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ORIGINAL ARTICLE

Domestic dog and alien North American mink as reservoirs of infectious diseases in the endangered Southern river otter

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ABSTRACT. Introduced alien carnivores are host to infectious diseases that may become an important threat for native carnivore species conservation. Canine distemper virus (CDV) is thought to be transmitted among individuals by direct contact and to present viral dynamics associated with a density-dependent multi-host carnivore community. In contrast, Canine Parvovirus (CPV) is mostly transmitted by indirect contact and does not depend only on the density, but also on the social behaviour of infected as well as susceptible hosts. The objective of this study was to assess how introduced American mink (*Neovison vison*) can act as a bridge-host between domestic dog (*Canis familiaris*) and Southern river otter (*Lontra provocax*) in different dog and mink population density scenarios. Our data show that otters are seropositive to both CDV and PV, as well as a molecular identity to Parvovirus in dogs and minks. Furthermore, a strong positive correlation between dog population density and observed seroprevalence of CDV in dogs, minks, and otters was recorded. For Parvovirus, the observed seroprevalence in mink and otters was not correlated to a higher dog population density, but instead a relationship between dog and mink population densities and social behaviour. Our results suggest that introduced American mink and domestic dogs are reservoirs of CDV and PV, both being diseases of major importance for the conservation of native endangered carnivores in Patagonia.

Key words: American mink, domestic dog, otters, Canine Parvovirus, Canine Distemper virus.

INTRODUCTION

Among the domestic hosts of infectious diseases, free-roaming dog populations are of interest in Chile because they are large and known to affect wildlife (González-Acuña *et al.*, 2003; Medina-Vogel, 2010; Acosta-Jamett *et al.*, 2011; Silva-Rodríguez & Sieving, 2012; Sepúlveda *et al.*, 2014). Furthermore, the intense migration dynamics of dog populations in Chile can modify the spread of an infectious disease in a way that is difficult to understand (Villatoro *et al.*, 2016). In south-central Chile, the average number of dogs ranges between 0.54 to 0.95 with a maximum of 1.28 dogs per rural household, with 1.6 to 2.4 males per 1 female and up to 60% of the local rural population may come from urban areas, as far as 1000 km away (Villatoro *et al.*, 2016). Silva-Rodríguez & Sieving (2012) found that households were the best predictor for dog occupancy during their study in rural sites in the south of Chile. Among domestic dog infectious diseases, there are two important viral diseases transmitted from them to wildlife species of conservation concern, Canine Parvovirus (CPV) and Canine Distemper Virus (CDV) (Frölich *et al.*, 2000; Acosta-Jamett *et al.*, 2011; Acosta-Jamett *et al.*, 2014; Millán *et al.*, 2015). Canine Parvovirus (CPV) is a DNA virus of the *Parvoviridae* family that is quite resistant to environmental conditions and can survive up to six months at room temperature (Parrish, 1990; Williams, 2001). It has been linked with mortality in mustelids in captivity (Gjeltema *et al.*, 2015), therefore, it might be able to threaten the viability of small carnivore isolated populations. On the other hand, Canine Distemper Virus (CDV) is a RNA virus, a member of the Morbillivirus genus of the Paramyxoviridae family; it is highly contagious among carnivores, it spreads rapidly in mustelids and induces a high mortality rate on unvaccinated mink (Hammer *et al.*, 2007). Another issue of wildlife conservation concern in Patagonia is the introduced American mink (*Neovison vison*). This semiaquatic mustelid registers stable populations since the 1970s, it is resistant to the presence of humans and its diet includes a substantial proportion of rodents (Medina, 1997; Medina *et al.*, 2013). Also, it cohabits with free-ranging domestic dogs associated with farming and housing near rivers as well as lakes shores and seashore. Minks are known to cause damage to hen houses and poultry, therefore, interspecies contact between American mink and domestic species is likely to occur (Philippa *et al.*, 2008; Sepúlveda *et al.*, 2014). American minks also share habitat with endangered Southern river otter (*Lontra provocax*) in freshwater and marine environments (Medina, 1997; Medina-Vogel *et al.*, 2013), suggesting that there is

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a potential function of mink as a bridge host of infectious diseases from domestic dogs to wild otter populations in Chile (Sepúlveda *et al.*, 2014). In North America, CDV has been reported in mink, otters, and domestic animals; North American river otters (*Lontra canadensis*) and Eurasian otters (*Lutra lutra*) have tested seropositive against CPV-2 and mink parvovirus (Kimber *et al.*, 2000); new antigenic types of CPV-2 have been isolated from stone martens (*Martes foina*) and other carnivores (Steinel *et al.*, 2001). Therefore, it is expected that similar situations of disease transmission of both CPV and CDV between domestic dog, alien American mink, and Southern river otter could happen in Southern Chile (Sepúlveda *et al.*, 2014).

