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**ZOONOTIC PARASITES IN FELINE COPROLITES FROM A HOLOCENIC
MORTUARY CONTEXT FROM EASTERN PATAGONIA (ARGENTINA)**

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ABSTRACT

Nowadays, wildlife is one of the most important sources of zoonoses, and it is a major concern for the public health. Nevertheless, little is known about the role of wildlife as a reservoir and source of infectious diseases in the past. South America presents a wide diversity of wildlife. In the south of the continent, Argentina shelters a large diversity of neotropical carnivores. Although the paleoparasitological studies on carnivores have been increasing in southern Argentina, most of the efforts have been focused in a handful of sites located in western Patagonia. In this paper, two coprolites of felid found in Cueva Galpón, an initial late Holocene mortuary site from northeast Patagonia (Argentina), were studied for paleoparasitological purposes. Samples were processed by rehydration, homogenization, filtered and processed by spontaneous sedimentation. The samples were assigned to *Puma concolor* (puma) or *Panthera onca* (jaguar). Microscopic observations revealed that both coprolites were positive for parasite remains. High parasite richness was observed. Six nematodes, one cestode and one coccidian morphotypes were reported. This is the first time that *Gnathostoma* sp. and *Spirocerca* sp. were recovered from holocenic times from Patagonia. This finding implies that some diseases such as taeniasis, spirocercosis, gnathostomosis, ascariasis and coccidiosis could be present in holocenic wildlife from Patagonia prior to the Spanish colonization and domestic animal introduction. The overall results suggest that felids could have played a role as reservoirs and source of some parasitic species, some of which are zoonotic. Therefore, this animal could have entailed a risk agent for human health in the site.

**KEYWORDS: PALEOPARASITOLOGY – ZOONOSES – HOLOCENE -
CARNIVORES**

INTRODUCTION

Paleoparasitology is the study of parasites from ancient times, and some of its main interests are the origin and evolution of infectious diseases and parasites-hosts-environment relationships (Araújo et al., 2003; Reinhard et al., 2013). On this basis, paleoparasitology is able to provide knowledge of the major conditions of health and illness related to parasites in the past (Bouchet et al., 2003; Reinhard, 1992). Zoonoses are defined as infectious diseases that are naturally transmitted between vertebrate animal species and humans. The 60% of human emerging infectious diseases can also be catalogued as zoonotic (Jones et al., 2008). Nowadays, wildlife is one of the most important sources of zoonoses, and it is a major concern for the public health (Woods et al., 2019). Nevertheless, little is known about the role of the wildlife as reservoir and source of infectious diseases in the past (Sianto et al., 2009).

South America presents a wide diversity of wildlife. In the south of the continent, Argentina shelters a large diversity of neotropical carnivores (Bárquez et al., 2006; Teta et al., 2018). Although the paleoparasitological studies on carnivores have been increasing in southern Argentina (e.g. Beltrame et al., 2010, 2018; Fugassa et al., 2009, 2018), most of the efforts have been focused in a handful of sites located in western Patagonia. In this paper, the results of the first paleoparasitological study of the wild felid coprolites found in “Cueva Galpón”, a late Holocene mortuary context from northeast Patagonia, are presented. Based on the parasitic richness found, their implications in term of zoonotic risk are discussed.

MATERIALS AND METHODS

“Cueva Galpón” (CG) is a mortuary archaeological context dated to the initial late Holocene (ca. 3000 years BP), located in the northeast of Patagonia region, Argentina. The site is located inside a rock shelter of ca. 50 m wide at the entrance and 10 m high, located on the eastern edge of the Pailemán hills. This shelter is filled with a sandy–silty deposit (60–65 cm), sealed by an overlying layer of sheep dung (~0.40 cm thick). Taking into account the origin of the sediments, four main sources of detrital components were defined: aeolian, from rocks detached from the bedrock roof, biological (dung) and anthropogenic (bones, wood, grasses and seeds). Most of the archaeological record of CG consists of scattered human remains, funerary goods (e.g. textiles, ornaments, a leather bag) and rock art, with no evidences of residential occupation (Carden and Prates, 2015; Prates et al., 2016).

