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Ancient parasites from endemic deer from "CUEVA PARQUE DIANA" archeological site, Patagonia, Argentina

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Abstract The narrow Andean-Patagonian temperate rainforest strip in the west of southern South America is inhabited by two endemic species of cervids, the southern pudu (*Pudu puda*) and the huemul (*Hippocamelus bisulcus*), both cataloged as near threatened and threatened species, respectively. One of the possible causes of their declined number is the susceptibility to livestock diseases. Significant zooarchaeological records of both deer have been found throughout the Holocene from Patagonia. The present contribution reports the first paleoparasitological results obtained from coprolites of endemic deer from the archeological site "Cueva Parque Diana," Neuquén Province, Argentina, and discusses the possible diseases found in ancient times. Thirty-four coprolites were fully processed, rehydrated, homogenized, sieved, subjected to spontaneous sedimentation, and examined by light microscopy. Thirty samples contained parasite remains. The presence of diverse parasitic diseases such as trematodioses, metastrongylosis, trichuriosis, strongylida gastroenteritis, dioctophymosis, and coccidiosis which could cause diseases in deer previous to the arrival of European livestock and the presence of zoonotic diseases in the hunters-gatherers and fishermen are discussed.

Keywords Paleoparasitology · Coprolites · Holocene · Cervid

Introduction

The narrow Andean-Patagonian temperate rainforest strip in the west of southern South America is inhabited by two endemic species of cervids, the southern pudu (*Pudu puda*) and the huemul (*Hippocamelus bisulcus*). The southern pudu is the smallest deer in the world reaching up to 40 cm in height. *Pudu puda* is a near threatened species (Silva-Rodríguez et al. 2016) and one of the less known South American deer (Meier and Merino 2007). The huemul is one of the most threatened deer in South America. This native deer is listed as an endangered species (Black-Decima et al. 2016) as a result of a significant decline in number and as a reduction of its original distribution range. The total population is estimated at approximately 1500 individuals (Smith-Flueck et al. 2011). Possible causes of the huemul's decline are poaching, habitat destruction, predation by dogs (*Canis familiaris*), competition with domestic animals, the introduction of exotic species and susceptibility to livestock diseases (Díaz and Smith-Fluek 2000; Serret 2001).

Parasitoses are one of the most studied diseases in ruminants around the world, especially in livestock. Host-parasite relationships most of the time do not result in disease. One

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way of recovering the emergence and disappearance of parasite infections through time and consequently provide new information on the evolution, paleoepidemiology, ecology, and phylogenetics of infectious diseases is the paleoparasitology (Reinhard 1992; Araújo et al. 2013). This discipline is the study of parasites present in ancient remains by the examination of archeological and paleontological materials (Ferreira et al. 1988).

Significant deer zooarchaeological records have been found throughout the Holocene from Patagonia, mainly remains of huemul. In recent years, diverse deer zooarchaeological analyses have been made focused on huemul ecology and human use (Pérez and Batres 2008; Fernández et al. 2015). However, studies on parasites from these populations in the past are lacking. The present contribution reports the first paleoparasitological results obtained from coprolites of endemic deer from Patagonia and discusses the possible diseases found in ancient times.

Materials and methods

Samples were collected from the archeological site "Cueva Parque Diana" (CPD), located at the Lanín National Park, Neuquén Province, Patagonia, Argentina (40° 19' S, 71° 20' W) (Fig. 1). The site is a rock shelter part of the archeological locality named Meliquina, at 964 m.a.s.l., and 50 m close to the Hermoso River. The archeological sequence was divided in 3 components representing different hunter-gatherer occupation processes. The Upper Component was dated from 760 ± 60 to 580 ± 60 ^{14}C years B.P. (vegetal charcoal), the Middle Component was dated from 990 ± 60 to 900 ± ^{14}C years B.P. (vegetal charcoal), and the Lower Component was dated at 2370 ± 70 ^{14}C years B.P. (vegetal charcoal). The site was occupied by hunters-gatherers and fishermen along the late Holocene, incorporating pottery from the Middle Component and the possible production of plant foods from the Upper Component. The climate in the area is cold and wet, with annual precipitations around 1500–2000 mm. The environment is mainly composed by colihue bamboo (*Chusquea culeou*), radal trees (*Lomatia hirsuta*), and coihues (*Nothofagus dombeyi*) and is inhabited by 166 native vertebrate species (Pérez 2010a; Pérez et al. 2015).

