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► **To cite this version:**

G. Acosta-Jamett, W.S.K. Chalmers, A.A. Cunningham, S. Cleaveland, I.G. Handel, et al.. Urban domestic dog populations as a source of canine distemper virus for wild carnivores in the Coquimbo region of Chile. *Veterinary Microbiology*, 2011, 10.1016/j.vetmic.2011.05.008 . hal-00724203

HAL Id: hal-00724203

<https://hal.science/hal-00724203>

Submitted on 20 Aug 2012

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Accepted Manuscript

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PII: S0378-1135(11)00272-0
DOI: doi:10.1016/j.vetmic.2011.05.008
Reference: VETMIC 5305

To appear in: *VETMIC*

Received date: 18-6-2009
Revised date: 28-4-2011
Accepted date: 6-5-2011

Please cite this article as: Acosta-Jamett, G., Chalmers, W.S.K., Cunningham, A.A., Cleaveland, S., Handel, I.G., Bronsvoot, B.M.C., Urban domestic dog populations as a source of canine distemper virus for wild carnivores in the Coquimbo region of Chile, *Veterinary Microbiology* (2010), doi:10.1016/j.vetmic.2011.05.008

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1 **Urban domestic dog populations as a source of canine distemper virus for wild**
2 **carnivores in the Coquimbo region of Chile.**

3
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20
21 **Running Title:** Urban dogs as a source of CDV in Coquimbo region, Chile.
22
23

24 **Abstract**

25 Urban areas can support dog populations dense enough to maintain canine distemper
26 virus (CDV) and can be a source of infection for rural dogs and free-ranging
27 carnivores. The aim of this study was to investigate the relationships between urban
28 and rural domestic dog and wild carnivore populations and their effects on the
29 epidemiology of CDV to explain retrospectively a CD outbreak in wild foxes in
30 2003. From 2005 to 2007 a cross-sectional household questionnaire survey was
31 conducted in Coquimbo and Ovalle cities, in three towns and in rural sites along two
32 transects from these cities to the Fray Jorge National Park (FJNP) in the Coquimbo
33 region, Chile. Blood samples were collected from unvaccinated dogs at surveyed
34 households and from free-ranging foxes in rural areas along the transects. The
35 seroprevalence of CDV in domestic dogs was higher in urban than in rural areas and
36 in the later was highest in dogs born before 2001-2002. The seroprevalence of CDV
37 in foxes was higher in areas closer to human settlements. A high seroprevalence in
38 dogs born before 2001-2002 further supports a link between CDV patterns in rural
39 dog and fox populations. In our study area, urban dogs are proposed to be the source
40 of CDV infection to wild carnivores. The large dog population size and density
41 detected in Coquimbo and Ovalle provides optimal conditions for maintaining a
42 large and dense susceptible population of dogs, which can act as a reservoir for
43 highly infectious diseases and could have been the source of infection in the CD
44 outbreak in wild foxes.

45

46 **Key words:** Canine distemper virus, urban areas, wild carnivores, risk factors,
47 domestic dog.

48

49

50 **1. Introduction**

51 Canine distemper is one of the most common and globally significant infectious
52 diseases of the domestic dog (*Canis familiaris*). In addition to causing disease in
53 domestic dogs, canine distemper virus (CDV) can cause high mortality rates in wild
54 carnivores and can threaten endangered carnivore populations (Funk et al., 2001).
55 Key aspects for the control of CDV and for minimising its threat to wildlife
56 conservation should include the identification of infection reservoirs, the
57 mechanisms by which infections are sustained within reservoirs, and the sources and
58 routes of transmission from reservoirs to species of concern (Woodroffe, 1999).
59 Following Haydon et al. (2002), a reservoir is defined as one or more
60 epidemiologically connected populations or environments in which the pathogen can
61 be permanently maintained and from which infection is transmitted to the defined
62 target species (e.g. wild carnivores). On the other hand, a source population is
63 defined as any population that transmits infection directly to the target population,
64 being by themselves maintenance populations or constitute all or part of a
65 transmission link from a maintenance population to the target population (Haydon et
66 al., 2002) .

67

68 Theoretical studies and empirical data suggest that infectious pathogens should only
69 persist in populations larger than a threshold or critical community size (CCS),
70 where the pathogen is maintained by an input of susceptibles by birth and/or
71 immigration (Swinton et al., 2002). On the other hand, in populations below the
72 CCS, such pathogens cannot persist because of the low probability of contact
73 between infectious and susceptible hosts (Anderson and May, 1991). However, even
74 those populations that are under a CCS (non-maintenance populations) if they are

75 epidemiologically and spatially connected with other non-maintenance or
76 maintenance populations (eg. through immigration), they can be part of larger
77 complex meta-population which can be part of a reservoir in which the pathogen can
78 persist in the long-term (Haydon et al., 2002; Almberg et al., 2010).

79

80 Domestic dogs are one of the most numerous carnivores in the world (Daniels and
81 Bekoff, 1989), and they are particularly abundant in urban areas of some developing
82 countries where they can be excellent reservoirs for pathogens, since they usually
83 live in large populations, are not vaccinated and are regularly allowed to roam freely,
84 facilitating contact between infected and susceptible hosts (WHO/WSPA, 1990). In
85 contrast, in rural areas, where dog densities and population size are often low (see
86 Acosta-Jamett et al., 2010), highly virulent pathogens cannot be maintained and the
87 infection fades out in the absence of the introduction of new infections from
88 neighbouring areas (Swinton et al., 2002). Furthermore, rural areas tend to be the
89 habitat of wild carnivores that may be susceptible to CDV (Appel, 1987; Funk et al.,
90 2001). Wild carnivores commonly have small populations and occur at low densities.
91 Therefore, they are often not suitable to maintain infections for highly pathogenic
92 generalist viruses like CDV (Cleaveland et al., 2002). Instead, these pathogens tend
93 to spill over from domestic dogs to wild carnivores through occasional contact (eg.
94 Cleaveland et al., 2000).

95

96 At the end of the austral spring in November 2002, wild foxes of the genus
97 *Pseudalopex* were seen with canine distemper-like signs by local people in the
98 surrounding areas of Tongoy town and after five months in the Fray Jorge National
99 Park (FJNP) in the semi arid Coquimbo region of north-central Chile, reporting a

100 peak of cases in the austral summer (January-March) of the next year (Moreira and
101 Stutzin, 2005; Acosta-Jamett, 2009) (see Figure 1). In this region the size and density
102 of domestic dogs is many orders of magnitude higher in urban (i.e. ~80,000 dogs and
103 2,000 dogs km⁻²) than in rural areas (i.e. ~2,000 dogs and 6 dogs km⁻²) and it has
104 been hypothesised that urban dogs, due to their high population size and density and
105 high population turnover, can act as reservoir of directly transmitted pathogens such
106 as canine distemper virus (Acosta-Jamett et al., 2010). CDV has been reported to be
107 present in domestic dogs and in wild carnivores in Chile (González-Acuña et al.,
108 2003). Whether domestic dogs inhabiting towns or cities in the Coquimbo region can
109 be reservoir populations for directly transmitted pathogens such as canine distemper
110 virus to domestic and wild carnivores inhabiting rural areas is unknown.

