

Seroprevalence and spatial distribution dynamics of *Yersinia pestis* antibodies in dogs and cats from plague foci in the State of Ceará, Northeastern Brazil

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Abstract

Introduction: In Brazil, the plague is established in several foci located mainly in the northeastern part of the country, where it alternates between active and quiescent periods. These foci in the State of Ceará have high epidemiological importance. In addition to other plague detection activities, plague areas can be monitored through serological surveys of dogs and cats (domestic carnivores), which, following feeding on plague-infected rodents, can develop mild to severe forms of the disease and produce long-lasting antibodies. This study aimed to characterize the circulation dynamics and spatial distribution of *Yersinia pestis* antibodies in dogs and cats in plague foci areas of Ceará. **Methods:** An ecological study was conducted to analyze the temporal series and spatial distribution of secondary data obtained from domestic carnivore serum surveillance in Ceará's plague areas from 1990 to 2014. **Results:** Joinpoint analysis revealed that the overall trend was a reduction in antibody-positive animals. The mean proportion of antibody-positivity during the whole study period was 1.5% (3,023/203,311) for dogs, and 0.7% (426/61,135) for cats, with more than 4% antibody-positivity in dogs in 1997 and 2002. Antibody titers ranging from 1/16 to 1/64 were frequent. Despite fluctuations and a significant reduction, in recent years, there were antibody-positive animals annually throughout the study period, and the localities containing antibody-positive animals increased in number. **Conclusion:** *Yersinia pestis* is actively circulating in the study areas, posing a danger to the human population.

Keywords: *Yersinia pestis*. Domestic carnivores. Reservoirs. Time series. Surveillance.

INTRODUCTION

The plague is a vector-borne disease found primarily in wild rodents, which can affect humans and other mammals, such as wild or domestic carnivores. Plague persists in natural foci in many countries worldwide. As yet, the accumulated technological advances and scientific knowledge have not been enough to eradicate it^{1,2}. Human cases of the disease are recorded annually in several countries in Africa, Asia, and the Americas³.

Currently, the plague is considered a reemerging disease by the World Health Organization (WHO), with a tendency to increase in case number and geographical dispersion⁴. The plague can spread quickly, significantly impacting public health. Pneumonic plague can generate an international public health emergency, thus requiring WHO notification in accordance with current International Health Regulations⁵.

Introduced to Brazil in 1899, the infection was established in several natural foci, and spread throughout the States of Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Bahia, Minas Gerais, and Rio de Janeiro. The zoonosis remains in these foci, alternating between quiescent periods and epizootics, a phenomenon expressed by high rodent mortality and sporadic human cases⁶.

Until the 1970s, human plague notification numbers in Brazil varied from 20 to 100 cases per year. Since 1990, human plague cases have been rare and sporadic. In the State of Ceará, Northeastern Brazil, the last laboratory-confirmed human cases occurred in the 1990s, with the exception of one case in 2005, which was initially misdiagnosed as dengue⁷.

Brazilian focal plague areas are continuously monitored through serological surveys of domestic carnivores [dogs (*Canis lupus familiaris*) and cats (*Felis catus*)], and the serological analyses are performed at the Central Laboratory of Public Health (LACEN) in the focal area states^{6,7}.

As predators of rodents, dogs and cats can become infected with *Y. pestis* either by infected flea bites or by ingesting infected prey. They develop mild to severe forms of the disease, and,

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after recovery, they produce antibodies that remain detectable for several months. This justifies their use as sentinels/detectors of *Y. pestis* circulation between the rodents/reservoirs in the foci areas⁸⁻¹¹. In nature, the time of antigen exposure is unknown; thus, it is generally accepted that high proportions of antibody-positive animals with high antibody titers indicate recent plague activity. It is estimated that sampling a few dogs is equivalent to sampling hundreds of rodents in serological surveys to detect anti-plague antibodies¹².

