansion of rodents' population, and epizootization.

In summary, the dynamics of plague in Brazil and in other countries where the disease was introduced during the third pandemic are quite alike, alternating from epidemics to decades of quiescence. It is, however, of most importance to study the local population of reservoirs and competent vectors to comprehend how these species are affected by *Y. pestis* and how they can cause spill over to humans. Combined with continuous serologic surveys, these data provide key information for proper plague control in the given region. It is important to highlight that the absence of human cases nowadays in Brazil does not mean that it is eradicated and plague remains an important epidemic disease of global concern. The existence of a large animal reservoir and competent vectors demonstrate a need for continuous surveillance to prevent new outbreaks of this disease in humans.

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APÊNDICE C - SPATIAL AND TEMPORAL DISTRIBUTION OF RODENTS DURING THE EPIZOOTIC AND ENZOOTIC PERIODS OF PLAGUE, WITH A FOCUS ON EXU, NORTHEASTERN BRAZIL

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Este trabalho analisa a ocorrência e a distribuição geográfica dos roedores que participam do ciclo da peste no município de Exu no período de 1966 a 2005. Importante flutuação nas populações de roedores foi observada com redução da fauna de roedores silvestres após o fim de um período de epidemia de peste, representada principalmente por *Necromys lasiurus* e aumento da espécie comensal *Rattus rattus*. Os resultados confirmam a espécie *Necromys lasiurus* como responsável por epidemias de peste nesta área focal de transmissão no nordeste do Brasil, uma vez que a redução na abundância desta espécie ao longo do tempo coincide com o período de quiescência da doença. Além disso, o aumento da abundância de *R. rattus* está diretamente relacionado ao processo de urbanização de pequenas localidades rurais. Apesar de sua abundância, eles não provocaram epidemias de peste como era de se esperar, especialmente considerando sua proximidade com os humanos. Uma maior abundância de ratos pode levar a uma maior exposição das populações humanas, favorecendo a propagação da peste e outras doenças transmitidas por roedores. Nossa análise contribuiu para confirmar o papel das espécies de roedores silvestres como hospedeiros amplificadores e dos ratos comensais (*Rattus rattus*) como hospedeiros preservadores no período quiescente naquela área de infecção de transmissão.

Tropical Medicine and Infectious Disease

Article

Spatial and Temporal Distribution of Rodents during the Epizootic and Enzootic Periods of Plague, with a Focus on Exu, Northeastern Brazil

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Abstract: The plague caused by the *Yersinia pestis* bacterium is primarily a flea-transmitted zoonosis of rodents that can also be conveyed to humans and other mammals. In this work, we analyzed the spatial and temporal distribution of rodent populations during epizootic and enzootic periods of the plague in the municipality of Exu, northeastern Brazil. The geospatial analyses showed that all the rodent species appeared through the whole territory of the municipality, with different occurrence hotspots for the different species. Important fluctuations in the rodent populations were observed, with a reduction in the wild rodent fauna following the end of a plague epizootic period, mostly represented by *Necromys lasiurus* and an increase in the commensal species *Rattus rattus*. A higher abundance of rats might lead to an increased exposure of human populations, favoring spillovers of plague and other rodent-borne diseases. Our analysis highlights the role of wild rodent species as amplifier hosts and of commensal rats (*R. rattus*) as preserver hosts in the enzootic period of a specific transmission infection area.

Keywords: Rodentia; plague; *Yersinia pestis*; zoonoses; disease reservoirs

1. Introduction

The plague caused by the *Yersinia pestis* bacterium is primarily a flea-transmitted zoonosis of rodents, the main hosts, that can also be conveyed to humans and other mammals [1]. Rodents constitute the most diverse order (Rodentia) of mammals, with almost 2600 species, representing 40% of the living mammal species [2]. Out of these, 279 species have already been found to be naturally infected by *Y. pestis* [3].

The plague caused three worldwide pandemics in the Christian era, claiming numerous lives, having a major impact on the course of our history, scientific development and culture [4,5]. The infection reached Brazil by sea in 1899, during the third pandemic, through the port of Santos, São Paulo state. The infection initially afflicted the brown rat population of *Rattus norvegicus* in seaports and the commensal species (*Rattus rattus*) in the rural zones of the Northeast Finally, it encountered susceptible autochthonous wild or sylvatic fauna and established several natural foci where the ecological conditions were suitable for its persistence [6,7]. These foci persisted until the present day, spreading through several mountain ranges and plateaus across the states of Ceará, Piauí, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Bahia, Minas Gerais and Rio de Janeiro [8,9].

By analyzing the records of human plague in the Brazilian plague foci, the municipality of Exu located in the Pernambuco State, Northern Brazil, was considered the epicenter of the focal area of Chapada do Araripe [10]. Based on the concepts of a natural-permanent focus and the telluric conservation of the plague bacillus inside the rodents' burrows, Baltazard [10] hypothesized that the plague activity would persist for longer there and reduce gradually to basal, undetectable levels, until reappearing in the same regions. Indeed, the Kernel density analysis (KDE) of the number of cases reported in Pernambuco revealed that the municipality of Exu is at higher risk for the occurrence of plague. Exu appeared at the epicenter of the Kernel patch, which radiates in decreasing intensity as it moves away from the plateau slope towards the plains and neighboring municipalities [11].