111

112 We hypothesise that the population size of rural dogs and wild carnivores in the
113 Coquimbo region is far below that required for maintaining CDV in the region and
114 that urban dogs were the source of CDV infection for wild carnivores during the CD
115 outbreak in 2003. We estimated the seroprevalence of CDV in domestic dogs
116 inhabiting urban and rural areas and in wild foxes in the Coquimbo region in central
117 Chile, determined age-specific seroprevalence, and identified and quantified risk
118 factors for CDV seropositivity that could explain retrospectively the 2003 CD
119 outbreak in foxes, by exploring whether CDV prevalence in wild carnivores relates
120 to urban domestic dog populations.

121

122

123 **2. Material and methods**

124 **2.1. Study area**

125 The study site comprised an area of ~1,600 km² of the Coquimbo region in North
126 Central Chile (71° 12' to 71° 40' W, 29° 58' to 30° 39' S). This area included two
127 cities, three towns and several small human settlements connected to the FJNP
128 through land use gradients (Figure 1). The cities are the capital of the region,
129 Coquimbo city, with a human population of ~148,500 inhabitants and an average of
130 3.4 people/household, and Ovalle city, with a human population of ~66,500, and an
131 average of 3.5 people/household (INE, 2005). The towns are Tongoy, Guanaqueros
132 and La Torre with a human population of less than 5,000 inhabitants (INE, 2005).
133 Rural human settlements are dispersed areas existing between cities and towns in
134 settlements with a low human density of 2.0 individual/km² (INE, 2005), and where
135 households are typically placed both sides of a main road in very isolated places.
136 Two fox species inhabit rural areas in this region, the chilla (*Pseudalopex griseus*)
137 and the culpeo (*Pseudalopex culpaeus*).

138

139 **2.2. Sampling design**

140 The sampling design is described in detail elsewhere (Acosta-Jamett et al., 2010).
141 Briefly, in order to assess CDV prevalence in an urban/rural complex, sampling was
142 carried out along gradients of urbanization by two transects from Coquimbo and
143 Ovalle cities to FJNP (Figure 1). The first transect ran for 80 km north-south from
144 Coquimbo to the FJNP and included Guanaqueros and Tongoy towns (sites B and C,
145 respectively) and the rural sites Lagunillas (A), El Tangué (D), and Punilla (E). The
146 second ran for 40 km east-west transect from Ovalle city to the FJNP. This transect
147 included the rural site Barraza (F) and La Torre town (G). The centroids were evenly

148 spaced out along the transects at intervals of 13 km and a circle of 6.5 km radius
149 drawn at each to demarcate the sampling site.

150

151 *Urban areas*

152 Within each of the two cities a cross-sectional design was used with stratification by
153 neighbourhood units (NU: i.e. geographically defined areas with relatively
154 homogeneous socioeconomic characteristics) as described by Ibarra et al. (2003) in
155 Santiago city, Chile. This method consists of calculating the number of household
156 questionnaires to conduct in each NU according to the percentage of households of
157 that NU within the overall household number in the city. In Coquimbo city there are
158 27 NU and in Ovalle city 15 NU (INE, 2005). Blocks were randomly selected within
159 each NU, using data provided by Coquimbo and Ovalle municipalities. A maximum
160 of 4 households were interviewed per block (an arbitrary and logistically affordable
161 number), which was used to calculate the number of blocks needed to complete the
162 proportional number of households calculated for each NU. In both cities, the
163 downtown area was less populated; therefore in these sites fewer questionnaires were
164 conducted. In towns the sampling design followed that of Cleaveland et al. (2000) in
165 which one in five of the households of each sampled village were interviewed. In our
166 study area, every street of each town was surveyed using available maps of the
167 towns.

168

169 In order to calculate the number of questionnaires to be conducted to obtain blood
170 samples, it was necessary to estimate the size of the dog population in both cities.
171 This was based on a previous study in Santiago city (Ibarra et al., 2003), since no
172 other reliable studies were available we used a conservative dog/household estimate

173 proportion of 0.72. Based on this the estimated dog populations were 31,611 and
174 13,850 for Coquimbo and Ovalle, respectively. The sample size was calculated using
175 Win Episcopo 2.0, based on a seroprevalence of 50% (which is selected when no
176 previous idea of the pathogen prevalence in the study population is found and will
177 result in the highest sample size) (Thrusfield et al., 2001), an accuracy of $\pm 5\%$ and a
178 95% confidence interval. The final sample size was 500 households within each city
179 to get blood samples from 385 dogs, allowing for a refusal rate of up to 20%.

180

181 *Rural areas*

182 The sample size in rural areas was calculated in a similar way to that for urban areas;
183 however, the size of the dog population was estimated using data from the human
184 population census provided by the National Bureau of Statistics (INE, 2005). Due to
185 the small household numbers within each rural site, when calculating the sample size
186 with the program Win Episcopo 2.0 for a disease with a seroprevalence of 50%, an
187 accuracy of $\pm 5\%$ with a 95% confidence interval, nearly all households existing in
188 rural areas should be sampled. This was close to the expected number of dogs that
189 would be found in all household and so all households were sampled.

190

191 **2.3. Questionnaire interviews**

192 A questionnaire was conducted at each household between 2005-2007. The
193 questionnaire was developed following the guidelines of the World Health
194 Organisation (WHO/WSPA, 1990), and from similar published studies (eg. Butler
195 and Bingham, 2000). Households were visited and re-visits were done if no
196 household members were available at the first visit. Only adult members of the
197 household were interviewed.

198

199 Questions intended to explore risk factors to CDV seroprevalence were divided in
200 two levels: dog and household. At the dog level, questions were: ‘*what is the sex of*
201 *your dog?*’ (i.e. male, female), ‘*what is the age of your dog?*’ (in months), ‘*what is the*
202 *function of your dog?*’ (i.e. guarding, pet, or herding), ‘*do you allow to your dog to*
203 *roam freely in the neighbourhood?*’ (i.e. always, sometimes or never). At the
204 household level question were: ‘*do you see neighbours’ dogs roaming in the*
205 *neighbourhood?*’, and ‘*do you see unknown dogs roaming in the neighbourhood?*’;
206 the responses to these questions were grouped on always, sometimes or never. The
207 questionnaire took between 30 and 40 minutes to complete. After each interview, all
208 dogs that were reported as “unvaccinated against CDV” during the questionnaire,
209 were blood sampled. Dogs were manually restrained and blood was collected from
210 the cephalic vein into plain 5ml vacuutainers, and centrifuged the same day with a
211 Mobilespine centrifuge. The serum was stored at -18° C in electrical freezers until
212 laboratory analysis.

213

214 The coordinates of each household were recorded with a GPS (Etrex, Garmin®) and
215 then transferred to a GIS (Arc View 3.3). Four additional spatial risk factors were
216 included in the analyses: ‘*distance to nearest city*’, ‘*distance to nearest urban area*’,
217 ‘*distance to nearest human settlement*’, and ‘*distance to nearest household*’, by
218 plotting the position of the household of each sampled dog and measuring the
219 straight line distance to the centre of the nearest city, urban area (i.e. city or town),
220 human settlement (i.e. city, town or centre of village) or nearest household,
221 respectively.

