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Tag, you're it!: viral diseases in native otters of south-central Chile due to coexistence with invasive American mink and domestic dogs

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Introduction: Biological invasions represent a significant epidemiological route for the introduction and dispersion of pathogens, facilitating disease emergence and transmission among native biodiversity. In the temperate rainforest ecoregion of south-central Chile, the native semiaquatic mustelid *Lontra felina* (marine otter) and *L. provocax* (southern river otter) coexist both sympatrically and syntopically with two invasive species—American mink (*Neogale vison*) and domestic dog (*Canis lupus familiaris*), that act as carriers and hosts of canine parvovirus and distemper.

Methodology: To assess the occurrence of both diseases, we: (1) collected serum and mucous membrane samples from four species across three sectors of this ecoregion; and (2) employed serological immunoassays (IgG) and genetic analyses (qPCR-HRM) to detect both active and past infections, and to genotypically characterize the two viral agents.

Results: 75% of *L. felina* individuals tested positive for parvovirus. The melting temperature (T_m) of the analyzed DNA fragment revealed two diverging groups, suggesting the presence of two genotypic variants of the virus within this mammalian assemblage. *L. felina* individuals carried the variant with the higher T_m, which was also detected in *N. vison* from the same locality. In contrast, *L. provocax* individuals carried the variant with the lower T_m, while dogs and minks hosted both viral variants. Canine distemper virus was detected only in dogs that also tested positive for parvovirus.

Discussion: Our results present the first report of parvovirus in *L. felina* and support the hypothesis that *N. vison* and dogs acts as metareservoir and mink also as a bridge host for its transmission. In the study area, the synanthropic behavior of *N. vison* and its interactions with domestic and native species may facilitate the diversification of emergent pathogens within Chilean native fauna.

KEYWORDS

parvovirus, canine distemper virus, bridge host, metareservoir, serological detection, genetic screening

Introduction

Biological invasions promote the biodiversity loss by intensifying predation and competition pressures, causing environmental disruption, and/or facilitating disease transmission (1–3). The diversification of exotic pathologies in native ecosystems is primarily facilitated by interactions between endemic fauna and invasive species, the latter serving as reservoirs for some diseases (4, 5). Therefore, assessing the prevalence of different diseases in native and invasive fauna not only enables the understanding the effects of species invasions, but also provides insight into their implications for human health, which is particularly relevant given that recent pandemics have arisen from interactions between humans and wildlife with zoonotic consequences (6–10).

The temperate rainforest of south-central Chile is an ecoregion situated toward the southern end of the Chile Central biodiversity hotspot (11, 12). Over the past three centuries, this biome has undergone significant environmental impacts, including habitat loss, fragmentation, forest fires, and reduction of biodiversity by introduction of invasive species driven by urbanization, industrialization, agricultural and forestry activities (13–15). Here the endangered southern river otter (*Lontra provocax*) is fund. It is a species notable for its extremely low density and dependence on pristine riparian habitats (16, 17). In addition, the congeneric and endangered marine otter (*L. felina*) is more frequently observed in relatively undisturbed rocky coastal systems (18, 19). Both species coexist with the American mink (*Neogale vison*), a highly dispersive exotic mustelid whose range in Chile has expanded 2,500 km northward from its center of origin in southern Patagonia over the past century (20–23). Further, the domestic dog (*Canis lupus familiaris*) is an invasive species intrinsically linked to human activities that combines high synanthropy due to domestication, with a tendency to expand their home range and form packs that prey on native species (24–30). Additionally, dogs are globally recognized as the primary reservoir of canine parvovirus and canine distemper viruses, serving as the principal source of origin and diversification of both pathogens in wildlife worldwide (31–35).

Both diseases have multi-host capacity facilitated by their ability to remain viable in the air for weeks and by their transmission routes, which include aerosolized secretions and passive spread via fomites (36–38). In south-central Chile, domestic dogs exhibit active movement dynamics between rural, semi-rural, and forested areas, with a canine parvovirus and canine distemper seroprevalences ranging between 50 and 70% (39, 40). Both diseases have also been detected in minks and southern river otters, suggesting that mink acts as an active transmission bridge, as they move through forested areas and interact with domestic dogs, particularly when they hunt in poultry farms. Furthermore, southern river otters are particularly vulnerable to infection by these pathogens, as various native and invasive mammal species co-occur at their latrines, where fecal and urine deposits serve as focal points of infection for this and other species attracted to these conspicuous places (41–43).