The examination of the external shape of feces was conducted according to Chame (2003) and Jouy-Avantin (2003). Thirty-four coprolites were whole processed, individually by rehydration in a 0.5% water solution of tris-sodium phosphate in a glass tube for at least 72 h (Callen and Cameron 1960), followed by homogenization. After this period, the samples were sieved through thrice-folded gauze for spontaneous sedimentation (Lutz 1919) and preserved in 70% ethanol. Up to 20 slides were prepared, along with the addition of one drop of glycerin and examined at 10× and 40× by light microscopy (Zeiss® Primo Star). Parasite remains were measured and photographed at ×40 magnifications and their dimensions and morphologies 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 for diet analysis.

Results

Table 1 presents dated and parasitological records of each sample. Coprolites were dark brown, cylindrical, usually pointed at one end and concave in the other extremity, with smooth surfaces (Fig. 2). Average measurements of feces (N = 34) were 12.31 ± 2.52 mm long by 6.70 ± 0.86 mm wide. The coprolites were identified by morphology, size, parasite findings, and biogeographic locality as belonging to huemul or southern pudu. Macroscopical observations showed vegetal remains indicative of an herbivore diet. Microscopic observations also revealed hairs, pollen grains, vegetal, and fungi remains.

Thirty out of 34 coprolites examined contained parasite remains. Representatives of 12 helminth species eggs and coccidian oocysts were recovered from samples. Positive coprolites belonged to upper, middle and lower components. All measurements (length × width) of the eggs and oocysts in the following descriptions are given in micrometers, with the means in parenthesis.

Eighteen of the 34 samples were positive for digenean eggs (Platyhelminthes). Positive coprolites were found in the upper, middle, and lower components (Table 1). The eggs were ellipsoidal, operculated, yellowish, and thin-shelled (Fig. 3). A total of 148 eggs were counted in all samples. Measurements (N = 102): 120.0–147.5 (131.7 ± 7.82) × 62.5–87.5 (72.8 ± 5.96).

Eggs of nematodes attributed to genus *Metastrongylus* sp. (Nematoda, Metastrongilidae) were found in coprolites 11, 13, 18, and 19 from lower and middle components (Table 1). The eggs were elliptical, larvated, with thick walls, and slightly corrugated surfaces (Fig. 4). Measurements (N = 6): 40–52.5 (48.75 ± 4.68) × 35–45 (37.92 ± 5.10).

Strongylid-type eggs (Nematoda, Strongyloidea) were found in samples 12, 22, 26, and 32. Positive coprolites were found in the upper, middle and lower components (Table 1). Eggs were thin-shelled, elongated, with flat sides, and embryonated (Fig. 5). Average egg measurements (N = 8): 62.5–72.5 (65.62 ± 4.28) × 25.0–30.0 (26.88 ± 1.55).

