

# Parasitic diversity found in coprolites of camelids during the Holocene

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**Abstract** Knowledge of parasitic infections to which fauna was exposed in the past provides information on the geographical origin of some parasites, on the possible dispersal routes and for archaeological fauna on the potential zoonotic risk that human and animal populations could be exposed. The aim of the present study was to examine the gastrointestinal parasite present in camelid coprolites collected from the archaeological site Cerro Casa de Piedra, cave 7 (CCP7), Patagonia, Argentina. Coprolites were collected from different stratified sequences dating from the Pleistocene-Holocene transition to the late Holocene. Paleoparasitological examination revealed the presence of eggs of Trichostrongylidae attributed to *Lamanema chavezii* or *Nematodirus lamae*, eggs of three unidentified capillariids, *Strongylus*-type eggs and oocysts of *Eimeria macusaniensis*. These parasites affected camelids living in the studied area since the Pleistocene-Holocene transition, about 10,000 years ago. Gastrointestinal parasite fauna of patagonian camelids did not vary significantly from Pleistocene-Holocene transition to late Holocene, although environmental conditions fluctuated greatly throughout this period, as indicative of the

strength and the stability of these associations over time. In this study, the zoonotic and biogeography importance of parasites of camelids are also discussed.

**Keywords** Paleoparasitology · *Lama guanicoe* · Patagonia · Holocene

## Introduction

Knowledge of parasitic infections to which the camelids were exposed in the past provides information on the geographical origin of some parasites, on possible dispersal routes of some diseases that affect the current fauna, on issues related to the evolution of the host-parasite relationship (Loirelle and Bouchet 2003; Dittmar and Teegen 2003; Santoro et al. 2003) and, in the case of fauna associated with archaeological sites, on the potential zoonotic risk that human populations could be exposed in the past.

The presence of South American camelids (SACs) in Patagonia goes back to late Pleistocene. These herbivores accompanied human populations from the beginning, constituting the guanaco the most important food source, even in periods in which environmental conditions were critical (Miotti and Salemme 1999). SACs are infected by specific parasites and by those shared with the domestic livestock, being the presence in current camelids associated with the introduced livestock.

Camelid coprolites are well represented in most layers of the archaeological site Cerro Casa de Piedra, cave 7 (CCP7) (De Nigris 2004), being the paleoparasitological examination of these coprolites an opportunity to study the ecological relationships between camelids and parasites along Pleistocene-Holocene transition (9640±

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190 years  $^{14}\text{C}$  before present (BP)) to the late Holocene ( $3920\pm 80$  years  $^{14}\text{C}$  BP) in the area and also to add valuable information to the knowledge of the patagonic paleoepidemiological context.

The aim of the present study was to provide information about the gastrointestinal parasite fauna present in camelid coprolites collected from different layers, dated from  $9640\pm 190$  to  $3920\pm 80$  years  $^{14}\text{C}$  BP, of the archaeological site Cerro Casa de Piedra, cave 7 (CCP7), located in Perito Moreno National Park, Santa Cruz Province, Argentina.

## Materials and methods

### Studied area

Cerro Casa de Piedra (CCP) is a volcanic outcrop located in the Perito Moreno National Park (PMNP), exhibiting a set of rock shelters and caves: CCP, cave 7 (CCP7). It shows a stratigraphic sequence of 19 levels with human occupation between ca.  $9700\pm 100$  and  $3600\pm 70$  years before present (BP) (Fig. 1) (Aschero 1996; Civalero and Aschero 2003). PMNP is located in the southern portion of the Cordillera de los

Andes, Santa Cruz Province ( $47^\circ 40' \text{ S}$  and  $72^\circ 30' \text{ W}$ ) (Fig. 1).

