LETTERS

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An Eight-Year Survey for Canine Distemper Virus Indicates Lack of Exposure in the Endangered Darwin's Fox (*Lycalopex fulvipes*)

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?1 ABSTRACT: No evidence of exposure to canine distemper virus (CDV) was detected in 70 samples corresponding to 58 wild-trapped Darwin's foxes (*Lycalopex fulvipes*) in Chile. Given its current endangered status and it being immunologically naïve, in the event of a CDV spillover from dogs to foxes, high population mortality is expected.

Canine distemper virus (CDV) is the causal agent of canine distemper, an infectious disease of dogs and wildlife worldwide. Highly contagious, CDV is most commonly spread by aerosol transmission. Clinical signs are variable and depend on the virus strain, the age and immune status of the host, and can include respiratory, gastrointestinal, and neurological signs (Sykes 2013). Exposure, infection, and mortality have been confirmed in a wide range of carnivores. For example, CDV was responsible for outbreaks of distemper in red foxes (*Vulpes vulpes*) in Europe (Origgi et al. 2012) and the near extinction of the black-footed ferret (*Mustela nigripes*) in the wild in North America (Williams et al. 1988). In these and other cases, free-ranging dogs (*Canis lupus familiaris*) were the putative origin of the outbreaks (Belsare and Gompper 2013). In rural areas of Chile, nonferal free-ranging dogs are widespread. Local surveys have shown that vaccine and sterilization coverages are low and seroprevalence against infectious agents, including CDV, is high (Acosta-Jamett et al. 2015). Darwin's fox (*Lycalopex fulvipes*) is a canid endemic to Chile and one of the most endangered carnivores. Darwin's fox occurs throughout the temperate coastal forest in at least three distinct populations: two small and isolated mainland populations located in Nahuelbuta Mountain Range (37°36′S, 73°3′W) and Reserva Costera Valdiviana-Parque Oncol (40°7′S, 73°33′W) containing about 230 mature individuals, and a metapopulation located on Chiloé Island (42°21′S, 74°2′W) composed of about 415 mature individuals. The risk of disease spillover from domestic dogs (mainly CDV, as Chile is rabies free) is considered one of the major threats for Darwin's fox persistence (Silva-Rodriguez et al. 2016). No information about exposure to viral diseases exists for this species.

We aimed to fill this gap by conducting the first large-scale disease survey on the species, from April 2011 to August 2018. Fifty-eight healthy free-living individuals were captured during 70 capture events throughout the known range of Darwin's fox (Fig. 1, Table 1). We used Tomahawk traps, which were activated in the evening and checked the next morning at dawn. Foxes were anaesthetized with a combination of either 1 mg/kg xylazine (Xilazina 2%, Centrovet, Santiago, Chile) plus

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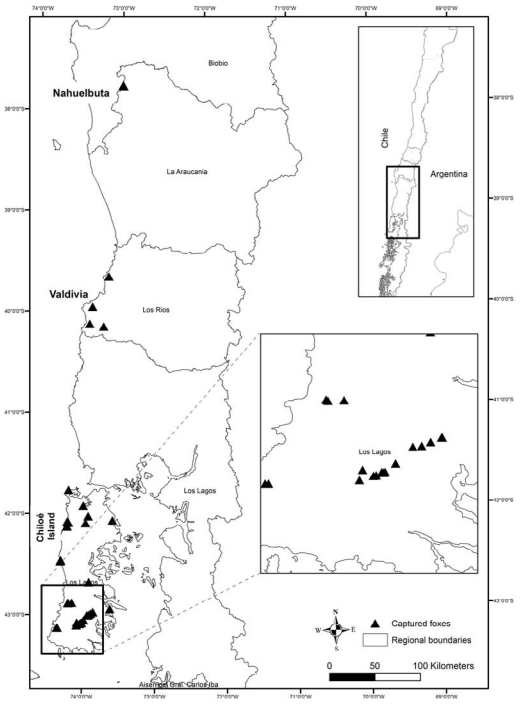

FIGURE 1. Map showing the capture location of 70 Darwin's foxes (*Lycalopex fulipes*) sampled during surveys for exposure to canine distemper virus conducted between April 2011 and August 2018 across the three known localities of Darwin's fox (Nahuelbuta, Valdivia, and Chiloé Island). Capture site (triangle) represents single or multiple captured foxes.