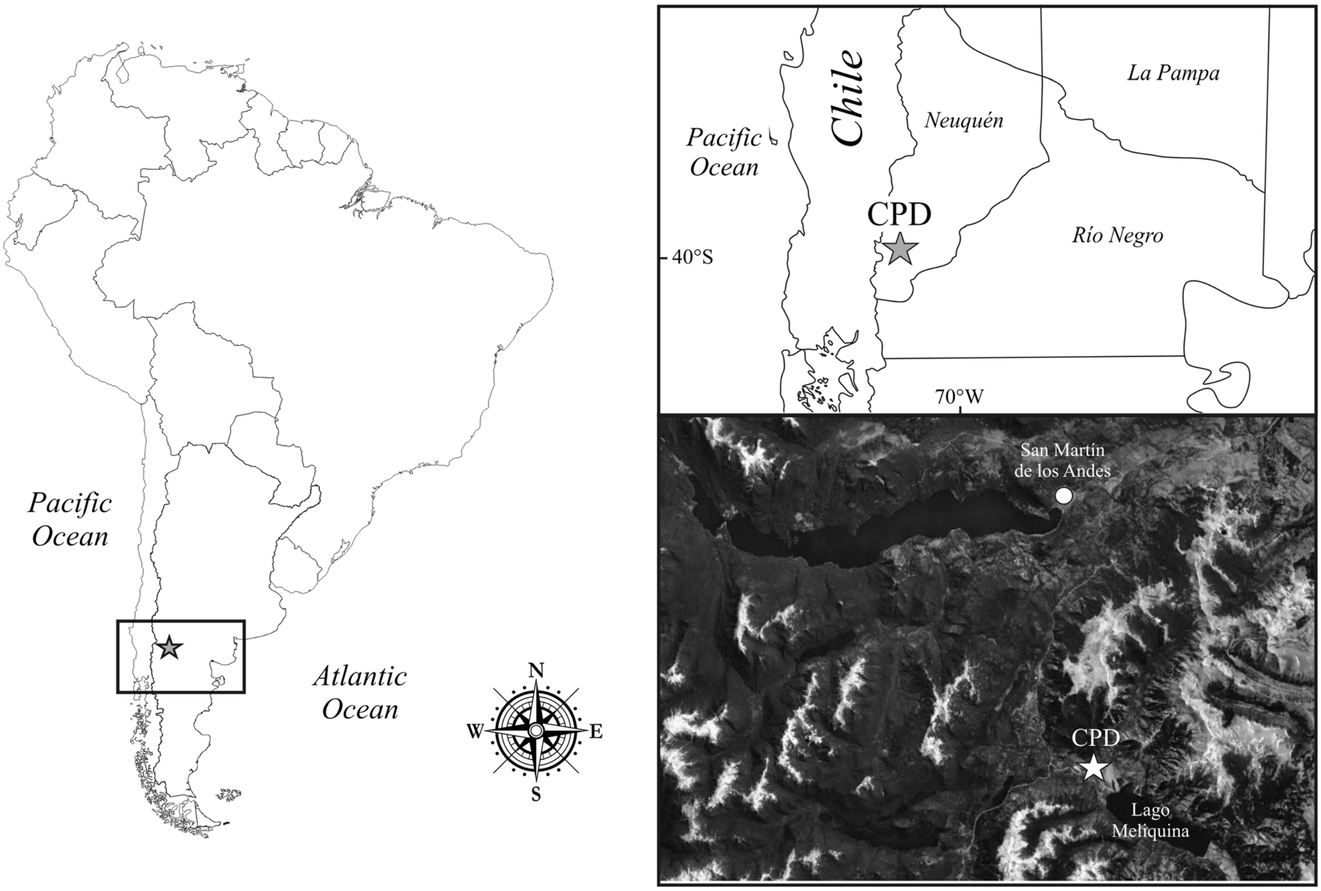

Fig. 1 Map of the "Cueva Parque Diana" archeological site, Patagonia, Argentina

Another strongylid-type egg was found in sample 13 (lower Component) (Table 1). The single egg was thin-shelled, rounded and larvated and measured 57 X 30 (Fig. 6).

Eggs of *Trichuris* spp. (Nematoda, Trichuridae) were observed in four coprolites belonging to lower and middle components (Table 1). These eggs were lemon shaped, with a smooth surface and polar plugs. Egg measurements were different among coprolites and were attributed to four different *Trichuris* spp. From sample 13, one egg was found, 75 × 40 (Fig. 7). One egg of 40 × 23 was found in sample 19 (Fig. 8). From sample 24 one egg of 55 × 40 was found (Fig. 9). From sample 26, egg measurements ($N = 5$) were 70.0–77.5 (73.46 ± 2.85) × 32.5–42.5 (37.82 ± 4.11) (Fig. 10).

