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RESEARCH

Human diphyllobothriasis in Argentina: assessing the epidemiological significance from historical records and reports of new cases

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Abstract

Between 1898 and 1940, eight human cases of diphyllobothriasis were reported in Argentina, always in recently arrived European immigrants. In 1982, the first autochthonous case was detected, and since then, 33 other autochthonous cases have been reported, totaling 42 cases of human diphyllobothriasis in Argentina before the present study. Our aim is to update the information on diphyllobothriasis in Argentina by identifying specimens from new cases using morphometrical and/or molecular methods. We also aim to assess the epidemiological relevance of this food-borne disease in the country. Anamnestic data were obtained from patients or professionals, along with 26 worms identified using morphometrical (21 samples) and molecular techniques (5 samples). All the patients acquired the infection by consuming freshwater salmonids caught in Andean lakes in Northern Patagonia. Morphometrics and DNA markers of worms were compatible with *Dibothriocephalus latus*. In total, 68 human cases have been detected in Argentina, 60 of which were autochthonous. The human population living North-western Patagonia, whose lakes are inhabited by salmonids, is increasing. Similarly, the number of other definitive hosts for *Dibothriocephalus dendriticus* (gulls) and for *D. latus* (dogs) is also increasing. In addition, salmonid fishing and the habit of consuming home-prepared raw fish dishes are becoming widespread. Therefore, it is to be expected that diphyllobothriasis in Argentina will increase further.

Keywords *Dibothriocephalus latus* · *Dibothriocephalus dendriticus* · Food-borne diseases · Zoonosis · Broad tapeworms · Biological invasions · Salmonids

Introduction

Adults of the genus *Dibothriocephalus* Lühe 1899, *Diphyllobothrium* Cobbold 1858 and *Adenocephalus* Nybelin 1931 are etiological agents of a non-lethal, fish-borne disease called diphyllobothriasis (Scholz et al. 2019). The spread of human cases to regions where the disease had not previously been recorded or where infected fishes had not been detected has been facilitated by the expansion of salmonid farming; fish originating from this aquaculture was shown to be infected with plerocercoids of *Dibothriocephalus latus* (Linnaeus 1758) Lühe 1899 in Chile (Torres et al. 2010). Another factor contributing to new infections is the worldwide popularization of raw fish meals used in Oriental dishes like sushi, nigiri and sashimi (Semenas 2013; Robertson et al. 2014; Kitaoka et al. 2020).

The Codex Committee on Food Hygiene (CCFH) requested that FAO and WHO review the current status of food-borne parasites to provide advice and guidance on those of global concern. Ninety-five species were analysed using seven criteria: the number of food-borne illnesses worldwide, the global distribution of food-borne infections, the severity of morbidity, the case fatality rate, the potential for an increase in disease rates and the trade and socio-economic importance of the parasite/food pathway. Finally, only

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24 parasite species were ranked in importance (Robertson et al. 2013), with diphyllobothriasis located in 23rd place. This indicates worldwide concern about the occurrence of this fish-borne disease.

The life cycle of *Dibothriocephalus* spp. involves planktonic copepods, which harbour the procercoid larva, and fishes of at least six orders where plerocercoids develop (Torres and Yera 2018). The natural definitive hosts are wild mammals and birds, but the low host specificity of these species means that humans can act as accidental definitive hosts (Waeschenbach et al. 2017). Also, paratenic transmission can occur when small prey fish are infected, which has been proven in Norway (Halvorsen and Wissler 1973) and suggested in Patagonia for the prey fish *Galaxias maculatus* (Semenas et al. 2021). Despite the complexity of their life cycles, *Dibothriocephalus dendriticus* (Nitzsch 1824) Lühe, 1899 (formerly *Diphyllobothrium dendriticum*), a circumboreal species, and *Dibothriocephalus latus* (formerly *Diphyllobothrium latum*), a Eurasian species, have successfully invaded and become established in Patagonia in birds (*D. dendriticus*, Casalins et al. 2015), dogs (*D. latus*, Roth et al. 2018) and humans (*D. latus*, Torres and Yera 2018, this study). It is assumed that diphyllobothriasis was introduced into Argentina and Chile during the final decades of the nineteenth century, probably by European immigrants (Scholz et al. 2019; Yamasaki et al. 2023). Although both species can parasitise humans, *D. latus* is the most frequent causative agent of human diphyllobothriasis (Torres and Yera 2018; Semenas et al. 2021). The fishes reported in Argentina as hosts for *Dibothriocephalus* spp. plerocercoids are the introduced salmonids *Oncorhynchus mykiss*, *Salmo trutta*, *Salvelinus fontinalis* and *Salmo salar*, as well as native species *Percichthys trucha*, *Galaxias maculatus*, *Galaxias platei* and *Olivaichthys viedmensis* (Semenas et al. 2021).