Immunologically, the infections with canine parvovirus and canine distemper trigger antigen–antibody binding reactions involving immunoglobulin G or IgG (44). This enables the use of specific serological immunoassays to detect past infections by identifying the presence of antibodies (45). Complementarily, the amplification of specific viral gene fragments enables the detection of the pathogenic agent currently present in the organism (46, 47). Quantitative real-time PCR (qPCR) combined with high-resolution melting analysis (HRM) enables the characterization of nucleotide sequences based on the temperature required to denature half of a double-stranded DNA strand, a property known as the melting temperature or T_m (48, 49). Thus, variations observed in T_m peak ranges indicate structural differences in gene sequences caused by specific mutations, which deviate from the known structure of a reference sequence (50, 51). Therefore, immunoassays are effective in detecting past infections; however, they lack the specificity required for detailed pathological characterization. Nevertheless, these techniques exhibit functional complementarity with genetic analyses based on DNA sequences (e.g., qPCR-HRM), which enable the detection of active infections as well as the genetic typing of the causative agents of parvovirus, particularly canine parvovirus (52–54).

We employed serological methods based on antigen–antibody reactions to test for parvovirus and distemper infections in dogs, as well as invasive mink, marine and southern river otters, across three distant locations within the temperate rainforest ecoregion of south-central Chile. Additionally, qPCR-HRM was performed to detect the presence of genetic material from these pathogens and to infer structural variability in the amplified fragment sequences based on their T_m peak ranges. These results will allow us to establish genetic-structural associations between viral agents affecting native fauna and to infer the role of invasive fauna as vectors of pathogen transmission.

Materials and methods

Sample collection

Between January 2018 and January 2020, blood and mucous membrane samples were obtained from domestic dogs, invasive American minks, and native otters at three hydrographic basins within a 200 Km long study area, where inhabits both otters and the rural activities contribute to the presence of free-ranging dogs and minks (55, 56). Toltén River basin has remnant riparian forest coexisting with introduced commercial plantations of *Pinus* spp. and *Eucalyptus* spp. This area is located near the current northern distributional limit of the southern river otter and has previously been used as a study site to investigate trophic, population, and genetic of the species (57). In Cunco (upper basin) we sampled dogs and southern river otters, while in Coipue (mid-basin) we sampled dogs only and Nueva Toltén (lower basin) we sampled dogs, minks, and southern river otters (Figure 1). Southward, in the Valdivia River basin we sampled dogs and minks in the lower valley around Valdivia city. Furthermore, Calfuco and Pilolcura are two remote coastal localities