One nematode egg (Fig. 11) was collected from the coprolite 34. This sample belongs to the upper Component (Table 1). The egg was elongated, with flat sides, thin shelled, and brown colored. It was identified as *Nematodirus* sp. (Nematoda, Trichostrongylidae). Egg measurements were 203 × 100.

One strongylid-type egg, 57 × 45 (Fig. 12), was present in coprolite 4. In sample 23, another strongylid-type was found; it measured 75 × 45. (Fig. 13). Both samples belong to the middle Component (Table 1).

One barrel-shaped egg, elliptical, with a thick, rough shell, and bipolar plugs attributed to the nematode *Dioctophyma* sp. (Enoplida, Dioctophymatidae), probably *D. renale*, was observed in sample 16 (lower Component). The measurements were 60 × 40 (Fig. 14).

Different coccidian oocysts (Apicomplexa, Eimeriidae) were found in 16 of the studied samples, attributed to *Eimeria* spp. and *Isospora* spp. Oocysts displayed differences of the wall and measurements and were observed sporulated and not sporulated. The sporocysts also displayed morphological differences (Fig. 15a–e). Positive coprolites for coccidians were found in the upper, middle, and lower components (Table 1).

Discussion

This paper reports the first paleoparasitological study on endemic deer coprolites from Patagonia. Only two paleoparasitological studies were done on South American deer. Ferreira et al. (1992) found *Eimeria* oocysts in deer

Table 1 Dated and parasites remains (eggs and oocysts) found from deer coprolites from the archeological site "Cueva Parque Diana," Patagonia, Argentina

Coprolite N°	Age (years B.P.)	Component	Parasites found
1	990 ± 60	Middle	Negative
2	990 ± 60	Middle	Negative
3	990 ± 60	Middle	Negative
4	990 ± 60	Middle	Digenean Strongylid
5	990 ± 60	Middle	Digenean
6	990 ± 60	Middle	Digenean coccidian oocyst
7	990 ± 60	Middle	*Eimeria* sp.
8	2370 ± 70	Lower	Negative
9	2370 ± 70	Lower	Digenean
10	2370 ± 70	Lower	Digenean
11	2370 ± 70	Lower	Digenean *Metastrongylus* sp.
12	2370 ± 70	Lower	Strongylid *Eimeria* sp. digenean
13	2370 ± 70	Lower	Strongylid *Metastrongylus* sp. *Trichuris* sp.
14	2370 ± 70	Lower	Digenean
15	2370 ± 70	Lower	*Eimeria* sp.
16	2370 ± 70	Lower	*Dioctophyma* sp.
17	2370 ± 70	Lower	Digenean
18	2370 ± 70	Lower	Digenean *Metastrongylus* sp.
19	2370 ± 70	Lower	digenean *Trichuris* sp. *Metastrongylus* sp. *Eimeria* sp.
20	2370 ± 70	Lower	Digenean coccidian oocyst
21	2370 ± 70	Lower	Digenean *Eimeria* sp.
22	900 ± 60	Middle	Digenean *Isospora* sp. strongylid
23	900 ± 60	Middle	Coccidian oocyst *Metastrongylus* sp. strongylid
24	900 ± 60	Middle	*Isospora* sp. *Eimeria* sp. *Trichuris* sp.
25	900 ± 60	Middle	*Eimeria* sp.
26	900 ± 60	Middle	*Trichuris* sp. strongylid *Eimeria* sp.
27	2370 ± 70	Lower	coccidian oocyst digenean
28	580 ± 60	Upper	*Eimeria* sp.
29	580 ± 60	Upper	digenean
30	580 ± 60	Upper	*Eimeria* sp.
31	580 ± 60	Upper	*Eimeria* sp.
32	580 ± 60	Upper	strongylid
33	760 ± 60	Upper	digenean *Eimeria* sp. *Isospora* sp.
34	760 ± 60	Upper	digenean *Nematodirus* sp.

coprolites (9000 years B.P.) from the site Perna I, São Raimundo Nonato, northeast Brazil and Sianto et al. (2012) found eggs of *Trichuris* sp. from cervid coprolites (1040 ± 50 years B.P.) from the archeological site Furna do Estrago, Pernambuco, Brazil.

