

Factors associated with adherence to annual rabies vaccination in dogs and cats  
in the municipality of Curuçá, Eastern Amazon

Rabies vaccination of dogs and cats in the Eastern Amazon.

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25 ABSTRACT

26 Dogs and cats maintain the urban cycle of rabies, and vaccination is the main form  
27 of prevention and control of the disease. Brazil has seen human rabies cases  
28 transmitted by dogs and cats infected with the bat variant in regions where annual  
29 campaigns no longer take place. Although the municipality of Curuçá has no cases of  
30 urban rabies and viral circulation in wild animals is unknown, there are informal  
31 reports of contact of animals and people with vampire bats. This study aimed to  
32 identify factors associated with immune response against the rabies virus in dogs and  
33 cats in this municipality. A total of 352 dogs and 46 cats were randomly selected for  
34 blood collection and their owners answered a questionnaire. The animals were mostly  
35 males, aged between 1-3 years, and with unrestricted access to the street. A total of  
36 48.8% of dogs and 32% of cats were not vaccinated in the last anti-rabies campaign,  
37 and 4.7% of dogs had been attacked by bats. Among the analyzed samples, only 21.1%  
38 had a titration  $\geq 0.5$  IU/mL. Risk factors for not participating in vaccination campaigns  
39 included species, presence of veterinary care, and participation in annual vaccination  
40 campaigns (OR = 0.46, 2.55, and 15.67 respectively). The animal population was  
41 estimated at 18,620 dogs and 4,556 cats. The human:dog ratio was 2.1:1 and the  
42 human:cat ratio was 8.7:1. This study revealed that the estimated population of dogs  
43 based on the human population was an underestimate for communities in the Amazon  
44 region. This was the first time that the number of dogs attacked by bats was  
45 determined. Health education with an emphasis on responsible ownership and periodic  
46 and biannual rabies vaccinations are recommended for the municipality.

47 AUTHOR SUMMARY

48 Rabies is a viral disease characterized by brain and spinal cord inflammation.  
49 It affects all mammals, being almost 100% lethal. Hematophagous bats are one of the  
50 main wild reservoirs responsible for outbreaks of human rabies in the state of Pará and  
51 other regions of Brazil. Vaccination is the most effective form of control and  
52 prevention, even where rabies is believed to be under control. The levels of antibodies

53 that fight the rabies virus must be constantly monitored through serological analysis  
54 to assess the effectiveness of vaccination programs. In the municipality of Curuçá,  
55 Pará, bats commonly attack people and their pets, and anti-rabies campaigns are not  
56 promoted every year. In the present study, we determined the number of attacks by  
57 bats on domestic dogs and estimated vaccination coverage and the canine and feline  
58 population in the municipality, which was underestimated. This information can be  
59 useful for future vaccination campaigns. The study identified factors associated with  
60 responsible ownership that interfere with the protective titration of animals against  
61 rabies and suggests promoting more than one annual anti-rabies campaign in this  
62 location.

63

## 64 **INTRODUCTION**

65 Stray or semi-domesticated dogs and cats are the main sustainers of the urban cycle  
66 of rabies, and when not vaccinated, they become important sources of infection for  
67 humans <sup>[1]</sup>.

68 Despite the decrease in cases of canine rabies since 1970 in Brazil <sup>[2]</sup>, between  
69 2000 and 2009 approximately 4,177,409 human rabies consultations were reported in  
70 the country. Data from the Notifiable Diseases Information System (*Sistema de*  
71 *Informação de Agravos de Notificação - SINAN*) show that among animals, dogs were  
72 the main human aggressor <sup>[3,4]</sup>.

73 Between 2007 and 2017 this condition increased in several regions of the country,  
74 <sup>[5]</sup> including the state of Pará. In the micro-region of Salgado, northeastern Pará, 74.1%  
75 of the notifications in SINAN came from aggression by dogs <sup>[6]</sup>.

76 The most recent cases of human rabies in Brazil were caused by the AgV3  
77 variant, which is typical of bats, but is transmitted by cats, demonstrating that there is  
78 contact between bats and domestic animals and between bats and humans <sup>[7]</sup>.

79 In the municipality of Curuçá, there are no records of human or canine rabies  
80 and the circulation of the rabies virus has not yet been studied. However, the lack of  
81 records does not necessarily indicate the absence of viral circulation, but rather that

82 the virus may be present in silent areas <sup>[8,9]</sup>.

83 It is known that in this municipality, in 2012 and 2013, about 10% of rabies  
84 treatment was required following attacks by bats against people living in rural areas,  
85 where domestic animals walk roam freely, and are under favorable conditions for this  
86 type of contact. <sup>[10,6]</sup>.

87 Considering the information above, this work aimed to investigate the  
88 epidemiological factors associated with the presence of neutralizing antibodies against  
89 the rabies virus in dogs and cats, vaccinated or not, owned in the municipality of  
90 Curuçá- Pará.

## 91 **METHODOLOGY**

### 92 **Ethical aspects**

93 The research project was approved by the Ethics Committee on Animal Use  
94 (*Comissão de Ética na Utilização de Animais - CEUA/UFPA*), under No.  
95 6363180918.

### 96 **Study area**

97 The municipality of Curuçá is located in the northwest of the Brazilian Amazon  
98 region, 130 km from Belém. More than half of its territory is part of the Mãe Grande  
99 de Curuçá marine extractive reserve, which consists of an environmental conservation  
100 unit that was established to ensure the sustainable use of its resources, which are used  
101 by most of its inhabitants for subsistence. This municipality includes the Curuçá River  
102 and a large coastal area mixed with Amazonian riverside zones, mainly occupied by  
103 mangroves. Due to its location, the municipality's economy is based on fishing, and  
104 trade of fish and shellfish collected in the environment.