Two coprolites (named coprolite A and coprolite B) from CG were examined for paleoparasitological purposes. Coprolites examined come from 9 cm inside the sheep dung layer, level 2, and were dated at ca. 2900 years ¹⁴C BP (Fernández et al. 2016). That means coprolites are almost contemporary (just later) than the mortuary deposit. The analysis started with the external observation of feces (color, texture, inclusions and measures) according to Chame (2003) and Jouy-Avantin (2003). Samples of 0.5 g from the interior of coprolites were rehydrated in a 0.5 % water solution of tris-sodium phosphate (TSP). The remaining sample was whole processed by rehydration in TSP in a glass tube for at least 72 hs, followed by homogenization, filtered and processed by spontaneous sedimentation (Lutz, 1919). Samples were preserved in 70 % ethanol. At least 40 slides of each sample were made with the aid of a drop of sediment mixed with one drop of glycerin and examined at 100 X and 400 X using a light microscope Zeiss® Primo Star. Egg dimensions and morphology were compared with data from the literature in order to identify the parasites at the lowest taxonomic level. The macroscopic remains were separated and dried at room temperature and were examined for diet analysis.

RESULTS

Coprolites showed a whitish coloration, smooth surface and a hard and compact consistency (Fig. 1). The measurements of coprolite A were 28.25 mm width by 47.93 mm long and those of coprolite B were 28.65 mm width by 39.77 mm long. After rehydration, the supernatant exhibited a clear and yellowish coloration and presented an intense smell. Abundant micromammal hairs and fractured bones were observed in both coprolites, indicative of carnivorous diet.

Microscopic observations revealed that both coprolites were positive for parasite remains. Five nematodes and one coccidian species in coprolite A, and three nematodes and one cestode species in coprolite B were found. A total of six nematodes, one cestode and one coccidian species in both coprolites were observed.

Two oblong, colorless and larvated eggs with a thin and smooth wall were found in coprolite A. Measurements were 137.5 µm by 75 µm and 145 by 62.5 µm, and were compatible to strongylid eggs (Strongylida, Trichostrongyloidea) (Fig. 2a). Their morphology and measurements were similar to some species of *Nematodirus* sp.

Thirty-five elliptical and larvated nematode eggs, with thick walls and slightly corrugated surfaces were found in coprolites A and B. Their measurements were 37.5 to 47.5 μm (43.97 ± 4.59) in length and 27.5 to 37.5 μm (31.47 ± 3.96) in width (n=20). This nematode was assigned to the superfamily Spiruroidea (Order Spirurida) (Fig. 2b).

Nematode eggs with thick walls and slightly corrugated surfaces were observed in both coprolites. Their measurements were 65.0 to 72.5 μm (68.26 ± 3.22) in length and 32.5 to 37.5 μm (35.75 ± 2.89) in width (n=4). Eggs were attributed to an ascaridid species (Ascaridida, Ascarididae), tentatively *Lagochilascaris* sp. (Fig. 2c).

Two nematode eggs with single thick wall and with a rounded pole and the other sharp, without operculum and larvated were found in coprolite A. The measurements (n=1) were 137.5 μm by 62.5 μm . and were assigned to *Heteroxyinema (Cavioxyura) viscaciae* (Oxyuroidea, Heteroxyematidae) (Fig. 2d),

Two nematode eggs, elongated, with parallel sides, thick and smooth shell and larvated were found in coprolite A. The measurements of both eggs were 37.5 μm long by 17.5 μm wide (Fig. 2e). The eggs were attributed to *Spirocerca* sp. (Spirurida, Spirocercidae)

Taeniid eggs were found in coprolite B (Cyclophyllidea, Taeniidae). Eggs were spherical with yellow-brown and striated shell and with three pairs of hooks inside. The measurements were 32.7 to 37.5 μm (33.62 ± 3.11) in width and 37.5 to 42.5 μm (39.89 ± 3.37) in long (n=12) (Fig. 2f). Eggs were identified as *Taenia* sp. or *Echinococcus* sp.

Two eggs of nematodes, oval, unembryonated, with a thin and pitted shell and one polar bulge were also found in coprolite B (Fig. 2g). Measurements were 60 by 42.5 μm and 52.5 by 37.5 μm . Eggs were attributed to *Gnathostoma* sp. (Spirurida: Gnathostomatidae).

One oocyst (Coccidia, Apicomplexa) was found in coprolite A (Fig. 2h). Their measurements were 25 μm by 26 μm .