### Material studied

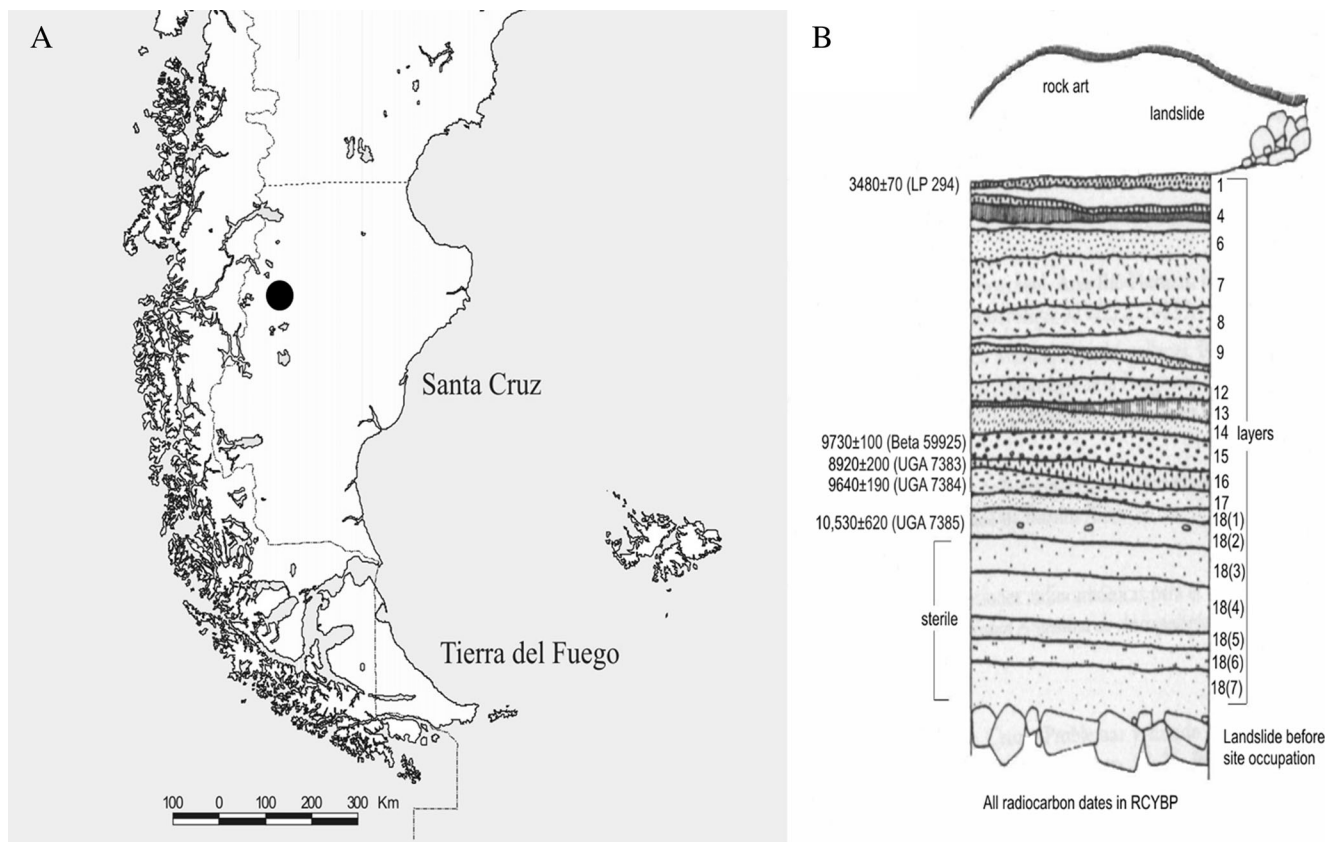
Camelid coprolites were found well preserved in most archaeological layers of CCP7, dated from Pleistocene-Holocene transition to late Holocene (De Nigris 2004).

Twenty four coprolites attributed to camelids collected from eight layers (three coprolites from each one), dated from 9600 to 3400 years  $^{14}\text{C}$  BP from the archaeological site CCP7, were examined for parasites (Table 1).

Pellets were described, weighted and photographed, and multiple shape and size predictor variables were calculated according to Taglioretti et al. (2014a). Coprolites were identified as camelids by the discriminant classification function performed by Taglioretti et al. (2014a).

### Paleoparasitological examination

Coprolites were whole rehydrated in an aqueous solution of trisodium phosphate 0.5 % (Callen and Cameron 1960) for a



**Fig. 1** a Map of the studied area. b Stratigraphy of the archaeological site CCP7 and the radiocarbon dates (from Civalero and Aschero 2003)

**Table 1** Radiocarbon dating and archaeological layers of CCP7 from which coprolites were recovered for paleoparasitological analysis (from Civalero and Aschero 2003)

Sample	Layer	Years <sup>14</sup> C BP
16	XVII	9640±190
32	XIV	8460±400
11	XIII–XII	7920±130–8300±150
19	XI	7880±150
12	VII	5610±110
29	IX	3920±80
38	V	6150±105
465	II	4270±90

week at 4 °C and subject to spontaneous sedimentation (Lutz 1919) following conventional paleoparasitological process for subsequent microscopic observation. Ten slides of each coprolite were observed.