### 105 **Sample size**

106 This research was based on the estimate of the Ministry of Health (*Ministério*  
107 *da Saúde* – MS; presented in the resolution No. 05/2013) that the canine population  
108 corresponds to 20% of the human population. Therefore there are approximately 6,859  
109 dogs in Curuçá, based on the last human census (34,294 inhabitants - IBGE, 2010).

110 The required canine sample size was calculated using the Statcalc tool of the Epi  
111 Info™ software version 7.2, with an expected frequency of 60% and a significance  
112 level of 5%, which resulted in a total of 350 animals.

### 113 **Sampling method**

114 The dogs were selected by random sampling, prioritizing the evaluation of one  
115 animal per household. The households were selected using the QGIS 3.2.2 software.  
116 The selected points were located using the MapIt-GIS Data Collector application. If  
117 there were no dogs in the randomly selected household or it was empty, the next  
118 household was promptly selected (Figure 1). Cats were selected for convenience in  
119 randomly selected households where dogs and cats were present.

120 **Figure 1.** Location of the study area and distribution of random points for collecting  
121 biological material from dogs.

### 122 **Questionnaire administration**

123 After signing an Informed Consent Form and adhering to the project protocol,  
124 owners were invited to answer a semi-structured questionnaire containing items  
125 identifying the animal, daily habits, and general care.

### 126 **Collection of biological material**

127 Whole blood was collected by a professional veterinary using a closed collection  
128 system or a needle-punched syringe, through puncture of the cephalic or external  
129 jugular vein, in a volume of 3 to 5 ml. Samples were placed in plastic tubes for blood  
130 collection and subsequent serum collection. Serum samples were stored in a freezer at  
131 -20°C until use.

### 132 **Serological analysis**

133 Samples of 374 animals were sent for serological analysis. Four canine samples  
134 arrived in poor condition and could not be evaluated. Thus, 370 samples were  
135 analyzed; 324 from dogs and 46 from cats.

136 The Rapid Fluorescent Focus Inhibition Test (RFFIT) was performed for

137 measuring neutralizing antibodies against the rabies virus <sup>[11]</sup>, with modifications.

138 First, the received sera were inactivated in a water bath at 56°C for 30 minutes  
139 and then six serial dilutions of each serum were carried out, in the ratio 2, starting from  
140 1:5. A total of 25 µL of serum was placed in the first opening with 37.5 µL of Eagle's  
141 Minimum Essential Medium (MEM) with Earle's salts, and it was supplemented with  
142 10% inactivated fetal bovine serum.

143 After dilution, 50 µL of virus of the Challenge Virus Standard (CVS) strain  
144 were added, and the plates were incubated in an oven with CO<sub>2</sub> at 37°C. After 1 h 30  
145 min of incubation, 50 µL of BHK-21 cells (2.5 X 10<sup>4</sup> cells/mL) were added and  
146 incubated again at 37°C in an atmosphere containing 5% CO<sub>2</sub> for 20 hours. The cells  
147 were fixed in an ice bath and the reaction was revealed by adding anti-rabies virus  
148 conjugate. The reading was performed under a LEICA® DMIL inverted fluorescence  
149 microscope at 200 X magnification. The titers were calculated using the Spearman-  
150 Kärber method of analysis, by comparison with the standard serum, and the results  
151 were expressed in International Units per milliliter (IU/ml), with animals with results  
152 equal to or greater than 0.5 IU/ml being considered immune and those with lower titers  
153 being considered non-immune.

## 154 **Data analysis**

### 155 *Statistical analysis*

156 Descriptive analysis of the variables addressed in the companion animals'  
157 questionnaire was performed using the SPSS v.24 software. The chi-square test ( $\chi^2$ )  
158 was used to test the associations between the variables. Associations were significant  
159 when  $p < 0.05$ . In these cases, the odds ratio (OR) was calculated.

160 Variables with significant associations and those with  $P \leq 0.02$  were included  
161 in the binary logistic regression model, using the "ENTER" method, with the  
162 following dependent variables: "was vaccinated last year", "usually vaccinates  
163 annually" and "result of serology". All covariates raised in the questionnaire were  
164 tested.

165 *Calculation of population estimate*

166 Sampling fraction methodology was used to estimate the population of dogs  
167 and cats in the municipality of Curuçá<sup>[12]</sup>, with modifications; where the ratio between  
168 the total number of animals counted in the visited households and the sample fraction  
169 was estimated, according to following formula:

$$170 \quad N = \frac{na}{nhv/tnh}$$

171

172 Where:  $N$  = number of estimated animals in the municipality;  $na$  = number of animals  
173 counted in the visited households;  $nhv$  = number of households visited;  $tnh$  = total  
174 number of households in the municipality.

175

176 **RESULTS**

177 **Characterization of the animal population**

178 A total of 352 dogs were selected. Of them, 56.7% were males and 43.3% were  
179 females, with most of them being aged between one and three years (48%) and being  
180 of mixed breed (93.8%).

181 Although most were companion animals (68.4%), there was also a percentage  
182 of guard animals (24.6%) and some intended for hunting (5.7%) and fishing (1.3%).  
183 The majority animals were adopted (76.4%), followed by those born at home (17.8%).  
184 As reported by the owners, 81.8% of the animals had access to the street on their own,  
185 spending the day outside the home and at night they returned and slept within the  
186 property (83.1%), either on the balcony (42.5%), in the yard/ open sky (45.5%) or in  
187 a dog house (12%). Also, among those who had free access to the street, 16.9% slept  
188 inside the house.

189 Most owners (68.5%) reported that they usually take their animal to the rabies  
190 vaccination campaign every year; however, 48.8% of the animals had not been  
191 vaccinated in the previous year. Among the reasons why not, some owners reported

192 that the animal was too young to receive the vaccine or had not yet been born (44.4%),  
193 and others reported other reasons (Figure 2).

194 **Figure 2.** Reported reasons for why dogs were not vaccinated in the previous year.