DISCUSSION

Coprolites determination

Both coprolites were identified as belonging to a large felid (Carnivora, Felidae) based on shape, coloration, size and content (Chame, 2003; Jouy-Avantin, 2003). Nowadays, the only species of large felid inhabiting the area is *Puma concolor* (puma, mountain lion or cougar). The distribution range extends from northern British Columbia (Canada) to southern Patagonia (Chile and Argentina). Nevertheless, the presence of another large felid, *Panthera onca* (yaguareté or jaguar), before 20th-century has been also proposed on the basis of ethnohistorical, archaeological and paleontological data (Cabrera and Yepes, 1960; Rusconi, 1967; De Angelis, 1972; Cardich, 1979; Redford and Eisenberg, 1992; Diaz, 2010). Some rock art motifs at the site suggest that humans could have represented a *P. onca*. In this regard, Carden and Prates (2015) described felid footprints and black empty circles with red dots inside, which closely resemble to the coat of that great felid. Similar motifs associated with a yaguareté-shaped figure have been recorded at El Ceibo site in southern Patagonia (Cardich, 1979). Although this archaeological information becomes relevant for the evaluation of human-felid interactions at the site, the morphological and parasitological results are not conclusive for determining at species level.

Paleoparasitological findings and zoonotic risk

Several parasites taxa were found at the site: six nematode species, one coccidian and one cestode. Our results show a high richness of gastrointestinal parasites. The variability in the parasitic contents of both samples, could suggest that they corresponded to separate events, despite exhibiting a common zoological origin.

Nematodirus spp., one of the most common parasitic nematodes in ruminants worldwide, occurs in the small intestine. Nematodes of this genus are important disease-causing parasites, on occasions causing severe pathology and even deaths. A characteristic of this genus is that development to the infective third larval stage (L3) occurs within the egg, allowing them to persist on pasture for long periods (Anderson, 2000). Their presence in carnivores is accidental by ingestion of an herbivore prey. *Nematodirus* spp. eggs were previously reported from Holocene samples from Patagonia, such as coprolites assigned to camelids (Taglioretti et al., 2015, 2017) and coprolites assigned to felines (Fugassa et al.,

2009). Although this genus is not considered zoonotic, their presence in the environment indicates the potential presence of the disease in the wildlife in the past.

Ascaridids are mainly parasites of terrestrial hosts and their transmission commonly involves terrestrial invertebrates and small mammal paratenic or intermediate hosts. However, the eggs of some species are directly infective to the definitive host (Anderson, 2000). Previous studies on carnivores from southern South America stated that ascarid species found were *Toxocara cati*, *Toxascaris leonina* and *Lagochilascaris major* (e.g. Beldoménico et al., 2005; González-Acuña et al., 2010; Martínez et al., 2010; Moleón et al., 2015; Scioscia et al., 2018; Vega et al., 2018). Species belonging to these three genera are zoonotic. The morphology of the ascaridid eggs found in this study was similar to those of *Lagochilascaris*. *Lagochilascaris minor* was found from *P. concolor* from Mexico (Falcón-Ordaz et al., 2016). The life cycle of *Lagochilascaris* is heteroxenous, involving natural definitive hosts (wild carnivores), accidental hosts (domestic carnivores and humans) and intermediate hosts. Human lagochilascariasis is a zoonotic disease with neotropical distribution. Lagochilascariosis is an emerging parasitic disease in the Americas caused by the nematode *Lagochilascaris* spp. and is distributed from Mexico to Argentina and the Caribbean Islands. Five species have been recognized in this genus. *Lagochilascaris minor* and *L. major* are the two most commonly reported species and both are biologically similar and produce similar injuries. *L. minor* is the etiological agent of human lagochilascariasis in South America and Mexico, where it is associated generally with purulent abscesses in the region of the ear, neck, jaw, orbit, mastoid process and retropharyngeal tissues. Humans can serve as a definitive host but the route of infection is unknown. The studies suggests that humans might acquire this strange nematode from eating uncooked or poorly cooked flesh of some rodents serving as intermediate hosts. There is, however, no explanation for the large number of worms at various stages of development reported in human infections (Anderson, 2000; Campos et al., 2017).

Heteroxynematidae includes nematodes that evolved in sciuriform, caviomorph and miomorph mammals. *Heteroxynema viscaciae* is a parasite found in the caecum and large intestine from *Lagidium viscacia* (mountain viscacia) (Hugot and Sutton, 1989) and wild viscachas *Lagostomus maximus* (Foster et al., 2002; Ferreira et al., 2007) from South America. *Heteroxynema viscaciae* was also found in ancient coprolites assigned to *L. viscacia* from Patagonia (Beltrame et al., 2014). Their presence in felines is accidental by ingestion of viscachas. *Lagidium viscacia* hair remains were found in the human mortuary

context of CG (Prates et al., 2016), indicating its presence and exploitation in the site. However, this species does not present any zoonotic risk.