Parasitological studies on actual samples from *P. puda* from Argentina and Chile were done. Endoparasites found were the cestodes *Cysticercus tenuicollis* and *Echinococcus granulosus*, the nematodes *Trichuris* sp., *Dictyocaulus* sp., *Strongyloides* sp., *Trichostrongylus* sp., *Nematodirus* sp., *Oesophagostomum* sp., *Capillaria* sp., *Ostertagia* sp., *Cooperia* sp., *Trichostrongylus* sp., *Trichostrongylus axei*, *Oesophagostomum asymetrica*, *Marshallagia* sp. and *Muellerius* spp., the trematode *Fasciola hepatica* and the coccidians *Sarcocystis* sp. and *Eimeria* sp. (Duval et al. 1990, in Bravo Antilef 2013; Cortés 2006; Bravo Antilef 2013). Recent parasitological studies on *H. bisulcus* from Patagonia mentioned the presence of *Ostertagia* spp., *Strongylus* spp., *Trichuris* sp., *Dictyocaulus viviparous, Dictyocaulus* sp., *Nematodirus* sp., *F. hepatica*, the cestode *Moniezia* sp., and oocysts of *Eimeria* sp. (Serret 2001; Pérez 2010b; Puen González 2013; Taglioretti 2015).

Fig. 2 Macroscopic aspect of the coprolites collected from the "Cueva Parque Diana" archeological site

Fig. 3 Digenean eggs found from "Cueva Parque Diana" archeological site (Platyhelminthes: Trematoda). *Bar* = 40 µm

Trematodioses are widespread helminthoses found in domestic and wild ruminants causing serious health problems over the world. In Argentina, digenean parasitism has been considered as one of the major constraints of livestock production. Fascioliosis and paramphistomosis are the most important trematodioses in domestic ruminants (Suárez et al. 2007; Sanabria and Romero 2008). Current knowledge of the digenean species on deer from Patagonia is limited. The unique species reported in wild deer was *F. hepatica*. The morphology and measures found in the present study are similar with that of this species. The presence of this digenean species from ancient times is discussed in Beltrame et al. 2017.

Gastrointestinal strongylids are within the most prevalent and important parasites affecting ruminants worldwide.

Fig. 4 Eggs found from "Cueva Parque Diana" archeological site, attributed to genus *Metastrongylus* sp. (Nematoda, Metastrongilidae). *Bar* = 20 µm

Fig. 5 Strongylid-type eggs (Nematoda, Strongyloidea) found from "Cueva Parque Diana" archeological site. *Bar* = 20 µm

Grazing ruminants are usually parasitized by one or more strongylid species which can cause parasitic gastroenteritis (Zajac 2006; Roeber et al. 2013). The life cycles are monoxenous and predominantly live in the gastrointestinal tract of their vertebrate hosts. Adult females produce relatively large numbers of typically ovoid, strongylid eggs, which are excreted in the feces into the external environment.

Metastrongylus lungworms are distributed worldwide and include six species reported. Metastrongylosis is an important parasitic respiratory disease especially affecting pigs and wild boars (*Sus scrofa*) with indirect life cycles in several earth worm species (Marruchella et al. 2012; Gassó et al. 2014). This genus exhibits a potential risk to both livestock and humans (Gassó et al. 2014; Calvopiña et al. 2016). They produce fully larvated eggs in the host respiratory system. Eggs are coughed up and swallowed and passed with the feces; in the soil the eggs hatch and release L1 larvae that are ingested by earthworms, the intermediate

Fig. 6 Strongylid-type eggs (Nematoda, Strongyloidea) found from "Cueva Parque Diana" archeological site. *Bar* = 20 µm