222

223 **2.4. Wild foxes**

224 Foxes were concurrently captured in rural areas to determine CDV seropositivity
225 and related risk factors. At each site, foxes were captured using padded leg-hold
226 traps (Victor Soft Catch No. 1.5, Chagnons Trapping Supply, Manistique, Michigan,
227 USA) and with homemade box traps. Animals were anaesthetised with a mixture of
228 2.5 mg/kg ketamine and 50 µg/kg medetomidine and reverted with 250 µg/kg
229 atipamezole, based on an estimated 3 kg of body weight for chillas and 7.5 kg for
230 culpeo foxes (González del Solar and Rau, 2004; Jiménez and Novaro, 2004).
231 Monitoring of anesthesia included temperature, heart rate, breathing rate and oxygen
232 saturation, which were recorded every five minutes, while blood samples were
233 taken. Before releasing the animals, they were marked with eartags to avoid re-
234 sampling during recaptures. Standard body measurements and sex were recorded,
235 and age was assessed on the basis of incisor wear and eruption (Gipson et al., 2000),
236 and body size and weight, classifying them as adults or juveniles. The trapping site
237 was georeferenced with a handheld GPS. Capture and handling procedures were
238 approved by the Ethical Committee at the Institute of Zoology, Zoological Society
239 of London.

240

241 Similarly to what was measured for domestic dogs, spatial risk factors were recorded
242 by plotting the capture of each sampled fox and measuring the straight line distance
243 to the centre of the nearest urban area, human settlement or nearest household
244 respectively. In rural areas all existing households were georeferenced, which
245 allowed us to measure exactly the distance of each trapping site to the nearest
246 household.

247

248 **2.5. Laboratory analyses**

249 Serum samples were analyzed to determine seropositivity to CDV using a
250 microneutralisation test (Appel and Robson, 1973) at Intervet, UK. This method
251 consisted of preparing 4-fold serum dilutions with tissue culture medium starting at
252 1:8 and incubating them at 37° C for 1 hr with an equal volume of virus suspension
253 (Bussel strain) containing between 32 and 316 TCID₅₀/ml of neutralizing antigen.
254 Each serum/virus suspension was then inoculated into freshly seeded Vero cell
255 cultures in 96-well microtitre plates. The inoculated plates were incubated at 37° C
256 for 4-6 days and then examined by microscopy for virus cytopathic effect. The titre
257 of neutralizing antibodies was obtained by counting the number of wells where the
258 cytopathic effect was and was not observed and entering into Lisa 1.6-Intervet
259 Statistical Application software that used the Spearman-Kärber formula for titre
260 calculation.

261

262 **2.6. Data analysis**

263 Data were entered into an Excel spreadsheet (Microsoft Excel 2003) and imported
264 into STATA 10 for windows software package (Stata Corporation, Texas, USA).
265 The associations between potential risk factors and seropositivity to CDV in dog and
266 foxes were examined using fixed effect univariable logistic regression. The test
267 results for antibodies for CDV for each animal were converted to a binary
268 positive/negative result based frequency distribution and served as outcome
269 variables. Categorical variables with more than two levels (k) were analysed using
270 k-1 dummy variables.

271

272 Predictor variables in dogs included ‘site’ (9 levels, with 5 urban sites: CQ, OV, B,
273 C, G and 4 rural sites A, D, E, and F), according to the area where dogs were
274 sampled; and six factors obtained from the questionnaire interview (i.e. sex, age, and
275 dog’s function, and if owners allow the sampled dog to roam freely, and if they
276 reported to see neighbour’s dog or unknown dogs roaming freely in the
277 neighbourhood) detailed above in section 2.3 and the spatial variables (i.e. distances
278 to nearest city, urban area, human settlement, and household) that were measured in
279 a digital map as detailed in section 2.4. In foxes, five potential risk factors including
280 two categorical: ‘age’ (2 levels: juvenile and adult) and ‘sex’ (2 level: male and
281 female) and three continuous factors: ‘distance to nearest urban area’, ‘distance to
282 nearest human settlement’ and ‘distance to nearest household’, were investigated.

283

284 Due to incomplete filling of some of the questionnaires, information regarding some
285 of the sampled dogs was missing (see table 1 for further details), therefore the final
286 sample size varied between variables. Factors with a likelihood-ratio test p -value of
287 <0.25 (a conservative value, since Hosmer and Lemeshow, 2000 recommend a p
288 value of 0.15) were considered for entry into a multivariable mixed-effects logistic
289 regression analysis using the function *xtnlogit* in STATA 10 for mixed logistic-
290 regression analysis. To control the effect of cross-infection between dogs within
291 households, households were included as a random effect in the model (Condon et
292 al., 2004). Initially, all selected variables were forced into the multivariable mixed-
293 logistic regression model. Manual backwards elimination was used for model
294 building, excluding variables with a p -value > 0.1 in the likelihood ratio test (Dohoo
295 et al., 2003). No variables were considered as confounders *a priori* but variables
296 were deemed to be confounders if the change in the odds ratio for the included

297 variable was 25% or greater (Dohoo et al., 2003). The fit of the fixed-effect models
298 were assessed using Hosmer-Lemeshow goodness-of-fit test (Hosmer and
299 Lemeshow, 2000), the area under the curve of the receiver-operating characteristic
300 (ROC) and the Pearson's χ^2 statistic. Regression diagnostic for identifying
301 influential covariate patterns were carried out by plotting the Pearson's residual
302 squared ($\Delta\chi^2$), the influence ($\Delta\beta$), and delta D (ΔD) against the predicted
303 probabilities of being seropositive as suggested by Hosmer and Lemeshow (2000).
304 The diagnostic measures ΔD and $\Delta\chi^2$ measure the effect of each covariate pattern on
305 the fit of the model by measuring the change in the deviance or χ^2 residual while $\Delta\beta$
306 measures the effect of each covariate pattern on the value of the estimated
307 parameters. The final logistic regression model residuals were examined for
308 evidence of spatial clustering by construction of a semivariogram (Kleinschmidt et
309 al., 2000).

310

311 Additionally, analyses of age-specific CDV seroprevalence were carried out to
312 assess retrospectively whether a higher prevalence existed in a given age in the rural
313 or urban dog populations or in wild foxes, which could be indicative of an increase
314 of exposure to CDV that could suggest the occurrence of a CDV outbreak in a given
315 year (eg. Packer et al., 1999; Cleaveland et al., 2000).

