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4 Associated factors and spatial patterns of the epidemic sporotrichosis in a high density  
5 human populated area: A cross-sectional study from 2016 to 2018.

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7 Epidemic sporotrichosis in a high-density populated area

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30 diagnosis

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## 33 **Abstract**

34 We carried out an epidemiological characterization of human and feline sporotrichosis,  
35 between 2016 and 2018, in a high density-populated area in Brazil. Professionals were  
36 trained to identify suspected cats and notified vets to interview the owners and collect  
37 swabs of the wounds from these animals. Mycological cultures were performed, and  
38 colonies identified for *Sporothrix* spp. Subsequently, data regarding the outcome from  
39 suspected animals were collected. Confirmed cases of human sporotrichosis (56) were  
40 also counted and analysed for spatial distribution. Regions with highest prevalence of  
41 feline sporotrichosis, had greater frequencies of both human and feline cases. 118  
42 (77.63%) animals were positive. Animals that lived only partially at home were 3.02  
43 times more likely of being positive (OR 3.02, CI 95% 1,96-10,43). The prevalence of  
44 feline sporotrichosis was 8.36 ‰ (CI 95%, 5.38 - 9.55 ‰). There was no statistically  
45 significant association between environmental variables and positive diagnosis,  
46 corroborating the hypothesis that direct transmission by infected cats plays a greater role  
47 in the occurrence and continuous outbreaks of sporotrichosis in Brazil. Among the  
48 positive animals, 61.90% (CI 95% 58.95 - 64.96) died, being 6.30 times more likely to  
49 die than negative animals ( $p < 0.05$ , OR 6.30, CI 95% 2,79-14,42). The lethality rate was  
50 55.08% in cats (CI 95% 49.20 - 51.15). The mortality for sporotrichosis was 4.6 ‰ cats  
51 (CI 95% 3.4 - 6 ‰). Only 7.62% (CI 95% 7.12 - 8.16) positive cats were treated and  
52 cured. Among dead positive animals, 29.23% were inappropriately discarded. This is  
53 the first report on the epidemic of sporotrichosis in Minas Gerais, Brazil. The free offer  
54 for treatment and veterinary care to these animals should be taken into consideration, as  
55 well as the collection and incineration of the dead ones, as measures of public health,  
56 followed by the guidance and care for the human patient.

## 57 **Introduction**

58 Sporotrichosis is a cosmopolitan zoonotic disease caused by fungi of the complex  
59 *Sporothrix schenckii*, with high relevance in Brazil. Since the first reports of its  
60 transmission to humans through contact with infected cats in the 1990s, the number of  
61 severe cases of the disease in humans has been increasing. Therefore, this zoonosis has  
62 become relevant to public health [1–4]. The infected cats carry the fungus in their  
63 injured sites, oral cavities, and nails, and the transmission is made by scratches, bites,  
64 and contact with the wound exudates [5–7].

65 Cases of the disease in cats and humans have been described in the northern, eastern,  
66 and southern states of the country [7–10]. In the state of Minas Gerais, there are no  
67 descriptions in literature about any epidemiological situation, although it shares  
68 boundaries with the state of Rio de Janeiro, a hyperendemic region for sporotrichosis.  
69 Only one study has reported the presence of *Sporothrix brasiliensis* in a human case and  
70 ten domestic cats in the state [7].

71 Considering infected cats as potential transmitters of the disease to humans and the  
72 increasing territorial expansion of sporotrichosis in the country, it is necessary to  
73 characterize the epidemiological situation of the disease in areas with potential spread of  
74 the infection.

75 Accordingly, the present study aimed to produce the epidemiological characterization of  
76 human and feline sporotrichosis in a high density-populated area located in the  
77 municipality of Belo Horizonte (Minas Gerais, Brazil), based on the first reports of  
78 sporotrichosis. This included the analysis of the spatial distribution, associated factors,  
79 and the identification of the most common outcomes of such cases in cats, in the period  
80 from August 2016 to June 2018.

## 81 **Material and Methods**

## 82 **Ethics approval**

83 This study was approved by the Universidade Federal de Minas Gerais, Comitê de Ética  
84 em Pesquisa da Universidade Federal de Minas Gerais (Committee for Ethics in  
85 Research of the Federal University of Minas Gerais) (number CAAE –  
86 67149517.5.0000.5149).

## 87 **Type and area of study**

88 We conducted a cross-sectional epidemiological study based on observation, laboratory  
89 diagnosis of sporotrichosis in domestic cats, and analysis of factors related to the  
90 disease and the respective outcomes. In addition, we incorporated the spatial analysis of  
91 cases in cats and humans, in the Barreiro region, Belo Horizonte, Minas Gerais,  
92 between 2016 and 2018.

93 Belo Horizonte, the capital of the state of Minas Gerais, is located in southeastern  
94 Brazil, and it is the sixth most populous city of the country. It has a geographic  
95 extension of 331.401 km<sup>2</sup> and a population density of 7,167 inhabitants per km<sup>2</sup>. The  
96 city is divided into nine regional administrative units. Of these regions, Barreiro (an  
97 area of study), which is subdivided into 20 areas, is covered with Health Centers. The  
98 selection of this study area was determined based on the fact that it has the largest  
99 population of cats compared to other regions of the municipality, and because the first  
100 reports of suspected sporotrichosis in the municipality were reported from Barreiro.