Eggs and cysts of parasites were identified, measured and photographed at 40× magnification. In the present study, the taxonomy criteria proposed by Moravec (1982) were applied to identify specimens attributed to the subfamily Capillariinae.

Parasitic abundance was also calculated for each sample and a multidimensional scaling ordination (NMDS) based on log-transformed ( $x+1$ ) abundances, and Bray-Curtis similarity matrix was performed to determine if coprolites collected from the same archaeological layer arranged closely by their parasitic abundance.

## Results

Among nematodes, the paleoparasitological examination reported the presence of eggs of three representatives of the family Trichuridae, subfamily Capillariinae, including 47 of barrel shape with dotted surface, polar plugs and double thick-walled, external ornamented with radial striations (64.9±4.2 μm in length (55–70) and 38.3±3 μm in width (32.5–45)) similar to *Calodium hepaticum* eggs (Fig. 2a) which it will be called capillariid morphotype I and two other unidentified large eggs of capillariids. Eggs of the second unidentified capillariid (morphotype II) ( $n=5$ ) were embryonated, oval, polar plugs slightly protruding, thin-walled with sculpture on egg surface indistinct (83±3.2 μm in length (80–87.5) and 49.5±3.2 μm in width (45–52.5)) (Fig. 2b). These eggs were only recovered from a coprolite of the archaeological layer V (6150±105 years <sup>14</sup>C BP). Seven eggs of the third unidentified capillariid (morphotype III) were found in four out of eight archaeological layers examined. These eggs of this

unidentified capillariids are wide with globe-shape, thin-shell with slightly dotted surface and prominent polar plugs (83.25±3 μm in length (80–87.5) and 52.5±5.3 μm in width (45–57.5)) (Fig. 2c).

Eggs of the superfamily Trichostrongylidae ( $n=95$ ) compatible with *Nematodirus lamae* or *Lamanema chavezii* (Fig. 2d) (178.5±8.3 μm in length (162.5–200) and 91±7.2 μm in width (77.5–112.5)) and *Strongylus*-type eggs ( $n=2$ , 65×52.5 and 87.5×50 μm) were also recovered (Fig. 2e).

Among protozoan, oocysts of *Eimeria macusaniensis* ( $n=27$ ) were found in samples (Fig. 2f). A large size variability was observed in *E. macusaniensis* oocyst examined from different time periods (Table 2).

Coprolites from the same layer were not necessarily grouped closely in the NMDS plot (Fig. 3), as coprolites collected from the same archaeological layer not necessary share the same parasites species and abundances. Capillariid morphotype I and trichostrongylid eggs were present in most layers examined (Table 3). *N. lamae* or *L. chavezii* eggs were present in 23 out of 24 coprolites observed and were also the most abundant parasites (Table 3).