10 mg/kg ketamine or 0.04 mg/kg dexmedetomidine (Dexdomitor©, Zoetis, Santiago, Chile) plus 5 mg/kg ketamine (Ketostop©, Drag Pharma, Santiago, Chile) reversed with 0.4 mg/kg atipamezole (Antisedan, Zoetis). Blood was obtained by venipuncture of the cephalic or jugular vein. The serum was separated from the cells and clot and then the whole blood and serum samples were stored in liquid nitrogen or kept on ice until reaching the lab, where samples were frozen at –80 C until analysis. Eight blood and serum samples were applied to FTA™ cards (Whatman, Maidstone, Kent, UK) and kept at room temperature. Nine individuals were recaptured once and one individual twice. Time between recaptures ranged from 2 to 32 mo (mean 16 mo). Serum samples (62) from 53 individuals and 46 blood samples from 39 individuals were analyzed, with 38 foxes analyzed both for antibodies and direct RNA detection.

TABLE 1. Source population information for the Darwin's foxes (*Lycalopex fulipes*) tested for canine distemper virus exposure in Chile, 2011–2018, either by reverse-transcription PCR or by virus neutralization (or both). Number of recaptures is indicated in parentheses.

	Number of captures (number of recaptures)			
	Sex		Age	
Area	Male	Female	Adult	Juvenile
Nahuelbuta	1 (1)	4 (1)	3 (2)	2
Valdivia	3	1	3	1
Chiloé Island	32 (7)	17 (3)	37 (8)	12 (2)
Total	36 (8)	22 (4)	43 (10)	15 (2)

Virus neutralization (VN) assay was used to detect antibodies against CDV. The eight samples preserved on cards were analyzed at the Animal Health Diagnostic Center of Cornell University (Ithaca, New York, USA), as reported in Millán et al. (2016) using the Onderstepoort strain. The remaining samples were analyzed by the Servicio Agrícola y Ganadero (Ministry of Agriculture, Government of Chile, Santiago, Chile) with minimal differences (MDCK instead of Vero cells). Viral RNA was extracted from the samples using TRIzol® LS (Invitrogen Life Technologies, Carlsbad, California, USA). Molecular detection was by reverse-transcription (RT)-PCR at the Faculty of Livestock and Veterinary Sciences, Universidad de Chile (Santiago, Chile) with the primers CDV-1 and CDV-2 (Rzezutka and Mizak 2002) using the SuperScript one-step RT-PCR with platinum Taq kit (Invitrogen®) and n-RT-PCR using the primers CDV-A and CDV-B. A sample from a CDV-positive dog was used as positive control. ?2

All samples were negative both for VN and RT-PCR (95% confidence interval: 0.0–6.2%). Maximum possible prevalence calculated using WinEpi (de Blas et al. 2000) was 4.9%. Nevertheless, sample size in the two mainland areas was low and might not be representative of what is happening in these areas. The lack

of detection of exposure indicated that the species is immunologically naïve, and suggested at least two possible scenarios. Foxes may not have had contact with the virus, although free-ranging dogs are ubiquitous in and around the areas occupied by Darwin's fox (Silva-Rodriguez et al. 2018), and serosurveys performed on Chilean rural dogs indicated seroprevalence above 40% (Acosta-Jamett et al. 2015). Since Darwin's fox is a forest specialist and CDV has low environmental survival, dog-to-fox transmission opportunities may be rare. Exposed foxes also may not have been detected because of unnoticed mortality in foxes that have had contact with the virus. The lack of wildlife health surveillance programs in Chile along with the rarity of Darwin's fox (i.e., low densities and habitat specificity, particularly dense forests; Yu and Dobson 2000) make carcass retrieval difficult (Wobeser 2007), which may jeopardize detection of infected animals. High mortality could be expected in a CDV spillover from dogs to foxes, given the lack of acquired immunity in Darwin's fox populations. A serological survey in Island foxes (*Urocyon littoralis*) in 1992 revealed no previous exposure to CDV in this species (Garcelon et al. 1992). A marked decline of the population was observed in 1999 due to a CDV outbreak (Timm et al. 2009). Given the small population size of some Darwin's fox metapopulations, especially those on the mainland, a CDV outbreak could be catastrophic for the species, both in terms of population size and also due to the loss of gene pool (Millán et al. 2009).

We strongly recommend continued health surveillance in the species, including satellite tracking to acquire better knowledge of Darwin's fox mortality causes, paired with management actions for domestic dogs in and around fox areas such as sustained dog sterilization, vaccination campaigns, and responsible pet ownership programs to prevent free-ranging dogs accessing protected areas where this charismatic species still thrives.

Captures were performed under the permits 1262/2009, 2263/2010, 206/2012, 3155/2013, 3363/2015, 3035/2016, 2288/2016, and 5029/2017 from the Servicio Agrícola y Ganadero. We thank all the volunteers who helped in fox captures during all those years. D.M.-A. thanks the Comisión Nacional de Investigación Científica y Tecnológica (FONDECYT Iniciación No. 11181180). C.N. acknowledges support from Conicyt Fondecyt Iniciación 11150934, Morris Animal Foundation D15ZO-413, National Geographic C309-15, and the Mohamed bin Zayed Species Conservation Fund 152510351.

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