The studies on the Rodentia and Siphonaptera faunas have become an important part of the plague control program activities and several field and laboratory studies have been carried out to understand the possible role of the different rodent and flea species in the maintenance, epizootization, and epidemization of plague in the Brazilian focal areas. It is worth noting that an important part of this work was the continuous trapping of rodents to detect plague activity among their wild species, especially *Necromys lasiurus* [10,12–17].

Here, we analyzed the spatial and temporal distribution of rodent populations in the municipality of Exu, northeastern Brazil, from 1966 to 2005, during epizootic and enzootic periods of plague in the region.

2. Materials and Methods

2.1. Study Area

The study was performed in the municipality of Exu (Figure 1C), State of Pernambuco (Figure 1B), Northeast Brazil (Figure 1A). This municipality lies in the mesoregion of *Sertão*; it encompasses an area of 1,336,788 km^2, contains an estimated population of 31,825 inhabitants (according to data from 2019) and scored 0.576 on the Municipal Human Development Index (2010). Its climate is warm and dry, with scarce and irregular rainfall (Biome *Caatinga*). Situated in the ecological complex of Chapada do Araripe, 600–700 m in altitude, about 200 km long and 30 km wide, it is bordered by the municipalities of Bodocó to the west, Granito to the south, Moreilândia to the east and to the north with Crato in the state of Ceará.

2.2. Data Collection

The data on the rodent collection was obtained by consulting the original documents available at the Nacional Reference Service of Plague (*Serviço de Referencia Nacional de Peste: SRP*) from the Institute Aggeu Magalhães (IAM), FIOCRUZ PE, located in Recife, PE, Brazil.

The collection of rodents and fleas was performed in order to follow the *Y. pestis* circulation in the focus area over the years. The animal capture and handling methods varied according to the recommendations in each period, and further details can be found in the original publications. In short, the rodent and flea collection was carried out overnight, using rodent live traps (Chauvancy, Tomahawk, and Sherman); the trapped animals were brought to a field processing site for the collection of ectoparasites, sexing, and identification to species or genus, then they were either kept in quarantine until death or euthanized [18–23]. In the period between 1966 and 1995, only the data about the number of animals collected per year in the municipality was available, without specifying the locality. From 1996 onwards, the localities of the collections became available. The localities were georeferenced in loco, with a GPS (Global Positioning System) receptor, model eTrex Vista Cx, Garmin (Kansas City, MO, USA), configured in a DatumWGS-84. A Landmark (house, church or gate) was standardized in order to georeference each of the localities.

For geospatial analyses, the vector data obtained were the municipal limits of Exu (2010) from the Brazilian Institute of Geography and Statistics (IBGE) [24]. The drainage (hydrography) was from the Mineral Resources Research Company (CPRM) [25]—*Instituto Nacional de Pesquisas Espaciais* (INPE). The Digital Elevation Model (DEM) data was obtained from the Shuttle Radar Topography Mission (SRTM) using the script on the Google Earth Engine (GEE) platform [26]. All the geospatial data were obtained from free access and use platforms.

