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BRIEF REPORT

Diphyllobothrium sp. in *Canis familiaris* from the subtropical area of Argentina (Puerto Iguazú, Misiones)

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Abstract This paper reports the first finding of *Diphyllobothrium* sp. eggs in *Canis familiaris* (domestic dog) from Puerto Iguazú, a subtropical city of Misiones province, Argentina. In 2013, two positive cases of *Diphyllobothrium* sp. eggs were detected during an annual parasitological survey of dogs. Dog feces were collected in vials containing 10% formalin and processed using Telemann's sedimentation and Sheather's flotation techniques. The two cases were detected in rural areas of the municipality. Since Misiones is not a part of the endemic area of diphyllobothriasis and given the fact that it is located in the three-border area of Argentina, Brazil and Paraguay, we consider this finding of great importance to public health. We stress the need for updating the current knowledge about the life cycle of these parasites considering the range of intermediate and definitive hosts, their zoonotic potential, and the epidemiological situation in non-endemic areas.

PALABRAS CLAVE
Diphyllobothrium;
Perros;
Tenias;
Zoonosis;
Subtrópico argentino

Diphyllobothrium sp. en _Canis familiaris_ de la región del subtrópico argentino (Puerto Iguazú, Misiones)

Resumen En este trabajo se informa el hallazgo de huevos de *Diphyllobothrium* sp. en ejemplares de *Canis familiaris* (perro doméstico) de Puerto Iguazú, una ciudad subtropical de la provincia de Misiones, Argentina. Durante 2013, en el marco de un relevamiento de la fauna parasitológica de los perros de Puerto Iguazú, se detectaron dos casos positivos en la búsqueda de huevos de *Diphyllobothrium* sp. La materia fecal de los perros fue recolectada en frascos

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con formol al 10% y procesada mediante las técnicas de sedimentación de Telemann y de flotación de Sheather. Dado que Misiones no forma parte de la zona endémica de difilobotriasis y considerando, además, su ubicación fronteriza, este hallazgo reviste gran importancia para la salud pública. Se señala la necesidad de actualizar el estado de conocimiento sobre el ciclo de vida de estos parásitos identificando el rango de hospederos intermediarios y definitivos, su potencial zoonótico y la situación epidemiológica en áreas no endémicas.

Diphyllobothrium tapeworms belong to the order *Pseudophyillidea*, which is characterized by complex triheteroxenic life cycles (comprising three obligated hosts).[14] Diphyllobothriasis is considered a metacyclic zoonosis since the first intermediate hosts are copepods and the second intermediate hosts are fish. The definitive hosts are fish-eating mammals and fish-eating birds,[14] which become infected after eating fish, either raw or insufficiently cooked, infected with plerocercoid larvae. For this reason the disease caused by species of the genus *Diphyllobothrium* is considered an ictiozoonosis. It is currently estimated that around 20 million people are infected worldwide.[9]

The adult parasite can eliminate up to one million eggs per day and it can reach variable lengths ranging from 4 to 25 m. Diphyllobothriasis is common in places with cold water such as the Palearctic region and some areas of North America. In South America, the infection is common in Chile and Perú, whereas in Argentina the disease is mainly restricted to the Andean Patagonia.[7] Out of the 80 species of *Diphyllobothrium* described,[6] only three have been detected in South America: *Diphyllobothrium latum*, *Diphyllobothrium dendriticum* and *Diphyllobothrium pacificum*. In Argentina, *D. latum* is identified as the etiologic agent causing diphyllobothriasis, all cases being autochthonous.[10] However, diphyllobothriasis in humans has been reported in non-endemic areas of Argentina. In Mendoza province, in the west part of the country, a case of human diphyllobothriasis was reported in 2012.[3] The origin of this case was attributed to the ingestion of raw salmon (*Oncorhynchus kisutch*) from northern Patagonia. In Buenos Aires, in the eastern part of the country, a case of diphyllobothriasis was attributed to the ingestion of contaminated sushi.[5]