195 A total of 4.7% of dog owners reported that their dogs had been attacked by  
196 bats at least once in their lives. These aggressions mostly occurred on islands in the  
197 municipality, where dogs accompanied their owners on fishing activities.

198 In the present study, 46 cats were selected, most of them being male (69.6%),  
199 aged from one to two years (44.4%), adopted (89.1%), having access to the street  
200 (89.1%), but sleeping inside the house (54.3%). About 32% of the owners reported  
201 not having vaccinated their cats in the last anti-rabies campaign that took place in the  
202 municipality. When asked about the reasons why not, 53.4% reported that they still  
203 did not have cats, or that their cat was too young to receive the vaccine. The others  
204 reported other reasons, which are described in Figure 3.

205 **Figure 3.** Reported reasons for why cats were not vaccinated in the previous  
206 year.

207 Animals that did not receive veterinary care such as deworming and other  
208 vaccines and whose owner was not used to adhere to anti-rabies vaccination  
209 campaigns in the municipality were more likely to have not been vaccinated in the  
210 previous year. However, being a dog (OR=0.5) and having another role besides  
211 companionship (OR=0.6) were protective factors for these animals to be vaccinated in  
212 the previous year. The study also revealed that the older the animal, the greater the  
213 chance of being vaccinated (Table 1).

214 When evaluating the candidate prediction variables, with rigorous annual  
215 vaccination as the dependent variable, the analysis revealed that older animals that do  
216 not receive care other than food are more likely to not participate in vaccination  
217 campaigns.



218 **Table 1.** Factors associated with owners' reports of rabies vaccination of their dogs  
 219 and cats in the last campaign in the municipality of Curuçá, Pará.

| VARIABLES          | CATEGORIE     | VACINATED LAST YEAR |     | P     | OR (IC 95%)         | VACINATED EVERY YEAR<br>OR (IC 95%) |
|--------------------|---------------|---------------------|-----|-------|---------------------|-------------------------------------|
|                    |               | NO                  | YES |       |                     |                                     |
| Species            | Dog*          | 166                 | 174 | 0,018 | 0,462 (0,24-0,88)   |                                     |
|                    | Cat           | 31                  | 15  |       |                     |                                     |
| Age                | < 1 Year      | 51                  | 17  | 0     |                     |                                     |
|                    | 1-3 Years     | 109                 | 81  |       |                     |                                     |
|                    | 4-6 Years     | 21                  | 54  |       |                     | 2,7 (1,44-5,22)                     |
|                    | 7-9 Years     | 8                   | 23  |       |                     | 3,6 (1,24-10,6)                     |
|                    | ≥10 Years     | 5                   | 13  |       |                     |                                     |
| Role_Companionship | No*           | 35                  | 50  | 0,037 | 0,596 (0,366-0,971) |                                     |
|                    | yes           | 162                 | 138 |       |                     |                                     |
| Resting place      | Balcony       | 50                  | 78  | 0,002 |                     |                                     |
|                    | Cat/dog house | 24                  | 23  |       |                     |                                     |
|                    | Yard          | 78                  | 49  |       |                     |                                     |
| Veterinary Care    | No*           | 168                 | 134 | 0     | 2,554 (1,528-4,268) |                                     |
|                    | yes           | 27                  | 55  |       |                     |                                     |
| Deworming          | No*           | 92                  | 62  | 0,004 | 1,83 (1,210-2,768)  | 1,7 (1,09-2,6)                      |
|                    | yes           | 103                 | 127 |       |                     |                                     |
| Other Vaccines     | No*           | 145                 | 127 | 0,049 | 1,645 (1-2,708)     |                                     |
|                    | Yes           | 34                  | 49  |       |                     |                                     |

220

221 \* Reference category

222 \*\* Dependent variable

223

224

225

226 **Serological analysis**

227 Of the total samples analyzed, only 21.1% showed protective titration.

228 Non-protective titration was present in 63.9% and 69.6% of the dog and cat  
229 samples, respectively.

230 When considering only dogs and cats that usually received the rabies vaccine  
231 every year in the campaign, only 61 (26.5%) dogs and 8 (33.3%) cats had titers  $\geq 0.5$   
232 IU/ml. Moreover, seven unvaccinated dogs and two cats had positive titers. As for the  
233 animals that received the vaccine in the previous year, most (58.7%) did not have  
234 sufficient protective titration.

235 The analyses revealed that age was a risk factor for the absence of rabies  
236 titration, with increasing age being associated with a greater chance of having a  
237 negative titer (< 1 year: OR=0.43 CI 95%=0.006-0.320; 7-9 years: OR=8.66 CI  
238 95%=3.59-20.88). Other associated risk factors were the place where the animal rests,  
239 lack of veterinary care, not participating periodically in anti-rabies campaigns, and  
240 non-vaccination in the last campaign, the latter having about seven times the chance  
241 of not being protected (Table 2).

242

243 **Table 2.** Analysis of associations between the variables of the questionnaire and the result of  
244 the rabies serological teste.