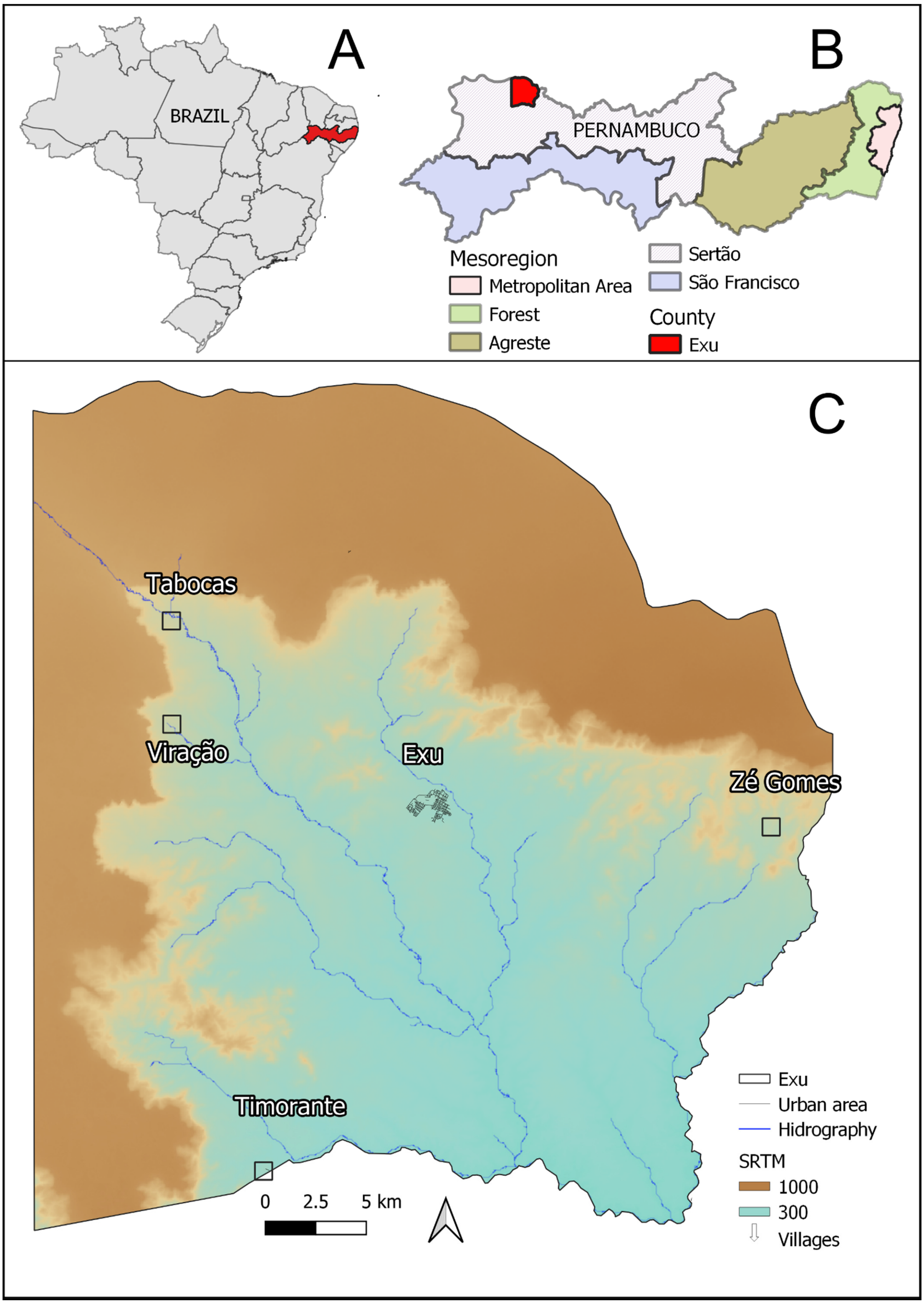

Figure 1. Identification of the study area: Exu, Pernambuco-Brazil. (**A**) Map of Brazil showing the state of Pernambuco highlighted in red. (**B**) Map of Pernambuco showing the mesoregions and the municipality of Exu highlighted in red. (**C**) Map of the municipality of Exu with hydrography and altitude (m), the urban area of Exu city (in the center) surrounded by other smaller rural settings the villages: Tabocas, Viração, Timorante and Zé Gomes. The shapefile of Exu was obtained from IBGE, the DEM from SRTM available online: http://www.dsr.inpe.br/topodata/ (accessed on 1 April 2021) and the hydrography from CPRM. These images are used for illustrative purposes only.

2.3. Data Analysis

The rodent species, the locality and the year of their collection were compiled and organized into a database (DB) using Excel software. While the analysis demonstrating the fluctuation of the rodent species from 1966–2005 comprised all the samples in the DB (Figure 2, n = 66,700), only the subset with data available on the location of the captures were included in the spatial analysis (Figures 3 and 4, n = 3724).