The province of Misiones in Argentina is almost entirely a part of the Atlantic Forest Ecoregion.[2] It is characterized by a subtropical climate with no dry season and abundant rainfall throughout the year (between 1600 and 2000 mm/year). Misiones has high temperatures with low annual variation (above 20 °C mean annual temperature).[2] This ecosystem differs radically from the one commonly associated to the development of the *Diphyllobothrium* tapeworm which is typically found in cold and temperate climates.[11]

Studies on mammals other than humans as definitive hosts of *Diphyllobothrium* in Argentina are scarce. The presence of *Diphyllobothrium* sp. has been reported in wild animals such as the freshwater otter (*Lontra provocax*) in southern Argentina.[12] *Diphyllobothrium* sp. adults and eggs were also detected in wild animals in captivity in the province of Corrientes, in northeastern Argentina. In this latter case, *Diphyllobothrium* sp. was detected in crab-eating raccoons (*Procyon cancrivorus*), the pampas gray fox (*Lycalopex gymnocercus*), and the maned wolf (*Chrysocyon brachyurus*).[4]

Therefore, it has been suggested that fish-eating mammals play a crucial role in water pollution and as part of parasite life cycles in wild environments either as definitive or paratenic hosts.[4] These findings highlight the importance of studying key aspects of parasitic zoonoses in interface areas (urban/rural with natural areas), and the contribution of wild and domestic animals in each case.

The first findings of *Diphyllobothrium* sp. in a domestic dog (*Canis familiaris*) in Argentina date back to 1952 in the province of Buenos Aires.[1] More recently, in 2002, the presence of eggs was reported in dogs from the riparian area of Buenos Aires,[7] as well as in dogs from Corrientes city in that same year.[6] In 2011, other cases were reported in Recreo city, 17 km from the capital city of Santa Fe province.[8] In the year 2000, *Diphyllobothrium* sp. eggs in dog feces were detected in the town of Lago Puelo, Chubut province, in the south of Argentina,[15] and also in 2010, in the rural area of Neuquén.[13] The most recent report is from Rio Negro province, where positive dogs feces were found in Bariloche city, one of the main tourist centers of the country.[11]

The objective of this brief study is to report the detection of tapeworm eggs belonging to the order *Pseudophyillidea* in Puerto Iguazú, Misiones, a non-endemic area of Argentina.

From the winter of 2013 to the winter of 2014 a cross-sectional study was conducted in Iguazú municipality in Misiones province, in order to survey the parasitological fauna of *C. familiaris* and to evaluate environmental contamination with parasitic structures in public areas. This municipality is surrounded by two large rivers, the Paraná in the west and the Iguazú in the north, and it comprises the city of Puerto Iguazú (25° 35′ 52″ S – 54° 34′ 55″ W), periurban and rural areas, and a large area covered by native forests mostly belonging to parks and reserves. Additionally, three aboriginal Mbyá Guarani villages, named Fortin Mbororé, Jasy Porá, and Yriapú are located in the rural area. This was a probabilistic study in which canine feces distributed on public sidewalks, vacant sites, parks and public trails of the municipality were selected by using a systematic random sampling. Up to five canine feces were collected

in labeled vials containing 10% formalin. Feces too dry or too old were not considered in the analysis. The samples were analyzed by classical coprological techniques, such as Sheather's sugar flotation and modified Telemann's sedimentation. The preparations were stained with Lugol's solution and observed in duplicate. We analyzed a total of 405 stool samples from domestic dogs. Stool observations were performed using a Primo Star Zeiss optical microscope with dry 10× and 40× objectives. Photographs were obtained with an Axion Cam ERC 5s camera adapted to the microscope. A micrometer slide with 1/100 mm graduation was used in combination with the Image J software for measuring eggs.