## 101 **Selection, samples collection, and data**

102 Between August 2016 and June 2018, the Escola de Veterinária da Universidade  
103 Federal de Minas Gerais (School of Veterinary Medicine of the Universidade Federal de  
104 Minas Gerais) (EV/UFMG), in partnership with Diretoria de Zoonoses (Council on

105 Zoonosis) (DZ/SMSA), in the Municipality of Belo Horizonte, received samples and  
106 cadavers of cats suspected of sporotrichosis, from the county of Barreiro.

107 The public health professionals in that region were then trained to identify nodular and  
108 ulcerative skin lesions in cats, potentially caused by sporotrichosis. After the suspected  
109 animals were identified, the vets of the DZ/SMSA were notified to interview the owners  
110 and collect samples from the animals. The period of search for suspected cases was  
111 extended until the end of the study (2016 and 2018), and the visits of veterinarians to  
112 the notified homes continued for the whole period.

113 The owners of the suspect animal answered a semi-structured questionnaire about the  
114 characteristics of the symptomatic animals and the home itself, to identify possible  
115 factors associated with the occurrence of sporotrichosis and conduct of those  
116 responsible for the animals face to that suspicion.

117 Swabs of the wounds of the suspected cats were obtained during the home visits and the  
118 corpses of the dead cats were forwarded by the DZ/SMSA to the Laboratory of  
119 Veterinary Pathology of the EV/UFMG.

120 Mycological cultures were then performed in the Laboratory of Mycology and  
121 Mycotoxins (LAMICO), of the School of Veterinary Medicine of the UFMG, following  
122 the cultivation into a solid media on Petri plates. Each sample was incubated in the  
123 media BHI/36 °C (BD, USA) and SDA/25 °C (Kasvi, Brazil). Colonies were identified  
124 by their macro and micromorphology [11,12].

125 From April to June 2018, the veterinarians of DZ/SMSA monitored the study area to  
126 identify cats suspected of sporotrichosis. The veterinarians investigated and collected  
127 data including as suspicious cats remained at their homes, progression of the clinical

128 signs, completion of treatment after confirmation of the disease and destination of the  
129 corpses in the event of death.

130 In addition, confirmed cases (with positive culture) of human sporotrichosis occurred in  
131 this same period were also counted and analysed for spatial distribution.

## 132 **Preparation and analysis of data**

133 The data from LAMICO and DZ/SMSA were entered in spreadsheets, and were  
134 grouped according to the four variables - Demographic, Environmental, Diagnosis, and  
135 Outcome. In cases where inconsistency was found in a record without the possibility of  
136 verification and correction, the record was excluded from the statistical analysis. For the  
137 analyses of environmental factors, lack of data on the environmental variables was  
138 considered one exclusion factor.

139 The descriptive analysis of the data included the calculation of averages, frequency of  
140 distributions, coefficient of prevalence, frequency of positivity, coefficient of mortality,  
141 and lethality rate, with their respective confidence intervals at 95% (CI 95%),  
142 considering the local feline census in 2018 as the total population.

143 The association analyses of the qualitative variables with the diagnosis was carried out  
144 through the chi-squared test (CI 95%;  $p < 0.05$ ) using the software Stata 14.0.  
145 Furthermore, Spearman correlation analysis (CI 95%;  $p < 0.05$ ) was performed between  
146 the variables of population density and prevalence of the disease in felines, using the  
147 packages *stats* and *ggpubr* of the software R.

148 For the analysis of the spatial distribution, the cases were geo-referenced based on the  
149 addresses of the households visited. Maps of population density, prevalence of  
150 sporotrichosis cases, and Kernel density were obtained using the QGIS® software 2.18.

## 151 **Results and Discussion**

### 152 **Zoonotic sporotrichosis**

153 Between 2016 and 2018, 56 human cases of sporotrichosis (Fig 1) were recorded in the  
154 study area. The regions where the rate of prevalence of feline sporotrichosis was above  
155 6.7%, were included 69.6% (39/56) of human cases and 66.4% (101/152) of feline cases  
156 (Fig 2). The areas of high prevalence of feline sporotrichosis, as highlighted in Fig 1,  
157 the calculation jointly accounted 42.8% of human cases. In two regions where no  
158 positive feline cases were found, human cases were recorded. This may indicate the  
159 presence of non-identified feline cases in those areas or that no animal transmission to  
160 humans occurred in the residence.

161 **Fig 1 - Spatial distribution of sporotrichosis cases in humans and cats in the county**  
162 **of Barreiro, Belo Horizonte, 2016 to 2018.**

163 **Fig 2 - Spatial distribution of human cases of sporotrichosis in relation to the**  
164 **prevalence of feline sporotrichosis per area of coverage in the county of Barreiro,**  
165 **Belo Horizonte, 2016 to 2018.**

### 166 **Feline sporotrichosis**

167 During the study period, samples were collected from 152 cats for laboratory diagnosis.  
168 One hundred and thirty seven (90.13%) cats were identified as suspect and the corpses  
169 of 15 (9.87%) dead cats were sent for necropsy. Sixteen animals with samples collected  
170 *in vivo* were excluded from statistical analyses of association, due to lack of data.  
171 Information regarding the 15 corpses was not included in the analysis of environmental  
172 variables, because questionnaires were not answered by the cat owners.