This study characterized the circulation dynamics and spatial distribution of *Y. pestis* antibodies in dogs and cats in the plague focal areas of the State of Ceará in Northeastern Brazil between 1990 and 2014.

METHODS

Ethical considerations

The project was approved by the Research Ethics Committee of the Federal University of Ceará (UFCE), registry CAAE 51371215.2.0000.5054, number 1 383 310.

Study design

The study was conducted using a time series analysis of sero-surveys among domestic carnivores in plague focal areas of Ceará from 1990 to 2014.

Study area

The study area included 44 (23.9%) of the state's 184 municipalities, located in seven focal plague areas: Serra de Ibiapaba, Serra de Baturité, Serra da Uruburetama, Serra das Matas; Serra da Pedra Branca, Serra do Machado, and Chapada do Araripe. The total area comprises 29,855 km², 20.4% of the state's surface^{6,7}.

Municipality inclusion criteria

Municipalities that performed plague serological surveillance of dogs and cats for at least one year between 1990 and 2014 were included in the study. Duplicate records in the *Sistema de Informação do Programa de Peste* (SISPESTE) were excluded.

Data collection

Secondary data were collected from Plague Control Program (PCP) reports in Ceará, SISPESTE, and the Plague Regional Laboratory in Fortaleza (CE), from 1990 to 2005 and from LACEN-CE from 2005 to 2014.

The field surveillance program was conducted during annual cycles of health agent visits and blood sample collections from dogs and cats for serological analyses¹³.

Serological analyses

Passive Hemagglutination Assays/Hemagglutination-Inhibition (HA/HI) assays were used to detect specific *Y. pestis* anti-F1 antibodies¹³. In brief, the tests were performed using 96-well U-shaped polystyrene plates. Twenty-five microliters of each serum was serially diluted (1/4 to 1/8,192) into 25µL of HA buffer containing sterile normal saline (NS) and 1/100 normal rabbit sera (NRS). The sera were simultaneously diluted

into the HI buffer containing 100µg/mL of F1-specific *Y. pestis* protein antigen suspension, as described above. Next, 25µL of a saline suspension (NS plus 1/250mL NRS) of sheep red blood cells (SRBCs) sensitized with 100µg/mL of F1 (SRBCs/F1) was added to each well. Samples were incubated overnight at 4-10°C. The test was considered positive when the HA endpoint titer was depressed by three or more dilutions in the HI test. A titer of $\geq 1/16$ was considered antibody-positive. The NRS used to prepare the buffers and all sera tests were previously inactivated at 56°C for 30 min in a water-bath and adsorbed for 30 min at room temperature with fresh sheep red blood cells¹³.

Data analysis

A database was built using MS Excel 2010 to organize, separate and categorize the information. The data were exported and analyzed using Stata software, version 11.2. Time trend analysis was performed using the Joinpoint Regression Program, version 4.5.0.1. The trend analysis included dog surveillance data from 1997 to 2014, and cat surveillance data from 1997 to 2009. To calculate the annual percentage change (APC) in the antibody-positive animal proportions, a trend analysis was performed using continuous linear regression with inflection points (Joinpoint method). This method allowed the time series data to be adjusted from the minimum number of joinpoints (i.e., 0 joinpoints, which is a straight line) and determined whether the inclusion of more joinpoints was statistically significant. The significance is represented by graphs and two indicators, the APC and the average annual percentage change (AAPC).

The trend analysis of the significant variables from Ceará and for each plague focus was determined. Each significant point that indicated a change in the increase or decrease (if any) was maintained in the final model¹⁴. The independent variable (Y) was the study year, and the dependent variables (X) were the proportion of antibody-positive dogs, the proportion of antibody-positive cats, and the proportion of antibody-positive animals (dogs and cats). Differences were considered statistically significant at a p value < 0.05.