| Factors       | Categories               | Serology |          | Total | p-value* | Odds ratio | CI (95%)    |
|---------------|--------------------------|----------|----------|-------|----------|------------|-------------|
|               |                          | Negative | positive |       |          |            |             |
| Place of Rest | Balcony                  | 68       | 35       | 103   | 0.003    | 2.435      | 1.353-4.382 |
|               | dog/cat house            | 27       | 8        | 35    | 0.841    | -          | -           |
|               | Yard                     | 96       | 18       | 114   | 0.005    | 0.414      | 0.223-0.769 |
| Age           | < 1 year <sup>a, b</sup> | 55       | 1        | 56    | 0.000    | 0.43       | 0.006-0.320 |
|               | 1-3 years                | 127      | 32       | 159   | 0.063    | -          | -           |
|               | 4-6 years                | 34       | 22       | 56    | 0.005    | -          | -           |
|               |                          |          |          |       |          |            | 3.593-      |

|                                       |                                      |        |         |          |                |            |                  |
|---------------------------------------|--------------------------------------|--------|---------|----------|----------------|------------|------------------|
|                                       | 7.0 years <sup>b</sup><br>≥ 10 years | 8<br>8 | 18<br>4 | 26<br>12 | 0.000<br>0.498 | 8.663<br>- | 20.883<br>-      |
| Veterinary care                       | No <sup>a</sup>                      | 195    | 55      | 250      | 0.033          | 1.902      | 1.046-3.460      |
|                                       | Yes                                  | 41     | 22      | 63       |                |            |                  |
| Participates in anti-rabies campaigns | No <sup>a</sup>                      | 103    | 9       | 102      | 0.0000         | 6.636      | 3.157<br>13.950  |
|                                       | Yes                                  | 119    | 69      | 188      |                |            |                  |
| Vaccinated in last year               | No <sup>a</sup>                      | 150    | 16      | 166      | 0.0000         | 7.266      | 3.936-<br>13.411 |
|                                       | Yes                                  | 80     | 62      | 142      |                |            |                  |

245 \*Pearson's chi-square test a Reference category b Multiple regression analysis

246

### 247 **Estimation of the population of dogs and cats in households**

248 Considering the numbers of dogs (703), cats (172), and households (321) selected  
 249 for this study and the number of households in the municipality according to the last  
 250 IBGE census (8,502), the animal population was estimated at 18,620 dogs and 4,556  
 251 cats, with an average of 2.19 and 0.53 dogs and cats per household, respectively.

252 In 2019, the human:dog ratio was 2.1:1 and the human:cat ratio was 8.7:1, that is,  
 253 in the municipality of Curuçá, there was one dog for every two people and one cat for  
 254 every eight people.

## 255 **DISCUSSION**

256 Dogs and cats are the most prevalent domesticated animals in the world, and  
 257 they coexist in great intensity with the human population <sup>[13]</sup>. This interaction can  
 258 cause the exchange of pathogens, leading to the development of zoonoses, including  
 259 rabies, in human populations <sup>[14]</sup>. It is estimated that the global load of dogs in the  
 260 world reaches 700 million animals <sup>[15]</sup>. Brazil is the second largest country with respect  
 261 to its number of domestic animals, with approximately 52.2 million dogs and 22.1  
 262 million cats <sup>[16]</sup>.

263 Most of the animals studied in both species were male, as reported in other  
 264 studies <sup>[17,18,19,20]</sup>. This suggests that the human population has a preference for this

265 gender since females can become pregnant which can increase the number of animals,  
266 and the methods of sterilization of females are more expensive <sup>[20]</sup>.

267 Despite the large percentages of dogs and cats for companionship (68.4% and  
268 100%, respectively), these animals are considered free-living or "from the  
269 neighborhood"; that is, they are semi-dependent and semi-restricted or even  
270 unrestricted. This means that although they do not always depend directly on the  
271 human to provide food and shelter, they have a reference family <sup>[21]</sup>.

272 The animal population is considered young, between 1 and 3 years old (48%),  
273 corroborating a study by Magnabosco <sup>[22]</sup> in São Paulo. Most (84%) had free access to  
274 the street, as reported in other studies in Brazil and other countries <sup>[17,18,23]</sup>, and which  
275 can increase the risk of accidents. Therefore, the animal population has a shorter  
276 lifespan and will constantly renew as new animals arrive. The free access to the street  
277 also allows contact with wild animals, increasing the chance of aggression and  
278 transmission of pathogens to humans <sup>[24]</sup>.

279 Studies show that most animal bites are caused by dogs and cats <sup>[25,6,26]</sup> from  
280 the neighborhood or even belonging to the victim <sup>[27,28,26]</sup>, in unprovoked conditions.  
281 In the present study, about 22% of dogs and cats, despite spending the day outside the  
282 house, sleep together with the family, inside the house, which increases the chances  
283 of aggression and transmission of pathogens to humans <sup>[23]</sup>. In this context, controlling  
284 animal movement is extremely important to reduce the risk of exposure to rabies.

285 In Curuçá, these domestic animals are also found in wild territory, since 22  
286 (5.7%) dogs were used by their owners to assist in hunting. This practice is common  
287 in many cultures in the world <sup>[29,30]</sup>, and involves letting the animal run free in search  
288 of prey <sup>[31]</sup>, without constant supervision by the hunter. At this moment, there is a  
289 connection between domestic and wildlife, allowing the transmission of pathogens  
290 between species <sup>[32,15]</sup> and involving the human population in this connection <sup>[33]</sup>.  
291 Moreover, interaction between hunters from different places and their dogs enables  
292 the transmission of pathogens between communities <sup>[31]</sup>.

293 Given its location and economic base, some residents of extractive reserve

294 (RESEX) develop fishing activities and many take their dogs, which explains the  
295 finding that the main role given for five animals was fishing. In this study, about 4.7%  
296 of the dogs had already been attacked by bats. Aggression occurs when people together  
297 with their animals move long distances, using temporary makeshift shelters and  
298 staying several days without protection against bats <sup>[34]</sup>, or when the animals live  
299 together with their owners in areas of mangrove forests <sup>[35]</sup>.

300 The study by Hughes & Macdonald <sup>[15]</sup> showed that, in several parts of the  
301 world, domestic dogs constantly interact with several wild species, including bats,  
302 either for predation, which can be in either direction, or in the transmission of  
303 pathogens.

304 Cats are also important in this context since most of them have access to the  
305 street (89.1%) and sleep inside the house (54.3%), in addition to being hunters by  
306 nature. According to Welch & Leppanen <sup>[36]</sup> cats are the species most responsible for  
307 attacks on bats, whether for predation or for fun <sup>[37]</sup>.