During the last century, the population of Southern river otters (*Lontra provocax*) declined dramatically in its former territory (Medina, 1996) and as a result the species is currently listed as endangered by the International Union for the Conservation of Nature and Nature Resources (UICN, 2013). Although these otters were once widely distributed, the remaining population is currently spread out over two different areas: i) a small and fragmented northern population associated to freshwater systems (38ºS to 43ºS Latitude) and; ii) a patchy, but relatively extended southern population associated to the marine habitat of Chilean fiords and channels, as well as Argentinian and Chilean Tierra del Fuego and Cape Horn region (43°S to 58°S) (Medina, 1996). Several factors have been involved in the population decline of this mustelid in Chile, including excessive hunting and trapping in the past and a substantial loss of habitat during the last 70 years (Medina, 1996). However, there is almost no information on pathogen prevalence or disease in this species (Medina-Vogel, 2010; Sepúlveda *et al.*, 2014; Barros *et al.*, 2018) and increasing concerns about the potential importance of infectious disease spill-over from dog to otter, having the introduced American mink as a bridge host (Sepúlveda *et al.*, 2014).

The increasing human intervention of natural habitats and globalisation, resulting in the transport and introduction of alien species into other regions, enhance the emergence of new diseases in wildlife and make the re-emergence of old diseases not surprising (Daszak *et al.*, 2000; Medina-Vogel 2010). For instance, in California, USA, foxes (*Urocyon cinereargenteus*) and bobcat (*Lynx rufus*) live close to towns where important populations of stray dogs and cats have recorded a significantly high seropositive reaction to both CPV and calcivirus (Riley *et al.*, 2004). Many infectious diseases that originate from domestic dogs, cats and livestock have been reported in mustelids, such as CPV (Steinel *et al.*, 2001; Gjeltema *et al.*, 2015) and CDV (Frölich *et al.*, 2000; Williams, 2001; Philippa *et al.*, 2008). The transmission of CDV is thought to be through direct contact since the virus can survive only some hours at 25°C and up to 14 days at 5°C under test conditions (Shen & Gorham, 1980); also, the transmission is primarily by aerosol or by contact with oral, respiratory, ocular fluids and exudates containing the virus. Due to the relative fragility of the virus in the environment, a close association between infected and susceptible animals is necessary, for example, some carnivore behaviours such as sharing carcasses or latrines are a potential source of inter and intraspecies infection making the inter and intraspecies transmission of CDV quite plausible (Craft *et al.*, 2011, Sepúlveda *et al.*, 2014). In this sense, dense populations of susceptible individuals with special or characteristic behaviours are necessary to sustain CDV dynamics on a multi-host system of carnivores.

In contrast, CPV can survive for months under cool and moist conditions when protected from sunlight, hence the infection dose required for CPV may be very low. The transmission within dogs (Steinel *et al.*, 2001) and other wild carnivores occurs via contact with the virus shed in faeces (faecal-oral route), suggesting that indirect transmission rather than direct contact with infected animals may play a key role in the maintenance of this virus in a population, particularly among wild carnivores characterised by low contact rates. Transmission of CPV between domestic and wild carnivores may also occur through close contact or predation on smaller carnivores, and across long distances by fomites (Miranda & Thompson, 2016). Moreover, free-ranging carnivores at low densities, even solitary individuals, may be exposed at marking sites, latrines or other sites contaminated by faeces deposited by a virus shedder (Bakker & Parrish, 2001).

If CDV and CPV are being transmitted between domestic dogs, American minks, and otters in Chile, then these species should report a higher CDV seroprevalence in places with a higher population of dogs and CPV seroprevalence should follow a different pattern (Deem *et al.*, 2000; Almberg *et al.*, 2010). To validate this, molecular evidence of those transmissions should be found. The present study aimed to carry out a seroprevalence and molecular cross-sectional survey in populations of domestic dogs, minks and river otters in Southern Chile.

MATERIAL AND METHODS

STUDY AREA

This study was carried out in Southern Chile, 39º S and 45º S latitude (figure 1). Eleven sites were chosen based on independence of the Southern river otter home range size, dispersion pattern and distribution (figure 1) (Medina, 1996; Sepúlveda *et al.*, 2014).

All sites were located within a region characterised by a temperate-humid-cool climate with 2,000 mm to 3,000 mm of rain per year and an average humidity of around 90%. Rivers, lakes and marine coastal vegetation in this area is characterised by a type of forest known as Valdivian rainforest and Norpatagonic Valdivian rainforest. The average annual temperature in this region is below 10°C (Veblen & Schlegel, 1982; Toledo & Zapater, 1989).