Spirocerca sp. is found worldwide especially in tropical and subtropical regions. This nematode has been found in many species, but affects mostly carnivores, especially Canidae (Mazaki-Tovi et al., 2002; Rojas et al., 2018). The life cycle of *Spirocerca lupi* is the most studied and involves intermediate and paratenic hosts. The adult worms are found coiled within nodules in the oesophageal wall. *Spirocerca lupi* eggs containing larvae (L1) are passed from the oesophagus through the gastrointestinal tract and into the feces or may be shed in the vomitus. Eggs are ingested by the intermediate host (coprophagous beetles) and the larvae encyst within the tissues and develop to infectivity (L3) within two months. The beetle is ingested by the final host or a paratenic host (which include birds, lizards and rodents). Spirocercosis is a disease caused by this nematode which has a variety of clinical presentations. Death is typically a result of malignant neoplasms or aortic aneurysms (Mazaki-Tovi et al., 2002; Yogeshpriya, 2016; Van der Merwe et al., 2008; Rinas et al., 2009). This is the first report of *Spirocerca* sp. from ancient times from South America. Human spirocercosis was not reported at the moment. Therefore, although it is not a zoonotic species, its presence indicates the potential presence of the disease in wildlife in ancient times.

Gnathostomosis, a neglected food-borne zoonotic parasitic disease mostly in tropical and subtropical regions, is spread all over the world. Members of the genus *Gnathostoma* generally occur in tumors in the stomach wall of carnivorous mammals but two species occur in the kidneys and the oesophagus. Dogs, felines and wild mammals serve as the definitive hosts in the life cycle of the nematode, which has two intermediate hosts (Anderson, 2000). The first intermediate hosts are crustaceans and copepods. The second intermediate hosts are fresh-water fishes, frog, turtles and snakes harboring the infective third-stage larvae. Gnathostomosis can cause an extremely wide range of symptoms, such as cutaneous lesions and visceral disease, which damage pulmonary, gastrointestinal, genitourinary, ocular, auricular and central nervous system. Infection occurs by ingesting raw or insufficiently cooked fresh fish meat contaminated with the third-stage larvae. There are numerous cases reported in humans (Moore et al., 2003; Herman and Chiodini, 2009; Katchanov et al., 2011; Bravo, 2018). This is the first time that *Gnathostoma* sp. is reported from ancient times. Therefore, the presence of this species in the past displays the potential presence of the gnathostomosis both in carnivores and humans from holocenic times from Patagonia.

The family Taeniidae is of great importance in the medical and veterinary fields, particularly in the tropics and subtropics. All taeniid parasites have complex life cycles that include a carnivorous (or omnivorous) definitive host and a second mammalian intermediate host (herbivores, mainly artiodactyls, rodents, and lagomorphs) in which the larval or metacestode develops. The cycle is completed when eggs voided with the feces of infected final hosts contaminate vegetation that subsequently is eaten by the intermediate hosts. Eggs of *Taenia* and *Echinococcus* spp. are impossible to identify because they are all quite similar (Samuel et al., 2001). Humans are an aberrant host that does not play a role in the natural cycle of the parasite. Wildlife is essential for maintaining the life cycle of some taeniids in nature. Cysticercosis/Taeniasis and echinococcosis are neglected and cosmopolitan zoonotic diseases of public health significance caused by *Taenia* spp. and *Echinococcus* spp., respectively. Nowadays, both diseases are an important public health problem in South America. Several socioeconomic and cultural factors influence the transmission (Acha and Szyfres, 2003). Living or having lived in rural areas where farming and animal husbandry are common is an important risk factor for these diseases; in some areas particularly where people frequently come in contact with domestic and wild animals as well as where wild animal hunting for food is a common practice (e.g., hunters and aboriginal people). Humans and wildlife who lived in this site may have been exposed to these mentioned diseases in ancient times.