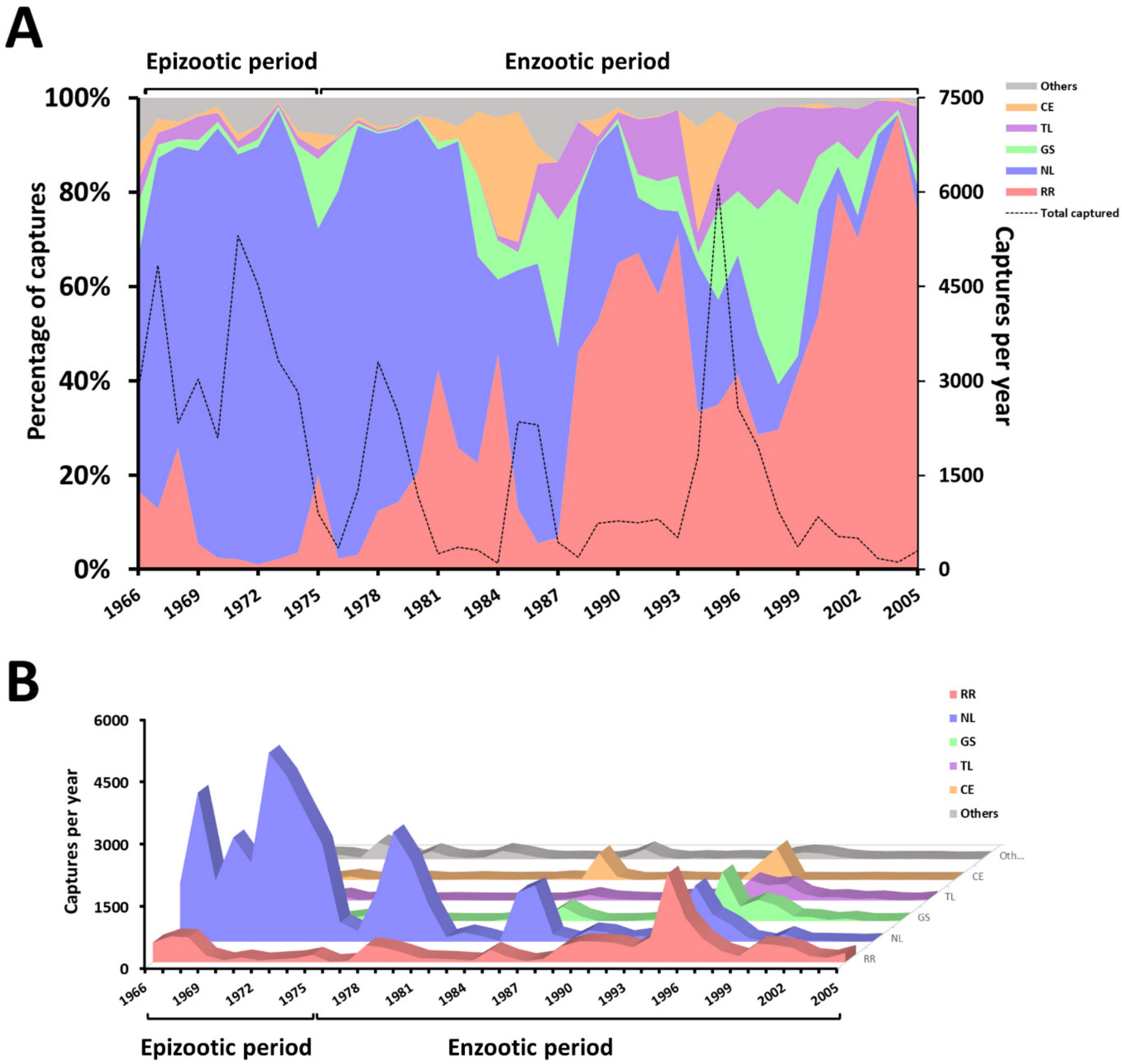

Figure 2. Abundance of rodents captured in Exu, Pernambuco, Brazil, 1966 to 2005. (**A**) The left axis displays the fluctuations in the proportion of captured rodent species as percentages, while the right axis shows the total number of captured animals per year. (**B**) The absolute number of captured animals per year according to the species.

The GPS data was transferred to a GPS TrackMaker Pro 4.9.603 (Geo Studio Technology, Belo Horizonte, Brazil) and the geographic coordinates were organized and stored in comma-separated values (CSV) and the shapefile format, which was then used to create the spatial database (SDB).

The spatial analyses performed were: (1) a map of the spatial distribution and abundance of the rodents to spatially visualize the localities and the number of animals collected in each locality (*sitio*, farm, village) of the municipality of Exu (choropleth maps); (2) Kernel density estimation (KDE) to identify the localization of clusters of animal occurrences. For the KDE, the following parameters were used: the bilinear interpolation method; the data classification method 'Natural breaks (Jenks)', with nine classes; grid cell size (bandwidth method), defined using an adaptive radius—as it is more applicable to the use of data from animals with different dispersion radii—with the area unit defined in m^2. The choroplethic maps of the localization and density of the rodents collected were produced by the software Qgis Desktop 3.16.5 [27]. The Kernel maps built with ArcGIS 10 [28].