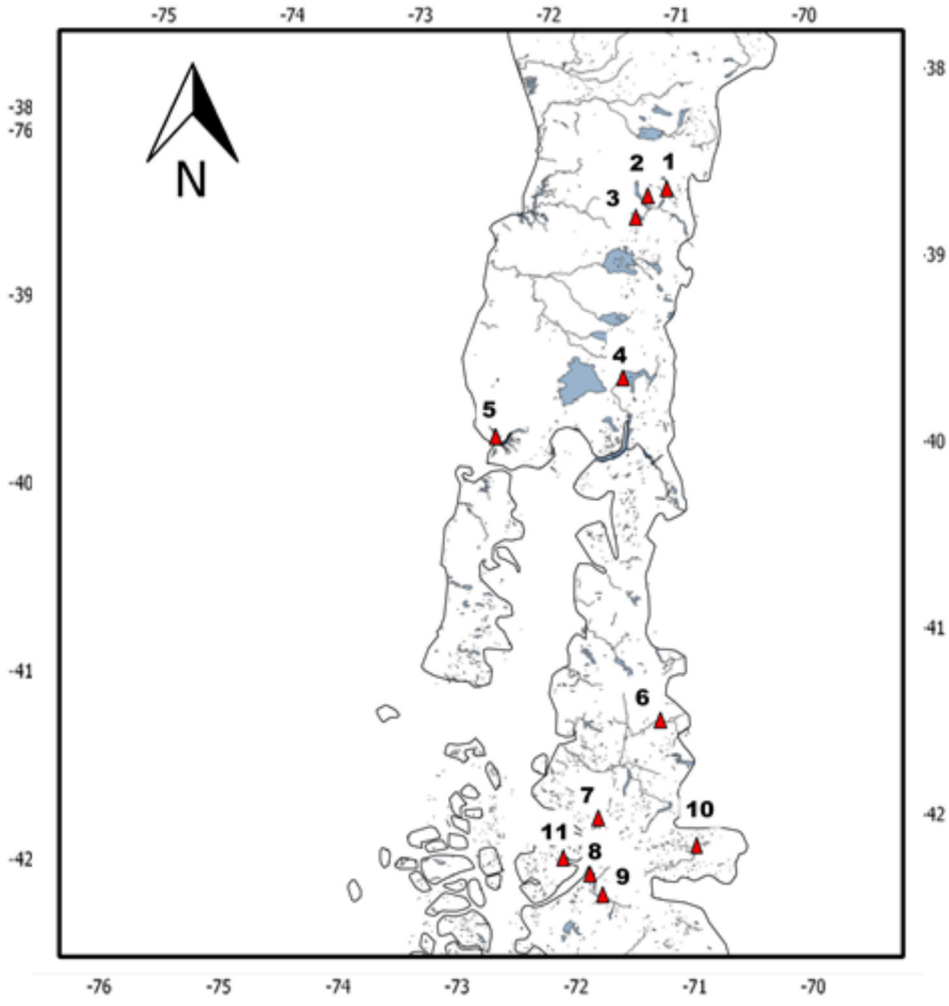

Figure 1. Geographic location of study sites: 1= Neltume Lake; 2= Liquiñe River; 3= Panguipulli Lake; 4= Todos Los Santos Lake; 5= Maullin River; 6= Palena River; 7= Cisnes seashore; 8= Cisnes Alto River; 9= Cisnes River; 10= Queulat Fiord; 11= Magdalena Island.

DOG AND AMERICAN MINK POPULATION DENSITY ESTIMATION

The sampling was defined in terms of the capture success (total individuals captured/number of traps) of American mink. We established a buffer zone of 4 km around each American mink captured in each sampling site, then these buffer zones merged to produce a wider area creating eleven sampling zones with an extension that depends on how separated and distant the minks were trapped. These sampling zones were divided into 1 km^2 cells which were then categorised as either having or not having human presence. Google Earth imagery was used to identify the presence of households. Cells with one or more households were designated as having a human presence and cells without households were designated as not having a human presence. To estimate the domestic dog population density, we counted the number of roofs per cell to have an approximate number of households per zone. In each of the zones we performed an on-ground survey of homeowners to estimate the number of dogs per house; the population density of domestic dogs was obtained as the ratio between the estimated number of dogs and the area (km^2) associated to the zones, following Acosta-Jamett *et al.* (2011) and Silva-Rodríguez & Sieving (2012). Finally, the estimated density of dogs per cell was divided into three categories of a similar sample size to denote study zones of low (≤4.0 dogs per km^2), medium (4.1 - 8.0 dogs per km^2), or high (> 8.1 dogs per km^2) dog population density. The population density of mink (minks/km) was estimated at each study zone using the following data: a) the number of trapped mink during the first 12 days of trapping (Harrington *et al.*, 2008; Medina-Vogel *et al.*, 2015); b) extrapolating the data of the length of home ranges of five male minks living in Cisnes River, providing an average of 2,213 m long (ranging between 1,422 m to 3,834 m), a female mink living in Magdalena Island,which had a lineal home range of 1,769 m, and another male living in Magdalena Island, whose home range was 2,069 m (Medina-Vogel *et al.*, 2013, 2015); and c) Minks intrasexual territoriality (Powell, 2000; Zuberogoitia

et al., 2010), assuming a proportion of males:female given by 1:1.2/km. Hence, the density of mink population in each study zone was obtained using the formula (TM/D) x 2.2, where TM=trapped minks and D=Total distance covered by the trapping transect (see Medina-Vogel *et al.*, 2015; for a detailed explanation).