In South America, the first report of diphyllobothriasis was made in Argentina from a worm eliminated by a European immigrant (Cantón 1898). In Argentina, previous to the report of Cantón (1898), cases had been reported in three Ph.D dissertations from the Medical School of the Universidad de Buenos Aires; these reported *D. latus* eggs expelled in the faeces of children (referred as *Bothriocephalus latus*) (Eguren 1837; Urquiza 1884; Lynch 1896). The eggs shown in photographs in these reports did not seem to have the shape or size of *D. latus* eggs, and no further information about the patients was included. Other cases were later registered in Chile (Neghme et al. 1950), Venezuela (Vogelsang and Potenza 1953), Brazil (Coutinho 1957; Oliveira et al. 2017) and Uruguay (Sanches et al. 2016). These records should be considered with caution because the taxonomic identification of the etiological agent was based only on morphological analysis, and this methodology may not always be conclusive. The first molecular identification of human cases of *D. latus* in South America was made by Mercado et al. (2010) in Chile.

Szidat and Soria (1957) reported the occurrence of plerocercoids of *D. latus* and *D. dendriticus* in salmonids from Nahuel Huapi Lake, in Northern Patagonia. Since then, no case of fish infected by plerocercoids has been reported outside the area of the Andean lakes of Northern Patagonia, so this region is established as the endemic area where people acquire the infection in Argentina (Semenas and Úbeda 1997; Abuin et al. 2012; Cargnelutti and Salomón 2012; Semenas et al. 2021). Salmonids (*Salvelinus fontinalis*, *Salmo salar* and *Oncorhynchus mykiss*) were introduced into Andean Patagonian lakes in 1904 as embryonated eggs, so they arrived without parasites (Tulián 1910). Currently, these self-sustaining salmonid populations contribute significantly to the Patagonia's world-class sport fisheries.

Because a declaration of this disease is not mandatory in Argentina, we believe cases in the literature are underestimated. Therefore, we aim to update the information on human diphyllobothriasis for this country. To this end, we explored previous citations of human cases in occasional reports and "grey" literature. We searched for new records of human cases in laboratories and hospitals, assessed epidemiological importance, and provide insights for the implementation of sanitary measures. We studied the specific identity by morphometry of proglottids and eggs and used molecular techniques for the ethanol-preserved specimens.

Materials and methods

Collection of information and specimens

To update the historical information on human diphyllobothriasis in Argentina, we reviewed scientific journals, books, Ph.D dissertations and scientific meeting communications. New data and worms were obtained from patients or professionals who brought or sent the eliminated worms to our laboratory from private and public health institutions of the Argentinean provinces of Buenos Aires, Mendoza, Neuquén and Río Negro. The cases of infection (previously reported and new) were divided into three groups: acquired abroad (Table 1), autochthonous and published in journals or informed in scientific meetings (Table 2) and new autochthonous unpublished cases (Table 3).