Eggs with morphological characteristics of the genus *Diphyllobothrium* were observed in two different samples. These positive samples were temporarily separated (one collected in the winter of 2013 and the other one in the spring of the same year), and spatially distanced (6.4 km apart from each other). One sample (sample I) was collected in the southern border of the rural area (25° 40′ 4.203″ S – 54° 33′ 49.511″ W) and the other one (sample II) was collected from a public road near Yriapú village, located near the urban area of Puerto Iguazú (25° 36′ 52.292″ S – 54° 32′ 55.535″ W).

Fifty eggs belonging to each sample were subjected to morphometric analysis. Eggs were brown and ovoid, with a smooth thin wall, and possessed an operculum at the narrower end and a minute terminal knob at the opposite end. They were unembryonated eggs containing vitelline cells of vague limits and granular cytoplasm (Fig. 1). Damaged eggs, having an open or absent operculum, as well as eggs covered totally or partially with fecal matter were excluded from the measurement analysis. The average and standard deviation values of eggs measured from sample I were 66.10 ± 3.35-μm long and 44.74 ± 1.99-μm wide. Measurements for sample II were 65.73 ± 2.68-μm long by 44.31 ± 2-μm wide. No significant differences between both samples were found ($t = 0.66$, $df = 98$, $p = 0.51$ for length, $t = 0.55$, $df = 98$, $p = 0.60$ for width). Hookworm eggs were also detected in both samples.

Stool routine diagnostic techniques of this tapeworm do not allow determining the *Diphyllobothrium* species involved. In fact, there is a large overlap between egg size among species and it has been indicated that the intensity of the infection can also influence egg size as reviewed in Scholz *et al.*[9] *Diphyllobothrium* species are most easily distinguished in adult individuals by the shape and size of the scolex, neck, and male genital organs.[9] However, due to the sampling methodology applied in this study, it was not possible to identify the infected dogs that gave rise to the positive samples so as to obtain *Diphyllobothrium* adults for species identification.

This work is the first report of *Diphyllobothrium* sp. in Misiones Province, Argentina and the northernmost record for this genus in the country. Considering the previous cases of *Diphyllobothrium* sp. in *C. familiaris* from Argentina and the findings reported here, it is possible to observe a widespread range of environments and temperatures in which eggs of this parasite were detected (Fig. 2). Although data are scarce and isolated, a pattern of wide distribution is observed and the species identification would significantly increase the knowledge of climate and socio-environmental determinant conditions defining the distribution of these parasites. Combining the distribution of this parasite with geographic information systems and spatial analysis would help to advance the understanding of these determinants. The importance of these findings for regional public health requires research on feeding practices and food handling for risk estimation, in addition to population parasitological studies.

The role of the dog in the spread of *Diphyllobothrium* sp. mainly through water contamination with feces should not be underestimated. Additionally, it has to be considered that fishermen often feed dogs with the viscera of infected