ANIMAL SAMPLING

To capture American minks, wire cage traps with a double entrance (81 cm long, 21 cm high, and 23.5 cm wide) were used along with fresh or canned fish as bait. The trapping period lasted from January 2009 to February 2013. Traps were deployed along main river stretches, as well as along lakeshores and seashores; traps were set regularly spaced (around 500 m), considering the existence of mink field signs such as scats and footprints during a period between 10 to 20 days. Once captured, American minks were introduced into a mesh to perform a mechanical immobilisation to later inoculate the anaesthesia in the semimembranosus - semitendinosus muscle applying one single combined injection of ketamine-dexmedetomidine in a dose of 10-0.025 mg/kg IM, and blood samples (3-5 mL) were collected with cranial vena cava and intracadriae venipuncture using tubes with EDTA. These animals were then euthanised with thiopental by intracardiac injection (Biosano S.A., Santiago, Chile) (about 2 mL per individual) for post mortem examination. Likewise, Southern river otters were trapped using soft-catch leg-hold traps (Blundell *et al.*, 1999; Sepúlveda *et al.*, 2007) and they were handled using previously developed protocols (Soto-Azat *et al.*, 2006). They were anaesthetised with one single combine injection of ketamine-dexmedetomidine in a dose of 5-0.025 mg/kg IM and blood samples were taken from the jugular vein. Afterwards, otters were released on the same capture site. Both American mink and otter traps were checked once and twice a day, respectively. Simultaneously, near the place where minks and otters were captured, we randomly selected houses and asked dog owners for their informed consent to take a blood sample from their domestic dog (*Canis familiaris*). Dogs were manually restrained and 5 mL of blood were taken from their brachiocephalic vein, the blood samples were then processed as described for mink and otters. To obtain serum, the blood without anticoagulant was centrifuged for 10 minutes at 1,200 x g. All samples were stored in liquid nitrogen during fieldwork. In addition, a short interview with the owners was conducted to know if their dogs were permanently confined or otherwise allowed to roam freely outside their places and they were also asked about the dog vaccination records. The identification and age of the dogs were provided by the owners. All animal trapping and handling were carried out following the ethical protocols of the Bioethics Committee of Universidad Andrés Bello and the National Commission for Scientific and Technology (CONICYT) (Fondecyt 1100139 - Letter from Bioetical Comittee, there was no protocol number before 2013; Fondecyt 1171417 - Bioethics Approval Nº 007/2017). Otter trapping was done under permit number 1228 delivered by The Undersecretariat for Fisheries and Aquaculture of Chile (Subsecretaria de Pesca, Chile). As additional information on sampling, minks were removed from each study site after being trapped and sampled because of their invasive alien species status. On the other hand, after being trapped the otters were released in the same study site and were not trapped again in the same place for the following year. Finally, dogs were sampled one each year for each study site. As a result, the year was not considered as a variable, thus pseudo-replications were avoided.

LABORATORY ANALYSIS

Serum samples were tested for CPV and CDV antibody titers. Seropositivity to PV was analysed using a haemagglutination inhibition test, and titer ≥ 1:350 (see table 1) was considered positive. On the other hand, seropositivity to CDV was analysed using a seroneutralisation test and titer ≥ 1:16 (table 1) was considered positive. The detection of genomic DNA from CPV and CDV was performed through PCR from blood samples.

For the molecular analysis, DNA was extracted with the QIAamp DNA Mini Kit (Qiagen, Germany). PCR was used to screen CPV as described by Touihri *et al.*, (2009). To detect CPV, a region of 583bp of capsid protein gene was amplified by PCR with CPV primers (tables 2 and 3).

To determine the variant of parvovirus present in the positive samples, a PCR was performed to amplify and sequence 1,195bp of VP2 gene of CPV using CPV primers (tables 2 and 3).

To detect CDV, a region of 419bp of nucleocapsid protein gene (N) of CDV was amplified by RT-PCR with CDV primers (tables 2 and 3).

All PCR products were visualised using electrophoresis on 1% agarose gels with GelRed Nucleic Acid Stain (Biotium). The analysis was carried out in the Molecular Biology laboratory of the School of Veterinary Science, Universidad Andrés Bello and the Faculty of Agronomy and Forestry, Pontificia Universidad Católica de Chile. The PCR products from the VP2 gene of CPV were purified and sequenced bi-directionally at Macrogen Inc., Seoul, South Korea. Sequences were aligned and polymorphic sites were confirmed by eye according to the chromatogram using Sequencher 5.1 (Gene Codes Corporation, Ann Arbor, MI, USA). Sequences were compared with the GenBank database to confirm the presence of CPV and to compare the similarity of the amplified fragments for the different animal species.

STATISTICAL ANALYSIS

Observed seroprevalence was defined as the proportion of positive individuals among the totality of those sampled

Table 1. Observed seroprevalences (%) for PV (1:16) and CDV (1: 350) and estimated population density for dogs (km^2) and mink (trapping transect) in each study sites.

Study site	Population Density		Sample size			Seroprevalence PV			Seroprevalence CDV		
	Dog	Mink	Dog	Mink	Otter	Dog	Mink	Otter	Dog	Mink	Otter
Cisnes seashore	20.0	4.0	15	6	2	80	0	0	80	0	0
Palena River	9.1	2.0	0	1	0		100			0	
Neltume Lake	8.7	3.0	12	6	1	83	18	0	17	17	100
Panguipulli Lake	6.9	6.0	13	7	3	69	14	0	69	33	33
Maullin River	6.3	6.0	7	6	0	57	0		57	67	
Liquiñe River	5.1	3.9	4	1	0	50	0		50	0	
Cisnes River	1.3	7.5	10	1	0	30	0		30	0	
Todos Los Santos Lake	1.1	6.0	7	4	3	43	25	0	43	25	0
Cisnes Alto River	0.8	8.4	5	4	0	40	50		40	50	
Queulat Fiord	0.5	6.5	4	4		25	75		25	0	
Magdalena Island	0.0	13.0	1	19	3	100	37	33	100	20	0

Table 2. Observed seroprevalences (%) comparing both positives agents (PV and CDV) with the total positive of one of them, in each species.