308 Cats come into contact with bats in their shelters <sup>[38]</sup>, or when they come across  
309 animals that fall while flying during the day <sup>[22]</sup>. According to Castilho et al. <sup>[39]</sup> this  
310 type of contact is responsible for cases of rabies in this species.

311 It is worth mentioning that the most recent cases of human rabies reported in  
312 Brazil had cats as transmitters, with one case in Jacaraú-PB, in 2015 <sup>[40]</sup>, and two more  
313 cases in Boa Vista-RR (2016) and Recife-PE (2017) in the following two years. In all  
314 these cases, the variant involved was AgV3, common in vampire bats. In 2019, there  
315 was a case in Santa Catarina, with transmission by a domestic cat, and in 2020 a case  
316 in Paraíba involving the AgV2 variant transmitted by a wild canid. Thus, there are  
317 predisposing conditions for the occurrence of human cases in the studied municipality.

318 In addition to establishing pre-exposure immunity, rabies vaccination of  
319 domestic animals protects individuals, and thus can prevent the spread of the virus to  
320 humans and other animals <sup>[41]</sup>. The state of Pará, among other states, has canine  
321 vaccination coverage below 70% <sup>[16]</sup>. Similarly, according to the present study, the  
322 municipality of Curuçá vaccinated 51.2% and 32.6% of dogs and cats (respectively)

323 in the most recent rabies vaccination campaign, conducted in 2018.

324 These results are below the level recommended by WHO and the levels  
325 described in other regions of the country <sup>[22,42,16]</sup> and are above levels reported in  
326 countries where urban rabies is considered endemic. In locations such as the  
327 Philippines, Mali, and the Democratic Republic of Congo, vaccination coverages  
328 ranged from 17% to 47% <sup>[20,43,44]</sup>.

329 The reasons for low vaccine rates most frequently reported in other locations  
330 were coverage, negligence, the belief that the vaccine is not effective, lack of time,  
331 lack of knowledge, distance from vaccination posts, and fear of adverse effects from  
332 the administered vaccine <sup>[45,20,46]</sup>.

333 In Curuçá, the rabies vaccination campaign takes place on a “D” day, where  
334 fixed posts are set up in the central region of the city and receive dogs and cats for  
335 vaccination. On other days, vaccination takes place door-to-door. However, the  
336 campaign is conducted in a decentralized manner, with each health unit being free to  
337 conduct the vaccination campaign in the way it deems most appropriate for its area of  
338 coverage. In some locations, this does not occur at all due to difficult access, so dog  
339 owners reported that vaccination did not take place in that area (39%) or that due to  
340 the distance there was some difficulty in taking the animal to the vaccination post  
341 (19%). Besides this situation, another 10% reported not having been informed about  
342 the vaccination campaign that year, reinforcing that communication efforts must be  
343 made constantly in these populations <sup>[47]</sup>.

344 Some cat owners also reported no campaign in their location (29%). However,  
345 what stands out is the fact that they do not care about vaccinating their animals (29%)  
346 and believe that the species does not need to be vaccinated against rabies.

347 In the study by Kongkaewa et al. <sup>[17]</sup> a part of the population reported that  
348 vaccination was useless in cats, as they did not believe that the species was also prone  
349 to rabies infection.

350 The absence of canine and feline rabies cases in the municipality may also  
351 contribute to the low adherence to vaccination, as according to Rodrigues et al. <sup>[42]</sup> this

352 absence generates a false sense of protection in the population and health agencies.  
353 The municipality of Curuçá conducts rabies vaccination campaigns annually, around  
354 the months of August and September. However, in 2016 and 2017 there were no  
355 campaigns according to the Information System of the National Immunization  
356 Program (*Sistema de Informações do Programa Nacional de Imunizações - SI-PNI*)  
357 <sup>[48]</sup>, which may have discouraged the population from vaccinating their animals.

358 About 6% of respondents reported not taking their pets to be vaccinated  
359 because, in their perception, when vaccinated against rabies, dogs and cats get sick  
360 and die. In fact, in 2010, the anti-rabies campaign was interrupted after successive  
361 deaths of animals with neurological signs. Until that year, the vaccine used in the  
362 campaigns was Fuenzalida & Palácios, consisting of an inactivated virus produced in  
363 the nerve cells of mice <sup>[49]</sup>. It was suspected that batches of the vaccine were  
364 compromised, as it has been previously associated with Guillain-Barré syndrome in  
365 humans <sup>[50]</sup>.

366 From 2010, the Ministry of Health changed the vaccines distributed in the dog  
367 and cat campaigns to a cell culture vaccine, which provides greater safety and efficacy  
368 in the conversion of protective titers in these animals <sup>[2]</sup>.

369 Possibly because of what happened, the population has become more resistant  
370 to the administration of vaccines to their animals and many have even reported this  
371 fact, which may also be associated with the high chance of dogs not having been  
372 vaccinated in the anti-rabies campaign in the previous years.

373 Moreover, participation in the vaccination campaign was closely associated  
374 with the interaction between the animals and their owners, since youngest animals  
375 were the ones more likely to be vaccinated, as reported by Mauti et al. <sup>[46]</sup>. Culturally,  
376 in this region, puppies are likely to receive more attention from their owners because  
377 at this stage they are more closely associated with the children in the house. This  
378 information is aligned with the association revealed by the study, that companion  
379 animals and those who receive other care such as veterinary care and periodic  
380 vaccination were more likely to have been vaccinated in the previous year,

381 corroborating what was reported by Davlin et al. <sup>[20]</sup>, that the chance of vaccination  
382 was greater among domiciled animals, due to the constant proximity between the  
383 animals and human beings.