173 The sample was divided into two groups: positive and negative. The inclusion in each  
174 category was based on epidemiological, clinical, and/or laboratory criteria (Fig 3).  
175 Among the samples with positive isolation, one was sent for molecular analysis, which  
176 was confirmed as *S. brasiliensis* through molecular diagnosis [13]. For the samples of  
177 corpses, diagnosis was based only in laboratorial results. Thus, 118 (77.63%) animals  
178 were considered positive while 34 (22.37%) animals were considered negative (Table  
179 1).

180 **Fig 3. Criteria for the diagnosis of feline sporotrichosis in the county of Barreiro,**  
181 **Belo Horizonte, Minas Gerais, August 2016 to June 2018.**

182 **Table 1 - Distribution of household cats in relation to the diagnosis of**  
183 **sporotrichosis in the county of Barreiro, Belo Horizonte, Minas Gerais, August**  
184 **2016 to June 2018.**

<b>Field collections of suspect cats</b>	
Laboratory positive	75 (54.74%)
Clinical-epidemiological positive with laboratory negative	16 (11.68%)
Clinical-epidemiological positive, going through prior treatment with laboratory negative	12 (8.76%)
Clinical-epidemiological and laboratory negative	34 (24.82%)
Total	137 (100%)
<b>Corpses of suspected cats</b>	
Laboratory positive corpses	15 (100%)
Laboratory negative corpses	-
Total	15 (100%)
<b>Final diagnosis</b>	
Positive	118 (77.63%)
Negative	34 (22.37%)
Total	152 (100%)

185

186 **Characteristics of the feline population**



187 The distribution of characteristics in the population composed of domestic cats studied  
188 was similar between the positives and negatives groups. Among the animals of known  
189 age, majority were young adults (aged between 1 and 3 years) (38.97%). There was  
190 predominance of animals without a defined breed (WDB) (98.53%), male animals  
191 (65.44%), not surgically spayed animals (69.12%), and animals with a lifestyle spent  
192 partially at home (68.38%) (S1 Table).

193 For animals with a positive diagnosis, there was predominance of young animals of  
194 known age (1 to 3 years) (33.33%) and males (64.76%). Similar results were obtained in  
195 studies on endemic and outbreak regions in Rio de Janeiro, Rio Grande do Sul, and  
196 Recife [9,10,14,15]. Majority of the animals was not spayed (68.57%); however, it was  
197 not possible to verify a significant association ( $p > 0.05$ ) between castration and positive  
198 diagnosis. Furthermore, in both groups, the proportion of animals that would regularly  
199 wander the streets was high (83.3% of non-spayed and 74.08% of spayed animals).  
200 Such information shows that, even for those guardians who decided spaying their cats,  
201 confinement is not considered an important measure for animal care.

202 Only the lifestyle presented a statistically significant association with positive diagnosis  
203 of sporotrichosis ( $p < 0.05$ ). Of the animals assessed, 86.76% (118/136) lived only  
204 partially at home. These animals were 3.02 times more likely of being diagnosed as  
205 positive for sporotrichosis compared to animals strictly confined to their homes (OR  
206 3.02, CI 95% 1,96-10,43). The finding is similar to that obtained in previous studies  
207 conducted in southern Brazil [16], which shows a considerable percentage of animals  
208 with free access to the street and a low degree of responsible ownership by their  
209 guardians. Such behavior exposes the animals to fights, copulation, and contact with  
210 sick animals. Additionally, the sick cat may act as a disseminator of the fungus to other  
211 animals and to the environment [14,16].

212 All the variables (young animals, male, non-spayed, and with access to the street) are  
213 known risk factors of the disease, according to previously published studies [5,14]. For  
214 the improvement of a responsible ownership, it is of great importance to spread the  
215 information about this serious disease presenting a risk of infection for the animals and  
216 for the families they live with.

217 As majority of the population included in the study comprised young, non-spayed cats  
218 with free access to the street, this homogeneity did not allow the quantification of the  
219 effect of these variables on the occurrence of the disease in this population.

## 220 **Spatial distribution of feline cases**

221 In relation to the spatial distribution of the sample, 16 (80%, CI 95%, 56.34% - 94.27)  
222 of the 20 areas showed at least one positive cat for sporotrichosis. The prevalence found  
223 in the county of Barreiro was 8.36 positive cats per one thousand cats (CI 95%, 5.38 -  
224 9.55 per one thousand cats – S2 Table).

225 The three areas with the highest prevalence were not in the group of areas with higher  
226 cat density per household (Fig 4). The cat population density and the prevalence were  
227 analyzed with the Shapiro-Wilk test, and the results showed that only population density  
228 exhibited normal distribution ( $p > 0.05$ ). In addition, Spearman correlation test was  
229 carried out between such variables, but no statistically significant correlation between  
230 them was found ( $p = 0.09$ ). This result may have occurred because the calculation of  
231 density did not include an estimate of the free roaming population, of which there were  
232 no available data, and they may contribute to the greatest infection rate in cats with  
233 access to the street. Another possibility is that the risk of infection is more related to the  
234 cultural habits of the owners, giving cats a free access to the street, than the local

235 density. This is because studies have shown that cats with access to the street may  
236 wander across large areas, up to 1.92 ha [17,18].