Species	Dog density	Positives CDV	Positives PV	Both positives
Dogs	Low	3 (50%)	5 (100%)	2 (33%)
	Medium	7 (33%)	11 (52%)	4 (19%)
	Hight	37 (73%)	38 (79%)	29 (57%)
Minks	Low	5 (22%)	9 (39%)	3 (13%)
	Medium	1 (11%)	4 (44%)	0
	Hight	5 (19%)	6 (22%)	1 (4%)
Otters	Low	0	1 (33%)	0
	Medium	0	0	0
	Hight	2 (33%)	0	0

Table 3. Primers used for detection and sequencing of Parvovirus and Distemper.

	Name	bp	Sequence	Sample size	Function
Parvovirus	CPV-F	583	CAGGAAGATATCCAGAAGGA	20	Detection
Parvovirus	CPV-R	583	GGTGCTAGTTGATATGTAATAAACA	25	Detection
Parvovirus	VP2-561-F	1195	GAGCATTGGGCTTACCA	17	Sequencing
Parvovirus	VP2-1755-R	1195	TTAATATAATTTTCTAGGTGCTAGTTGAGA	30	Sequencing
Distemper	N-F	419	GTTAGCTAGTTTCATCCT	18	Detection
Distemper	N-R	419	GGTCCTCTGTTGTCTTGG	18	Detection

within each species (Philippa *et al.*, 2008). Exposure status (presence/absence) was recorded as binary outcomes (1/0) (Courchamp *et al.*, 2000). The differences in observed seroprevalence between more than two variables [species host, sex, age, study sites and both mink and dog population densities (Low, Medium, High)] were assessed applying Generalized Linear Models (GLM) by SYSTAT, where the tested variables were considered as the predictors and the exposure status obtained as the frequency of positive (1) or negative (0) seroprevalence (binary) was considered as the dependent variable. Subsequently, a non-parametric Mann-Whitney *U* test was used to assess differences between two variables. The Pearson correlation matrix was used to assess the correlation between two tested variables. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

The questionnaire for dog owners provided an approximation of how closely domestic animals interact

with wildlife. For each question, the percentages were calculated from the totality of the sample. A 64% of the dogs were male (usually adults), 87% were neutered, and 73% were allowed to roam free during part of the day. It was found that free or enclosed management of dogs has no significant effect on vaccination (P=0.18). Out of the 238 dog population surveyed, 38 (16%) were vaccinated against CDV and CPV at some point in their life.

Out of those individuals that were found seropositive to CPV, three were positive to CPV by PCR: one dog from Neltume Lake and two American minks from Cisnes Alto River, and Puerto Cisnes. As previously described, to characterise the virus sequencing and amplification of 1,195bp fragments were performed, however, after editing and cleaning the sequence for the analysis, these fragments were shortened to 438bp. Although this length cannot differentiate between CPV, Feline Panleukopenia, and mink Enteritis virus these short sequences showed that the two mink samples correspond to CPV, showing a 100% identity with CPV sequences at Genbank (Acc. Number: gb|HQ413321.1|). Interestingly, the dog sample showed 99% identity with CPV, 99% identity with Feline Panleukopenia, and 99% identity with Mink Enteritis virus (Acc. Number: gb|KP881687.1|). An alignment between these sequences showed 100% identity between minks and 99% identity between mink and dog samples. No positive sample to CDV was recorded. Since the number of positive samples to PCR was small, a statistical differentiation between infecting viruses with dogs and minks was not possible by molecular analysis, therefore, we will use Parvovirus (PV) to designate an exposure to CPVs for which the specific identification was unknown.

An average of 5.5 dogs/km^2 and 6.0 minks/km^2 population density per mink trapping transect were estimated in our study areas (table 1). Population size trends were different between dogs and minks: indeed, mink populations were smaller in areas where dog populations were bigger (Pearson´s coefficient equal to -0.85). None of the mustelids and dogs sampled showed any clinical signs neither of CPV nor CDV disease. Dogs had the highest seroprevalence for both diseases, followed by minks, and finally otters ($F_{2\text{-}291}$:34.7; P<0.01) (figures 2 and 3). CPVs recorded a higher prevalence than CDV, with a difference close to significant (Mann-Whitney U test 9.6; df: 1; P=0.06). Out of the total, 35 (47% of) dogs were seropositive to both PV and CDV, and 4 (7% of) minks were seropositive to both PV and CDV (table 2). Observed seroprevalence of PVs in dogs and minks showed no difference between gender or age, but a significant difference for PV seroprevalence in dogs regarding their population density was recorded ($F_{2\text{-}71}$:3.9; P=0.03), dogs had a higher observed seroprevalence in those zones with a higher population density. However, no relationship between dog population density and PVs seroprevalence in mink was inferred (figures 2 and 3, table 1).