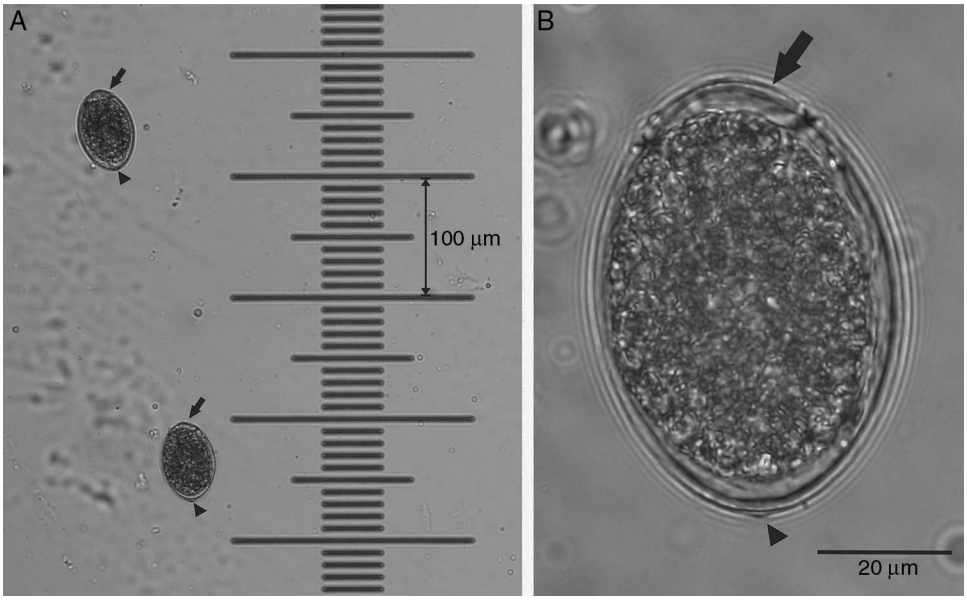

Figure 1 Eggs of *Diphyllobothrium* sp. in feces of *Canis familiaris*. (A) Eggs of *Diphyllobothrium* sp. at 100×. On the right, a micrometer ruler 1/100 mm. (B) Eggs of *Diphyllobothrium* sp. at 400×. Reference bar, 20 μm. In both images the black arrow indicates the operculum and the arrowhead the small knob at the opposite end.