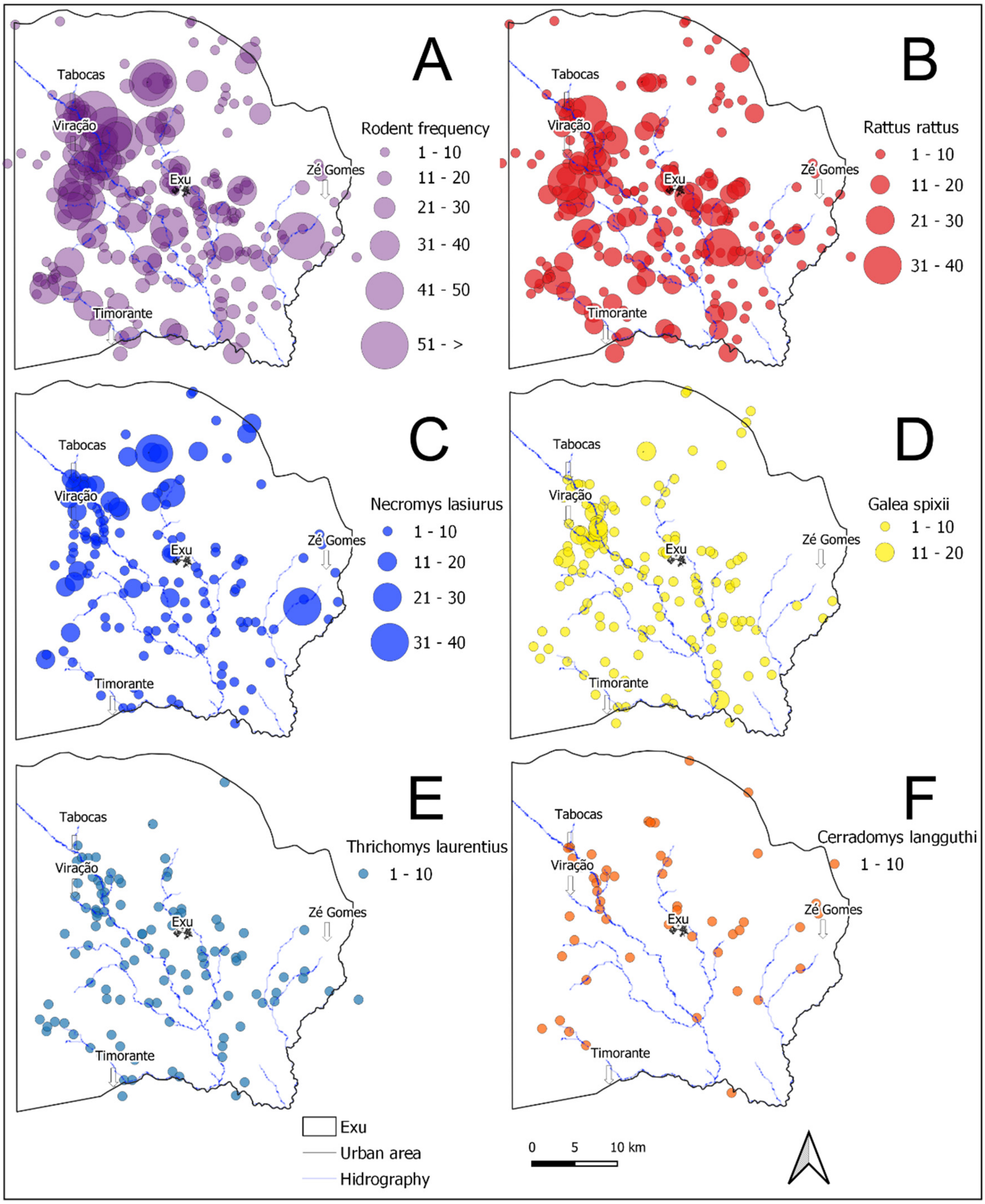

Figure 3. Distribution and abundance maps of the rodent species captured in the localities of Exu, Pernambuco, Brazil, 1996 to 2005. (**A**) Total rodents captured. (**B**) *R. rattus*. (**C**) *N. lasiurus*. (**D**) *Galea spixii*. (**E**) *T. laurentius*. (**F**) *C. langguthi*.

