

Serosurvey of canine distemper virus in culpeo (*Lycalopex culpaeus*) and chilla (*Lycalopex griseus*) foxes of the Araucanía region, Chile

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ABSTRACT. Our goal was to assess whether free-ranging foxes have been exposed to canine distemper virus (CDV) in the Araucanía region in Chile. The study was conducted at three sites in rural areas where free-ranging foxes were trapped and bled from 2009 to 2012. We sampled two species of foxes: the culpeo (*Lycalopex culpaeus*) ($n=13$) and the chilla (*Lycalopex griseus*) ($n=14$). A serum virus neutralization assay was used to detect the presence and magnitude of functional systemic antibodies to CDV. Overall, CDV seroprevalence in culpeo and chilla foxes was 7.7 and 21.4%, respectively. Exposure to CDV did not differ among the sites. Despite the relatively low seroprevalence found in free-ranging foxes, the presence of CDV-seropositive dogs previously reported in rural sites nearby, suggests a potential risk of pathogen spill over from domestic dogs to foxes in the area.

Keywords: Araucanía, canine distemper virus, seroprevalence, *Lycalopex griseus*, *Lycalopex culpaeus*, pathogen spill over.

INTRODUCTION

Several disease outbreaks have affected wild carnivores worldwide, involving several multi-host pathogens carried by domestic dogs (*Canis familiaris*), such as canine distemper virus (CDV) (Alexander *et al.*, 1996; Berentsen *et al.*, 2013; Cleaveland *et al.*, 2000; Lembo, 2006; Roelke-Parker *et al.*, 1996). For example, pathogen spill over from dogs was suggested as the likely cause of CDV emergence in lions (*Panthera leo*) (Cleaveland *et al.*, 2000; Lembo, 2006; Roelke-Parker *et al.*, 1996) and African wild dogs (*Lycaon pictus*) (Alexander *et al.*, 1996), which caused a drastic reduction in these wild carnivore populations. Similarly, spotted hyenas (*Crocuta crocuta*) and African wild dogs have shown relatively low CDV exposure in and around protected areas, potentially originated from dogs (Berentsen *et al.*, 2013). Despite the compelling evidence of CDV spill over between domestic and wild carnivores worldwide, additional studies are needed to understand the exposure to this pathogen through rural landscapes in the Neotropics (e.g. Fiorello *et al.*, 2007; Furtado *et al.*, 2016; Megid *et al.*, 2009, 2010), where contact between dogs and free-ranging carnivores is likely to occur.

The Araucanía region of Chile consists of a formerly forested landscape that is currently fragmented by farmlands, exotic plantations, pastures and human settlements (Echeverría *et al.*, 2006). This region is inhabited by three species of free-ranging foxes: the culpeo (*Lycalopex culpaeus*), the chilla (*Lycalopex griseus*) and one of the most endangered canid species in the world, the Darwin's fox (*Lycalopex fulvipes*). The latter is classified as endangered by IUCN (Silva-Rodríguez *et al.*, 2016¹) and inhabits the Nahuelbuta National Park (NNP) (37° 47' S, 72° 59' W). Recent studies have reported its presence in the locality of Lastarria (Gorbea district, 39° 11' S, 72° 6' W – D'elía *et al.*, 2013) and other areas south to the NNP (Fariás *et al.*, 2014; Vilà *et al.*, 2004). Culpeo and chilla foxes are solitary species occurring mainly in mountainous areas and lowland sites, respectively (Jimenez *et al.*, 1995). Chilla foxes are habitat-generalists regularly approaching households and getting into contact with domestic dogs and therefore at high-risk of infection with pathogens (Acosta-Jamett *et al.*, 2011; Acosta-Jamett *et al.*, 2015a, Hernández *et al.*, 2021).

We previously reported that urban and rural domestic dogs were exposed to CDV in the Araucanía (Acosta-Jamett *et al.*, 2015b), but we still lack data on disease exposure on free-ranging foxes to estimate the potential risk of pathogen spill over from domestic to wild canids in the region. In this study, we explored whether free-ranging foxes inhabiting rural sites of the Araucanía have been exposed to CDV, a pathogen of potential conservation concern for threatened carnivores such as Darwin's fox.