384 The seroprevalence of antibodies against the rabies virus is quite  
385 heterogeneous, as it depends on the method of analysis, the animals involved, and the  
386 region where these animals are located <sup>[51,52,53]</sup>. In the present study, the global  
387 seroprevalence of neutralizing antibodies was around 20%, which, despite being low,  
388 is in accordance with other reports for farm animals <sup>[46]</sup>. Although there are reports  
389 that cats have higher titers than dogs, possibly due to particularities of the immune  
390 system that are not yet well understood <sup>[53]</sup>, the present study did not show any  
391 differences in immune response between species ( $p=0.97$ ).

392 However, it was possible to notice a difference between vaccinated and  
393 unvaccinated animals in the previous year, with most dogs and cats that participated  
394 in the vaccination campaign not having a protective titer and some unvaccinated  
395 animals having neutralizing antibody titers  $\geq 0.5$  IU/ml.

396 The low titer of neutralizing antibodies in vaccinated animals may be  
397 associated with factors such as the number of doses received by the animal in its  
398 lifetime <sup>[54,55,56,57]</sup>, the time between vaccination and collection for analysis <sup>[53,58,59]</sup>,  
399 the age of the animal that received the vaccine <sup>[60]</sup>, and the type of vaccine used <sup>[61,41]</sup>,  
400 because according to Kennedy et al. <sup>[62]</sup>, the differences in the formulation and  
401 production of different vaccines can produce different failure rates and immune  
402 responses.

403 The time elapsed between vaccination and collection for serological testing  
404 can also influence the response to antibody production, as the chances of testing  
405 positive tend to decrease as this period increases. The ideal time to perform the  
406 collection is between 4 and 8 weeks after vaccination <sup>[53,58,59]</sup>, and the collections of  
407 the present study took place between 10 and 12 months after the last rabies vaccination  
408 campaign, and therefore may be associated with the small proportion of animals with  
409 positive titer.



410           According to Gold et al. <sup>[63]</sup>, multiple exposures to small doses of the virus can  
411 stimulate the development of antibodies, preventing future infections. This type of  
412 non-lethal exposure has been reported in humans in an isolated indigenous tribe who  
413 never received rabies prophylaxis and were exposed to bat attacks <sup>[64]</sup>. People and their  
414 animals in Curuçá are in constant contact with hematophagous bats, which can make  
415 the local population acquire antibody titers, and helps to explain the 7.6% animals who  
416 have never been vaccinated and yet produced antibodies at protective levels.

417           Moreover, the sensitivity and specificity of antibody detection methods can  
418 impair the detection of protective titers <sup>[63]</sup> and the presence of antibodies can result  
419 from an alternative course of exposure, where the elimination of the virus by the  
420 organism occurs even before it invades the CNS, causing a subclinical infection but  
421 with antibody production <sup>[65,66]</sup>.

422           Young and old animals are more prone to immunological failures, as the  
423 former do not have an immune system mature enough to produce an adequate response  
424 and the latter are already entering the cellular senescence phase, decreasing their  
425 immune capacity <sup>[41,67]</sup>.

426           Some of the animals in the study were not vaccinated because they were  
427 newborn, and one year later it would be expected that they would not have a titration.  
428 As older animals are less likely to be vaccinated every year, it is possible that many of  
429 them received only one dose as puppies, which could explain the fact that age was a  
430 risk factor for the absence of rabies titration, and younger and older animals had a  
431 greater chance of not showing protective titration.

432           It is recommended that puppies receive a booster dose after primary  
433 vaccination and periodic reinforcements from then on because even with these animals  
434 being born from vaccinated bitches, they cannot acquire sufficient titration for  
435 protection even 30 days after vaccination <sup>[55,57]</sup>.

436           This study showed that the animals in Curuçá received only the essential for  
437 their survival, such as food, water, and shelter, but not veterinary care nor other  
438 vaccines. Although the rabies vaccine is available throughout the year in agricultural

439 stores, pet shops, and food stores, most of the population only seeks the vaccines  
440 donated through campaigns, which take place once a year throughout Brazil.

441         When planning a vaccination campaign, counting or estimating the population  
442 of animals is essential, as it allows the identification of the necessary resources and  
443 the best methods <sup>[68]</sup>. Currently, in most of Brazil and other countries, the estimation  
444 of animal population for rabies vaccination campaigns is based on the calculation of  
445 the human population <sup>[69]</sup>. However, adopting a single ratio or percentage for  
446 population estimates for a continent can lead to errors in future, as each location has  
447 particularities that must be taken into account <sup>[23,16]</sup>. This study presents population  
448 estimates and epidemiological characterization of dogs and cats in a municipality in  
449 the state of Pará and evaluates the titration of antibodies against rabies in animals,  
450 which can help vaccination and disease control programs in the municipality and the  
451 state.

452         According to data provided by the 3rd Regional Health Center of the Public  
453 Health Department of the State of Pará (*3º Centro Regional de Saúde da Secretaria*  
454 *de Saúde Pública do Estado do Pará - 3ºCRS/SESPA*), in 2018 the municipality of  
455 Curuçá reached 136.3% and 97.5% of the vaccination goal for dogs and cats,  
456 respectively. However, such results close to and above 100% are common and were  
457 obtained because the anti-rabies vaccination campaigns in Brazil are programmed and  
458 organized while considering the information established by WHO <sup>[21]</sup> and the Pasteur  
459 Institute <sup>[49]</sup>, which estimate the human:dog ratio at 10:1 and 7:1, and also by the  
460 Ministry of Health, which estimates that the canine population corresponds to between  
461 10 and 20% of the human population.