237 **Fig 4 - Map of feline population density (cats per 100 residences, A), and the**  
238 **prevalence of sporotrichosis (cases per thousand cats, B) in the county of Barreiro,**  
239 **in Belo Horizonte, 2016 to 2018.**

240 Five areas of coverage amounted to a participation of 71.19% (CI 95% 70.29 - 72.07)  
241 of the total number of positive cats. The Kernel analysis showed the existence of three  
242 areas where cases were centered (Fig 5). These results may be indicators of areas where  
243 actions for the prevention and control of the disease should be prioritized and  
244 intensified.

245 **Fig 5 - Map of the intensity of sporotrichosis cases in the county of Barreiro, Belo**  
246 **Horizonte, 2016 to 2018.**

## 247 **Environmental Conditions**

248 The distribution of characteristics related to the homes of the analyzed cats (S3 Table)  
249 was similar between positive and negative animals groups, with predominant houses  
250 (94.21%, CI 95% 89.52 - 98.16) having a backyard (90.98%, CI 95% 87.18 - 97.05).  
251 Organic matter was observed in 49.59% (CI 95% 40.74 - 59.26) of the households. The  
252 presence of debris (27.27%, CI 95% 20.28 - 37.27), trees (24.79%, CI 95% 18.02 -  
253 34.54), vegetable gardens (18.18%, CI 95% 12.28 - 27.29), and grass (15.70%, CI 95%  
254 10.07 - 24.19) was less common.

255 There was no statistically significant association between environmental variables and  
256 positive diagnosis for sporotrichosis. It was observed that the characteristics of  
257 households in the sample studied, as well as the characteristics of cats, were quite

258 homogeneous, which did not allow the quantification of the association between them  
259 and the diagnosis of sporotrichosis.

260 It is important to emphasize the existence of the only article in literature, which reported  
261 that it was possible to isolate *S. brasiliensis* from environmental samples, a more  
262 frequent species in the outbreaks taking place in Brazil [19]. These results seem to  
263 corroborate the hypothesis that direct transmission by infected cats plays a greater role  
264 in the occurrence and continuous outbreaks of sporotrichosis in Brazil [20], although the  
265 possibility of a classical infection route in these places should not be discarded.

## 266 **Outcome of positive domestic cat cases**

267 It was possible to get data on the outcome of 96.32% (95% CI 91.63 - 98.80, 131/136)  
268 of the cats assessed (S4 Table); however, 62.49% (95% CI 53.79 - 70.65, 85/136) were  
269 not present at the residence after the period between the first and second visits to the  
270 site. Among the positive animals, 61.90% (CI 95% 58.95 - 64.96) died, with a lethality  
271 rate of 55.08% in the studied population (CI 95% 49.20 - 51.15), which is considered  
272 high, and proves the severity of the disease. The mortality coefficient for sporotrichosis  
273 was 4.6 deaths for every 1000 cats (CI 95% 3.4 - 6 per 1000 cats) of the population of  
274 the Barreiro's county. Animals with a positive diagnosis were 6.30 times more likely to  
275 die than negative animals ( $p < 0.05$ , OR 6.30, CI 95% 2,79-14,42).

276 Among the positive animals, 82.85% (CI 95% 82.10 - 83.58) showed an unfavorable  
277 progression of the disease and only 7.62% (CI 95% 7.12 - 8.16) were cured, after  
278 undergoing treatment. Notably, there were reports of deaths caused by both natural  
279 causes and by the actions of those responsible for the animals (such as poisoning or  
280 setting the animals on fire).

281 A number of factors may be related to the reduction in the cure of the disease, such as  
282 the high cost of medication, socioeconomically poor region, prolonged period of  
283 treatment, weak accountability of the cat owners, and an absence of a public policy that  
284 permits access to treatment and free veterinary monitoring of these animals.

285 In cases of death or euthanasia of the animals, we tried to find out what happened to the  
286 corpses. The percentage of positive cases that resulted in death and sent to incineration  
287 was 46.15% (CI 95% 33.70 - 58.97, 30/65). This result was possible because, at the  
288 time of the first visit, the owners were informed about the need to incinerate the corpse  
289 of the infected animals and free access to this service was provided by the UFMG.  
290 Despite this, a considerable percentage of dead positive animals (29.23%) (CI 95%  
291 17.31 - 40.19, 19/65) were inappropriately discarded. Among these, 63.2% (CI 95%  
292 50.20 - 74.72) were thrown in barren land, 31.2% (95% CI 28.40 - 34.14) were  
293 discarded into household trash, and one of the owners did not inform the exact location  
294 of disposal. Therefore, public policies of environmental education and actions that will  
295 emphasize the correct disposal of animal corpses with sporotrichosis are of great  
296 importance to prevent contaminating the environment, given the saprophytic nature of  
297 *Sporothrix* sp.

## 298 **Conclusion**

299 According to previous literature, this is the first report on the epidemic of sporotrichosis  
300 in Minas Gerais. Sporotrichosis is present in the municipality of Belo Horizonte with  
301 wide distribution, affecting humans and cats, and a high mortality in domestic feline  
302 cases has been observed in the county of Barreiro. Suspected animals with free access to  
303 the streets have a greater chance of a positive result for sporotrichosis; therefore, public  
304 environmental education is urgently required to modify the relationship between cat

305 owners and their animals. The disease presented an unfavorable prognosis for cats, even  
306 though a treatment exists. Additionally, inappropriate disposal of corpses was also seen.  
307 Therefore, the free offer for treatment and veterinary care to these animals should be  
308 taken into consideration, as well as the collection and incineration of the dead ones, as  
309 measures of public health, followed by the guidance and care for the human patient.