Received: 13.01.2023.

Accepted: 21.03.2023.

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¹ Silva-Rodríguez E, Fariás A, Moreira-Arce D, Cabello J, Hidalgo-Hermoso E *et al* (2016) *Lycalopex fulvipes* (errata version published in 2016). The IUCN Red List of Threatened Species 2016: e.T41586A107263066. <http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T41586A85370871.en>. Accessed 9 January, 2023

MATERIAL AND METHODS

The study was conducted between 2009 and 2012 in the Araucanía region in south-central Chile, including three sites: a) a more remote site inserted at the Santa María forestry company lands located 32 km north-west of Angol city and 15 km north of the Nahuelbuta National Park ($37^{\circ} 39' S$; $73^{\circ} 03' W$) (hereafter Angol); and two sites nearby towns as b) Conguillío National Park, located 15 km south-east of Curacautín city ($38^{\circ} 42' S$; $71^{\circ} 37' W$) (hereafter Curacautín), and c) Arauco forestry company lands located close to Lastarria locality, 20 km south-west of Gorbea city ($39^{\circ} 11' S$; $72^{\circ} 46' W$) (hereafter Gorbea) (Figure 1). Angol site was chosen instead of NNP since a capture license was not available from the National Forest Service (CONAF) that administers the Chilean protected areas.

We captured foxes with padded leg-hold traps (Victor Soft Catch No. 1.5, Chagnons Trapping Supply, Manistique, MI, USA) and Tomahawk traps (Tomahawk Live Trap Co., Tomahawk, WI), which were consistently checked for captures every four hours (i.e., two checks/trapping night), to avoid injuries. Animals were anesthetised as reported by Acosta-Jamett *et al.* (2010) and blood was sampled from the cephalic vein. Foxes were marked with unique ear tags to avoid re-sampling during recaptures,

and all of them were safely released at the corresponding capture site. To determine humoral immune response to CDV infection, neutralizing antibody titres were obtained from serum samples through a virus neutralization (SVN) assay using the reference strain Onderstepoort from a commercial CDV vaccine (Novibac Puppy DP; Intervet International B.V., Netherlands). Neutralizing antibody titres at serum dilution $\geq 1:12$ was considered positive for CDV exposure.

DATA ANALYSIS

Ninety-five percent confidence limits were calculated for all seroprevalence values. Data analysis was carried out using the software R v. 4.1.0 (R Core Team, 2021).

RESULTS AND DISCUSSION

Overall, 27 foxes were trapped, of which 13 were culpeos (5 males/8 females; 4 juveniles/9 adults) and 14 chillas (7 males/7 females; 4 juveniles/10 adults). One (7.7%; CI 0.2-36.0%) of the 13 culpeos and 3 (21.4%; CI 4.7-50.8%) of the 14 chillas were CDV seropositive (Table 1). CDV-seropositive foxes were evenly distributed according to sex and age; the CDV-seropositive culpeo corresponded to an adult female captured at Curacautín,