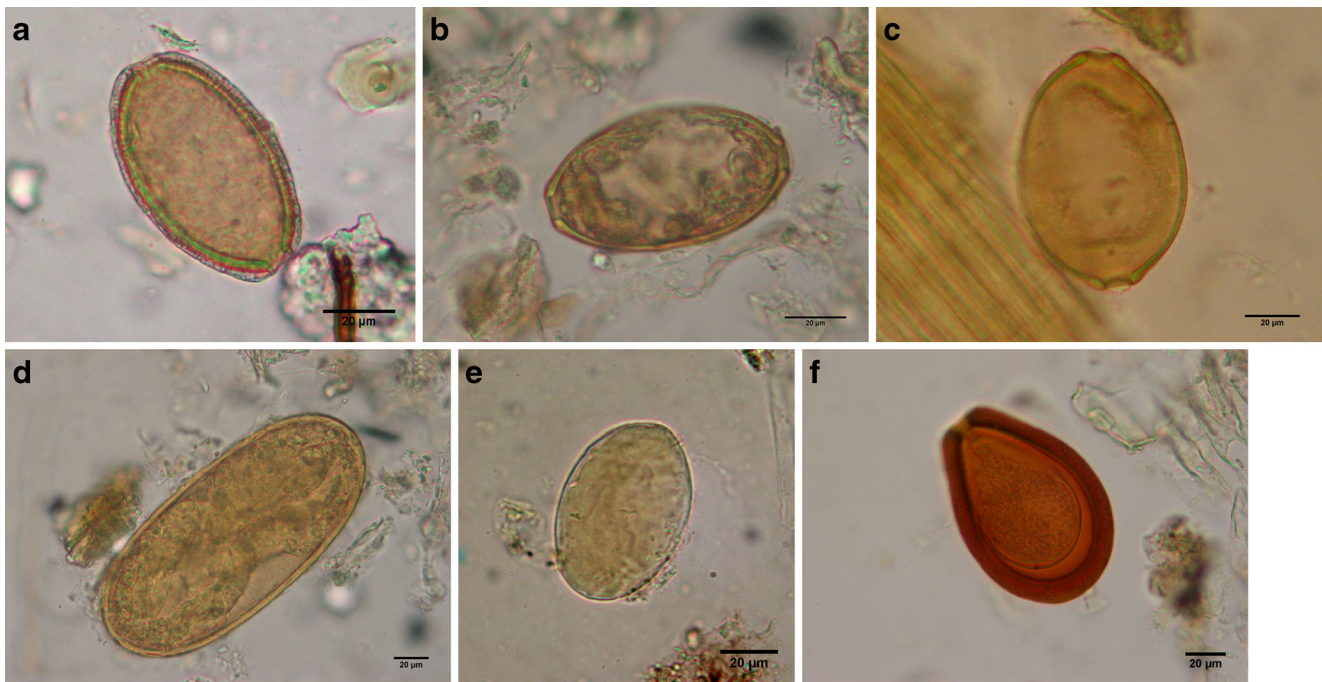
## Discussion

The presence of *E. macusaniensis*, a specific parasite of the South American camelids, corroborates the zoological origin of samples that were also identified by the morphological analysis (Taglioretti et al. 2014a). This eimeriid was previously registered in prehispanic camelids of Patagonia and Peru (Leguía et al. 1995; Fugassa 2007); however, the present finding is one of the most ancient records (9640±190 years <sup>14</sup>C BP) for this eimeriid.

The large biometric variability of *E. macusaniensis* oocyst observed in the present study is according to Fugassa et al. (2008), who found a temporal trend in *E. macusaniensis* oocyst dimensions, which involves a decrease in oocyst dimension through the time.

*Strongylus*-type eggs are easily confused, and the specific identification from eggs is not possible (Leguía 1999). The finding of only two *Strongylus*-type eggs in the present study was probably due to a low parasitic load or to taphonomic processes, since the eggshell of strongyles is thin, probably not goodly preserved in the archaeological record, as it was suggested by Leguía (1999).

Eggs of Trichostrongyloidea found in the current research could correspond to *N. lamae* or *L. chavezii*; both are specific parasites of current SACs (Becklund 1963; Leguía 1999). These eggs have similar shape and size (Leguía 1999), so the identification of these eggs is difficult to achieve from archaeological samples. At present, neither *L. chavezii* nor *N. lamae* was found in camelid from Patagonia (Navone and Merino 1989; Karesh et al. 1998; Beldoménico et al. 2003).



**Fig. 2** a Egg of unidentified capillariids, morphotype I. b Egg of unidentified capillariid, morphotype II. c Egg of unidentified capillariid, morphotype III. d Egg of Trichostrongylidae, compatible with *N. Lamae* or *L. chavezii*. e *Strongylus*-type egg. f Oocyst of *E. macusaniensis*

In the present study, Trichostrongylidae were present in all the coprolites examined from the Pleistocene-Holocene transitions to the late Holocene, despite substantial fluctuations in climate and landscape during the Holocene in Patagonia (Mancini 1998), showing these parasite-infected camelids along variable environmental conditions. This fact could be related with the ability of these eggs to resist extreme environmental conditions (Poole 1956; Soulsby 1987; Leguía 1999).

Capillariid morphotype I eggs found in the present study were similar to that of *Calodium* sp. of intestinal or stomach location found in human, feline, canid and rodent coprolites from Patagonian archaeological sites (Taglioretti et al. 2014b). Eggs of capillariid morphotype I were also found in most archaeological layers examined. These eggs had a double egg-shell, like *C. hepaticum*, which probably conferred high resistance to the environmental conditions of Patagonia.