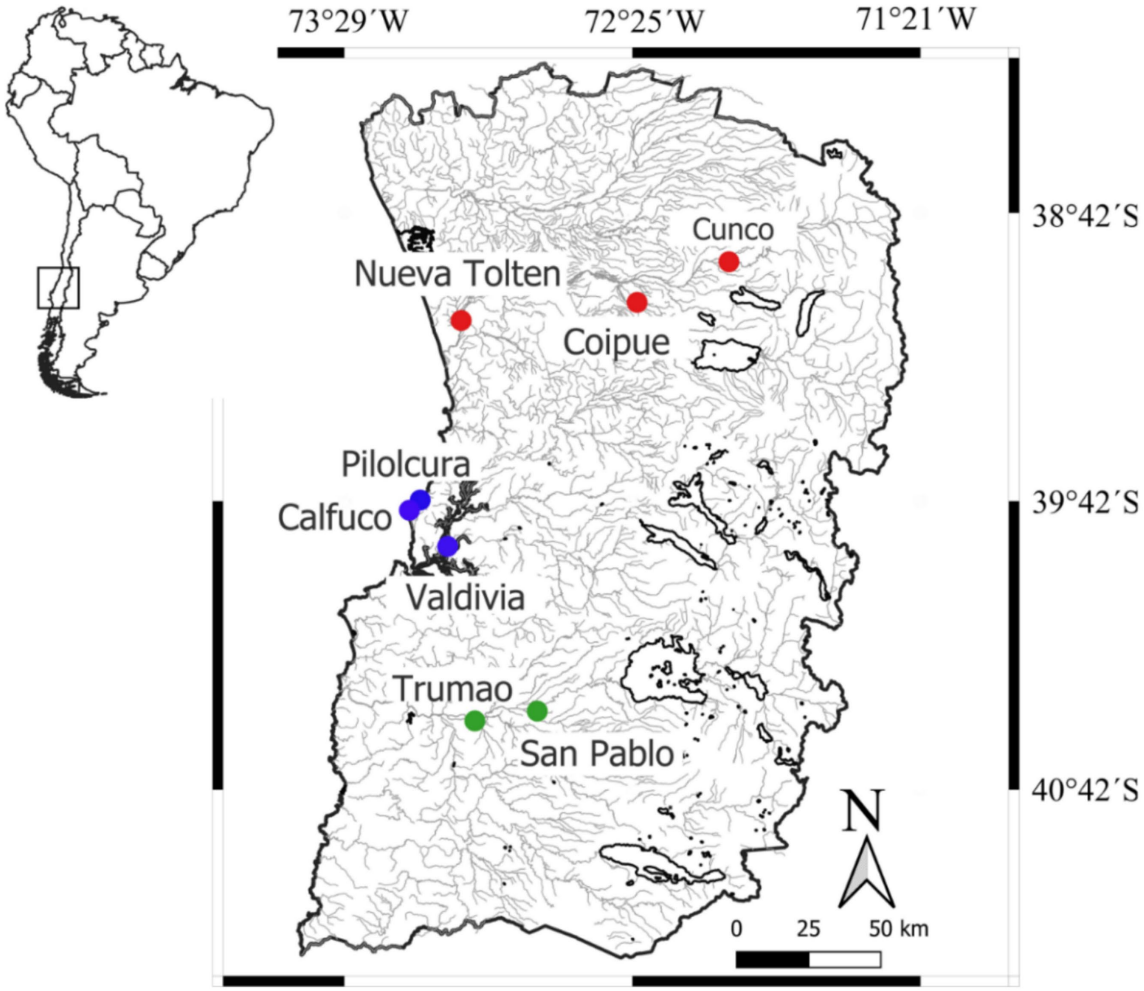

FIGURE 1
Location of the sampling sites within the temperate rainforest ecoregion of south-central Chile. The sampling sites in the Toltén River, Valdivia River and Río Bueno basins are indicated with red, blue and green circles, respectively.

characterized by a diverse rocky intertidal and subtidal community, which supports a stable population of marine otters (58, 59). Here we sampled only marine otters in Pilolcura and marine otters with minks in Calfuco (Figure 1). Finally, in Río Bueno basin (Figure 1) we sampled minks and dogs in San Pablo-Trumao (mid basin). Although the riparian forest in this area remains relatively undisturbed and provides important breeding and reproductive habitat for southern river otter (42, 60), no southern freshwater otter were captured.

Otters were lived captured using Victor® No 1.0 padded traps (Woodstream Corp., Lancaster, United States) to minimize the risk of injury. The sampled otters were previously analyzed for leptospirosis and toxoplasmosis (61). Minks were captured using single-door modify Tomahawk traps of 60 cm × 13 cm × 13 cm (Hazelhurst, United States) baited with canned fish or mink anal gland lures (62, 63). Both trap types were checked every 12 h (63–65). To minimize the likelihood of recapturing the same individual, sampling points were situated more than 10 km apart, exceeding the reported home range for these species (41, 66). Captured otters were anaesthetized with a combination of 5.3 mg/kg ketamine hydrochloride (Ketamil 111.56 mg/mL; Ilium Veterinary Products, Glendenning, Australia) and 26.5 µg/kg dexmedetomidine hydrochloride (Dexdomitor® 0.5 mg/mL; Zoetis, Parsippany, United States), administered via intramuscular injection (67, 68). Cardiorespiratory rate, oxygen saturation, body temperature, blood pressure, and depth of anesthesia through muscle relaxation and reflex responses were continuously monitored (67). Serological samples were obtained by venipuncture of the cephalic, jugular, or cranial vena cava to extract 6 mL of blood with anticoagulant-free tubes centrifuged *in situ* at 3000 rpm for 10 min within three hrs. of collection. In addition, conjunctival, tonsillar, and rectal mucosal swabs were obtained using sterile cotton-tipped applicators, which were placed into cryotubes containing viral transport medium and immediately frozen in liquid nitrogen (69). Thus, the samples were transported in liquid nitrogen to Health Ecosystem Laboratory, Universidad Andrés Bello (Santiago, Chile), where it was stored at −80 °C until processing.