Regarding CDV, seroprevalence in dogs and minks showed no difference between gender or age, but dogs had a significantly higher seroprevalence ($F_{2\text{-}75}$:6.3; P<0.01) in those areas with higher dog population density (figures 2 and 3), namely in those areas with smaller mink population density ($F_{2\text{-}75}$:3.3; P=0.04).

Otters did not show significant differences in PV or CDV seroprevalence concerning the sampling area, gender or age. Observed seroprevalence of PV in otters had a positive tendency towards those areas with higher mink population density (Pearson correlation Matrix= 0.43). In contrast, CDV seroprevalence observed in otters had a higher tendency to occur in those areas with higher dog population density (Pearson correlation Matrix= 0.43) (figures 2 and 3).

When grouping the data of the studied species, both diseases showed a higher observed seroprevalence in those sampling areas with larger dog population density ($F_{2\text{-}146}$:6.5; P<0.01), and in lower mink population density ($F_{2\text{-}146}$:3.7; P=0.03) (table 1). Although there was no significant difference, both diseases registered higher observed seroprevalence (%) in males than in females: dog (69/63), mink (27/16), and otter (17/11).

DISCUSSION

Domestic and introduced alien animals may act as amplifiers of infectious diseases and as a source of a pathogen for diseases that could otherwise not be maintained by native species with already low-density wild populations (Grenfall & Dobson, 1995; Woodroffe, 1999; Medina-Vogel, 2010). Pathogen spillover from domestic animals can occur when they are near wild ones (Lembo *et al.*, 2008; Sepúlveda *et al.*, 2014). Indirect or direct contact must exist for spillover to occur in diseases such as PV and CDV, also, population abundance of the domestic reservoir seems to be a very important issue. Several studies have found significant positive relationships between urbanisation, proximity to farms, presence of domestic dogs, and CPV and/or CDV seropositive foxes, wolves, and mustelids (Frölich *et al.*, 2000; Acosta-Jamett *et al.*, 2011; Acosta-Jamett *et al.*, 2014; Millán *et al.* 2015). Therefore, it is plausible that the host population density as well as how the species interact with each other affect the pattern of transmission of infectious diseases like CDV and CPV. For instance, Millán *et al.*, (2015) did not record any wolves positive to CDV, but 76% of the wolves that they studied presented evidence of exposure to CPV. Long term studies in the USA have shown that CPV is already enzootic in wolf populations (Mech *et al.*, 2008; Almberg *et al.*, 2009) where it can be maintained in the absence of reintroductions, but not CDV. This is consistent with the fact that CDV is an acute, highly immunising pathogen that requires high densities, and a large population of hosts for long term persistence; although CDV might also persist among terrestrial carnivores with small, patchily

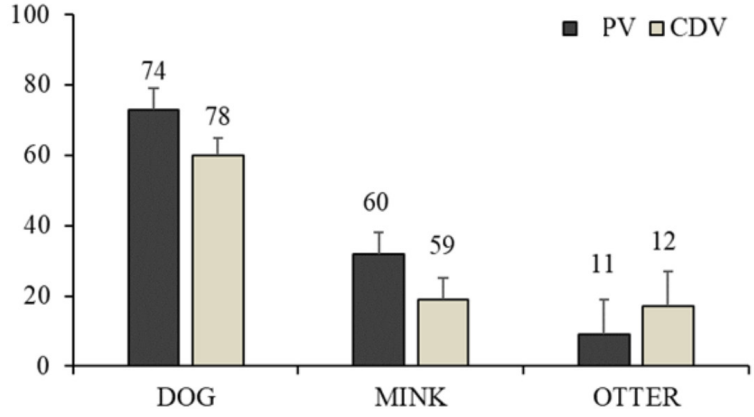

Figure 2. Observed seroprevalence (%) in domestic dogs, American mink, and Southern river otter in Southern Chile. Numbers indicate sample size; error bars indicate standard error.