One of the most remarkable features of CG is that the site was occasionally and almost exclusively visited by humans with symbolic (mortuary and artistic) purposes, with no evidence of human residential use (Carden and Prates, 2015; Prates et al., 2016). Since the cave offers appropriate conditions for both human occupation and archaeological visibility/preservation (see discussion in Straus, 1990; Prates et al., 2013), the lack of archaeological remains of residential use is indeed an unexpected attribute. Though different causes could be related to this feature, two suggestive hypotheses emerge from our results. On the one hand, as pointed above, CG could have been a zoonotic risk zone, due to the presence of zoonotic parasites. However, it does not seem to explain the human reticence to the residential use of the cave. Not only because it is unlikely that the hunter-gatherers could have recognized this potential zoonotic risks, but also because most of the residential archaeological sites of Patagonia come from similar sheltered environments (caves and overhangs). On the other hand, spatial competition between humans and great felids could have discouraged the latter from settle in the cave.

CONCLUSION

The samples studied in the present work were assigned to a large felid, *Puma concolor* (puma) or *Panthera onca* (yaguareté). The paleoparasitological study shows high parasite richness and new parasite species for archaeological contexts were finding. Six nematode species, one cestode and one coccidian were reported. *Gnathostoma* sp. and *Spirocerca* sp. are the first records of these genera for Holocene contexts from Patagonia. On the other hand, diseases such as taeniasis, spirocercosis, gnathostomosis, ascariasis and coccidiosis could be present in wildlife from southern South America prior to the Spanish colonization and domestic animal introduction due to the presence of the parasite remains mentioned previously. Also, this means that felids could have played a role as reservoirs and sources of some parasitic species, some of which are zoonotic. Therefore, this animal could have entailed a risk agent for human health in the site.

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Figure 1: Studied samples from Cueva Galpón, Patagonia, Argentina.