The joinpoint analysis was performed using the number of antibody-positive animals as the numerator, and the number of tested animals as the denominator, multiplied by 100. Data were converted logarithmically. Errors were considered heteroskedastic, and the regression coefficients were estimated by weighted least squares. For both the proportion of antibody-positive animals, and the temporal evaluation of the data, an adjusted autocorrelation model of the errors based on the data was also used.

The APC and the AAPC confidence intervals 95% (95% CI) were calculated using the empirical quantile method. This method generates re-sampled data by (i) generating re-sampled residuals as the inverse function values of uniform random numbers over 0% to 100%, where the function is the empirical distribution function of the original residuals, then (ii) adding re-sampled residuals to the original fit. A total of 10,000 re-samples were used for the analyses. A maximum of two joinpoints for the periods were considered.

RESULTS

From 1997 to 2014, serum samples from 203,311 dogs from seven plague foci in Ceará were analyzed for *Y. pestis* anti-F1 antibodies using HA-HI testing, and 3,023 (1.49%) were antibody-positive. Of the serum samples from 61,135 cats analyzed from 1997 to 2009, 426 (0.7%) were HA-HI-positive (Table 1).

Joinpoint analysis showed two different trends, both for dogs and for cats (Figure 1A, Figure 1B and Figure 1C). The proportion of antibody-positive dogs displayed a non-significant positive trend (APC = 10.7, 95% CI: -14.2 to 42.9, $p = 0.402$) in the period of 1997 to 2002. From 2002 to 2014, the trend changed, and the proportion of antibody-positive dogs decreased significantly (APC = -17.2, 95% CI: -24.6 to -9.1, $p < 0.001$). The AAPC for the entire period displayed a significant decreasing trend (AAPC = -9.8, 95% CI: -18.4 to -4.4) (Table 2 and Figure 1A). Joinpoint analysis showed that the proportion of antibody-positive cats increased significantly from 1997 to 2001 (APC = 40.5, 95% CI: 10.3 to 78.9, $p = 0.011$), then decreased significantly from 2001 to 2009 (APC

= -29.1, 95% CI: -35.6 to -22.1, $p \leq 0.001$). The AAPC for the entire period displayed an overall downward trend, but the model was not statistically significant (AAPC = -11.0, 95% CI: -38.1 to 3.4) (Table 2 and Figure 1B).

In the accumulated period from 1997 to 2014, 16 (36.4%) municipalities had up to 25 antibody-positive dogs, 19 (43.2%) had 25 to 100, five (11.4%) had 100 to 200, and four (9%) municipalities had over 400 antibody-positive dogs. Over the years, both antibody-positive dogs per locality and the number of localities with antibody-positive dogs decreased. Therefore, after 2003, no municipality had more than 40 antibody-positive dogs. The number antibody-positive cats per municipality also decreased, as did the number of municipalities with antibody-positive cats. Throughout the entire study period, 11 (25%) municipalities had no antibody-positive cats, 15 (34%) had up to five antibody-positive cats, 10 (22%) had five to 15, six (14%) had 15 to 40 and two (5%) municipalities had 40 to 140 antibody-positive cats.

The median proportion of antibody-positive dogs was higher in the foci of Chapada do Araripe (0.87), followed by Serra do Machado (0.85) and Serra Uruburetama (0.81). The highest proportion was found in Serra das Matas (3.33)

TABLE 1: Distribution of antibody-positive dogs and cats from the plague foci in Ceará from 1997 to 2014.

Year	Dogs			Cats*		
	tested	positives	proportion of positives	tested	positives	proportion of positives
1997	8,367	417	4.98	5,970	67	1.12
1998	10,191	45	0.44	6,469	17	0.26
1999	10,491	131	1.25	6,132	31	0.51
2000	8,714	65	0.75	4,991	6	0.12
2001	9,581	378	3.95	4,995	170	3.40
2002	6,581	313	4.76	3,596	30	0.83
2003	9,096	183	2.01	5,159	23	0.45
2004	9,466	176	1.86	4,640	16	0.34
2005	11,135	387	3.48	4,535	28	0.62
2006	12,261	139	1.13	4,362	7	0.16
2007	13,258	207	1.56	5,137	19	0.37
2008	12,153	125	1.03	4,689	11	0.23
2009	14,342	109	0.76	460	1	0.22
2010	14,586	51	0.35	-	-	-
2011	14,005	109	0.78	-	-	-
2012	12,476	63	0.50	-	-	-
2013	13,257	69	0.52	-	-	-
2014	13,351	56	0.42	-	-	-
Total	203,311	3,023	1.49	61,135	426	0.70