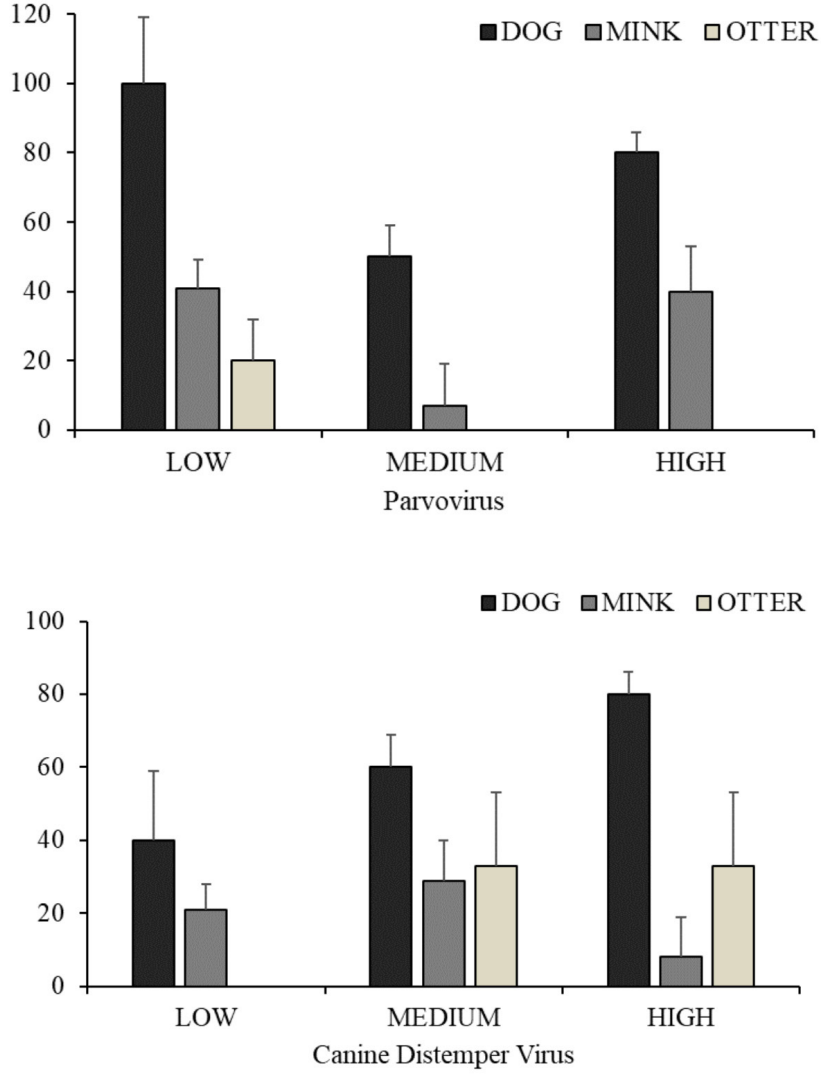

Figure 3. Observed seroprevalence (%) for PV and CDV in domestic dogs, American mink and Southern River otter in Southern Chile, according to estimated dog population size. Error bars indicate standard error.

distributed groups (Almberg *et al.*, 2010). Moreover, CDV is a pathogen with a short infection cycle that requires either large scales of hosts or multi-host transmission for its persistence, and it seems that wild carnivore species with naturally induced small populations cannot maintain CDV by themselves (Cleaveland *et al.*, 2000). However, the presence of a second competent host species can substantially increase the probability of long-term CDV persistence in a region (Almberg *et al.*, 2010). There are only a few studies concerning the spillover of CDV and CPV from domestic dogs to wild carnivores, where domestic as well as wild carnivore population densities have been estimated and their dynamics elucidated. Empirical studies seeking to identify disease population thresholds in wildlife find recurring obstacles, like small sample sizes and confounding factors (Lloyd-Smith *et al.*, 2005; Mech *et al.*, 2008, Cleaveland *et al.*, 2007; Almberg *et al.*, 2010). For instance, otters are susceptible to CDV and such is the case of American river otter (*Lontra canadensis*) (Kimber *et al.*, 2000) and captive Asian clawless otter (*Aonyx cinereus*) (Geisel, 1979; Madsen *et al.*, 1999; Mos *et al.*, 2003; De Bosschere *et al.*, 2005), however, only vague accounts of clinical canine parvovirus (CPV-2 variant) in otter species have been reported in the literature (Famini *et al.*, 2013; Gjeltema *et al.*, 2015; Miranda & Thompson, 2016). Another problem is the failure to find wild otters and minks with clinical signs of either CDV or CPV, this is probably because sick individuals remain in their dens during the disease and some die there (Barker & Parrish, 2001; Williams, 2001). This suggests that our results must be considered with caution due to the small sample size of the otters, the number of animals sampled per sampling zone, and the limitation of cross-sectional studies (Gilbert *et al.*, 2013). Moreover, we failed in finding molecular evidence for CDV, since infectious periods of this disease are short (Deem *et al.*, 2000); nevertheless, our sample size was comparable to that of Sobrino *et al.* (2008) and Kimber *et al.* (2000), but smaller than that of Delahay & Frölich (2000), none of which found antibodies against CDV.

The results we obtained regarding the observed seroprevalence for CDV were similar to those found by Sepúlveda *et al.* (2014) using 1:16 titer cut-off for mink (21.7% them, 17% us) and dogs (41.6% them, 60% us), but higher than those reported by Kimber *et al.* (2000) for river otter (*Lontra canadensis*) in North America because they recorded 4.7% (3 of 64) positive for CDV (1:8-1:768), and 7 of 64 (10.9%) otters positive for CPV-2 (range of titers 1:20-1:640). However, higher seroprevalences were found when compared to those obtained for American mink and other mustelids in France by Philippa *et al.* (2008) who recorded 9% of 127 European mink (*Mustela lutreola*), 20% of 210 polecats (*Mustela putorius*), 5% of 112 American mink, 33% of 21 stone marten (*Martes foina*), and 5% of 20 pine marten (*Martes martes*) in regions with almost no presence of free-ranging domestic dogs (Doherty *et al.*, 2017), although they considered positive a 1:10 titer. In our case, the observed seroprevalence in American mink was 33% for CDV with a titer 1:350, and 16% for CPV with a titer 1:16. Besides, these results are useful to begin the understanding of the ecology of CDV and CPV in a carnivore community where domestic dogs and alien American mink might be playing an important role (Cleaveland *et al.*, 2000; Gilbert *et al.*, 2013; Sepúlveda *et al.*, 2014); our cross-sectional assessment provides reliable information about exposure to viral infectious diseases of wild American minks and Southern river otters, adding support to the hypothesis of Sepúlveda *et al.* (2014) about behavioural aspects of transmission, with invasive species as host bridges from domestic to native species. Moreover, for the first time, CPV serological evidence in Southern river otters was documented in this study, as well as CPV serological and molecular evidence in wild American mink, and CDV serological evidence in otter from South America. Statistical differences in the observed seroprevalence of CDV and CPV in Southern river otter were not found, probably because of the small sample size. Nevertheless, we recorded a tendency of increased CDV seroprevalence in otter in those study areas with higher dog estimated population density, and similarly for CPV seroprevalence in otter in those study areas with higher mink estimated population densities (tables 1 and 2; figures 2 and 3).