Morphological analyses

Of the worms obtained, 21 preserved in 5% formalin were used for morphological analyses. Following Torres and Yera (2018) and Leštinová et al. (2016), two mature proglottids

Table 1 Human cases of diphyllobothriasis recorded in Argentina but acquired abroad, published in books and journals

Author	Reported as	Number of cases	Sex	Age	Infection date	Treatment	Patient nationality	Symptoms
Cantón (1898)	*Bothriocephalus latus*	1	Female	NI	1892	NI	Russian	NI
Greenway (1936)	*Diphyllobothrium latum*	3	NI	NI	1911	NI	NI	NI
Greenway (1936)	*Diphyllobothrium latum*	1	NI	NI	1932	NI	Polish	NI
Malamud et al. (1940)	*Bothriocephalus latus*	1	Female	32	1937	Ethereal extract of fern	Polish	Abdominal pain
Malamud et al. (1940)	*Bothriocephalus latus*	1	Female	40	1937	NI	Polish	Asymptomatic
Malamud et al. (1940)	*Bothriocephalus latus*	1	NI	NI	1937	Antihemintic	NI	Abdominal pain

NI not informed

and 10 eggs from each proglottid were measured (length and width in micrometres; the range is followed by the mean in parentheses) from each of these worms. The presence of operculum and knob on the eggs was also observed. The remaining proglottids of the specimens were immersed in lactophenol to clear them out, thus facilitating the observation of features of taxonomic importance.

Molecular analyses

Five specimens were fixed in 96% ethanol and processed through molecular procedures. DNA was extracted from an approximately 3 mm segment of each adult using a 5% Chelex solution protocol (Walsh et al. 1991) with some modifications (Semenas et al. 2021). One microliter of supernatant was used to amplify a region of the cytochrome *c* oxidase subunit 1 gene (COI) using a polymerase chain reaction (PCR) multiplex approach (with a concentration 0.3 µM for each primer) following Wicht et al. (2010). Briefly, we used MulRevCom (Common Reverse: ATGATA AGGGAYAGGRGCYCA), MulLat3 (*D. latum* Forward: GGGGTGTTACGGGTATTATACTC) and MulDen4 (*D. dendriticum* Forward: GTGTTTTTCATTTGATGATGA CCAGTC) with the following conditions for the thermal cycle: 94 °C for 15 min; 25 cycles of 94 °C for 30 s, 60 °C for 1 min 30 s, 72 °C for 1 min 30 s; and final extension at 72 °C for 10 min.

The initial identification of *Dibothriocephalus* species relied on analysing the size of the amplicon fragments. A multiplex PCR technique was employed, utilizing species' specific forward primers and a shared reverse primer. This method produced distinct amplicon sizes: 437 bp for *D. latus* and 318 bp for *D. dendriticus* (Wicht et al. 2010). DNA samples previously isolated from specimens, which had been confirmed by Sanger sequencing for both *D. dendriticus* and *D. latus,* were subjected to PCR amplification and used as positive controls. The resulting amplicons were sent to Macrogen in Seoul, Korea, for Sanger sequencing. Newly obtained sequences in this study and related sequences of *Dibothriocephalus* retrieved from the GenBank, i.e. *D. latus* ($n=50$), *D. nihonkaiensis* ($n=36$), *D. dendriticus* ($n=12$) and *D. ursi* ($n=2$) (see Supplementary Material), were curated (i.e. primer sequences, initial and final low-quality peaks were trimmed) and aligned using CLUSTAL W (Thompson et al. (1994), resulting in an alignment of 343 bp. A phenogram showing the distances for the partial COI sequenced region was constructed using Maximum Likelihood with standard settings but using 1000 bootstrap analysis (MegaX, version 10.1.8: Kimura 1980; Tamura and Nei 1993, Kumar et al. 2018; Stecher et al. 2020). All new sequences were submitted to GenBank.

Results

The morphological characteristics of the worms from the new autochthonous cases were as follows: the body consists of several hundred proglottids that are wider than they are elongated; there is the presence of constriction between proglottids; each segment contains a single set of reproductive organs; the testes and vitelline follicles are numerous; the genital (cirro-vaginal) pore is located in the first half of the ventral surface, anterior to the uterine pore; the uterus exhibits a posterior portion that is closely coiled, while the anterior one forms four to seven (most frequently five) loops at the midline; and there is a posterior bilobed ovary. Eggs operculated with a knob on the opposite side, 50–82 µm (70.6 µm; $n=420$) in length and 46–55 µm (49,8 µm; $n=420$) in width. These characteristics enabled us to assign all the analysed specimens to *D. latus*.