Thirty minutes after the collection of serum and mucous samples, an intramuscular injection of 26.5 µg/kg atipamezole (Antisedan® 5.0 mg/mL; Zoetis, Parsippany, USA) was administered as an anesthetic reversal agent. During anesthesia and recovery, the captured individuals were housed in a dark,

thermally controlled tubular containment cage until their release at the capture site, once full recovery had been achieved (70, 71). Captured mink were anaesthetized with a combination of 10 mg/kg ketamine hydrochloride and 25 µg/kg dexmedetomidine hydrochloride, and subsequently euthanized with an intracardiac injection of 5 mL sodium thiopental (67, 68). These actions are consistent with population control of invasive species programs based on capture/euthanasia and the application of trapping strategies specific to this species (62, 72). The samples from domestic dogs were collected with the consent of the owners, located no more than 10 km from the capture sites described above (41, 66). These samples were collected from brachiocephalic vein without anesthesia in unvaccinated individuals > 5 months old to avoid interference with maternal antibodies (73). The handling, capture, and sampling procedures for the animals analyzed were conducted in accordance with the protocols of Bioethics Committee of the Universidad Andrés Bello (Mustelids; Chile), Universidad Santo Tomás (dogs; Chile) and the Agencia Nacional de Investigación y Desarrollo de Chile (ANID), Fondecyt 1,171,417—Bioethics Approval No 007/2017. Additionally, the capture of otters was conducted under permit No. 1228 from the Subsecretaría de Pesca y Acuicultura de Chile.

Serological and genetic analysis

A rapid ImmunoComb Canine test (BioGal Galed Laboratories Acs. Ltd., Kibbutz Galed, Israel) was applied to all blood serum samples following the manufacturer's instructions. This commercial, ELISA-based immunoassay qualitatively detects immunoglobulin G (IgG) that reacts with the antigens of canine parvovirus and canine distemper virus. For the genetic detection of both diseases, the corresponding rectal, tonsillar, and conjunctival samples were separately subjected to DNA extraction and purification for the detection of canine parvovirus, and RNA extraction followed by reverse transcription to detect canine distemper (46, 74). DNA extraction was performed with the QIAamp DNA Mini kit (75), while RNA extraction was performed with the RNA-Solv Reagent kit (Omega Bio-Tek Inc., Norcross, United States) followed by the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, United States) for the reversal of RNA into cDNA (76). All extraction, purification, and reverse transcription procedures were carried out in accordance with the manufacturer's instructions.

Polymerase chain reaction (PCR) was employed for the detection and cyclic amplification of a specific DNA sequence in a highly sensitive manner, enabling, among other applications, the diagnosis of infectious diseases by recognizing the genetic material of microorganisms in biological samples (47). Conventional PCR for parvovirus and distemper was performed by preparing a PCR 1X buffer containing 2 mM MgSO4, 0.1 mM dNTPs, 0.25 µM forward primer, 0.25 µM reverse primer, 1 U/25 µL Taq polymerase (M0267S, New England Biolabs, Inc., Ipswich, United States), and 1 µL template DNA, to a final volume of 25 µL per tube. CPV primers amplify an 83 bp fragment of the capsid VP2 protein, flanked by the forward primer 5′-ACAAGATAAAAGACGTGGTGTAACTCAA-3′ and the reverse primer 5′-CAACTTCAGCTGGTCTCATAATAGT-3′, while CDV primers detect and amplify a 388 bp fragment of the H protein gene, flanked by the forward primer 5'-TTTGGGGCAACACCTATG GATCAAGT-3′ and the reverse primer 5'-CTCCGGATGGCTT ACCAT-3′ (46). For both sequences, the PCR program in the thermocycler consisted of an initial denaturation at 94° C for 5 min, followed by denaturation at 94° C for 30 s, annealing at 49° C for 30 s, elongation at 72° C for 30 s, elongation at 72° C for 10 min, and holding at 4° C for 15 min, all this repeated for 35 cycles. The Nobivac Puppy DP vaccine (Merck and Co., Inc., Rahway, United States), containing attenuated active strains of CPV (strain 154) and CDV (strain Onderstepoort), was used as a positive control, while molecular biology-grade distilled water was used as a negative control and nuclease free water as negative control. Subsequently, an electrophoretic run was performed on a 2% agarose gel in TAE 1X buffer at 100 volts for 30 min for qualitative DNA analysis, and for 40 min in the case of PCR products. A 100 bp DNA ladder was used as a molecular weight marker. The bands were stained with Gel and visualized under a trans illuminator Ultraviolet Viewer model UV1 (Extragene Inc., Taichung, Taiwan).