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## 378 **Supporting information**

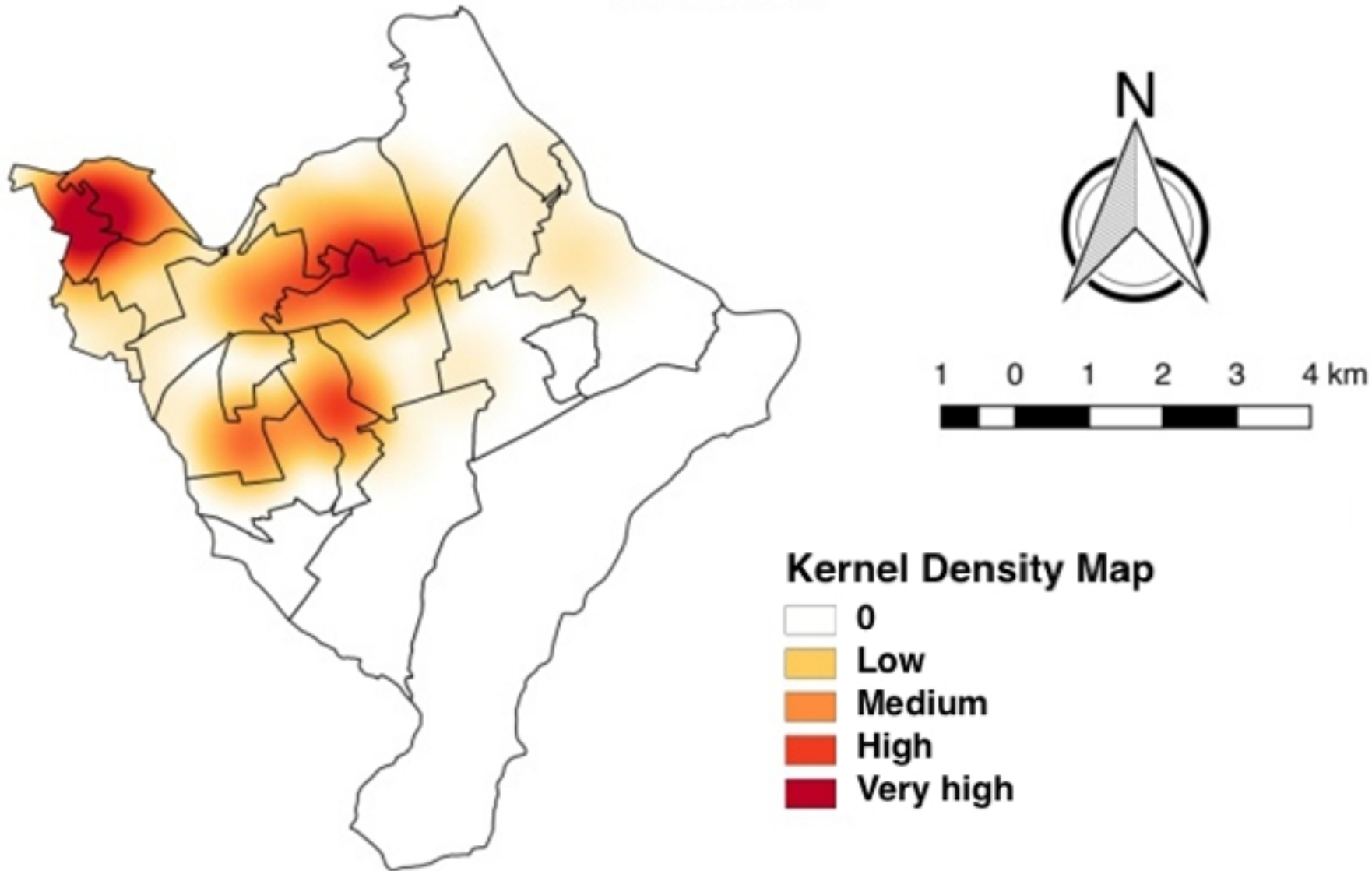
379 **S1 Table - Distribution of household cats analyzed as per the demographic**  
380 **variables, county of Barreiro, Belo Horizonte, Minas Gerais, August 2016 to June**  
381 **2018.**

382 **S2 Table - Distribution of household cats suspected of sporotrichosis analyzed,**  
383 **according to coverage areas, county of Barreiro, Belo Horizonte, Minas Gerais,**  
384 **August 2016 to June 2018.**