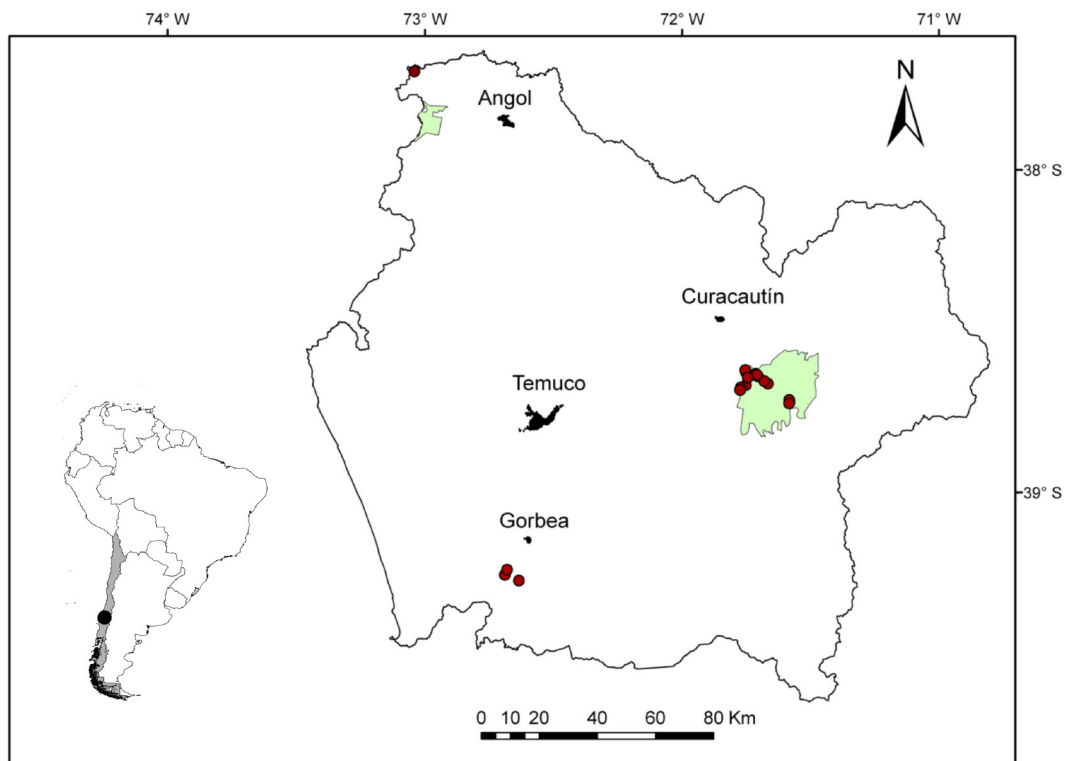


Figure 1. Location of sampled free-ranging foxes (red circles) at three sites of the Araucanía region (Chile) (2009-2012). Zones colored in black denote cities and zones colored in green denote protected areas (Nahuelbuta National Park and Conguillío National Park) in proximity to sampling sites.

Table 1. Percentages of CDV seropositive culpeo and chilla foxes sampled at three sites of the Araucanía region (Chile) (2009-2012). NA denotes that no individual of a particular species was sampled.

Site	CDV		
	Culpeo	Chilla	Overall
Angol	0/1	NA	0/1
Curacautín	1/12 (8.3)	2/11 (18.2)	3/23 (13.0)
Gorbea	NA	1/3 (33.3)	1/3 (33.3)
Total	1/13 (7.7)	3/14 (21.4)	4/27 (14.8)

while the CDV-seropositive chillas were 2 juvenile males captured at Curacautín, and 1 adult female captured at Gorbea. Due to the limited number of sampled animals, CDV seroprevalence data of both culpeos and chillas were pooled to compare pathogen exposure among foxes across sites (Table 1). Overall, exposure to CDV did not differ among the sites.

Our study is the first attempt to explore whether free-ranging foxes (i.e., culpeo and chilla) have been exposed to CDV in the Araucanía region of Chile. Our findings confirmed the exposure to CDV in both culpeos and chillas, similarly to previous studies that reported exposure to these pathogens in other fox populations (Acosta-Jamett *et al.*, 2011; Acosta-Jamett *et al.*, 2015a; Martino *et al.*, 2004). Habitat-generalists as chillas may have increasingly higher opportunities for interaction with domestic dogs compared to culpeos (Hernández *et al.*, 2021), likely enhancing the likelihood of interspecific pathogen exposure (i.e., CDV-exposed wild foxes corresponded to 1 culpeo vs. 3 chillas), which may be relatively recent in the two CDV seropositive juvenile chillas of Curacautín.

Compared to previous studies, our CDV seroprevalences in culpeo (7.7%) and chilla (21.4%) foxes were lower than the values reported in north-central Chile (i.e., 42% in both chillas and culpeos combined) (Acosta-Jamett *et al.*, 2015a), but higher than the values reported for free-ranging culpeo and chilla foxes in a remote area of the Argentinean Patagonia, where 3.6% of tested foxes had CDV antibodies (which corresponded to chillas only) (Martino *et al.*, 2004). This could be explained by a higher closeness to urban dog populations in foxes from the first study compared to the second study, but limited sample size prevents us to overinterpret our findings. Thus, our numbers of foxes sampled were too limited for meaningful comparisons of CDV seroprevalences across the study area (particularly at the remote Angol area), and only relying on serosurvey data we cannot determine whether CDV was actually transmitted between wild and domestic canids.