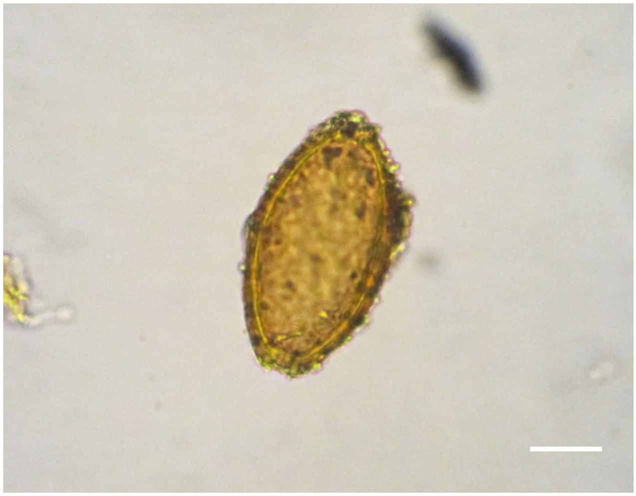

Fig. 7 Eggs found from "Cueva Parque Diana" archeological site, attributed to genus *Trichuris* sp. (Nematoda, Trichuridae). *Bar* = 20 μm

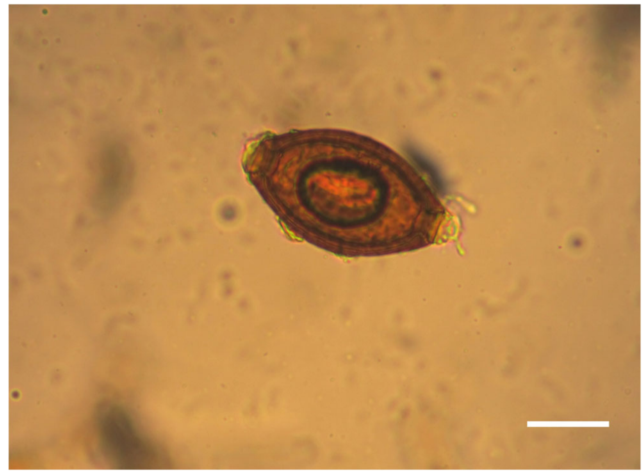

Fig. 9 Eggs found from "Cueva Parque Diana" archeological site, attributed to genus *Trichuris* sp. (Nematoda, Trichuridae). *Bar* = 20 μm

hosts. The final host acquires the parasite by ingesting infected earthworms which are very abundant in the environment. The infection causes dyspnea due to the destruction of interstitial tissues, obstruction and ultimately the consolidation of lungs in affected animals resulting to the progressive weight loss and increasing the mortality of weaker young and adult animals (Anderson 2000). This species was found in the analyzed coprolites, but it has not been recorded in actual individuals of deer at the moment.

Nematodes of the genus *Trichuris* were found in the present study and were also found in actual parasitological studies of *P. puda* and *H. bisulcus* and in deer samples from the archeological site Furna do Estrago, Brazil. These species are soil transmitted parasites that reside in the caecum of their hosts. They have a cosmopolitan distribution and parasitize a broad range of mammalian hosts, such as ruminants, marsupials, rodents and primates, including humans (Anderson 2000). These parasites produce a diseased called trichuriasis. *Trichuris* spp. also infects domestic and wild ruminants, causing clinical signs. Larval stages cause hemorrhages and local edema when they penetrate the intestinal wall, often leading to secondary bacterial infection. The adult worms are not pathogenic, unless present in large numbers when they may cause abdominal pains, diarrhea, anemia, loss of body mass and rarely, death. The zoonotic potential of *Trichuris* spp. such as *T. vulpis* and *T. suis* was documented (Acha and Szyfres 2003). It is probable that *Trichuris* spp. of Patagonic deer could have been a threat to human health in ancient times.