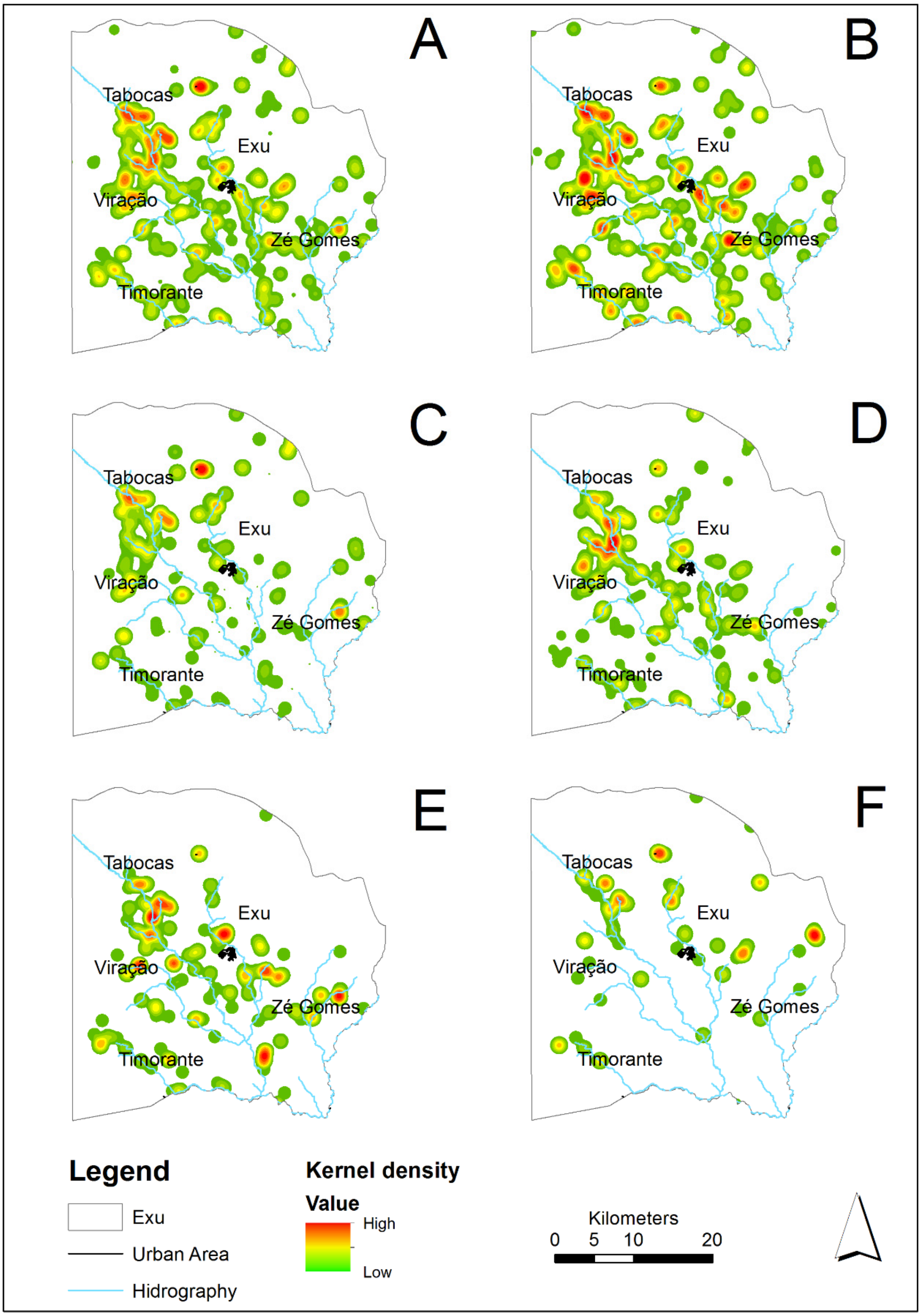

Figure 4. Risk maps for occurrence and density of rodent species based on the Kernel density estimator, in the localities of Exu, Pernambuco, Brazil, 1996 to 2005. (**A**) Total rodents captured. (**B**) *R. rattus*. (**C**) *N. lasiurus*. (**D**) *Galea spixii*. (**E**) *T. laurentius*. (**F**) *C. langguthi*.

3. Results

3.1. Fluctuation in the Abundance of Rodent Species from 1966 to 2005

Through long-term monitoring (1966–2005) of the plague activities in the municipality of Exu (Figure 1A–C), 66,700 rodents from eight species were captured: *Necromys lasiurus* (=39,797), *Rattus rattus* (=13,132) *Galea spixii* (=4581), *Thrichomys laurentius* (=3195), *Calomys expulsus* (=2696), *Cerradomys langguthi* (=2481), *Oligoryzomys nigripes* (=680) and *Wiedomys pyrrhorhinos* (=138). Figure 2 shows the fluctuation in the abundance of rodent species in percentage (2A) and absolute values (2B) per year, over the 40-year' period between1966 and 2005.

The species *N. lasiurus* and *R. rattus* were the most abundant throughout the study period (1966 to 2005). Until 1987, the rodent *N. lasiurus* was the predominant species (≅40 to 97% of the catches) but from 1988 onwards the rat (*R. rattus*) became predominant (≅28 to 96% of the catches), while the number of *N. lasiurus* decreased to 0–37% of the catches. The species *W. pyrrhorhinos* and *C. expulsus* occurred constantly in basal numbers and from 1990 onwards, no *O. nigripes* were captured (Figure 2A,B).

Due mostly to the reduction in the *N. lasiurus* population, there was a substantial decline in the overall number of captured animals between 1966 and 1981. However, population spikes were observed during the intercalated periods of 1985–1986 and 1994–1997. Notably, with the exception of *W. pyrrhorhinos* and *O. nigripes*, there was an increase in the total number of most species captured from 1994 to 1997 (Figure 2A,B; Supplementary Table S1).

3.2. Spatial Distribution of the Rodents' Populations in the Period Analyzed (1996–2005)

Regarding the geographical distribution in the period analyzed (analysis limited to 1996–2005), all the species occurred in the same *sitios* or farms scattered through the whole territory of the municipality (Figures 3A and 4A). Figure 3B–F and Figure 4B–F show the spatial distribution and frequency hotspots of the species *R. rattus*, *N. lasiurus*, *G. spixii*, *T. laurentius* and *C. langguthi* from 1996 to 2005. Due to the small quantity in this period, the species *C. expulsus* (=15), *W. pyrrhorhinos* (=23) and *O. nigripes* (=0) were not included in the maps.