*Surveillance of cats was discontinued after 2009.

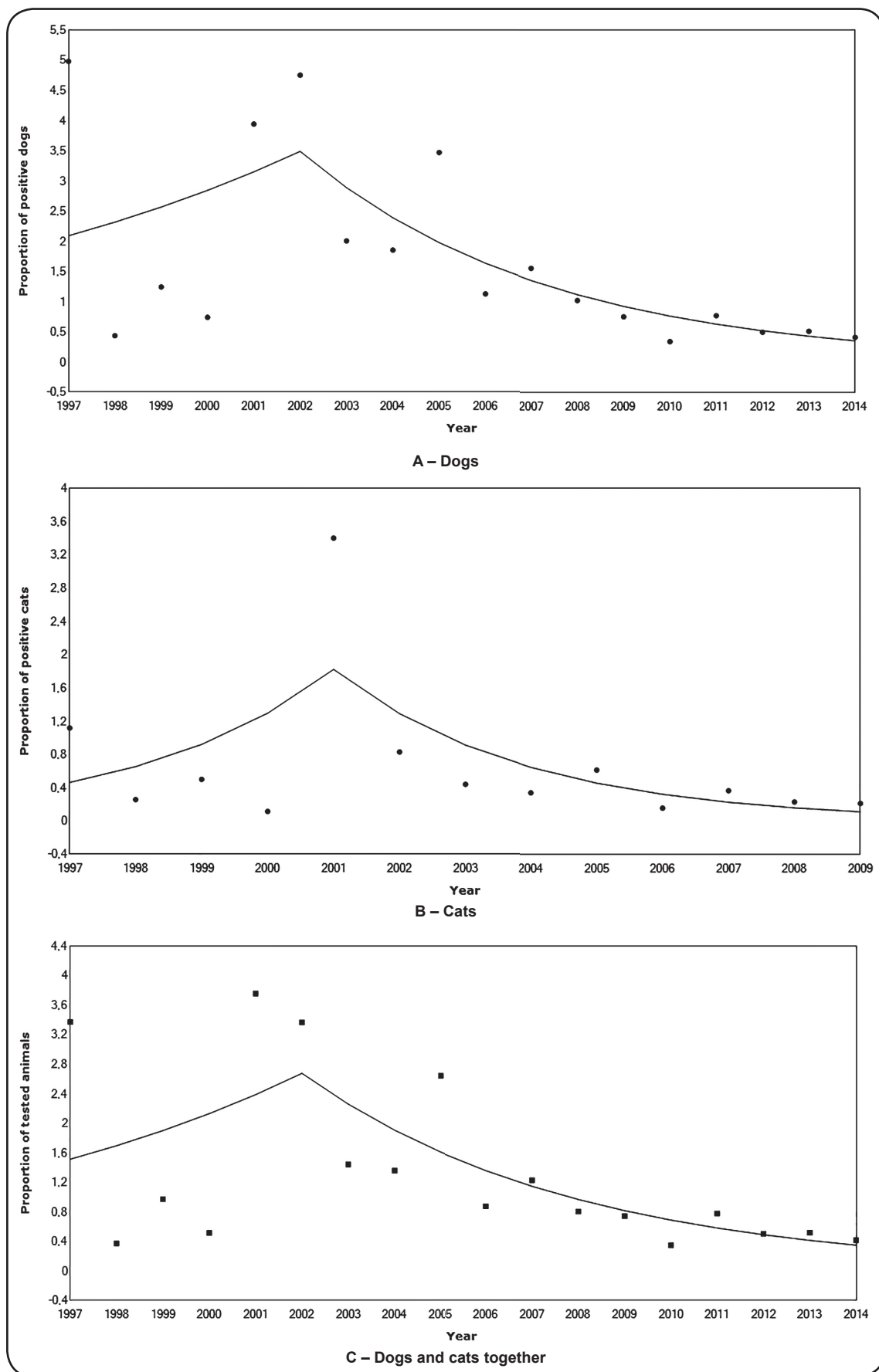


FIGURE 1 - Temporal trends of the proportion of antibody-positive: **A:** dogs, **B:** cats, and **C:** dogs and cats together, from the plague foci in Ceará from 1997 to 2014, using joinpoint analysis.

TABLE 2: Joinpoint regression analysis of the proportion of positive animals in Ceará from 1997-2014 (dogs, and dogs and cats together) and from 1997-2009 (cats).

Segment	Lower endpoint	Upper endpoint	APC	Lower CI	Upper CI	p-value
Dogs						
1	1997	2002	10.7	-14.2	42.9	0.402
2	2002	2014	-17.2	-24.6	-9.1	<0.001
AAPC	1997	2014	-9.8	-18.4	-4.4	
Cats						
1	1997	2001	40.5	10.3	78.9	0.011
2	2001	2009	-29.1	-35.6	-22.1	<0.001
AAPC	1997	2009	-11.0	-38.1	3.4	
Dogs and cats together						
1	1997	2002	12.1	-12.6	43.7	0.340
2	2002	2014	-15.6	-23.8	-6.5	0.003
AAPC	1997	2014	-8.2	-17.2	-3.0	

APC: annual percentage change; CI: confidence interval; AAPC: average annual percentage change.

(Table 3). During the study period, the proportion of infected dogs decreased over time in the seven plague foci. However, there was a notably high proportion of antibody-positive dogs in Serra do Machado almost every year (Table 4).

Regarding the HA/HI titer distribution in the historical series from 1990 to 2014, a total of 3,631 dogs and cats had anti-plague antibodies, with titers ranging from 1/16 to 1/8,192. The most frequent titers were 1/32 (n = 1,100 animals), 1/64 (n = 997) and 1/16 (n = 916), representing 