Dioctophyma sp. can be found worldwide especially parasitizing domesticated and wild carnivores and mustelids. Other mammals that can act as definitive hosts of the parasite include: canines, mink, wolves, foxes,

Fig. 8 Eggs found from "Cueva Parque Diana" archeological site, attributed to genus *Trichuris* sp. (Nematoda, Trichuridae). *Bar* = 20 μm

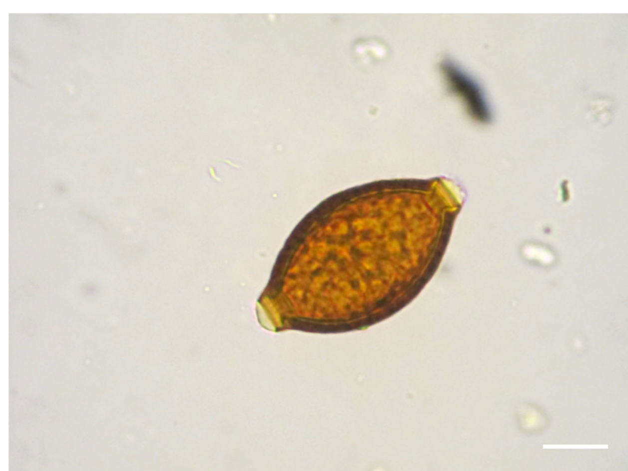

Fig. 10 Eggs found from "Cueva Parque Diana" archeological site, attributed to genus *Trichuris* sp. (Nematoda, Trichuridae). *Bar* = 20 μm

Fig. 11 Eggs found from "Cueva Parque Diana" archeological site, attributed to genus *Nematodirus* sp. (Nematoda, Trichostrongylidae). *Bar* = 40 μm

Fig. 13 Strongylid-type eggs (Nematoda, Strongyloidea) found from "Cueva Parque Diana" archeological site. *Bar* = 20 μm

jackals, coatis, otters, skunks, ferrets, weasels, rats, raccoons, wolverines, pumas, cats, seals, pigs, and horses (Anderson 2000). They can occasionally infect humans (Chauhan et al. 2016). In the parasite life-cycle, eggs are eliminated through the urine of the definitive host to reach their infective stage in the external environment. The intermediate host is an aquatic oligochaete annelid that ingests eggs containing first stage larvae of the parasite and the primary host is infected by ingesting the infected annelid or paratenic host, which can be a fish or frog (Anderson 2000). Dioctophymosis is a worldwide renal parasitosis caused by the *D. renale*, which generates the progressive destruction of the kidney, reducing the organ to a fibrous capsule. The presence of a dioctophymatid egg in a deer coprolite is probably due to carnivore urine contamination and indicates a potential zoonotic risk to humans in this archeological site.

Coccidiosis of small ruminants is a protozoan infection caused by coccidian parasites of the genus *Eimeria* and *Isospora,* which develop in the small and the large intestine and particularly affect young animals. The life cycle of these species is direct with an oral-fecal cycle involving three phases: schizogony or merogony, gametogony and sporogony. The infective transmission stage is the oocyst which contains, when sporulated, four sporocysts each containing two sporozoites (*Eimeria* spp.) or two sporocysts each containing four sporozoites (*Isospora* spp.). Nearly all of the species of ruminants are extremely host specific, able to complete development and reproduction in the digestive tract of a specific host species

Fig. 12 Strongylid-type eggs (Nematoda, Strongyloidea) found from "Cueva Parque Diana" archeological site. *Bar* = 20 μm

Fig. 14 Eggs found from "Cueva Parque Diana" archeological site, attributed to genus *Dioctophyma* sp. (Enoplida, Dioctophymatidae), probably *D. renale*. *Bar* = 20 μm