462         Data from the present study revealed that the human:dog ratio in the  
463 municipality of Curuçá was 2:1, that is, the canine population corresponded to 50% of  
464 the human population. These results are far from the recommended for emerging  
465 countries by the WHO, of one dog for seven or eight inhabitants <sup>[21]</sup>. In studies  
466 conducted in Southeastern Brazil, this relationship varied by location. Studies have  
467 reported the human:dog ratio as 7:1 <sup>[70]</sup>, 5:1 <sup>[71,72]</sup>, and 4:1 <sup>[73,18]</sup>. These ratios can be  
468 considered while planning vaccination campaigns in the corresponding regions, but

469 should not be used for planning vaccination campaigns in other regions of the country.  
470 If the numbers of the present study are taken into account, together with the fact that  
471 only 51.2% of the studied dog population was vaccinated in the previous year, it can  
472 be inferred that the 80% vaccination coverage recommended by the WHO was not  
473 achieved, as also described by De Lucca et al. <sup>[74]</sup> and Rodrigues et al. <sup>[42]</sup> in São Paulo,  
474 and Fernandes et al. <sup>[75]</sup> in Santa Maria/Rio Grande do Sul.

475           Until 2018, due to delays in the delivery of vaccines and in the programming  
476 of campaigns, the vaccination coverage rates varied greatly in almost all Brazilian  
477 municipalities. In 2019, the campaign took place only in higher risk areas, in the states  
478 of Maranhão, Ceará, Rio Grande do Norte, Mato Grosso do Sul, Mato Grosso,  
479 Rondônia, and Acre <sup>[2]</sup>.

480           Southern Brazil is considered controlled for urban rabies <sup>[16,75]</sup>, which led to  
481 the cancellation of anti-rabies campaigns in the region since 1995. Until 2015, only  
482 the state of Paraná promoted campaigns and only in municipalities bordering Paraguay  
483 <sup>[2]</sup>.

484           However, the absence of vaccination has caused animals to lose the immune  
485 reinforcement provided by it and, despite the large decrease in cases of canine and  
486 feline rabies since 1999, there was an increase in the infection of dogs and cats <sup>[75]</sup>  
487 with consequent transmission to humans. This demonstrates the importance of these  
488 animals as secondary transmitters and of maintaining vaccination even where the  
489 disease has already been eliminated <sup>[76]</sup>. Furthermore, the duration and size of  
490 outbreaks tend to be longer in unvaccinated populations <sup>[77]</sup>. It is worth mentioning  
491 that the most recent cases of human rabies were reported in Santa Catarina, where it  
492 was transmitted by domestic cats in 2019, Angra dos Reis/Rio de Janeiro, where it was  
493 transmitted by bats, and Catolé do Rocha/Paraíba, where it was transmitted by  
494 domestic dogs, both in 2020 <sup>[2]</sup>.

495           Therefore, the maintenance of current campaigns is suggested, in order to  
496 achieve both a greater proportion of truly immunized animals <sup>[62]</sup> and the promotion  
497 of education programs for responsible ownership, since some factors associated with

498 general care are associated with the acquisition of antibodies against the rabies virus.

## 499 **CONCLUSION**

500 This study revealed that the estimate of the population of dogs based on the  
501 human population was an underestimation for communities in the Amazon region,  
502 where the animals were semi-restricted or unrestricted, having free access to the wild  
503 environment. This was the first time that the number of dogs attacked by bats was  
504 determined. These dogs are indicators of the possible re-emergence of urban rabies in  
505 the region. Factors associated with responsible ownership were identified as risk  
506 factors for periodic vaccination of these animals and, therefore, the region should  
507 promote health education with an emphasis on responsible ownership and periodic and  
508 biannual rabies vaccination.

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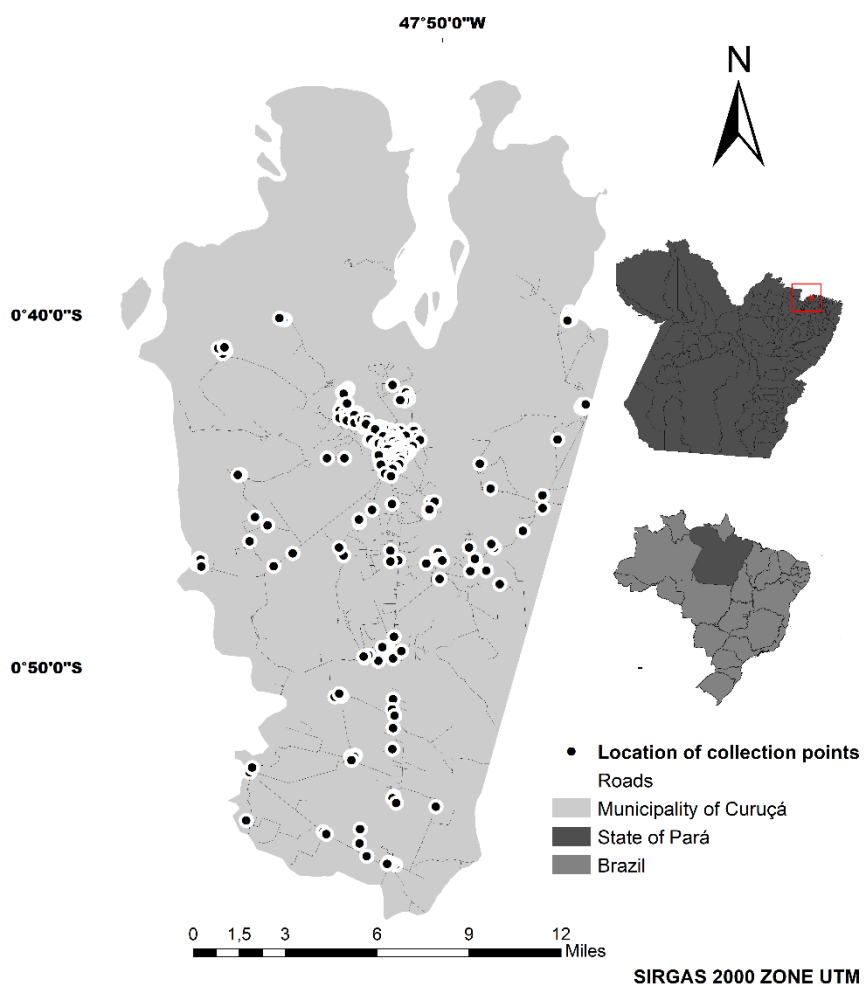
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797 **Figures**