316

317

318 **3. Results**

319 A total of 1,315 households were visited of which only 1,063 were interviewed, of
320 these 654 (61%) were dog-owning-household (DOHH). Overall, 292 households had
321 unvaccinated dogs, and blood samples were taken from 392 non-vaccinated dogs of

322 the 1,168 dogs reported in the interviewed households. In 88% of households, all
323 dogs reported in questionnaires were observed at the time of the interview. Detailed
324 analyses of the dog population has been published elsewhere (Acosta-Jamett et al.,
325 2010). Briefly, the proportion of dogs aged less than a year was higher in cities
326 (29%) than in towns and rural areas (19%). Also, the dog population growth in cities
327 was 20%, 19% in towns and 9% in rural areas. As expected the highest dog
328 population density was found in Coquimbo city with over 2,400 dogs km⁻², followed
329 by towns with a density between 100-1,500 dog km⁻² and was lowest in rural areas
330 with <24 dogs km⁻². From demographic data obtained during the questionnaires, the
331 susceptible dog population was estimated for each of the selected sites (Table 1).
332 According to results from the questionnaire survey, 42% (40-45%, 90% CI) of the
333 dog population was reported to be vaccinated against CDV (Table 1).

334

335 A high proportion of households reported vaccinating their dogs. To avoid problems
336 of interpreting tests results only unvaccinated dogs were sampled but this approach
337 resulted in a smaller than calculated sample size. Furthermore, not all the owners
338 allowed blood samples to be collected from their dogs, further reducing the final
339 sample size. Taking these factors into account, 90% confidence intervals (CI) for
340 seroprevalence results have been estimated throughout.

341

342 The frequency distribution of antibody titres demonstrated a demarcation between
343 seropositive and seronegative dogs and foxes at a dilution of >1.2 log₁₀ (Figure 2),
344 which was used as the cut-off point and was consistent with similar studies
345 (Cleaveland et al., 2000; Courtenay et al., 2001).

346

347 *Canine distemper virus in dogs*

348 The CDV seropositivity ranged from 0.34 to 0.76 in the different sites in the study
349 area. The sites with higher seropositivity were urban areas (i.e. cities and towns),
350 Coquimbo (0.74, 90% CI 0.60-0.85) and Ovalle cities (0.76, 0.61-0.87), showed the
351 highest and the lowest were rural sites, site F (0.34, 90% CI 0.25-0.44) and site D
352 (0.34, 90% CI 0.25-0.44) (Table 2 and figure 3). The overall estimate of
353 seropositivity, weighted for the sampling design, was 0.73 (CI 0.644-0.83).

354

355 Analyses of the age-specific CDV seroprevalence, shows a high seroprevalence in all
356 age classes in urban dog populations. Given the lifelong immunity from exposure,
357 the simplest interpretation is a recent CDV outbreak. On the other hand results
358 obtained in rural dog populations suggest an increase in seroprevalence in the 4-5
359 age class (i.e. dogs born before 2001), which suggests higher CDV infection between
360 2001-2002 in domestic dogs in rural sites (Figure 3B).

361

362 Of the eleven variables analyzed in the univariable fixed logistic regression analysis,
363 two did not pass the initial screening criteria and were dropped, '*what is the sex of*
364 *your dog*' and '*do you allow to your dog to roam freely in the neighbourhood?*',
365 thus nine variables were passed for inclusion in the final model (Table 3).

366

367 In the final model, $y = \beta_0 + \beta_1 * \text{Site} + \beta_2 * \text{Age} + \beta_3 * \text{DNH}$, in which Site (nine
368 groups) and Age (three groups) were fitted as categorical variables and distance to
369 nearest household (DNH) as a continuous variable (see table 4 for further details),
370 there was no improvement in fit from including a random effect for household
371 ($p > 0.1$), therefore results are presented only for the fixed effects model. The

372 multivariable fixed effect model for risk factors for seropositivity to CDV is given in
373 Table 4. The odds of a dog being CDV seropositive were similar in Coquimbo city
374 and in each of the urban sites, i.e. Ovalle city, and the towns placed in sites B
375 (Guaqueros), C (Tongoy), and G (La Torre). There was an increased risk of being
376 seropositive associated with being in urban rather than rural areas. The odds of a dog
377 being CDV seropositive were 2.21 times higher in the age classes 1-2 years, and 4.73
378 time higher in the age class >2 years compared to the baseline age class 0-1 years.
379 Finally, the odds of a dog being CDV seropositive were higher when the household
380 of the sampled dog was close to another household. There was no statistically
381 significant spatial autocorrelation in the final multivariate logistic regression model
382 residuals except in the distance band around 18 km, which showed an increased
383 variance at approximately the same order as the inter-site separation distance (Figure
384 4). This is likely to be a difference between sites rather than a primary spatial effect.

385

386 *Canine distemper virus in wild foxes*

387 CDV seroprevalence in wild foxes ranged widely from 0.17-0.80 in the study sites.
388 Overall, the CDV prevalence was 0.46 (90% CI 0.32-0.62) for *P. griseus* (n=28) and
389 0.20 (90% CI 0.06-0.58) for *P. culpaeus* (n=5). No statistical significant differences
390 were detected between the CDV seroprevalence between *P. griseus* and *P. culpaeus*,
391 therefore further analyses were performed using data pooled from both species. Of
392 the five variables analyzed in a univariable logistic regression analysis all were
393 selected for initial inclusion in the multivariable logistic regression analysis (Table
394 5).

395

396 In the multivariable logistic regression analysis, three variables were associated ($p <$
397 0.1) with an increased risk of CDV seropositivity. The odds of adult foxes being
398 CDV seropositive was 34 times that of a juvenile (OR 34.4, 90% CI 2.03-92.8),
399 since only one of the 12 samples of juveniles was seropositive to CDV. Finally, the
400 odds of foxes being CDV seropositive was higher when animals were captured in
401 areas closer to human settlements such as towns or villages (OR 0.25, 90% CI 0.08-
402 0.80) and closer to the nearest urban area (OR 0.91, 90% CI 0.83-0.99) (Table 6).

403

404 **4. Discussion**

405 In this study of seroprevalence of CDV in Chile, domestic dogs in cities and towns,
406 had a similar higher risk of being seropositive compared to rural sites, suggesting
407 increased force of infection in non rural dog populations due to higher densities and
408 contact rates between dogs. Although a cross-sectional study gives only partial
409 information about the patterns of infection, the differences in seroprevalence
410 between urban and rural areas supports the hypothesis that CDV is endemic in cities
411 and transmitted to rural sites by occasional contacts with urban-originated dogs. Our
412 observations and personal communications with officials of the local municipalities
413 indicate that urban dogs are abandoned in small towns and rural sites by households
414 when they can no longer manage the animal. This could help to spread infections and
415 might explain similar CDV prevalence between cities and small towns.

416

417 The higher CDV seroprevalence in adult dogs could be explained by a) a constant
418 force of infection in an endemic area, b) differential rates of exposure in a population
419 experiencing sporadic outbreaks, c) an increase in disease exposure with age, or d) a
420 recent epidemic. Similar CDV age-seroprevalence patterns have been reported for

421 domestic dogs living in high-density areas in villages near the Serengeti National
422 Park (SNP) in Tanzania where it is thought that CDV is maintained in these densely
423 populated areas (Cleaveland et al., 2000). Similarly, the age-seroprevalence pattern
424 found in rural sites is similar to those found in the low-density populations near the
425 SNP where younger animals have no or low CDV seroprevalences, suggesting low
426 recent pathogen circulation (Cleaveland et al., 2000). In Tunisia by contrast, no
427 differences in CDV seroprevalence were detected between adults and juveniles in a
428 domestic dog population where CDV was thought to be circulating regularly
429 (Chabchoub et al., 2008).