The *R. rattus*, the most abundant species found during the period (1966–2005), was widely disseminated throughout the territory and occupied a higher number of localities (Figure 3B). However, the areas with the highest density and considered hotspots for the occurrence of this species were in the boundaries of the villages Tabocas, Viração, Timorante and Zé Gomes (Figure 4B). *N. lasiurus* was also found throughout the territory and presented several hotspots near the villages Tabocas and Viração, as well as aa hotspot near the village of Zé Gomes (Figure 4C). The relatively abundant population of *G. spixii* was also disseminated throughout the territory and presented hotspots in the boundaries of the villages Tabocas and Viração, in the southern part of the municipality, as well as another hotspot on the plateau of the Chapada do Araripe (Figure 4D). The *T. laurentius* hotspots occurred in the boundaries of the villages Tabocas and Viração and of the city of Exu and others in the southeast of the municipality (Figure 4E). *C. langguthi*, the least numerous species and with the lowest dispersion (Figure 4F), presented a distribution of hotspots different from the others occurring along the slope of the Chapada do Araripe and in the boundaries of the village Zé Gomes.

Importantly, marked differences were observed in captures from traps set at household or field environments. The proportion of traps set in fields or household environments was standardized in 3:1, respectively. While the proportions of *N. lasiurus* and *R. rattus* in field captures were 44% and 6.5%, respectively, 99% of household captures were *R. rattus* and no *N. lasiurus* were found in this environment (Figure 5).

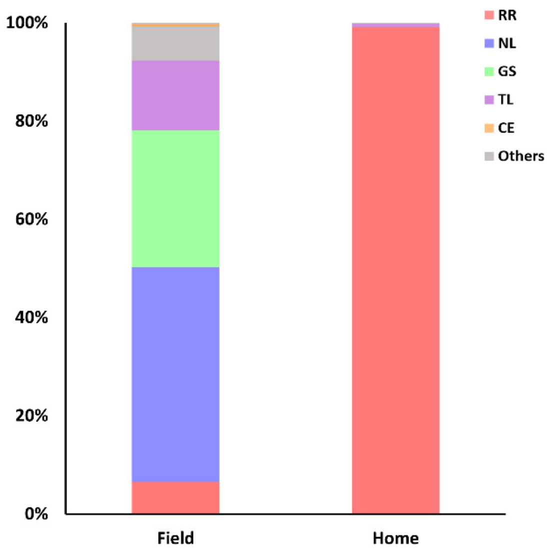

Figure 5. Proportion of species captured in field or household environments.

4. Discussion

Practically since the arrival of the plague in Brazil in 1899, during the third pandemic, a surveillance and control program adjusted to the epidemiological situation, ecological and demographic characteristics and scientific and technological conditions has been carried out [7,10,17]. For several decades, the rodents were trapped for the detection of the plague bacillus and/or anti-plague antibodies [22,23,29]. The surveys among the rodents were discontinued in 2007 due to new evidence that the serological survey of plague antibodies among roaming dogs is a more efficient and cost-effective tool for plague surveillance [17,20]. By compiling the data from 40 years (1966 to 2005) of monitoring in the plague focus region of Chapada do Araripe, we were able to observe an important fluctuation in the number of captured rodents (Figure 2A,B). It is important to highlight that while the period that saw the predominance of *N. lasiurus* comprises the years in which human cases of plague were noted in the region (1966–1976), the period that saw the predominance of *R. rattus* overlapped with the enzootic period of plague [17].

Rodent populations are known to undergo significant fluctuations over both seasonal and multiannual cycles, which also impacts on the risk of zoonosis spillovers to humans [30,31]. Here, we observed four-to-seven-year intervals in the pendular *N. lasiurus* population spikes. However, from the last years of human cases of plague onwards, their abundance peaks progressively decreased both in frequency and abundance. From the 1995 peak until the end of the study period (2005), no *N. lasiurus* population growths were observed. The decline of these populations might have been due to the important and continuous plague deaths of susceptible species over many years, climate change and environmental alterations created by agriculture [9,17,32].

From 1996 to 2005, no *O. nigripes* were observed and *C. expulsus* and *W. pyrrhorhinos* were captured in small numbers (Figure 2A,B). The reduction or disappearance of these species does not qualify them as endangered species at risk of extinction because this is only a local event [30,33]. It is noteworthy that some species may multiply suddenly and explosively, a phenomenon popularly known as "ratadas". This phenomenon is generally correlated with an unusual availability of specific food that occurred in the State of Bahia, involving the species *W. pyrrhorinos* in 2002 and *C. expulsus* in 2015 [34].

As observed in Figures 3A and 4A, all the species appeared in the whole territory of the municipality. The wild species lived off agricultural products, which they consumed in situ. Although occupying the same places (*sitios* or farms) dispersed throughout the territory, the different species did not occupy the same habitats. The species *N. lasiurus*, *C. expulsus*, *C. langguthi*, *O. nigripes* and *W pyrrhorhinos* usually shelter in sites covered by low and dense vegetation, where they make their nests. Besides, *C. langguthi*, *O. nigripes* and

W. pyrrhorhinos can make nests in small trees or rock walls. Others (*G. spixii*, *T. laurentius*) shelter in the cracks and crevices of rocks, further away from humans [10,34]. Along with field observations, these results are not suggestive of attraction or avoidance patterns, with implications for competitive relationships and plague transmission among these species [35].