Accepted

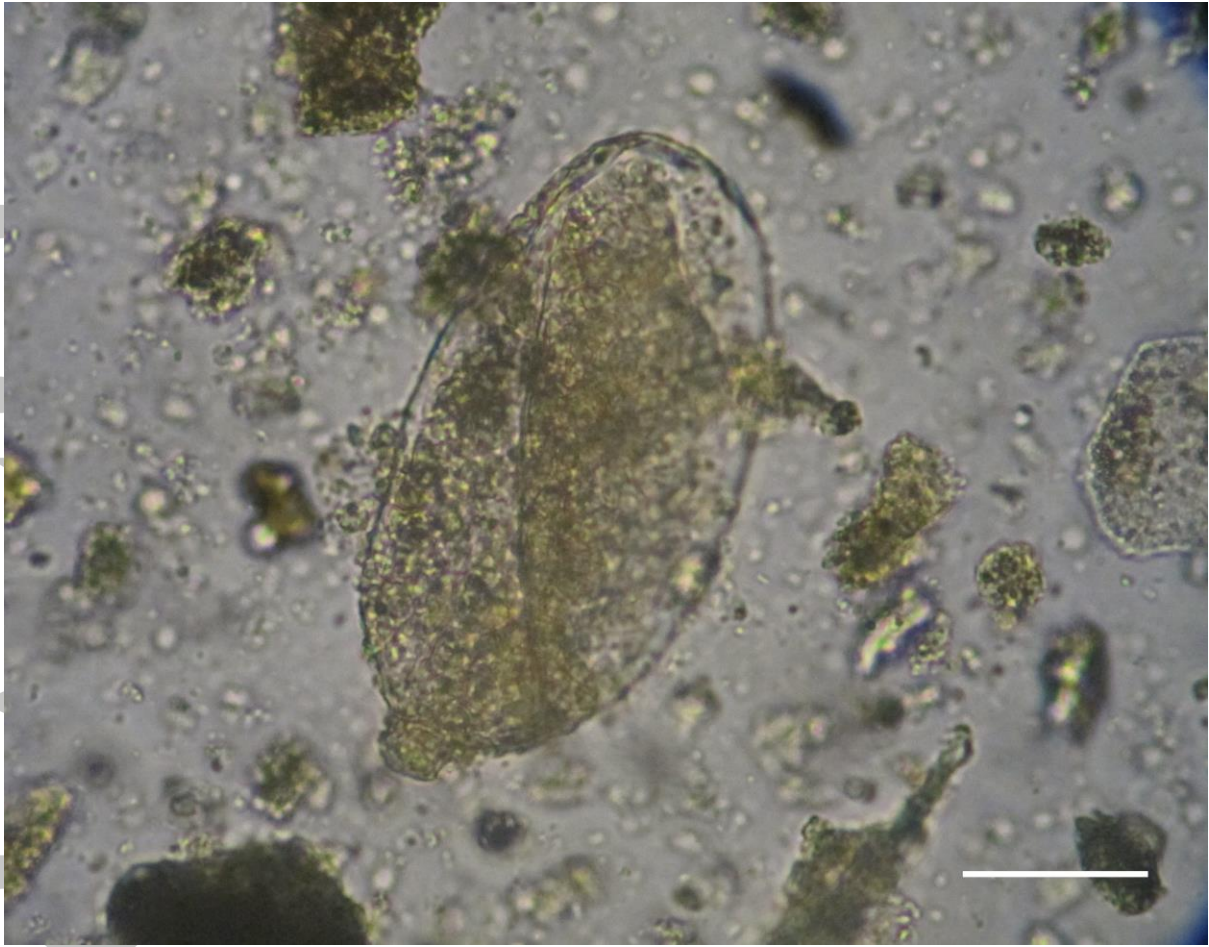


Figure 2a: Helminth egg found in Cueva Galpón, Patagonia, Argentina, compatible to *Nematodirus* sp. (Strongylida, Trichostrongyloidea). Bar = 40 μ m.

Accepted



Figure 2b: Helminth egg found in Cueva Galpón, Patagonia, Argentina, assigned to the superfamily Spiruroidea (Order Spirurida). Bar= 20 μ m.

Accepted

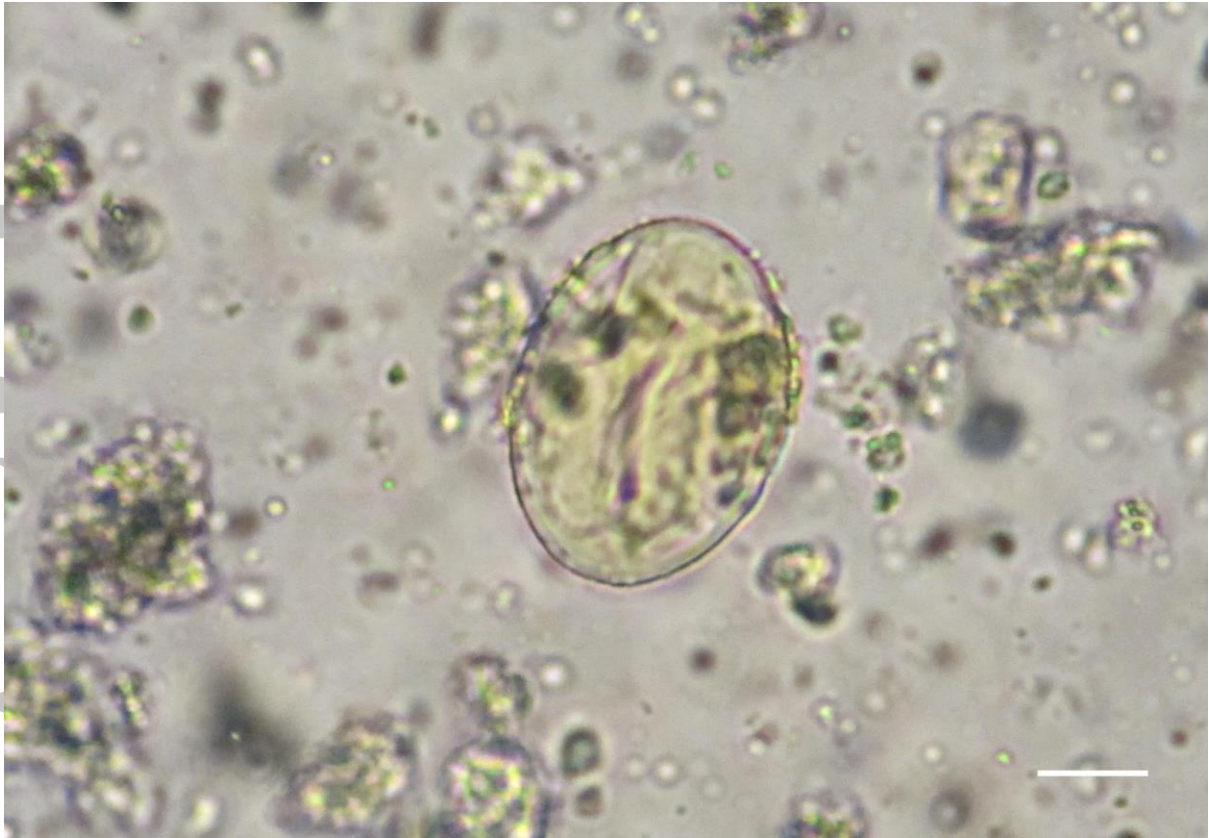


Figure 2c: Helminth egg found in Cueva Galpón, Patagonia, Argentina, attributed to an ascaridid species (Ascaridida, Ascarididae). Bar= 20 μ m.