Quantitative real-time PCR with high-resolution melting analysis (q-PCR-HRM) was applied for parvovirus in order to differentiate amplicons based in their melting temperature (T_m) to detects changes that can occur even in a single position of the sequence (48, 49, 77). HRM analysis establishes a high-resolution dissociation curve that differentiates DNA sequences based on their T_m, defined as the temperature at which half of the DNA fragment is denatured or separated. This value depends on the conformation of the base pairs in the sequence. We used a mixture of the q-PCR Brilliant II SYBR Green qPCR Master Mix Kit 2X (Agilent Technologies, Santa Clara, USA), diluted to 1X, with the same primers used in conventional PCR at a concentration of 0.25 µM, and a reference dye at 30 nM. Added to this was 1 µL of DNA, with the final volume per tube adjusted to 20 µL using molecular biology-grade water. The q-PCR program was performed on an Agilent Technologies AriaMx and consisted of an initial denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 49 °C for 30 s, and both initial and final elongation at 72 °C for 30 s. The amplification reaction was monitored in the FAM channel for the samples and in the HEK channel for the reference dye. The peak T_m of the sample amplicons was compared with that of the positive control to verify whether there was molecular correspondence between the two types of sequences (77). The HRM procedure consisted of one cycle at 95 °C for 30 s, 65 °C for 30 s, and 95 °C for 30 s, with temperature increments of 0.2 °C from 65 °C to 95 °C. All serological and molecular analyses described were conducted in the Clinical Biochemistry Laboratory of the Medical Technology Department at Santo Tomás University, Osorno Campus, Chile.

For each species, the prevalence of parvovirus, distemper, and co-infection was defined as the proportion of seropositive individuals relative to the total number of animals captured. The uncertainty associated with prevalence was quantified using exact binomial 95% confidence interval calculated according to the Clopper–Pearson method (78). Potential statistical differences between age classes (juvenile, adult), sexes (female, male), and hydrographic basins were assessed using Fisher's exact test (79). Both test were performed in the base package of R 4.1.1 (80).

Results

A total of 81 individuals were sampled in the study area, from which blood serum and mucous membrane samples were collected. Of these, 86.4% (70 individuals) were invasive species, with *N. vison* and *C. lupus familiaris* representing 51.9% (n = 42) and 34.6% (n = 28) of the total sample, respectively. The remaining 11 individuals belonged to native mustelids, comprising eight marine otters and three southern river otters. All marine otters were captured in Valdivia River basin (4 in Calfuco and 4 in Pilolcura), where 62% of the mink individuals (n = 26) were also captured. In contrast, 50% of the dogs in the sample were obtained from the Toltén River basin (Table 1).