Table 2 Previous autochthonous human cases of diphyllobothriasis recorded in Argentina and published in journals or informed in scientific meetings

Authors	Sex	Age	Year of Infection	Treatment	Symptoms	Source of Infection	Dish preparation	Reported as
Journals								
Semenas and Úbeda (1997)	Female[a]	40	NI	Niclosamide	NI	NI	NI	*Diphyllobothrium* sp.
	Male	33	1983	NI	Asymptomatic	Patagonian trouts	Roasted, smoked	*Diphyllobothrium* sp.
	Male	32	1985	Niclosamide	Asymptomatic	Patagonian trouts	Roasted, smoked	*Diphyllobothrium* sp.
	Female	12	1986	Praziquantel	NI	NI	NI	*Diphyllobothrium* sp.
	Male	50	1989	Praziquantel	NI	NI	NI	*Diphyllobothrium* sp.
	Male	53	1991	NI	Abdominal pain	Patagonian trouts	Roasted, smoked	*Diphyllobothrium* sp.
	Male	40	1991	NI	NI	Patagonian trouts	NI	*Diphyllobothrium* sp.
	Male[b]	32	1992	Praziquantel	Asymptomatic	Patagonian trouts	Roasted	*Diphyllobothrium* sp.
	Female	27	1993	Niclosamide	Abdominal pain	NI	Roasted	*Diphyllobothrium* sp.
	Male	36	1995	Niclosamide	NI	Patagonian trouts	Roasted	*Diphyllobothrium* sp.
Semenas et al. (2001)	Male	45	1994	Niclosamide	Weight and appetite loss	Patagonian trouts	NI	*Diphyllobothrium latum*
	Female	8	1997	NI	Asymptomatic	Patagonian trouts	NI	*Diphyllobothrium latum*
	Male	41	1997	Praziquantel	Asymptomatic	Patagonian trouts	NI	*Diphyllobothrium latum*
	Male[b]	39	1997	Praziquantel	Asymptomatic	Patagonian trouts	NI	*Diphyllobothrium latum*
Abuin et al. (2012)	Male	21	2002–2006	Praziquantel	Asthenia, reflux	Patagonian trouts	NI	*Diphyllobothrium* sp.
	Male	46	2002–2006	Praziquantel	Asthenia, diarrhoea	Patagonian trouts	NI	*Diphyllobothrium* sp.
	Female	44	2002–2006	Praziquantel	Asymptomatic	Patagonian trouts	NI	*Diphyllobothrium* sp.
	Male	10	2002–2006	Praziquantel	Asymptomatic	Patagonian trouts	NI	*Diphyllobothrium* sp.
	Male	44	2002–2006	Praziquantel	Asthenia, diarrhoea	Buenos Aires restaurant	Sushi	*Diphyllobothrium* sp.
	Male	44	2002–2006	Praziquantel	Meteorism, diarrhoea	Patagonian trouts	NI	*Diphyllobothrium* sp.
Menghi et al. (2006)	Female	42	2006	Praziquantel	NI	Buenos Aires restaurant	Sushi	*Diphyllobothrium* sp.
Cargnelutti and Salomón (2012)	Male	20	2010	Mebendazole, Praziquantel	Meteorism, diarrhoea, colic	Patagonian trouts	NI	*Diphyllobothrium latum*
Scientific meetings								
Garaguso (1983)	Male	30	1982	Chlorosalicylamide	Digestive disorders	Patagonian trouts	Roasted	*Diphyllobothrium latum*

Table 2 (continued)

Authors	Sex	Age	Year of Infection	Treatment	Symptoms	Source of Infection	Dish preparation	Reported as
Garaguso (1991)	Male	26	1985	Praziquantel	NI	Patagonian trouts	NI	*Diphyllobothrium latum*
	Male	9	1986	Praziquantel	NI	Patagonian trouts	NI	*Diphyllobothrium latum*
Garaguso (2000)	NI	5 Adults	1987–2000	NI	NI	Patagonian trouts	NI	*Diphyllobothrium latum*
9 people	NI	4 Children	1987–2000	NI	NI	Patagonian trouts	NI	*Diphyllobothrium latum*