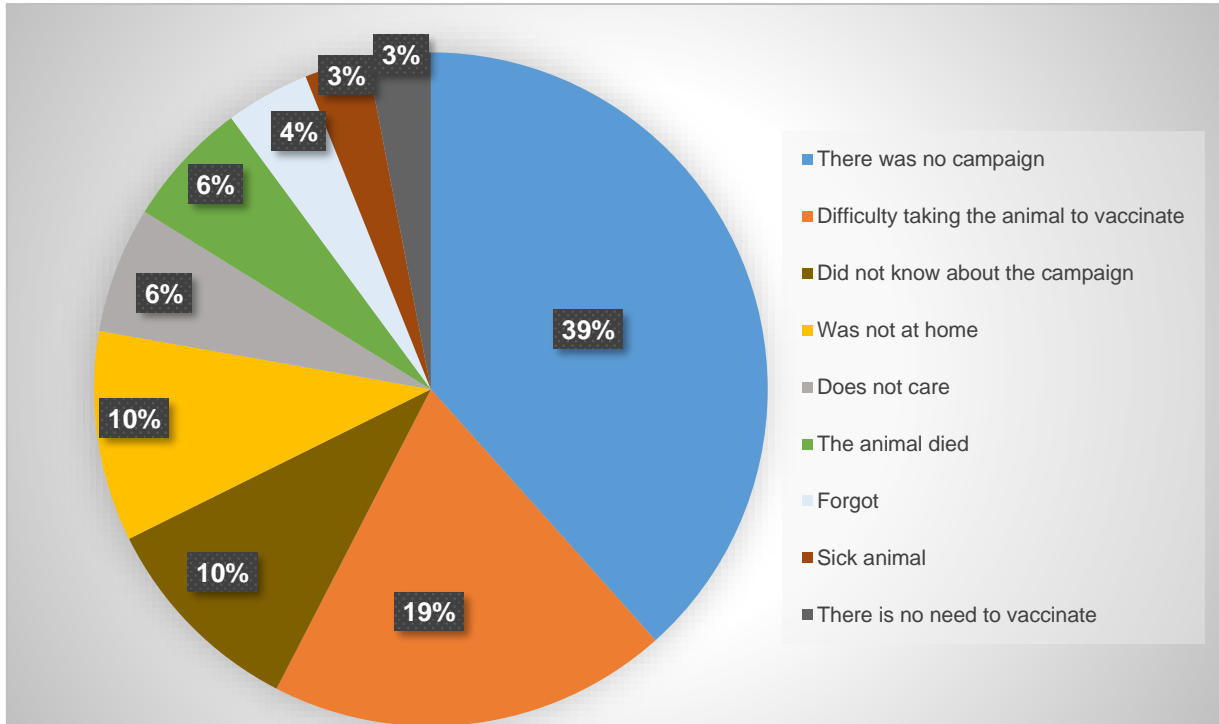


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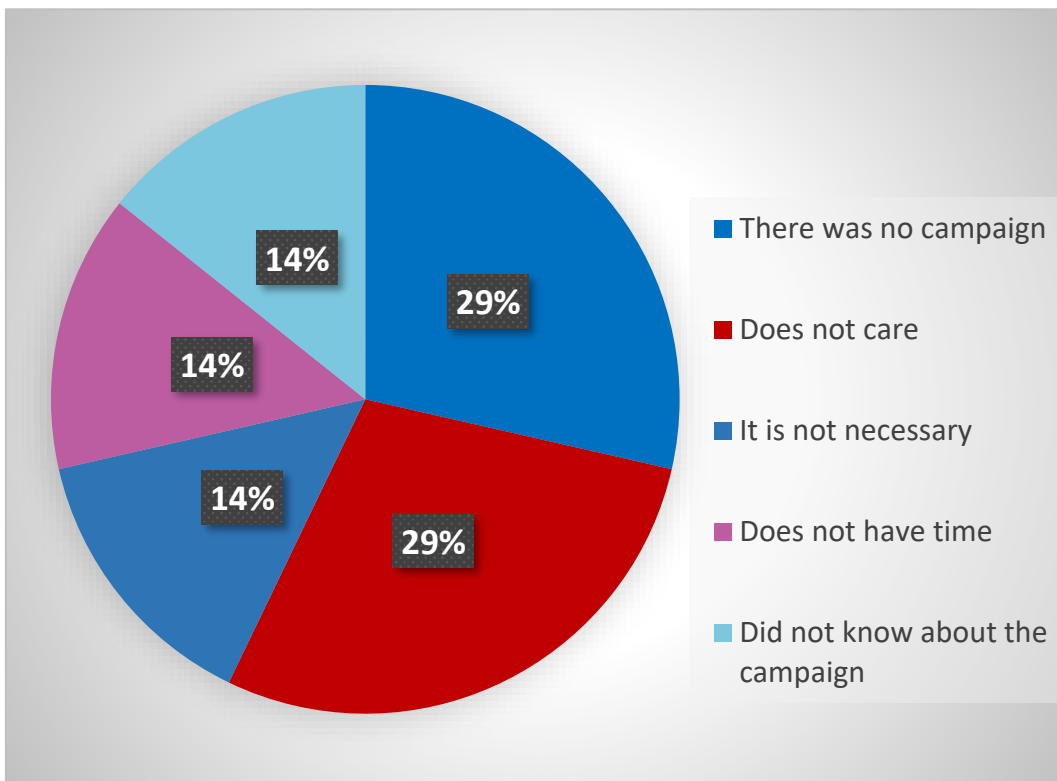
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812 Fig. 2.

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814 Fig. 3.

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