All serological samples from dogs in Valdivia River basin, 92.9% of dogs from Toltén River basin (13 of 14 individuals), and 60% of dogs from Río Bueno basin (3 of 5 individuals) tested seropositive for either parvovirus alone or parvovirus in combination with distemper. In addition, canine distemper was consistently detected together with parvovirus in 21.4% of the dogs from Toltén River basin (3 of 14 individuals) and 66.7% of the dogs from Valdivia River basin (6 of 9 individuals). Of the 26 minks captured at Valdivia River basin, only one (3.8%) tested seropositive for parvovirus, but 75% of marine otters (4 in Calfuco and 2 of 4 in Pilolcura) tested seropositive for parvovirus (Table 1). Furthermore, 66.6% of southern river otters tested from Toltén River basin (2 of 3 individuals) were positive for parvovirus by conventional PCR, although they were negative with serological analysis. In the sample from Valdivia River basin, all dogs, 1 of 26 minks, and 7 of 8 marine otters tested positive by both serological analysis and conventional PCR. In Río Bueno basin, one dog tested negative for both the serological reaction and positive for conventional PCR, while one mink was positive by serological and conventional PCR analysis (Table 1). All samples serologically positive for canine distemper were negative for conventional PCR. Intraspecifically, no significant differences were detected in the seroprevalence of either pathology with respect to age class (juvenile vs. adult), sex (female vs. male), or study site (Fisher's exact test, $p > 0.11$). An exception was observed for parvovirus and distemper seropositivity, which differed significantly between dogs from the Valdivia River basin (66.7%) and those from the Río Bueno basin (0%; Fisher's exact test, $p = 0.031$).

In the qPCR-HRM analysis, all parvovirus-positive samples identified by conventional PCR exhibited melting temperatures (T_m) that differed markedly from the T_m range of the positive control. Based on this pattern, two distinct genotypic profiles were identified for the nucleotide fragment analyzed in parvovirus. A group exhibiting higher T_m values than the control sample included dogs from Río Bueno basin, as well as minks and marine otters from Valdivia River basin. In contrast, parvovirus DNA fragments from minks in Río Bueno basin and southern river otters in Toltén River basin displayed lower T_m values than the control. Finally, both genotypic profiles were detected in dogs tested from Toltén River basin and Valdivia River basin (Figure 2).

TABLE 1 Sample size (N) and serological and genetic prevalence of parvovirus and distemper (mean % and exact binomial 95% confidence intervals) for four species across three river basins in the temperate rainforest ecoregion of south-central Chile.

Hydrographic basin		Southern river otters	Marine otters	Minks	Dogs
Tolten River	N	3	0	8	14
	Seropositivity parvovirus	0 [0% (0–70.8%)]	-	0 [0% (0–36.9%)]	13 [92.9% (66.1–99.8%)]
	Seropositivity distemper	0 [0% (0–70.8%)]	-	0 [0% (0–36.9%)]	3 [21.4% (4.7–50.8%)]
	Seropositivity both diseases	0 [0% (0–70.8%)]	-	0 [0% (0–36.9%)]	3 [21.4% (4.7–50.8%)]
	Parvovirus (S+/PCR+)	0 [0% (0–70.8%)]	-	0 [0% (0–36.9%)]	4 [28.6% (0.1–58.1%)]
	Parvovirus (S-/PCR+)	2 [66.7% (9.4–99.2%)]	-	0 [0% (0–36.9%)]	0 [0% (0–23.1%)]
	Distemper (S-/PCR-)	0 [0% (0–70.8%)]	-	0 [0% (0–36.9%)]	4 [28.6% (0.1–58.1%)]
Valdivia River	N	0	8	26	9
	Seropositivity parvovirus	-	6 [75% (34.9–96.8%)]	1 [3.8% (0.01–38.5%)]	9 [100% (66.4–100%)]
	Seropositivity distemper	-	0 [0% (0–36.9%)]	0 [0% (0–13.2%)]	6 [66.7% (29.9–92.5%)]
	Seropositivity both diseases	-	0 [0% (0–36.9%)]	0 [0% (0–13.2%)]	6 [66.7% (29.9–92.5%)]
	Parvovirus (S+/PCR+)	-	2 [25% (3.2–65.1%)]	1 [3.8% (0.01–38.5%)]	6 [66.7% (29.9–92.5%)]
	Parvovirus (S+/PCR-)	-	4 [50% (15.7–84.3%)]	0 [0% (0–13.2%)]	3 [33.3% (7.5–70.1%)]
	Parvovirus (S-/PCR+)	-	0 [0% (0–36.9%)]	3 [11.5% (2.4–30.1%)]	0 [0% (0–33.6%)]
	Distemper (S-/PCR-)	-	0 [0% (0–36.9%)]	3 [11.5% (2.4–30.1%)]	0 [0% (0–33.6%)]
Río Bueno	N	0	0	8	5
	Seropositivity parvovirus	-	-	0 [0% (0–36.9%)]	3 [60% (14.7–94.7%)]
	Seropositivity distemper	-	-	0 [0% (0–36.9%)]	0 [0% (0–52.2%)]
	Seropositivity both diseases	-	-	0 [0% (0–36.9%)]	0 [0% (0–52.2%)]
	Parvovirus (S+/PCR+)	-	-	4 [50% (15.7–84.3%)]	0 [0% (0–52.2%)]
	Parvovirus (S-/PCR+)	-	-	0 [0% (0–36.9%)]	1 [20% (0.5–71.6%)]
	Distemper (S-/PCR-)	-	-	0 [0% (0–36.9%)]	0 [0% (0–52.2%)]