Accepted



Figure 2d: Helminth egg found in Cueva Galpón, Patagonia, Argentina, assigned to *Heteroxy nema (Cavioxyura) viscaciae* (Oxyuroidea, Heteroxy nematidae). Bar = 40 μm .

Accepted

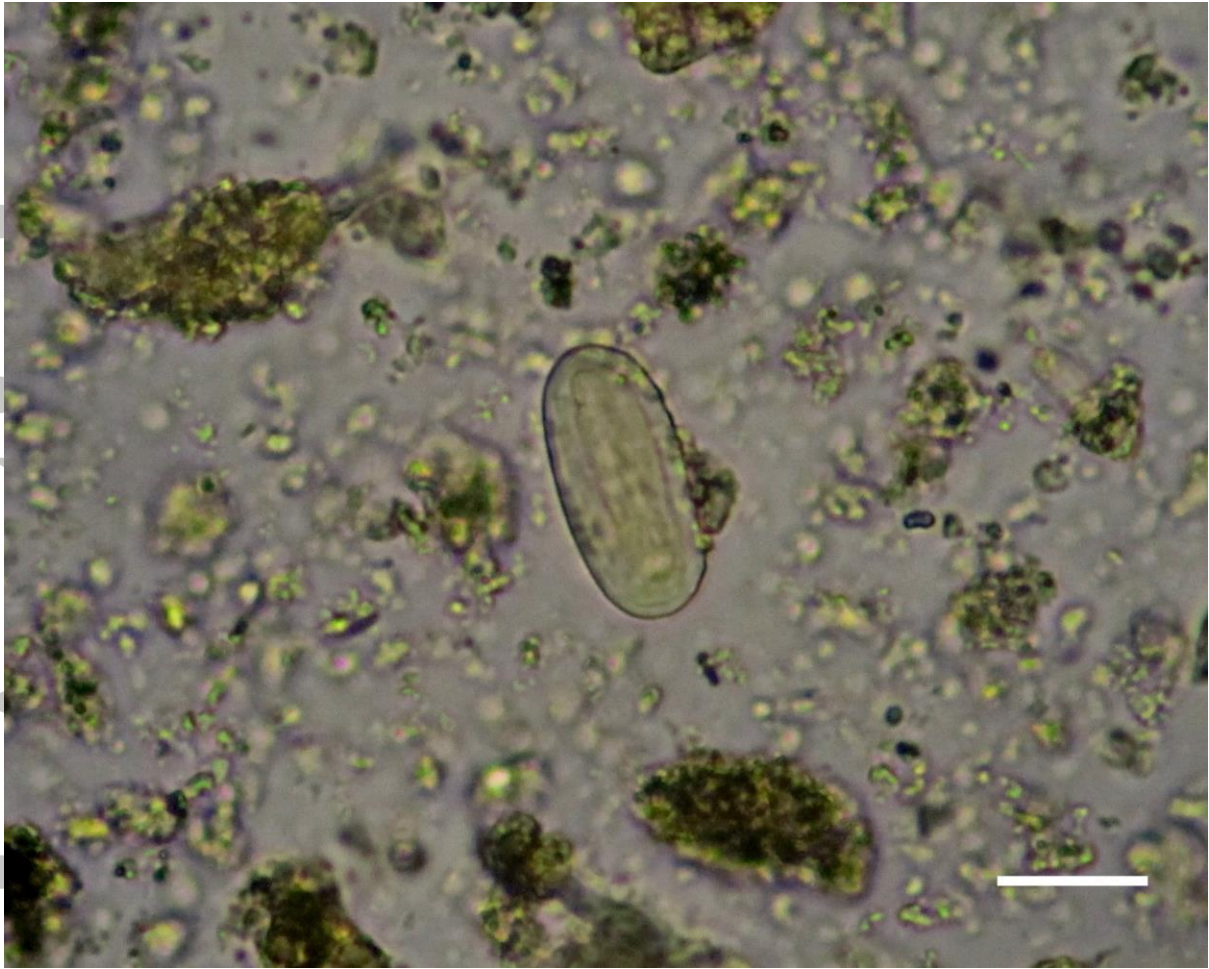


Figure 2e: Helminth egg found in Cueva Galpón, Patagonia, Argentina, attributed to *Spirocerca* sp. (Spirurida, Spirocercidae). Bar= 20 μ m.