430

431 In this current study in Chile, the results of the seroneutralization test indicated that
432 almost all CDV positive foxes were adults. Given that foxes only breed once a year
433 (González del Solar and Rau, 2004; Jiménez and Novaro, 2004), and assuming that
434 juveniles (non exposed) were born one year before sampling (i.e. 2005), we conclude
435 that only those foxes born at least before 2004 were in contact with the virus. The
436 higher seropositivity in foxes in the rural areas near Tongoy town, where the first
437 cases of CDV like-symptoms in a *P. griseus* were reported (Moreira and Stutzin,
438 2005), supports the hypothesis that the CDV epidemic in 2003 could have originated
439 in this area. Incursions of *P. griseus* into open rubbish dumps on the town's borders
440 (pers. obs.), provide potential points for inter-species transmission. In addition, the
441 report of *P. culpaeus* with similar signs after five months in the FJNP 50 km south of
442 Tongoy (Moreira and Stutzin, 2005), suggest a north-south spread of the CDV
443 epidemic; however, reliable data of the temporal dynamics in wild carnivores was
444 not available for further analysis. Although other wild carnivores, such as mustelids,
445 have been reported to be susceptible to CDV and being important for maintaining

446 CDV infection worldwide (Appel, 1987; Funk et al., 2001), it is unlikely that the
447 other four wild carnivore species inhabiting the Coquimbo region (two mustelids and
448 two felids: Muñoz-Pedrerros and Yañez, 2000), could have had a role during the
449 CDV epidemic, since these species are less abundant and not widely distributed
450 compared to the foxes (Acosta-Jamett, 2009).

451

452 In this paper, by combining official disease reports, published demographic studies
453 on domestic dogs and seroprevalence data in domestic and wild canids, we
454 hypothesise that urban dog populations are the most likely source of infection for
455 wild carnivores in 2003. In the Coquimbo region, the transmission events probably
456 occurred in the rural interface near Tongoy town, where a high density and an
457 elevated number of dogs allowed to roam freely exist (Acosta-Jamett et al., 2010).

458 Although the question whether a spill-over from domestic dogs to wild carnivores
459 really occurred is not easily answered with retrospective data, the seroprevalence
460 data from rural dogs with high seroprevalences found in the 4-5 age class, also
461 support the hypothesis of an epidemic occurring between 2001-2002 in domestic
462 dogs in rural sites, before the CDV outbreak in wild foxes.

463

464 High-density domestic dog populations have been proposed as the likely
465 maintenance population for rabies and canine distemper virus in the Serengeti
466 ecosystem in Africa (Cleaveland et al., 2000; Lembo et al., 2008). Also, domestic
467 dogs were identified as the source of CDV that affected lions (*Panthera leo*) in the
468 Serengeti (Roelke-Parker et al., 1996; Cleaveland et al., 2000), and also the probable
469 source of rabies in the side-striped jackals (*Canis adustus*) (Rhodes et al., 1998).

470

471 Although in this study, the maintenance of CDV infection was not addressed
472 directly, it is probable that the rural dog population and our target population, the
473 population of wild foxes, are both non-maintenance populations (Haydon et al.,
474 2002), because their population sizes are well below the suggested CCS necessary to
475 maintain a morbillivirus (Swinton et al., 2002).

476

477 If the CDV infection in the Coquimbo region follows a hierarchical transmission
478 dependent on the size and density of the susceptible population, it is likely that this
479 pathogen is maintained in a metapopulation, comprised mainly by maintenance
480 patches of dog populations in big cities and non-maintenance populations in small
481 towns and rural areas, all of them connected by the movement of potentially infected
482 dogs originated from cities, which are commonly left abandoned in both towns and
483 rural areas, following the measles city-village model (Anderson and May, 1991;
484 Grenfell and Bolker, 1998) (see Figure 5 for a schematic diagram of this theoretical
485 model in the Coquimbo region). However, this theoretical model needs further
486 research, for instance for assessing whether differences in incidence rate when
487 comparing urban and rural areas exist and/or if local cities are maintenance
488 populations by themselves or are part of a metapopulation with other cities in other
489 regions.

490

491 Although the wild carnivores studied in this paper are not classified as endangered,
492 this study should be viewed as a model that could be applied to other species of
493 conservation concern in Chile and elsewhere. For example, in the Serengeti,
494 although domestic dogs have been identified as a maintenance population for rabies
495 (Lembo et al, 2008), the exact extent of the domestic dog reservoir population is

496 difficult to determine as village populations are connected to nearby urban centres,
497 such as Mwanza (200 km from the Serengeti), which may act as the ultimate source
498 of infection. Similarly, in Kenya, a CDV epidemic in 1990 was thought to have
499 originated in the capital Nairobi (Alexander and Appel, 1994) and in Namibia, cities
500 were also thought to be the origin of the 2003 CDV epidemic in jackals (Gowtage-
501 Sequeira et al., 2009). A major consideration for large-scale disease control
502 programmes aimed to protect CDV outbreak in wildlife is therefore whether control
503 measures (such as dog vaccination) should be targeted primarily to high-density
504 urban centres instead of rural sites neighbouring protected areas.

505

506

507 **Acknowledgements**

508 G.A-J. was supported by a grant from the Ministry of Development and Planning of
509 Chile (MIDEPLAN). The Field Veterinary Program, Wildlife Conservation Society,
510 the Small Project Grant at the University of Edinburgh Development Trust, Idea
511 Wild, the Birrell-Gray Traveling Scholarship at the School of Veterinary Studies,
512 University of Edinburgh provided funding for the fieldwork and travel expenses. Dr.
513 D. Shaw and E. Fevre provided advice on early stages of this study. Dr. F. Astorga,
514 Dr. F. Hernández and Dr. D. Donoso and Mr. G. Vega for field assistance. The
515 Chilean Animal Health Service (SAG), the Corporación Nacional Forestal
516 (CONAF), Dr. Julio Gutiérrez and Mr. Julio Bonilla provided logistical support for
517 which we are most grateful.

518

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Table 1. Breakdown of interviewed households, vaccination coverage, estimated susceptible population, and number of blood samples obtained by site.

SITES	Number of interviewed households	Percentage of vaccinated dogs	Susceptible dog population ¹	No. of blood samples
<i>Urban</i>				
CQ	326	43	20,041±1,542	38
OV	242	40	6,427±415	37
B	81	40	264±40	24
C	147	38	698±78	53
G	49	30	421±165	25
<i>Rural</i>				
A	27	22	156±132	18
D	47	7	127±93	63
E	53	11	86±35	62
F	89	22	335±112	72
Total	1,063	33	28,555±1,543	392

¹ Obtained after multiplying the estimated population reported in Acosta et al. (2010) with the unvaccinated percentage.

Table 2. Estimated CDV seroprevalence of domestic dogs by each site.