NI not informed

[a]Misidentified as *Fasciola hepatica*

[b]Same person

All the adult samples of alcohol-preserved *Dibothriocephalus* obtained from humans were molecularly analysed and showed visible bands matching the expected size for *D. latus* of 437 bp. Moreover, Sanger sequencing from the five individuals and two controls matched 100% of the published sequences for the region to *D. latus* (Accession nos. OQ436022 to OQ436028) with no variability between conspecifics. Levels of intra- and interspecific variation of the remaining species, measured as genetic distances, are shown in Table 4: within-species variability was 3% or less, and between-species variability was more than 7%.

Following the report from Cantón (1898), another seven cases were reported in European immigrants between 1911 and 1937 (Greenway 1936; Malamud et al. 1940) (Table 1), but it was not until 1982 that the first autochthonous case was detected by Garaguso (1983) (Table 2). The author pointed out that the patient had not made any trip abroad, but frequently fished in Andean Patagonian lakes, where fishes infected with *Dibothriocephalus* spp. (cited as *Diphyllobothrium* spp.) plerocercoids had been reported 30 years before (Szidat and Soria 1957). Following this, 33 autochthonous cases were published; in most of these cases, the source of infection was the ingestion of salmonids from Andean Patagonian lakes (Garaguso 1991, 2000; Semenas and Úbeda 1997; Semenas et al. 2001; Abuin et al. 2012; Cargnelutti and Salomón 2012), and a few cases were due to eating sushi in Buenos Aires (Menghi et al. 2006; Abuin et al. 2012). In all these cases, broad tapeworms were identified by morphological, anatomical and/or histological characters and were referred as *D. latum* or *Diphyllobothrium* sp. (Table 2).

We added 26 new human cases of infection by worms, whose morphology ($n=21$) and the nucleotide sequence of COI ($n=5$) were compatible with *D. latus* (Table 3); the longest specimen recovered was 14 m in length. The primary cause of infection was the ingestion of salmonids from Andean Patagonian freshwater environments. The fish preparation methods of these cases were reported as follows: 1 fried, 3 smoked and 8 sushi. These infections were produced by only one worm each, and the age of the people varied. Evaluating published and new cases, it can be seen that most of the cases occur in men (men 54%, women 26%, not informed 20%; see Tables 1, 2 and 3). Generally, the parasite was eliminated spontaneously; only a few patients reported mild symptoms. In two families (the cases in 2008 and 2023), two members were infected. A variety of anthelmintic drugs were administered to the patients, but praziquantel was the most common (Table 3). Haematological (to check for anaemia) and post-drug coprological analysis (testing for persistence of infection) were infrequent. Fourteen of the 26 new cases were detected in patients living in the region of Andean Patagonian lakes (11 from Rio Negro, three from Neuquén), eight live in Buenos Aires Province, and four in Mendoza. In total, 60 autochthonous cases of human diphyllobothriasis have been registered in Argentina since 1982.

Discussion

Up to now, 68 cases of human diphyllobothriasis have been reported in Argentina, of which 60 were autochthonous (34 recorded before this update and 26 new cases reported in the present work). Thousands of tourists annually visit North Andean Patagonian lakes for sport fishing, the sole region in Argentina where *Dibothriocephalus* spp. plerocercoids have been detected in salmonid fish. It is not mandatory for health authorities to report this zoonosis, suggesting that diphyllobothriasis may be underdiagnosed in the country. This highlights the need for increased awareness among tourists and local health authorities to mitigate the risk of diphyllobothriasis transmission.