30%, 27% and 25% of the total, respectively. The highest titers were less frequent or rare: 1/128 (n = 290) 1/256 (n = 154), 1/512 (n = 148), 1/1,024 (n = 15), 1/2,048 (n = 8), 1/4,096 (n = 2) and 1/8,192 (n = 1). This may be due to the laboratory routine, which usually

tests up to 6 dilutions (1/128) and only occasionally up to 12 dilutions (1/8,192).

DISCUSSION

Dogs and cats participate in the epidemiological cycle of the plague and are good indicators of infection activity in the focal areas. They can carry infected rodents and fleas to human premises, thus increasing the risk of human infection¹⁵.

Dogs in highly enzootic areas may be repeatedly exposed to plague-infected rodents and fleas and develop high *Y. pestis* antibody titers¹⁶. In China, serological surveys of dogs are an important tool for plague surveillance, as these animals are notable plague carriers¹⁷.

TABLE 3: Minimum, median, and quartiles of the proportion of antibody-positive dogs in Ceará from 2008 to 2014.

Foci	Minimum	1 st Quartile	Median	3 rd Quartile	Maximum
Chapada do Araripe	0.31	0.35	0.87	1.01	1.47
Serra da Ibiapaba	0.34	0.45	0.48	0.65	1.02
Serra da Pedra Branca	0.26	0.29	0.43	0.63	0.8
Serra das Matas	0	0.23	0.7	1.52	3.33
Serra de Baturité	0.32	0.35	0.5	1.11	1.54
Serra de Uruburetama	0.23	0.29	0.81	1.05	1.34
Serra do Machado	0	0.54	0.85	1.26	1.77
Ceará State	0.35	0.42	0.52	0.78	1.03

TABLE 4: Distribution of the proportion of antibody-positive dogs from the plague foci in Ceará from 2008-2014.