According to our previous spatial analyses at the Araucanía, there was a wide presence of domestic dogs exposed to CDV in both urban and rural areas across the region (Acosta-Jamett *et al.*, 2015b), and they are present

even in close proximity to protected areas and sites where CDV-seropositive chilla and culpeo foxes were trapped. Dogs sampled at rural sites had a higher overall seroprevalence of CDV (47%) (Acosta-Jamett *et al.*, 2015b) than free-ranging foxes (14.8%), which would be a factor of concern given the high mortality rates caused by pathogens as CDV in chillas and culpeos (Acosta-Jamett *et al.*, 2011; González-Acuña *et al.*, 2003; Moreira & Stutzin, 2003). Considering that dog population has grown and spread continuously in Chile's urban and rural areas over the last decades (Acosta-Jamett, 2015), it is highly probable that pathogens such as CDV are transmitted from dogs to wild foxes through occasional interspecific contact. Alternatively, a guild of wild carnivores may naturally maintain and transmit generalist pathogens among species; however, because foxes, felids and mustelids generally exhibit lower densities than dogs through their geographic distribution, this hypothesis seems to be less likely (Acosta-Jamett, 2009; Acosta-Jamett *et al.*, 2011; Acosta-Jamett *et al.*, 2015a).

Our findings suggest that free-ranging foxes, such as culpeo and chilla, have been exposed in some cases recently to CDV across their range in the Araucanía. Domestic dogs from urban areas had a higher risk of being CDV seropositive than rural dogs, suggesting increased force of infection in more dense urban dogs with increased contact rates (Acosta-Jamett *et al.*, 2015b); however, whether pathogen exposure in wild foxes has occurred due to spill over from domestic dogs living in proximity is unclear yet. Although we did not sample Darwin's foxes, continued health surveillance should be also recommended for this endangered, immunologically naïve wild canid, given high population mortality could be expected in an eventual CDV spill over event from dogs (or potentially other foxes or carnivores acting as "bridge hosts") to Darwin's foxes in the region (Hidalgo-Hermoso *et al.*, 2020; Sepúlveda *et al.*, 2014). In this regard, Hidalgo-Hermoso *et al.* (2020) suggested that lack of exposure to CDV in Darwin's foxes may be related to either their forest specialist habits, or the lack of detection of exposed individuals due to unnoticed mortality in foxes that already had contact with the virus. Further long-term longitudinal studies using a combination of PCR and serological tests to assess potential routes of infection from domestic dogs to free-ranging foxes with emphasis in the endangered Darwin's fox are needed to understand the spatial dynamic of infection and prevent CDV outbreaks in wild carnivores at the Araucanía region of Chile.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing interests.

ETHICS STATEMENT

Animal capture and handling procedures were approved by the Ethics Committee at the Universidad Austral de Chile and authorized by the Animal Health Service (SAG: N°3245/2011).

AUTHOR CONTRIBUTIONS

GAJ conceived the ideas of the study. DS, AV and GAJ carried out the field work and sampling. MC, IMA and EAM carried out the laboratory assays. FAH and GAJ led the writing of the manuscript. GAJ obtained the funding for this study. All authors revised the manuscript, contributed critically to the drafts and approved the final version for publication.

FUNDING

This work was funded by ANID/CONICYT Fondecyt Iniciación N° 11100303, The Rufford Small Grants Foundation N° 39.07.09 and DID-UACH S-2010-58. FAH was funded by ANID/CONICYT Fondecyt Postdoctorado N° 3180111. MC was funded by ANID Becas Doctorado N° 21201298.

ACKNOWLEDGEMENTS

The support and permits provided by the National Forest Service (CONAF) and Animal Health Service (SAG) personnel were essential for conducting the trapping of foxes and questionnaire survey. We thank the logistic support provided by the Santa María forestry company, which allowed the fieldwork within their lands. Finally, we thank the support of more than forty volunteers from seven different countries within America and overseas.

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