Areas		Sites	<i>n</i>	Proportion positive (90%CI)
Urban	City	CQ	38	0.74 (0.60-0.85)
	City	OV	37	0.76 (0.61-0.87)
	Town	B	24	0.71 (0.55-0.87)
	Town	C	53	0.74 (0.62-0.85)
	Town	G	25	0.64 (0.50-0.78)
Rural	RA	A	18	0.50 (0.29-0.71)
	RA	D	63	0.48 (0.37-0.59)
	RA	E	62	0.53 (0.40-0.66)
	RA	F	72	0.33 (0.25-0.42)

Table 3. Univariable logistic regression model of factors associated with CDV seropositivity (n=392).

Risk factor	Sero (+)	Sero (-)	Odds ratio	90% CI	p-value ²
Site					<0.001
Urban					
CQ	28	10	1.00		
OV	28	9	1.11	0.46-2.66	0.843
B	17	7	0.87	0.33-2.25	0.806
C	39	14	0.99	0.45-2.20	0.992
G	16	9	0.63	0.25-1.58	0.414
Rural					
A	9	9	0.36	0.13-0.96	0.085
D	30	33	0.31	0.15-0.66	0.010
E	33	29	0.41	0.20-0.85	0.044
F	24	48	0.18	0.09-0.37	<0.001
Missing	0	0			
What is the sex of your dog?					
Male	169	135	1.00		
Female	51	31	1.32	0.87-2.01	0.274
Missing	4	2			
What is the age of your dog?					<0.001
0-1	20	43	1.00		
1-2	28	31	1.94	1.05-3.60	0.077
>2 year	174	92	4.00	2.44-6.55	<0.001
Missing	2	2			
What is the function of your dog?					0.007
Guarding	79	46	1.00		
Pet	97	68	0.83	0.87-2.18	0.446
Herding	38	51	0.42	0.54-1.50	0.002
Missing	10	3			
Do you allow your dog to roam freely in the neighbourhood?					0.667
Always	137	102	1.00		
Some times	40	37	0.81	0.53-1.25	0.425
Never	37	26	1.07	0.67-1.71	0.821
Missing	10	3			
Do you see neighbours' dogs roaming in the neighbourhood?					0.051
Always	158	99	1.00		
Sometime	33	34	0.61	0.39-0.96	0.072
Never	30	32	0.57	0.35-0.91	0.048
Missing	3	3			
Do you see unknown dogs roaming in the neighbourhood?					<0.001
Always	101	39	1.00		
Sometime	42	52	0.31	0.20-0.50	<0.001
Never	78	74	0.40	0.27-0.61	<0.001
Missing	3	3			
Distance to nearest city			0.98	0.96-0.99	0.001
Distance to nearest urban area			0.96	0.95-0.97	<0.001
Distance to nearest human settlement			0.91	0.85-0.98	0.041
Distance to nearest household			0.63	0.49-0.82	0.004

² Bolded p-values correspond to variables kept for multivariable analysis

Table 4. Multivariable logistic regression model of factors associated with CDV seropositivity at the dog level (n=377).

Risk factor	Odds ratio	90% CI	p-value
Site			
Urban			
CQ	1.00		
OV	0.96	0.38-2.48	0.965
B	0.73	0.26-2.03	0.611
C	0.94	0.39-2.29	0.911
G	0.52	0.20-1.39	0.276
Rural			
A	0.20	0.07-0.60	0.016
D	0.25	0.11-0.56	0.005
E	0.37	0.16-0.84	0.046
F	0.15	0.07-0.34	<0.001
What is the age of your dog?			
0-1 years	1.00		
1-2 years	2.21	1.13-4.32	0.052
>2 years	4.73	2.75-8.15	<0.001
Distance to nearest household	0.73	0.56-0.96	0.058

AUC=0.74; Pearson's $\chi^2=176$ (p=0.06); Hosmer- Lemeshow $\chi^2=5.65$ (p=0.69).

Table 5. Univariable logistic regression model of factors associated with CDV seropositivity in foxes (*Lycalopex spp.*) (n=33).

Risk factor	Sero (+)	Sero (-)	Odds ratio	90% CI	<i>p</i>-value³
Age					
Juvenile	1	11	1.00		
Adult	15	6	13.71	2.03-92.8	0.024
Sex					
Male	11	9	1.00		
Female	4	9	0.32	0.09-1.15	0.141
Distance to nearest urban area			0.93	0.88-0.99	0.071
Distance to nearest human settlement			0.55	0.30-0.99	0.092
Distance to nearest household			0.61	0.35-1.06	0.144

³ Bolded *p*-values correspond to variables kept for multivariable analysis

Table 6. Multivariable logistic regression model of factors associated with CDV seropositivity in wild foxes (n=33).

Risk factor	Odds ratio	90% CI	<i>p</i>-value
Age			
Juvenile	1		
Adult	34.4	2.9-413	0.019
Distance to nearest urban area	0.91	0.83-0.99	0.064
Distance to nearest human settlement	0.25	0.08-0.80	0.050

AUC=0.91; Pearson's $\chi^2=18.01$ (p=0.93); Hosmer- Lemeshow $\chi^2=4.98$ (p=0.73).