The etiological agent of all the new cases of human diphyllobothriasis was identified as *D. latus* by proglottid

Table 3 New human cases of diphyllobothriasis acquired in Argentina

Locality of detection	Sex	Age	Year	Treatment	Symptoms	Source of infection	Dish preparation
Bariloche, Río Negro	Female	4	NI	NI	NI	Andean Patagonian trouts	NI
Bariloche, Río Negro	Male	30	1982	Chlorosalicylamide	Digestive disorders	Andean Patagonian trouts	NI
San Martín de los Andes, Neuquén	Female	32	1995	Niclosamide	Nervousness	Andean Patagonian trouts	NI
San Martín de los Andes, Neuquén	Male	38	1997	Praziquantel	NI	Andean Patagonian trouts	NI
Bariloche, Río Negro	Male	46	2005	Praziquantel	NI	Andean Patagonian trouts	Smoked, roasted
Bariloche, Río Negro	Male	17	2007	Praziquantel	Cyclical diarrhoea	Andean Patagonian trouts	NI
CABA*, Buenos Aires	Male**	5	2008	Paromomycin sulfate	NI	Andean Patagonian trouts	Sushi
CABA*, Buenos Aires	Female**	NI	2008	Paromomycin sulfate	NI	Andean Patagonian trouts	Sushi
San Martín de los Andes, Neuquén	Male	40	2009	NI	NI	Andean Patagonian trouts	NI
CABA*, Buenos Aires	Male	55	2010	NI	NI	Andean Patagonian trouts	NI
CABA*, Buenos Aires	Male	40	2011	NI	NI	Andean Patagonian trouts	NI
Mar del Plata, Buenos Aires⌘	Male	52	2012	NI	NI	Andean Patagonian trouts	NI
Mar del Plata, Buenos Aires	Male	55	2012	NI	NI	Andean Patagonian trouts	NI
Bariloche, Río Negro	Female	66	2013	Mebendazole, praziquantel	Weight loss	Andean Patagonian trouts	NI
Bariloche, Río Negro	Female	42	2015	Mebendazole, Praziquantel	Abdominal pain, swelling	Andean Patagonian trouts	NI
Bariloche, Río Negro	Male	50	2017	Praziquantel	Diarrhoea, abdominal pain	Andean Patagonian trouts	smoked
Mendoza, Mendoza	Male	70	2019	Praziquantel	NI	Andean Patagonian trouts	NI
Olavarría, Buenos Aires	Female	51	2020	Nixoran	Sporadic diarrhoea	Andean Patagonian trouts	Sushi, ceviche
Bariloche, Río Negro⌘	Male	36	2020	Praziquantel	Sporadic diarrhoea	Andean Patagonian trouts	NI
Mendoza, Mendoza⌘	Male	40	2021	Praziquantel	Asymptomatic	Andean Patagonian trouts	Sushi
Mendoza, Mendoza	Male	20	2022	Praziquantel	Asymptomatic	Andean Patagonian trouts	Sushi
CABA*, Buenos Aires⌘	Male	53	2021	Praziquantel	Abdominal pain, swelling	Andean Patagonian trouts	smoked
Bariloche, Río Negro⌘	Female	67	2022	Nixoran	Digestive disorders	Andean Patagonian trouts	fried
Bariloche, Río Negro	Female**	47	2023	Praziquantel	Asymptomatic	Andean Patagonian trouts	Sushi
Bariloche, Río Negro	Male**	45	2023	Praziquantel	Abdominal swelling	Andean Patagonian trouts	Sushi
Mendoza, Mendoza	Male	47	2023	NI	Abdominal swelling	Andean Patagonian trouts	Sushi

NI not informed

*Ciudad Autónoma de Buenos Aires

**Members of the same family

⌘*Dibothriocephalus latus* confirmed by sequencing

Table 4 Number of base substitutions per site, averaging over all sequence pairs within each group. Upper part (grey) "within species distances". Lower part (white) "between species genetic distances". Retrieved sequences to perform these analyses from NCBI are shown in the Supplemental Material

	D. latus (this work)	*D. latus*	*D. dendriticus*
D. latus (this work)	**0.0000**		
D. latus (NCBI)	0.0000	**0.0000**	
D. dendriticus (NCBI)	0.0792	0.0792	**0.0190**
D. ursi (NCBI)	0.0641	0.0641	0.0513
D. nihonkaiensis (NCBI)	0.0791	0.0791	0.0755

morphology, egg measurements, and molecular analyses. The primary source of human infection in Argentina is salmonids from sport fishing in Patagonia, cooked in various ways, but mainly as home-prepared raw or undercooked fish dishes.