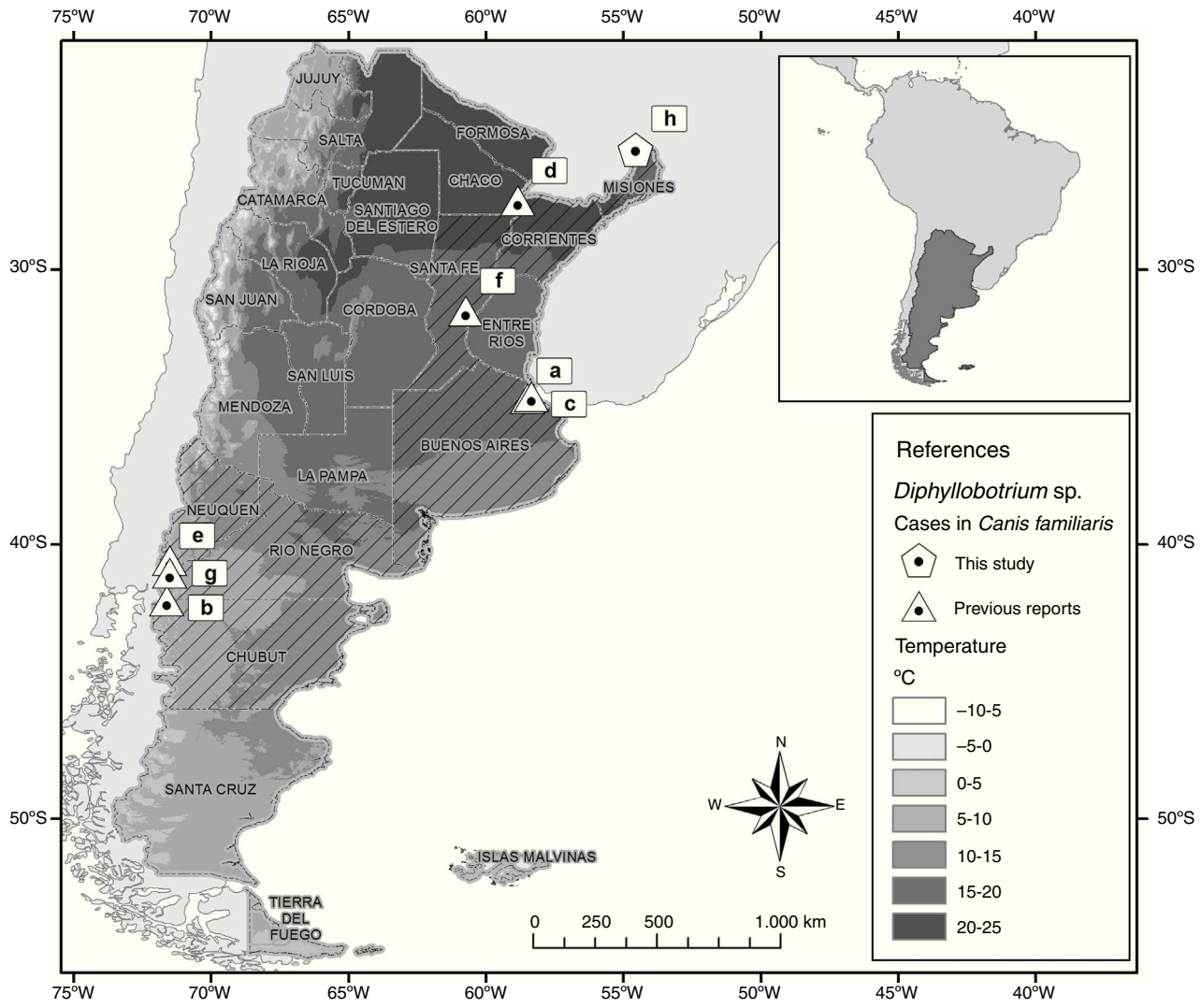

Figure 2 Map of temperatures of Argentina. The gray scale shows the average annual temperature ranges throughout the country. Triangles indicate the points where *Diphyllobothrium* sp. was reported in *Canis familiaris* (listed chronologically): (a) Villa Celina, Buenos Aires; (b) Lago Puelo, Chubut; (c) Riparian zones, Buenos Aires; (d) Corrientes city, Corrientes; (e) Traful lake, Neuquén; (f) Recreo city, Santa Fe; (g) Bariloche city, Río Negro. The pentagon symbol indicates the findings reported here, Puerto Iguazú, Misiones (h). Parallel lines indicate the Argentine provinces where positive dogs to this tapeworm were diagnosed. The inset in the upper right corner shows the locations of Argentina (dark gray) in South America (light gray).

fish.[11] Likewise, from an epidemiological perspective, relevant information on the fish consumed by the inhabitants of this subtropical region may lead to the detection of the most important sources of plerocercoid larvae and the parasite ability to complete the cycle in non-endemic areas. Furthermore, it is important to investigate potential native fish of Paraná and Iguazú rivers that could act as intermediate hosts in this region, which will allow shedding light on the tropicalization of the *Diphyllobothrium* sp. life cycle. Therefore, this research may help to explain the possible spread to other subtropical provinces and eco-regions crossed by these rivers. Regulation of these rivers by dams may also influence at the level of first and second intermediary hosts, and it should also be considered in future analyses.

Another aspect of great epidemiological value is that the Iguazú waterfalls are considered the main tourist attraction of Puerto Iguazú in the province of Misiones, and more than 1.5 million tourists from all over the world visit this region annually. This has greatly increased the international gastronomy offer, boosting the consumption of smoked, raw, or undercooked products, and, consequently, the potential consumption of contaminated products. This issue undoubtedly demands further investigation.

Dogs transmit large amounts of zoonotic diseases of diverse origins. Their role in the transmission and maintenance of infectious diseases, especially in tropical and subtropical areas, further highlights the need for identifying the broad spectrum of zoonotic agents that involve the domestic dog. The finding described here reinforces the uncompromising commitment to educate on good practices regarding responsible pet ownership as an essential pillar in public health and infectious disease control.

Developing studies to understand the true incidence and contribution to morbidity of *Diphyllobothrium* zoonosis in this region will be required in order to consider in deworming prevention and control programs, since praziquantel must be incorporated to the group of drugs commonly used for its treatment.[8] *Diphyllobothrium* lacks a known endemic cycle in this region of Argentina. Therefore a comprehensive characterization of the cycle is urgently required to reveal the conditions needed for its development and to determine autochthonous or imported cases of parasitism in this subtropical area of the country.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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