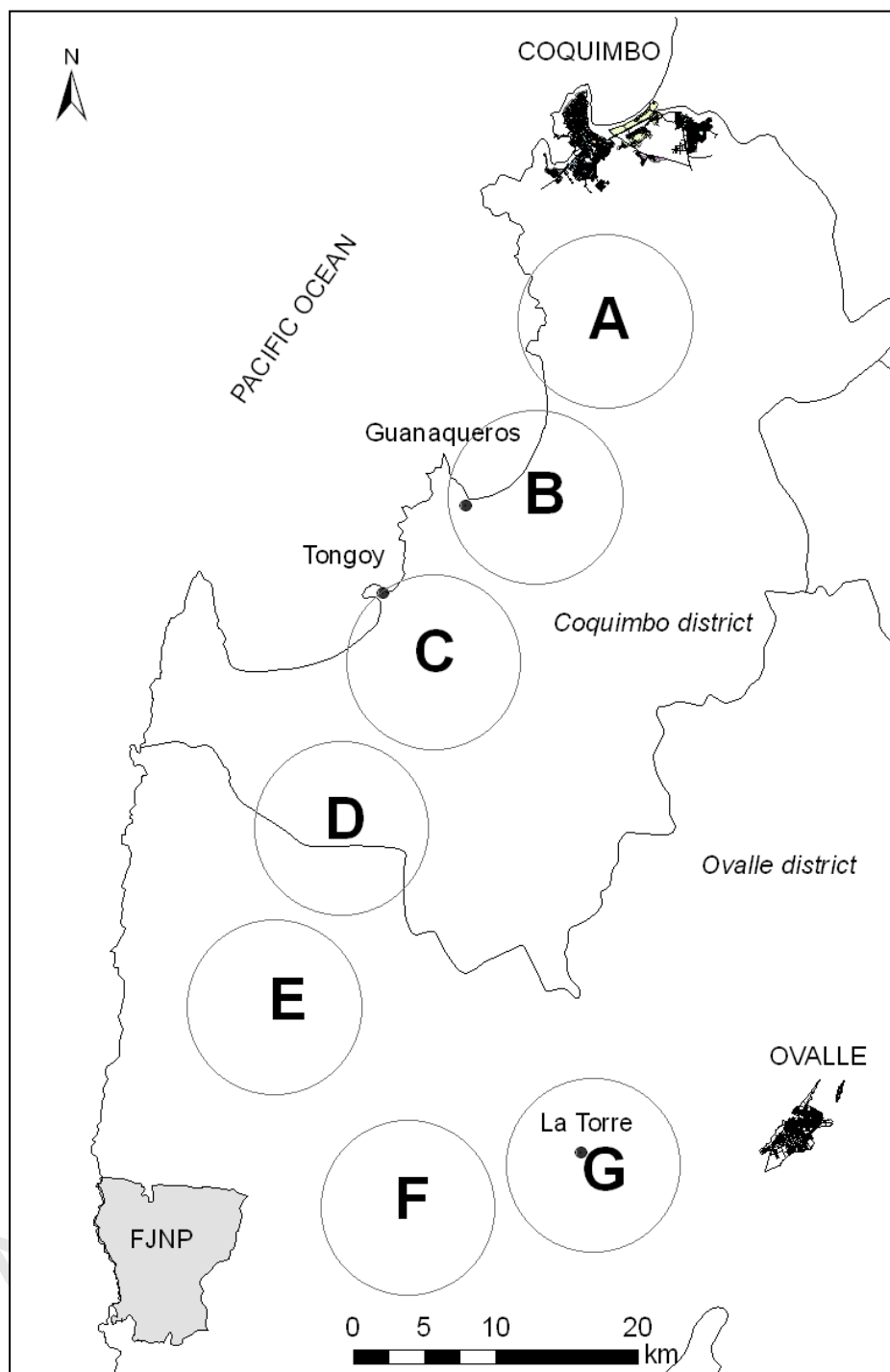


Figure 1. Study area. Two transects from Coquimbo and Ovalle cities through rural sites are shown. Nine sites, including Coquimbo and Ovalle cities, were delimited within which the questionnaire survey was conducted. In gray is the Fray Jorge National Park. Black dots show towns in the area.

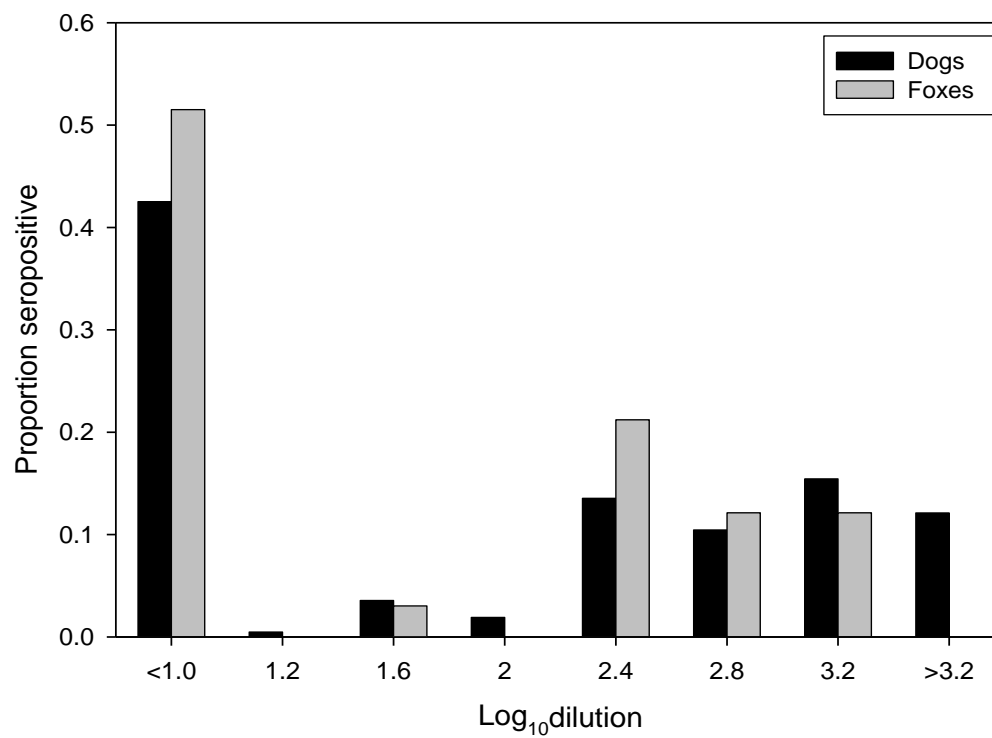


Figure 2. Distribution of antibody titres to canine distemper virus in domestic dogs (n=392) and free-ranging foxes (*Pseudalopex spp.*) (n=33).