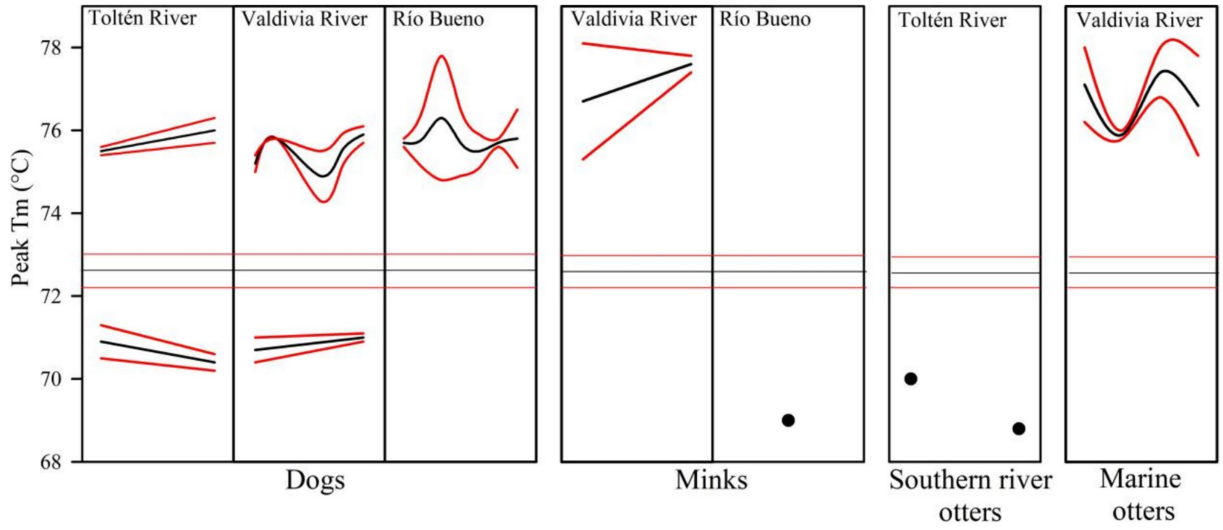

FIGURE 2
Melting temperature (T_m) peaks obtained by qPCR-HRM for the VP2 gene of parvovirus in four species across three river basins. Mean (black lines) and 95% confidence intervals (red lines) are shown. Black dots denote individual T_m values, and horizontal lines indicate T_m values in the control kit.

Discussion

Our findings support that the coexistence of domestic dogs and American mink facilitates the diversification of canine parvovirus among native aquatic mustelids in the temperate rainforest of south-central Chile. This pattern was evidenced not only by the presence of both structural variants of parvovirus in minks and dogs (detected through qPCR analysis of the DNA fragment and inferred from the T_m values), but also by the detection of genotypic variants between native otters from south-central Chile.

Between 2009 and 2016, both parvovirus and distemper were detected in southern river otters. During this period, the role of mink as a reservoir of these pathogens, and as a transmission bridge facilitated by its interactions with domestic dogs was described (25, 41). However, these findings contrasted with the absence of canine parvovirus reported in minks from samples collected between 2015 and 2016 within its current distribution in Chilean Patagonia (81). Both types of responses suggest that the infection and dispersion of parvovirus in minks was associated with population growth and expansion of its geographical distribution, which intensifies the intraspecific and community interactions and thereby increase the probability of infection by emerging diseases (81–84). In this sense, interactions between minks and dogs had likely favored the spread of canine parvovirus in both aquatic native otters from central- southern Chile. In addition, the high serological prevalence and low percentage of genetic detection of parvovirus in marine otters is indicative of its establishment in the population and the capacity of the hosts to become infected and recover (85–87).