Year	Plague foci							Ceará State
	Chapada do Araripe	Serra da Ibiapaba	Serra da Pedra Branca	Serra das Matas	Serra de Baturité	Serra de Uruburetama	Serra do Machado	
2008	0.87	1.02	0.80	3.33	1.11	1.34	1.77	1.03
2009	1.47	0.45	0.26	1.52	1.54	1.05	0.85	0.76
2010	0.31	0.34	0.29	0.00	0.35	0.44	0.54	0.35
2011	0.98	0.65	0.43	0.70	0.76	0.93	1.26	0.78
2012	1.01	0.48	0.43	0.90	0.48	0.29	0	0.50
2013	0.35	0.51	0.63	0.23	0.50	0.81	1.18	0.52
2014	0.53	0.48	0.48	0.41	0.32	0.23	0.59	0.42
Total	0.75	0.57	0.46	0.56	0.67	0.59	0.78	0.62

All animals in our study were domesticated, living in direct contact with humans. In the rural areas included in this study, dogs are commonly used for hunting, and cats help to control the rodents in homes, and in grain and food storage buildings. Therefore, they are exposed to *Y. pestis* through contact with rodents and their fleas.

The historical time series analysis in our study revealed similar trends for both positive dogs and cats, with a reduction in positivity from 2001 among cats and from 2002 among dogs. The proportion of positive dogs increased from 1997 to 2002 (not significantly) and declined significantly from 2002 to 2014. Among the cats, the trend increased from 1997 to 2001 and decreased from 2001 to 2009, both being statistically significant (Table 2, Figure 1A, Figure 1B and Figure 1C).

The human plague prevalence has also declined in the Ceará plague foci since the 1990s⁷. Our findings corroborate those of other studies on temporal associations with human plague incidence¹⁸⁻²⁰. Parmenter et al.²¹ found that the cyclical pattern of the annual distribution of plague cases in New Mexico (USA) was associated with fluctuations in the rodent population or in the rodents' responses to environmental variations such as food and shelter availability.

During our study period, the prevalence of antibody-positive dogs was 1.49% (3,023/203,311), with more than 4% antibody-positivity in 1997 and 2002, and the overall prevalence for cats was 0.70% (426/61,135), with higher antibody-positivity (3.40%) in 2001 (Table 1). Previous studies found that antibody-positivity prevalence among dogs and cats from the Ceará plague foci, and from Chapada da Borborema (PB), were similar^{13,22}. During a plague outbreak in a village in Yunnan Province, China serum-positivity rates in domestic cats and dogs were 23.5% and 26.5%, respectively²³. This could be because many diseased cats do not survive the infection, while dogs are more resistant and more likely to recover¹⁰.

Because it was assumed that plague antibody detection is more sensitive in dogs than in cats and rodents, the plague

surveillance of cats was discontinued in the Brazilian foci areas²⁴. Despite the low antibody-positive prevalence (< 1%) during the study period (with the exception of 1997 and 2001), cats tested antibody-positive in all years investigated. Therefore, serological surveillance in cats should not be discontinued, as it may be valuable for the plague monitoring program. Almeida et al.²² recorded 76% positive samples from cats, and 49.1% from dogs in Serra de Baturité (CE) during a human plague outbreak. The high positivity among cats was likely due to animals being infected and then surviving the disease during those events. Therefore, to improve the monitoring of *Y. pestis* in plague areas, implementing surveillance for cats is recommended.