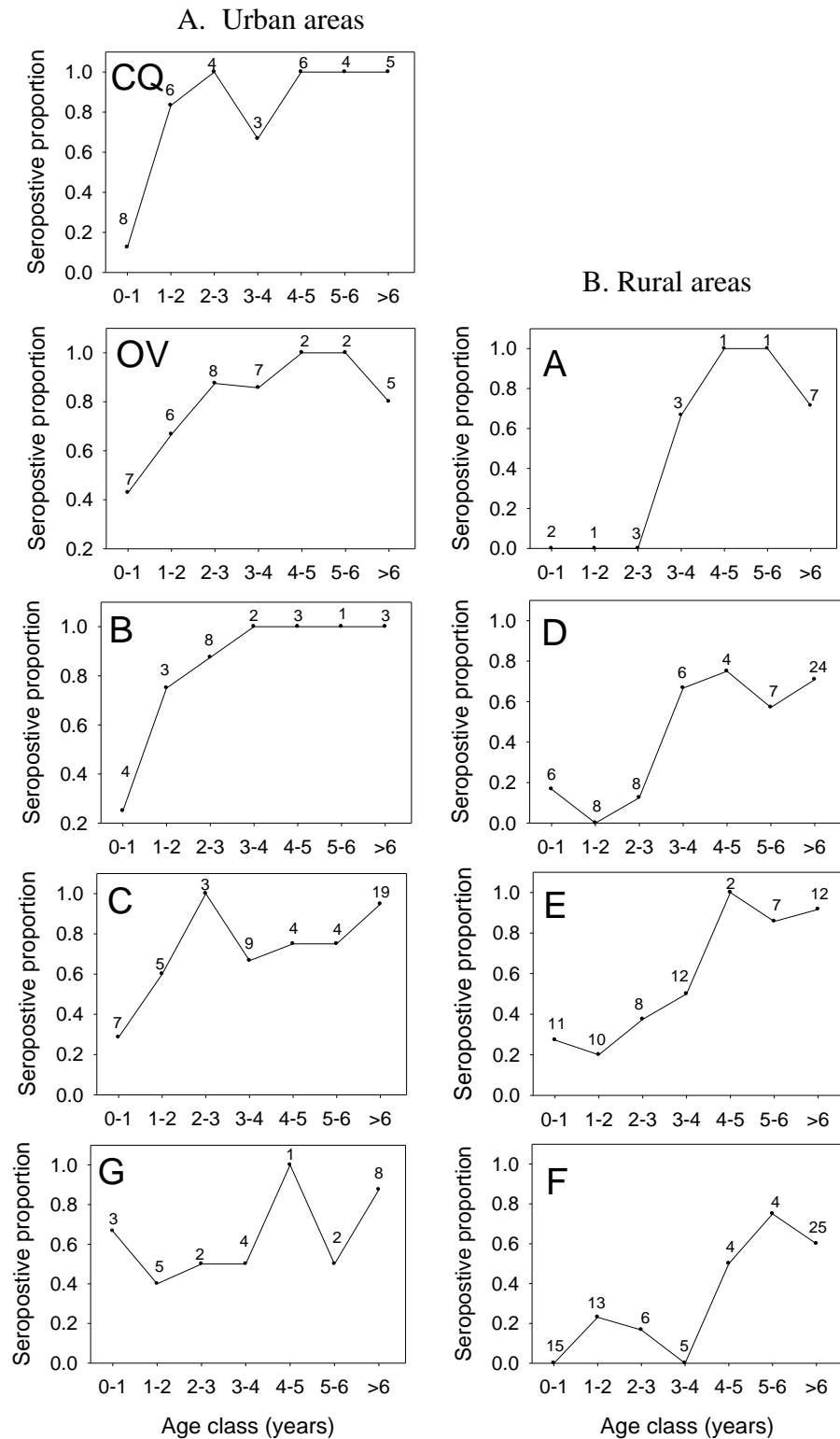


Figure 3. Age-seroprevalence of CDV in domestic dogs in urban (A) and rural areas (B). The numbers above the line correspond to the samples analyzed in each class.

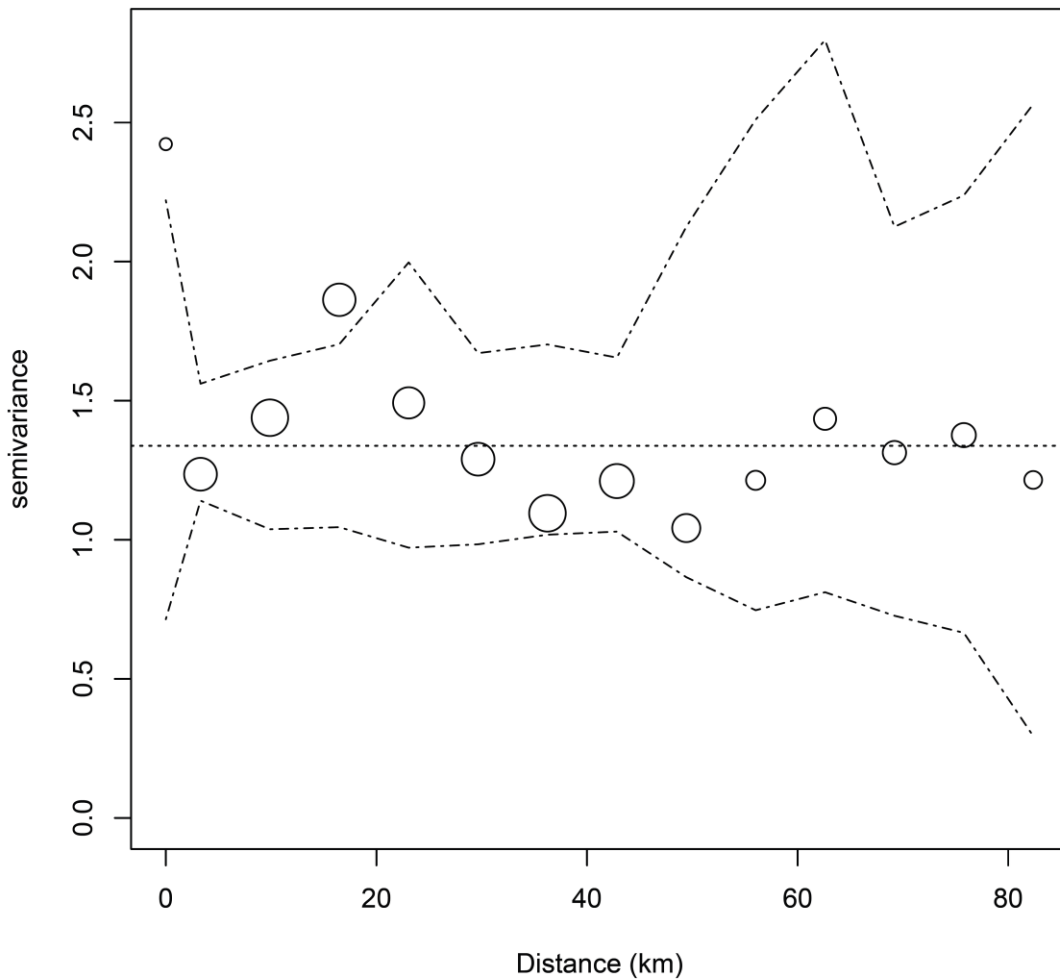


Figure 4. Semivariogram of residuals from final logistic regression model of risk factors for dog CDV seropositivity. The circles show the variance of residuals for pairs of observations in each distance band. The size of the circle is proportional to the number of pairs available for that distance band. The dotted lines show the 95% confidence envelope giving the range of results expected in the absence of spatial autocorrelation. If there was local spatial autocorrelation the shorter distance pairs would have a lower semivariance than the mean.

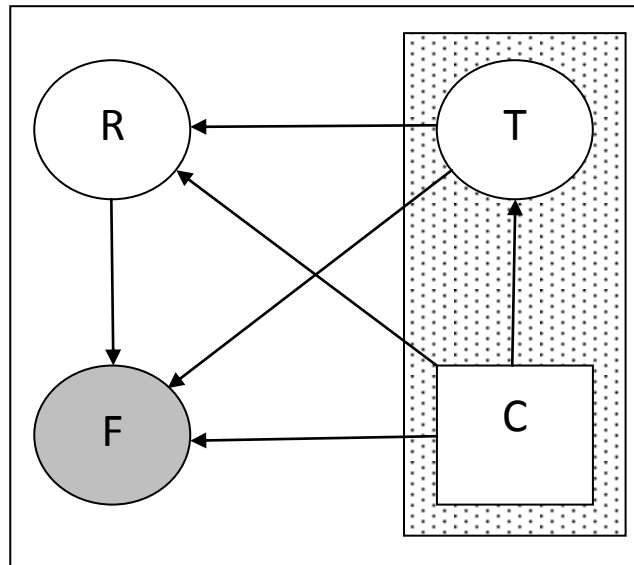


Figure 5. Pathway theoretical model for the maintenance of CDV infection in the study area. In circles are the proposed non-maintenance populations of dogs in towns (T) and rural areas (R), and in gray the target non-maintenance population of free-ranging foxes. In a white square is the proposed maintenance population of dogs in cities (C), and in a dotted rectangle is the reservoir population proposed for the study area, which could include dogs inhabiting urban areas. CDV transmission across the study area may be maintained by urban dogs left abandoned in neighbouring rural areas [Modified from Haydon et al. (2002)].