The VP-2 gene fragment of parvovirus from marine otters captured in Valdivia River basin exhibited T_m values similar to dogs and minks from the same locality. Indeed, dogs registered all parvovirus sequences detected in our study in nearly all study sites, minks also had all sequences but with differences between study sites. These genotypic and study site correspondence suggests the ecological interactions between otters and minks in the transmission of parvovirus, and domestic dogs as the main reservoir. In the freshwater environments of this ecoregion, southern river otters latrines are landscape features that attract a host of species and create interspecific connectivity scenarios in which minks and southern river otters frequently co-occur, thereby constituting a potential source of pathogen transmission between native and invasive species (25, 42).

In the coastal environments of south-central Chile, frequent co-occurrence between native fauna and minks (and potentially with domestic dogs) is also expected to occur at marine otter latrine sites. However, by addressing the challenges associated with accessing the complex locations of their latrines and deploying camera traps within them, it will be possible to document their co-occurrence with other mammals, including rodents, minks, and dogs (19, 88–90). Furthermore, the fact that mink from Río Bueno basin carried the genotypic variant with the lowest T_m value, which was also detected in southern river otters from Toltén River basin, supports the notion that minks may function not only as a transmission bridge for canine parvovirus, but also as a reservoir of genotypic variants present in rural dogs (38, 91, 92). Therefore, as the exposure rate to parvovirus depends on the density and/or prevalence of infection in the invasive host, the most probable interpretation our results is a meta-reservoir created by invasive mink and domestic dogs associated with population densities and frequency of contact (41).

Within the Chilean context, minks are susceptible to infection and capable of transmitting multiple viral, bacterial, and parasitic diseases, some of which are zoonotic (61, 81, 93, 94). The synanthropy of minks, associated with attacks on farms and animal breeding centers (72, 95), would favor interaction with domestic dogs and rodents. In the temperate rainforest ecoregion of south-central Chile, 11 endemic rodents species have been described coexisting actively with three introduced rodent species (rats [*Rattus rattus, R. norvegicus*], and house mice [*Mus musculus*]). This interaction has likely generated a complex network of disease transmission, including hantavirus, toxoplasmosis, and rabies (42, 93). Additionally, since the home range of minks can exceed 5 km (96, 97), the same individuals may move between riverine and coastal areas, co-occurring with either species of native otters through the attractant effect of their latrines. Under

this premise, biological control of mink in south-central Chile is recommended. For example, to intensify current capture and euthanasia efforts (62, 63), especially toward the northern limits of their current distribution to reduce their geographic dispersal rate. Also is advisable to incorporate serological and genetic analyses into these programs, in order to generate a geo-referenced database that enables the identification of spatio-temporal patterns in disease transmission (98, 99). Furthermore, regulatory measures concerning the implementation of vaccination schedules and territorial restrictions for rural domestic dogs, alongside controls on the access of minks to farms, could substantially reduce dog–mink co-occurrence. This, in turn, would lessen their pathological impact as both a transmission bridge and a key genetic reservoir for canine parvovirus, canine distemper, and other diseases linked to the coexistence of native and invasive species in south-central Chile.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal studies were approved by Bioethics Committee of the Universidad Andrés Bello (Mustelids; Chile), Universidad Santo Tomás (dogs; Chile) and the Agencia Nacional de Investigación y Desarrollo de Chile (ANID), Fondecyt 1,171,417—Bioethics Approval No 007/2017. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

AS: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing, Data curation, Formal analysis, Project administration, Software, Validation. CC: Conceptualization, Supervision, Writing – original draft, Writing – review & editing. EB: Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. SH: Formal analysis, Methodology, Software, Writing – original draft, Writing – review & editing. MB: Data curation, Methodology, Writing – original draft. CC-M: Data curation, Methodology, Writing – original draft. GM-V: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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