Accepted



Figure 2f: Helminth egg found in Cueva Galpón, Patagonia, Argentina, identified as *Taenia* sp. or *Echinococcus* sp. (Cyclophyllidea, Taeniidae). Bar= 20 μ m.

Accepted

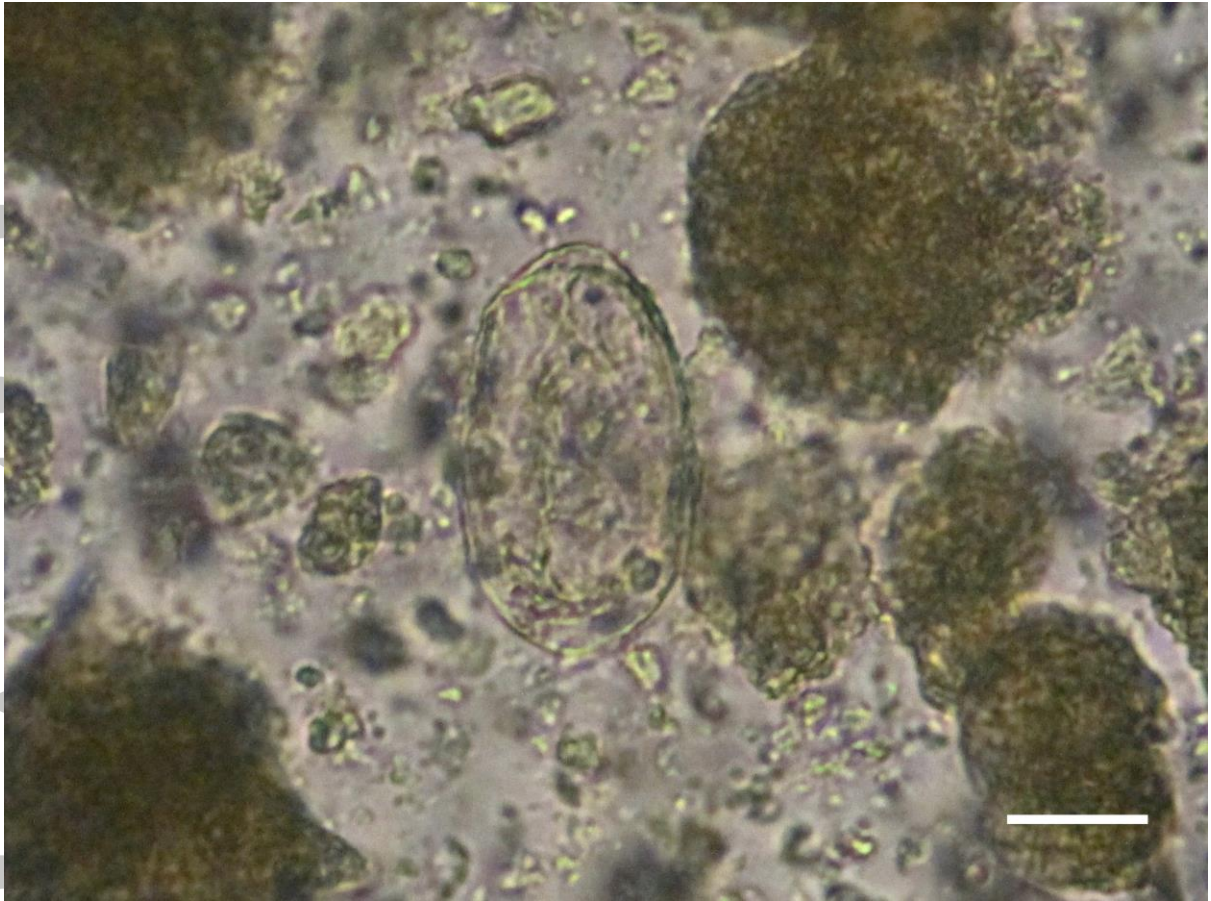


Figure 2g: Helminth egg found in Cueva Galpón, Patagonia, Argentina, attributed to *Gnathostoma* sp. (Spirurida: Gnathostomatidae). Bar= 20 μ m.

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Figure 2h: Oocyst (Coccidia, Apicomplexa) found in Cueva Galpón, Patagonia, Argentina.
Bar= 20 μ m

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