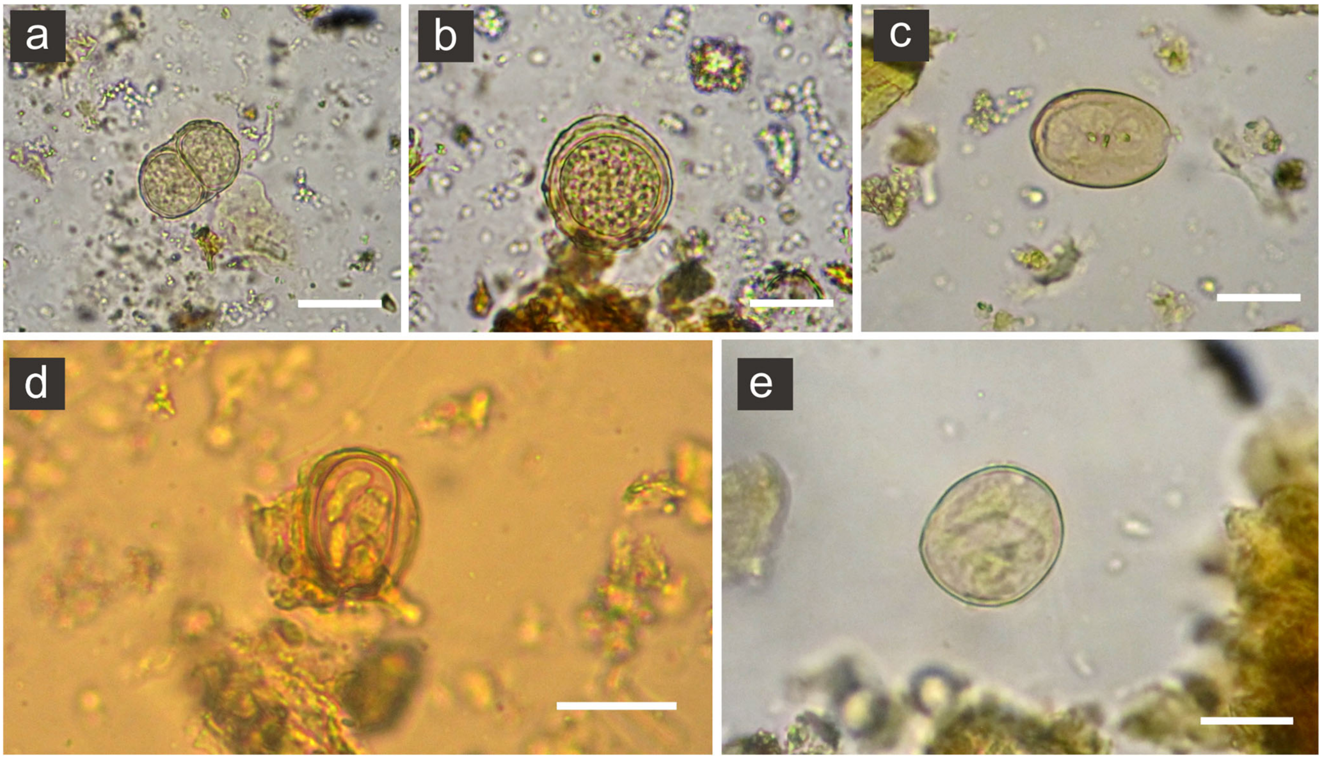

Fig. 15 Coccidians oocysts (Apicomplexa, Eimeriidae) found from "Cueva Parque Diana" archeological site, attributed to *Eimeria* spp. and *Isospora* spp. *Bar* = 20 μm

(Chapman et al. 2013). Clinical symptoms of coccidiosis begin with weakness, anorexia, and emaciation, with or without respiratory distress (Jolley and Bardsley 2006). Diverse *Eimeria* spp. and *Isospora* spp. were found in the present study but little is known about coccidiosis on deer from Argentina at present.

Conclusion

In the present study, the presence of parasitic species which can cause diverse diseases such as trematodioses, metastrongylosis, trichuriosis, strongylida gastroenteritis, dioctophymosis, and coccidiosis previous to the arrival of European livestock were reported. One of the possible causes of the huemul's decline proposed is the susceptibility to livestock diseases. However, diverse potential diseases were present before their arrival.

The close interaction between deer and human populations suggests the presence of zoonotic diseases also in the hunters-gatherers and fishermen population inhabiting this archeological site in the past.

The knowledge of animal natural history is relevant to achieve management and conservation decisions. The zooarchaeological record from Patagonia is a valuable tool to understand the natural history of the endemic deer. This is the first paleoparasitological study on endemic deer coprolites from Patagonia that contributes to the knowledge of their natural history.

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