The main economic activity in practically all the rural land of the municipality of Exu is dedicated to agriculture practiced in the "*sitios*", which are mainly located along the hydrographic network on the slopes of the Chapada do Araripe (seen in the satellite image in Figure 1C), where remnants of native vegetation (*caatinga*) are also found. The term "*sitio*" means a rural land division usually including housing, functional buildings (barns, garages, storage areas) and a parcel for cultivating and/or raising stock. The human dwellings are generally unpaved or cemented or composed of brick floors, clay or brick walls and a roof of tile, zinc, grass or straw. They are often used as both housing and storage for crop products (maize, beans and cotton grains). Unlike the *R. rattus*, wild rodents rarely enter these dwellings.

The urbanization of some rural communities living with precarious sanitary infrastructure has created the ideal conditions for the expansion of the commensal rat [36]. Therefore, *R. rattus* were more abundant inside household captures (Figure 5) or in the boundaries of the villages Tabocas, Viração, Timorante and Zé Gomes.

The high abundance of rats in these villages might lead to more contact between them and the inhabitants, favoring plague and other rodent-borne diseases [37]. Therefore, some preventive measures should be implemented in these villages, including surveillance and rodent and insect control [20]. Commensal rat (*R. rattus*) control includes educative measures for proper grain storage; eliminating rats by clearing the land around houses, thereby making the environment unsuitable for them; and rat extermination, using rodenticides [7,20]. Flea control was carried out using the insecticides DDT (Dichlorodiphenyltrichloroethane) and BHC (Benzene hexachloride), which unfortunately led to the selection of resistance, by continuous pressure, of rat fleas (*Xenopsylla cheopis*) and human fleas (*Pulex irritans*) explaining the ineffectiveness of preventive measures based on the continuous use of these insecticides [10,11,16,34].

In a previous study in this same plague area, the transition of the infection from urban to rural areas was observed [11]. The plague reappeared in rural areas after a six-year inter-epizootic period and disseminated among the wild fauna practically throughout the municipality territory. According to Figure 3A–F, the dispersal area of the rodents in the present study overlapped with the sites of the distribution of the human cases shown by Fernandes et al. [11].

The plague disappeared suddenly in this focus area from 1975 [11]. This may have been associated with the rarefaction of the susceptible species, mainly the population of *N. lasiurus*, which is considered the amplifier host. In spite of the increase in the *R. rattus* population during the 1990′s, plague activity was no longer detected in rodents or humans, as *Y. pestis* bacterium was last isolated in 1987, and serologic testing for anti-plague antibodies in sentinel animals has declined over time [17].

Rats are relatively resistant to fatal plague infection and do not suffer from major death rates that could lead to epizootization in the absence of susceptible species [10,15,38,39]. On the other hand, the *R. rattus* might act as a preserver host by keeping plague dormant until eventual flea re-infection reactivates the epizootic cycle after the restoration of susceptible wild hosts populations [3,32,40].

The *X. cheopis* is the prevalent flea among *R. rattus* that may harbor wild rodent fleas, but in small numbers [16]. The wild rodent species primarily harbor two species of fleas (*Polygenis bohlsi jordani* and *Polygenis tripus*) that likely play an important role in plague spread in the ecosystem and the occurrence of human cases. Previous results established the species *N. lasiurus* and its fleas as the epizootic amplifier hosts, spreading the infection to other species, even less susceptible ones, such as the commensal rat, eventually causing spillovers to human populations [10].

5. Conclusions

The data presented in this study highlight that *N. lasiurus* might be responsible for plague epidemics in this focal area of transmission in northeastern Brazil, since the reduction in the abundance of this species over time coincided with the enzootic period of the disease. Furthermore, the increase in the abundance of *R. rattus* is directly related to the urbanization of small rural localities. In spite of their abundance, the rats did not drive plague epidemics as might be expected, especially considering their proximity to humans. As the plague infection cycle can reactivate after several years of epidemiological silence, an enzootic period must not be misinterpreted as the extinction of a plague focus [1,5,32]. Therefore, continuous surveillance is required and preventive measures focused on driving rodents away from houses, along with protection against flea bites, should not be overlooked.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/tropicalmed6040195/s1, Table S1. Rodents captured in Exu, Pernambuco, Brazil, 1966 to 2005.

Author Contributions: Conceptualization, D.L.R.d.S.F. and E.C.d.S.G.; methodology, D.L.R.d.S.F.; M.F.B. and E.C.d.S.G.; formal Analysis, D.L.R.d.S.F.; M.F.B. and A.M.P.d.A.; investigation, B.M.D., M.P.d.F.S., H.d.A.S.; data curation, B.M.D., M.P.d.F.S., H.d.A.S.; writing—original draft preparation, D.L.R.d.S.F. and E.C.d.S.G.; writing—review and editing, M.F.B., E.C.d.S.G. and A.M.P.d.A.; supervision, E.C.d.S.G. and A.M.P.d.A.; project administration, E.C.d.S.G. and A.M.P.d.A. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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