There was a notable lack of intraspecific genetic variability in *D. latus* from the 50 sequences retrieved from GenBank and the 7 new sequences added in this work (2 control and 5 human samples). Recent haplotype analysis of *D. latus* revealed that H1 in COI is the main haplotype common to *D. latus* worldwide, including Chile (Yamasaki et al. 2023). These authors used the sequences published by Kuchta et al. (2019) to infer that *D. latus* specimens from Argentina have a European origin. However, this suggestion needs to be studied further because the COI region used by Kuchta and his team in the Argentinian samples is shorter than the one used by Yamasaki and lacks the variable sites necessary to compare and draw conclusions about their origin (Kuchta et al. 2019, Yamasaki et al. 2023). Therefore, we can conclude that the genetic distances, with an average of 7% substitutions per site, with respect to the remaining species (*D. dendriticus, D. ursi* and *D. nihonkaiensis*) support the use of the COI region to identify individuals at the species level; however, this fragment is not suitable to obtain the haplotypes used in Yamasaki et al. (2023) to establish the origins.

Kuchta and colleagues (2014) highlight a potential risk of confusion between *D. latus* and *Adenocephalus pacificus* (formerly known as *Diphyllobothrium pacificum*). If we had confused *A. pacificus* with *D. latus* and performed the multiplex PCR, we would have noticed the disparity in amplicon sizes (727 bp for *A. pacificus* and 437 bp for *D. latus*). Therefore, the amplicon's size is adequate for distinguishing the two species directly on the gel. On the other hand, having sequenced the PCR fragment definitively rules out the possibility of misidentification. The genetic distance between any of the *Dibothriocephalus* genus species was examined in our study, and *A. pacificus* exceeds 15% (data not shown).

The new cases of human diphyllobothriasis are epidemiologically similar to the cases previously published in Argentina (Semenas and Úbeda 1997; Semenas et al. 2001; Menghi et al. 2006; Abuin et al. 2012; Cargnelutti and Salomón 2012), except that the frequency of eating sushi-style fish is higher for the new cases (Table 3). For two previous cases detected in Buenos Aires caused by eating sushi in restaurants, it cannot be ruled out that the sushi eaten was prepared using farmed salmonids from Patagonia (Menghi et al. 2006; Abuin et al. 2012; Semenas 2013; data in this paper). The fact that the majority of cases are men would be explained because sport fishing is a predominantly male sport in Argentina. From the scant information available on post-drug haematological and coprological analyses, we cannot determine the incidence of anaemia or the success of anthelmintic treatments.

Oncorhynchus mykiss (rainbow trout) is the most captured of the sport-fishing salmonid species in North Patagonia and has the highest prevalence and mean intensity of plerocercoids of *D. latus* and *D. dendriticus* (Semenas et al. 2021). Sport fishing is an increasingly popular activity: An average of approximately 18,000 fishing licenses is sold per Patagonian province during each fishing season. This activity brings in nearly 50 million US dollars annually, representing a very high income for Patagonia. These lakes are visited by fishers not only from other countries but also from many provinces of Argentina, so the detection of human diphyllobothriasis in only two provinces outside Patagonia (Buenos Aires and Mendoza) is not likely to reflect the actual distribution of the disease in Argentina.

The existence and persistence of diphyllobothriasis in Patagonia are related to many factors, which are generally associated with the increasingly widespread custom of eating raw fish meat; the high reproductive potential of broad tapeworms; the high abundance of plerocercoids in introduced salmonids; the preference shown by sport fishers for salmonids, especially *O. mykiss* (Semenas et al. 2021); the discharge of untreated sewage into these lakes (Madariaga 2007; UGCA 2015); and the abundance of definitive hosts like dogs (Roth et al. 2018; Garibotti et al. 2021). Gull populations are also increasing (Frixione et al. 2012; Lyons et al. 2015), and they are definitive host for *D. dendriticus* (Casalins et al. 2015), which also can parasitize humans (Kuchta et al. 2013). The presence of fish hatcheries in floating cages located in reservoirs where the infection occurs in wild fishes is also a situation to be considered in the circulation of diphyllobothriasis. Therefore, it is likely that in the near future, diphyllobothriasis cases will increase in both intermediate and definitive hosts, including humans.