In the USA, serological surveillance is performed on a wide variety of mammals, as it is thought that this procedure is more sensitive, and allows better assessment of plague behavior and circulation in focal areas²⁵⁻²⁶. Lowell et al.²⁷ state that surveillance involving many species provides better coverage of plague activity than monitoring a single species. Plague surveillance programs monitoring die-offs and serum prevalence in reservoir animal populations can provide an early and timely warning of possible human cases^{10,27}.

In the surveillance routine of sentinel animals, including coyotes (*Canis latrans*), badgers (*Taxidea taxus*), and raccoons (*Procyon lotor*) in American plague areas, an overall prevalence of 16% (3,481/21,826) was found, varying by region and species. The prevalence rate was higher in mustelids (43% - 504/1,173), followed by bears (23% - 95/459) and canids (15% - 1,079/7,291)²⁸. Malmlov et al. reported a 28% (7/25) serum prevalence in coyotes²⁶.

Despite the reduction in antibody-positive dogs per municipality, the number of municipalities with antibody-positive animals increased, and after 2003, the number of municipalities with antibody-positive dogs also increased. These results suggest that the infection is spreading geographically (i.e. few animals dispersed over larger areas). This change could

result from eco-epidemiological factors favoring dissemination, such as the densities of natural vectors and rodent hosts, in addition to possible climatic changes, such as temperature and precipitation¹⁹. Comparing the proportional distribution of antibody-positive dogs in the seven plague foci, the prevalence was reduced in all but Pedra Branca, where antibody-positivity was instead increased. Serra do Machado had the highest prevalence in dogs during the study period.

Analyzing the focal associations, statistically significant correlations were found between Serra da Uruburetama and the year; Chapada do Araripe and Serra das Matas; Serra de Ibiapaba, Serra da Pedra Branca and Serra do Machado; Serra de Baturité and Serra de Uruburetama and between Serra de Uruburetama and Serra do Machado (**Table 3** and **Table 4**). The prevalence rates among the different plague focal areas may have been influenced by factors common to the seven foci. Thus, we assume a common pattern exists for actively circulating *Y. pestis* in the focal areas. These results agree with those of Baltazard²⁹, who considered that the geographical massifs of Ibiapaba, Baturité, Machado, Uruburetama, Pedra Branca and Matas constitute a single focus. Giles et al.³⁰ confirmed that plague is focal in northeast Brazil, with a discontinuous distribution. These studies showed that those foci depend on a broad set of conditions, and are not necessarily correlated with their elevation, but likely to the presence of the hairy-tailed bolo mouse, *Necromys lasiurus*, which is the primary plague host in these foci, and is responsible for epizootics.

The proportion of antibody-positive animals fluctuated in all foci, however, these fluctuations were only statistically significant in Serra das Matas. A prevalence higher than 1% was observed in some years, which increases the risk for human cases to emerge¹².

Antibody titers among dogs and cats in the study period showed a higher frequency of 1/32, followed by 1/64 and 1/16. Titers $\geq 1/64$ suggest recent infections and recent contact with *Y. pestis*, signaling a potential risk for human infection^{12-13,22}. Paul-Murphy et al.³¹ reported anti-*Y. pestis* antibody titers $\geq 1/128$ in 15 pumas, *Felis concolor*; and a range of 1/1,024 to 1/4,096 in four pumas in rural areas of California (USA). In coyotes from urban areas in California, titers ranged from 1/16 to 1/1,024²⁶. In plague areas of China, titers varying from 1/40 to 1/1,280 were found in 49 domestic dogs²³.

In conclusion, during the study period, the prevalence of antibody-positive dogs and cats fluctuated, with a significant reduction in recent years. However, our results also revealed annual antibody-positive animals, providing evidence that *Y. pestis* is persisting in active circulation. Thus, even the quiescent areas with no human cases have the potential for transmission risk, and despite the decrease in antibody-positive domestic carnivores, continual surveillance is needed in these areas. Due to the plague's cyclical characteristics - alternating periods of activity and quiescence depending on a series of complex factors - plague can reemerge and reach the human population. Hence, surveillance, monitoring, and control actions must be continued.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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