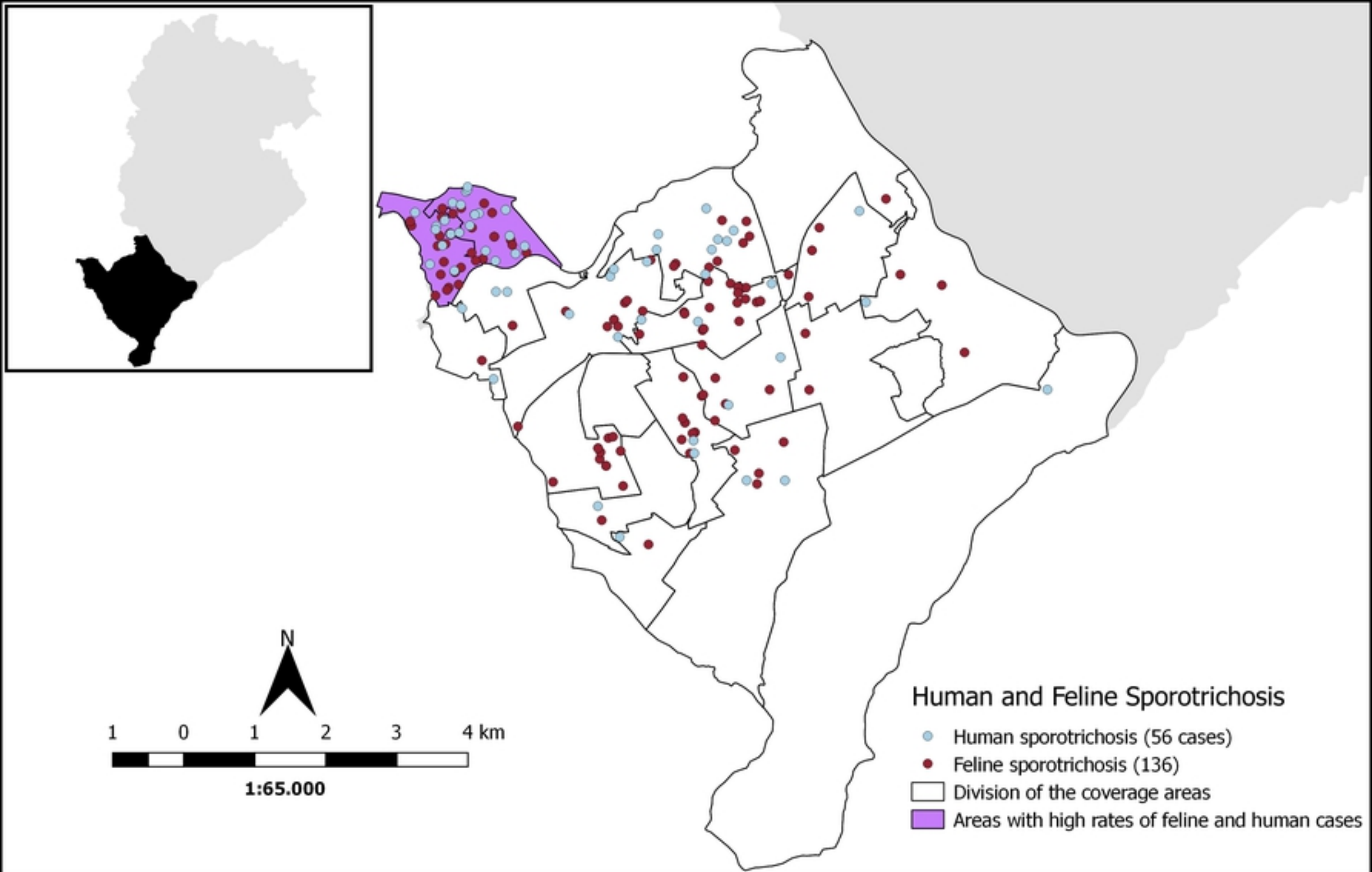
385 **S3 Table - Distribution of domestic felines analyzed as per the environmental**  
386 **variables.**

387 **S4 Table - Distribution of domestic cats collected according to the outcome and**  
388 **association of the variables with the positive diagnosis of sporotrichosis, in county**  
389 **of Barreiro, 2017/2018.**