In addition, recommendations about safe fish consumption and adequate offal disposal have been included since 2009 in the Continental Patagonia General Sport Fishing Rules and Regulations (Comisión Consultiva de Pesca Continental Patagónica 2023). However, communication with fishers, local people, and restaurants should be intensified regarding the most effective practices for the safe consumption of fish caught in lakes near cities, which have a higher risk of parasite transmission (Semenas et al. 2021). Furthermore, if clinical laboratory technicians, doctors, and veterinarians were better informed about diphyllobothriasis, this would result in a more efficient diagnosis of this zoonosis and more effective sample preservation. The anterior half should be fixed in 5% formalin, including scolex, if available, and strobila for morphological identification, and the remainder of the specimen should be fixed in 96% ethanol for molecular analysis. Well preserved samples facilitate

taxonomic determination, not only through morphology but also molecular analysis; this is important considering that in Argentina, in addition to *D. latus*, another three zoonotic species of broad tapeworms have been identified by molecular procedures in different hosts: the marine *A. pacificus* (Hernández-Orts et al. 2015; Cantatore et al. 2023), the freshwater *D. dendriticus* (Casalins et al. 2015; Semenas et al. 2021), and a recently described species, the marine *Diphyllobothrium sprakeri* (Hernández-Orts et al. 2021), although the zoonotic potential of this last species remains unknown.

Estimates of the number of people infected worldwide vary between nine and 20 millions (Stoll 1947; von Bonsdorff 1977; WHO 1979; Stürchler 1982; Chai et al. 2005; Scholz et al. 2019). Perhaps one of the reasons for the large variation in this number is the lack of systematic reporting of this zoonosis, even in developed countries. In Argentina, some factors that contribute to this are mild or no symptoms, inadequate training in egg detection, and particularly, poor knowledge of this fish-borne zoonosis.

Our knowledge of the circulation of this zoonotic species benefits from this updated information on human cases of diphyllobothriasis in Argentina, along with previous knowledge of the distribution of *Dibothriocephalus* spp. and its occurrence in various hosts in urban and natural environments in Andean Patagonian lakes (Ortubay et al. 1994; Casalins et al. 2015; Rauque et al. 2018; Roth et al. 2018; Garibotti et al. 2021; Semenas et al. 2021). This information constitutes a valuable foundation for understanding the current epidemiological situation of diphyllobothriasis in Argentina and will enable us to improve ways of estimating not only its occurrence in humans, but also in other definitive and intermediate hosts around freshwater lakes and rivers in Argentina.

The sanitary measures applied worldwide are related to the detection of larvae during handling prior to cooking, the freezing of fish meat before it is eaten raw, and the treatment of wastewater in modern sewage plants. However, none of the known sanitary measures will be effective if applied in isolation, and therefore, a comprehensive approach is necessary if we are to counteract this increasingly common fish-borne zoonosis. In particular, in Argentina, the following main actions should be taken: (1) those who do sport fishing in Northwestern Patagonian lakes and rivers should be informed of the correct way to dispose of salmonid viscera, to prevent the spread of parasites when ingested by dogs or gulls, (2) physicians and clinical laboratory technicians should be given advice on storing samples in alcohol for molecular identification, (3) the public health agenda should specify the risks and give recommendations for safe fish consumption, (4) clinical laboratory technicians, physicians, veterinarians, and biologists should be provided with information about the presence of this zoonosis in the country. All these actions, taken together, will reduce the risks associated with this zoonotic disease.

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Data availability Data on which the conclusions are based are provided within the article and the supplementary material.

Declarations

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