Theoretical models suggest that whenever strong spatial segregation leads to distinct sub-grouping within a population, as it is the case for territorial species, interspecies transmission may be the dominant transmission pathway and the presence of an alternative host is required for pathogen establishment (Holt *et al.*, 2003; Keeling, 2005). Also, the influence of social hierarchy on disease dynamics becomes more important at low disease prevalence (Davidson *et al.*, 2008) and this seems to be the case for minks and otters, which have territorial as well as social hierarchical behaviour (Powell, 2000). Sepúlveda *et al.* (2014) found significant interactions between introduced American mink and both otter and dogs, either directly (harassment) or indirectly (latrines co-use). The indirect interactions between mink and dogs in latrines were not separated by more than two days (Sepúlveda *et al.*, 2014), an interval in which a pathogen, such as CDV and CPV, can remain viable in the environment (Shen & Gorham, 1980; Parrish, 1990; Williams, 2001). Similar observations were reported by Medina-Vogel *et al.* (2013) with a variable space overlap as well as latrines co-use between Southern river otter and mink, and aggressive encounters between both species in a dog free habitat. These facts led Sepúlveda *et al.* (2014) to theorise the feasibility of transmission of infectious diseases like CDV from dogs to River otter with mink acting as a *bridge host*, allowing our results to support this hypothesis. Moreover, the interspecies interactions mentioned above suggest a directional transmission from dog to mink, and from mink to otter.

With regard to interactions between domestic dogs and domestic cats with wild carnivores, transmissions were reported in a recent study in Madagascar, due to an important overlap in habitat use and specific sites (Rasambainarivo *et al.*, 2017). Frölich *et al.* (2000) found a significant difference in the number of seropositive foxes between urban, suburban and rural areas, indicating that free-living foxes can become infected with CDV by contact with domestic dogs. These authors conclude that dogs are contaminating the habitat of wild carnivores and, therefore, dog density would influence the seroprevalence of CDV antibodies in wild carnivores. Our results support the hypothesis that CDV and CPV are maintained in wild carnivore species due to the permanent presence of domestic dogs, we did not find molecular evidence of CDV in otters and mink but our results suggest that the exposure of mink to PVs could be the result of CPV infections.

In our study, dog-to-mink transmission seems to occur when minks visit farms attracted by rats, mice and poultry, and then can be infected by dogs (Philippa *et al.*, 2008; Sepúlveda *et al.*, 2014). The other possibility seems to be when dogs visit mink latrines (Sepúlveda *et al.*, 2014). Therefore, dog-to-mink transmission seems to take place as a result of the dynamics of scent communication since dogs use their faeces as territorial marks, and minks become in contact with those marks. These transmission rates appear to depend on domestic dog population size and density (table 1) and on the possibility that minks can get in contact with an infected spot. Instead, CPV and CDV transmission between mink and Southern river otters may occur mainly by direct interaction or when they are in close contact within a commonly used spraint site (Sepúlveda *et al.*, 2014; Medina-Vogel *et al.*, 2013). Transmission rates do appear to depend on the frequency of these contacts and on mink population densities, but not on Southern river otter population densities which are significantly lower: southern river otters in freshwater habitats in Chile record densities below 0.5 individuals per km of river (Sepúlveda *et al.*, 2007). Male mustelids have larger home ranges than females, leading to increased exposure to infections. Additionally, male increased stress during the breeding season may immunosuppress and increase their susceptibility to diseases (Powell, 2000; Cross *et al.*, 2009). Although we did not record gender statistical differences between observed seroprevalence in all three-study species, we found that seroprevalence was higher in males than in females. Male mink larger home range, strongest territorial behaviour, and larger displacement patterns could have increased the probability of becoming in contact with male dogs as well as male otters, which is supported by our results showing that this pattern is stronger for CDV than for PV.

As previously mentioned, our analysis supports the hypothesis of Sepúlveda *et al.* (2014) of American mink acting as a bridge host between domestic dogs and wild Southern river otter in Patagonia for infectious diseases such as PV and CDV, which is a matter of important conservational concern. These results raise a concern about the conservation of Patagonian otters because, from the perspective of the pathogen, one of the susceptible populations namely southern river otter and marine otter (*Lontra felina*) is made up of a small group of hosts (Sepúlveda *et al.*, 2007; Medina-Vogel *et al.*, 2007).

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