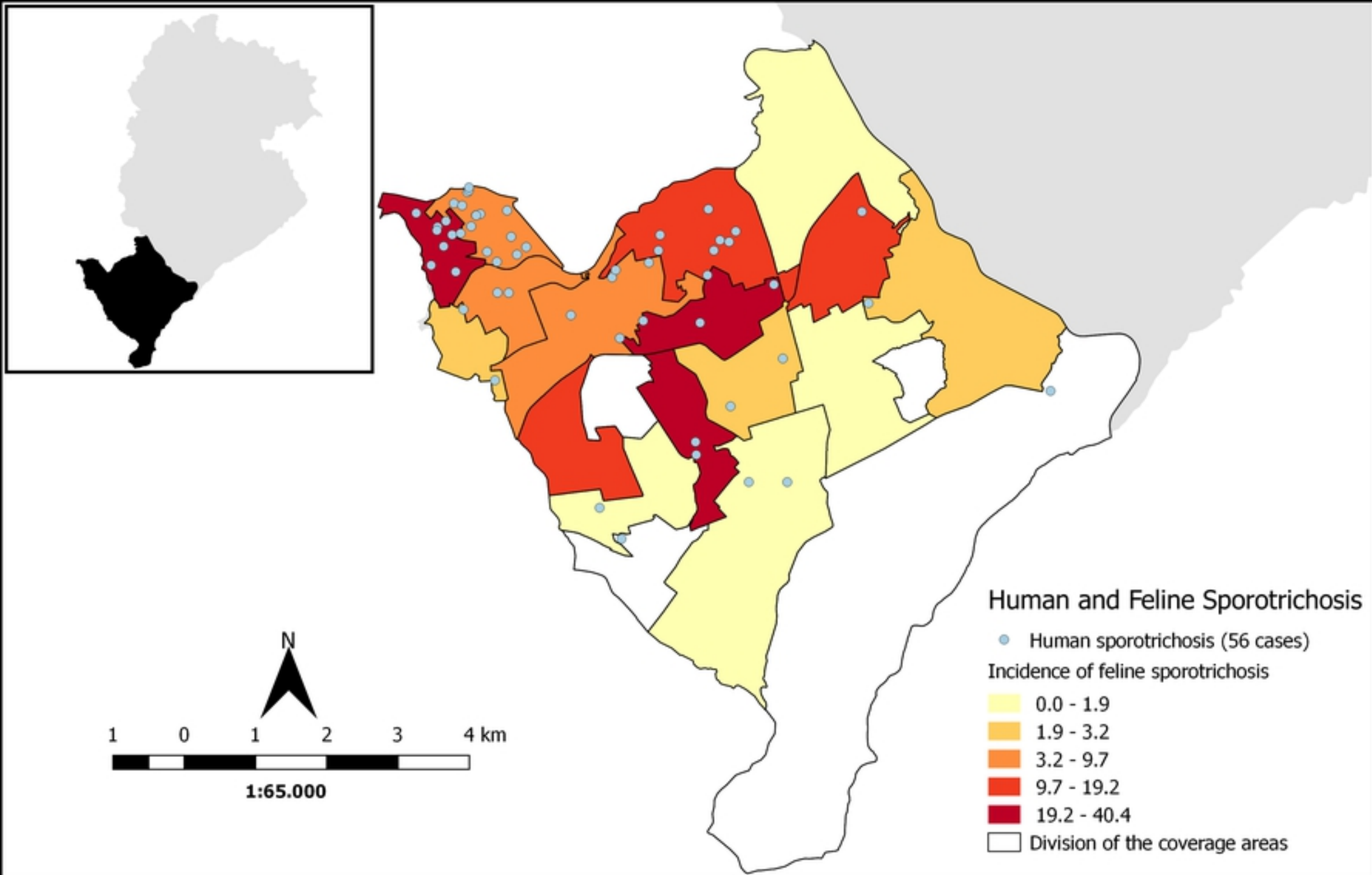




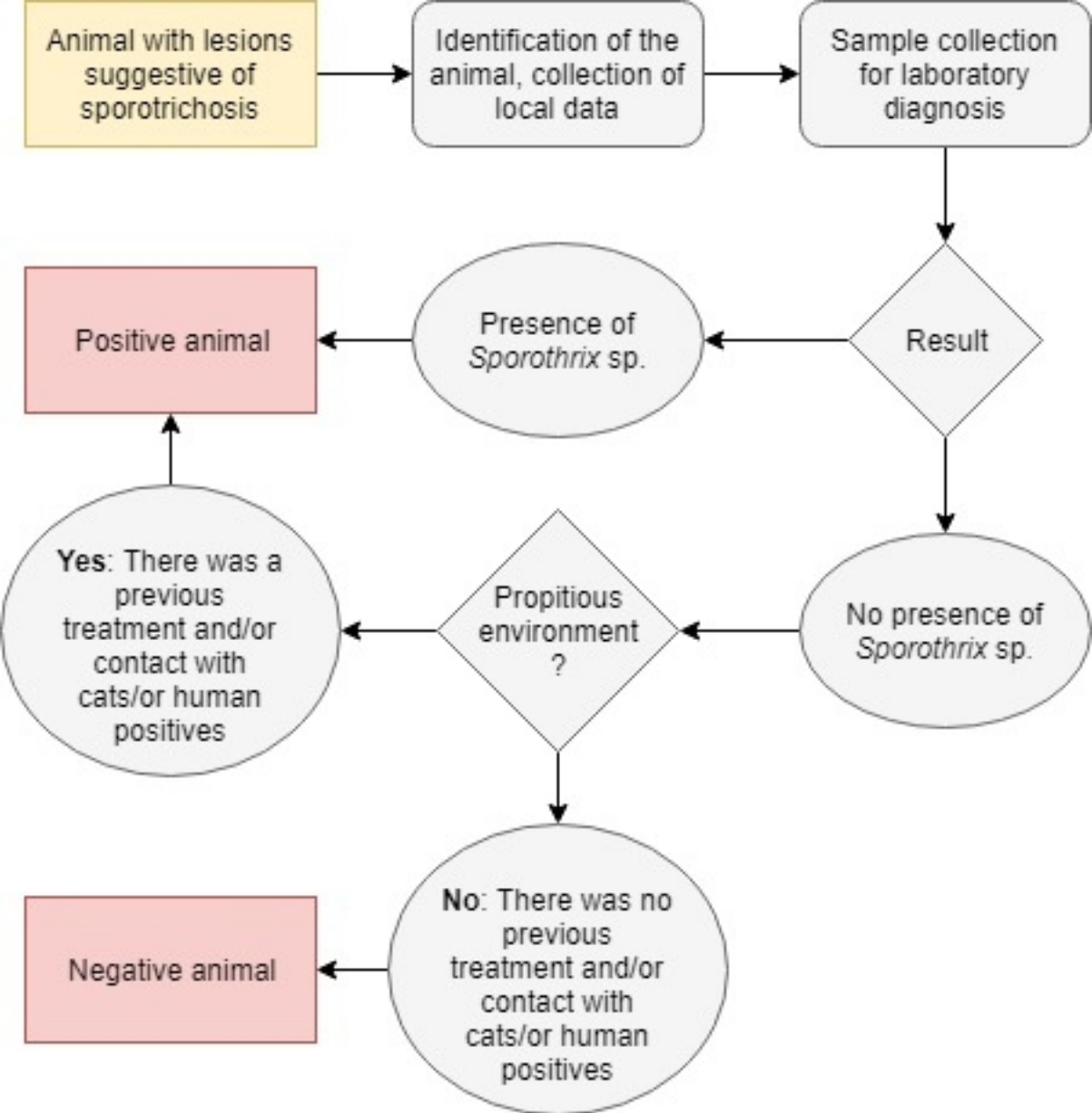
Figure



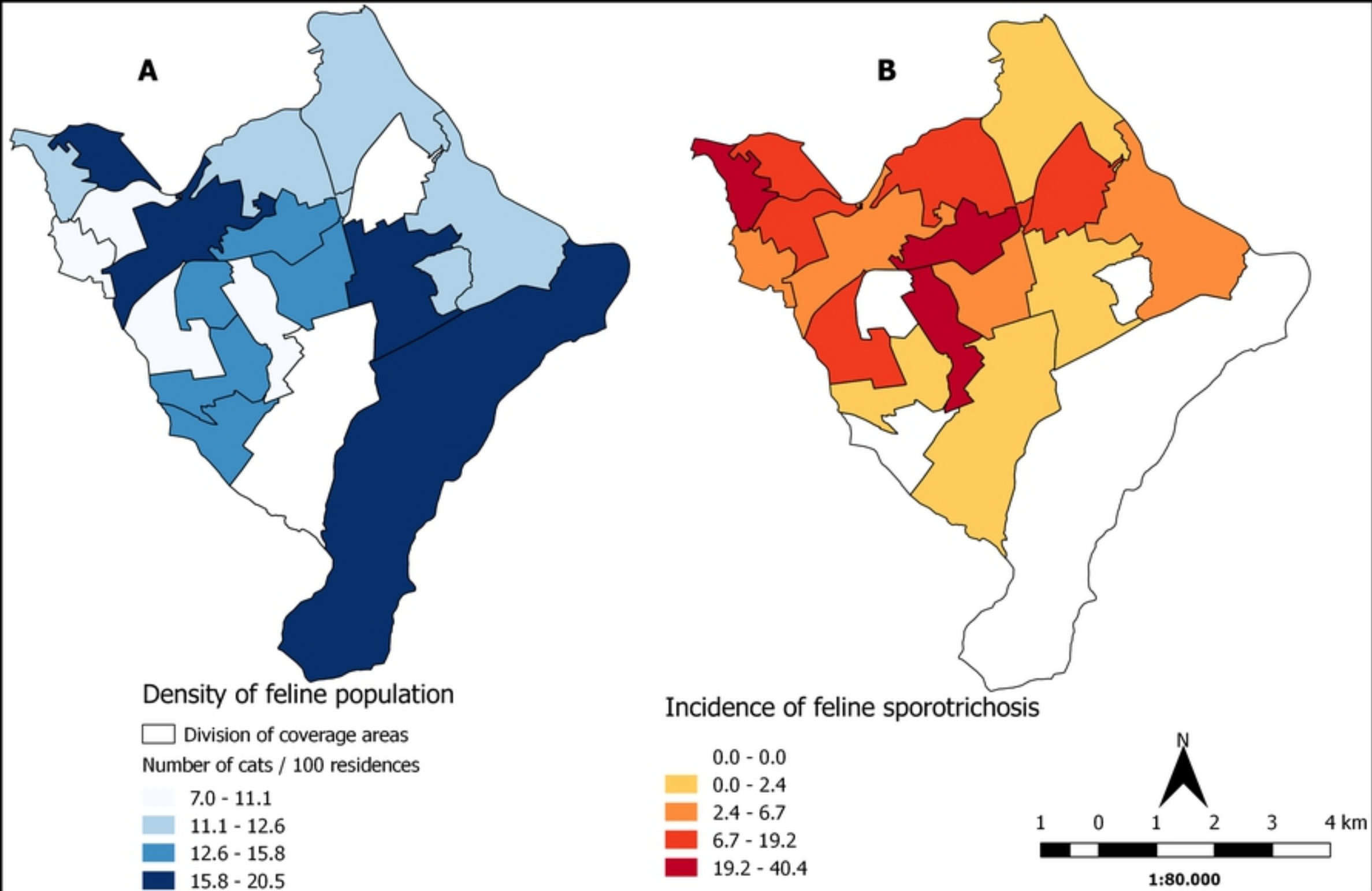
Figure



Figure



Figure



**Production:**  
 Department of Preventive Veterinary Medicine  
 Veterinary School - Universidade Federal de Minas Gerais

**Geographical Projection System: Transverse Cylindrical**  
**DATUM: SIRGAS 2000 / UTM zone 23S**

Figure