Eggs of capillariid type III found in the present study are similar to representatives of the genus *Eucoleus*, either of gastrointestinal location, like *Eucoleus* n. sp. reported in rodents

by Robles (2008), or of respiratory location, like *Eucoleus boehmi* or *Eucoleus tenuis* (Moravec 2000). Nevertheless, its morphometry exceeds the biometric range of these species, and at present, ruminants are not included among the host range of these species.

Among capillariids known as parasites of extant camelids are *Aonchotheca bovis* and representatives of the genus *Capillaria* (Leguía 1999; Moravec 2000), whose species identification remains unknown as in the case of many other capillariids. Unidentified eggs of capillariids recovered in the present study do not correspond to any of those reported in camelids. Large capillariid eggs found could correspond to capillariid parasites of gastrointestinal or respiratory location, not yet identified.

The zoonotic trichostrongylosis is produced by several species of *Trichostrongylus*. However, other trichostrongyles such as *Ostertagia ostertagi*, *Ostertagia circumcincta*, *Haemonchus contortus* and *Nematodirus* sp. had been found in humans (Acha and Szyfres 1989; Botero and Restrepo

**Table 2** Dated and measurement of *Eimeria macusaniensis* oocysts found in the present study

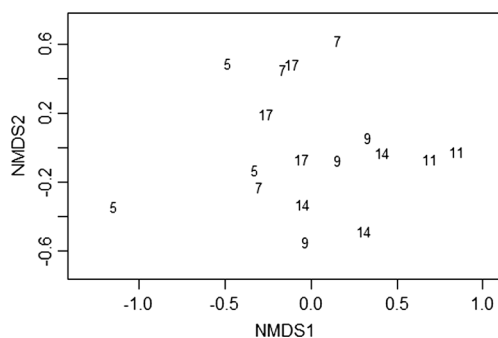
Archaeological layer		Width (µm)			Length (µm)			N
Layer	Years <sup>14</sup> C BP	Mean	SD	Range	Mean	SD	Range	
XVII	9640±190	88.75	16.1	77.5–112.5	113.75	15.5	95–135	4
VII	5610±110	71.2	4.7	60–82.5	99.4	9.2	72–115	18
V	6150±105	70.625	7.1	65–80	98.75	13.3	82.5–115	4
II	4270±90	67.5	–	–	97.5	–	–	1

**Table 3** Parasite remains found in different archaeological layers

Layer	Parasite remains					
	<i>E. macusaniensis</i>	Capillariid type I	Capillariid type II	Capillariid type III	<i>Strongylus</i> type	Trichostrongylid
XVII	+	+	–	–	–	+
XIV	–	+	–	+	–	+
XII– +	XIII	–	+	–	+	–
XI	–	–	–	–	+	+
IX	–	+	–	+	+	+
VII	+	+	–	+	–	+
V	+	+	+	–	–	+
II	+	+	–	–	–	+

2003; Pestechian et al. 2014). Among trichostrongylids with *Strongylus*-type eggs infecting SAC are those of the genera *Teladorsagia*, *Haemonchus* and *Trichostrongylus* which also infected humans (Pestechian et al. 2014). Moreover, most capillariids are known as zoonotic (Moravec 2000, 2001). Although animal and human occupation of the archaeological sites were not simultaneous, some parasites could remain infective during a long time, the presence of camelid dung in archaeological sites being a potential source of parasitic infection for humans by the ingestion of food or water contaminated with faeces of infected camelids.

Parasites like Trichostrongyloidea attributable to *Nematodirus* sp. or *Lamanema* sp., *Calodium* sp. and *E. macusaniensis* affected South American camelids, mainly guanaco, living in Patagonia since the Pleistocene-Holocene transition. Furthermore, gastrointestinal parasite fauna of patagonian camelids did not vary greatly from Pleistocene-Holocene transition to late Holocene, although environmental conditions fluctuated throughout this period (Mancini 1998), indicative of the strength and the stability of this association over time.



**Fig. 3** NMDS ordination diagram of coprolites based on log-transformed ( $x+1$ ) abundances and Bray-Curtis similarities (stress=0.15). Numbers identify archaeological layers

South American camelids originated in the plains of North America around 9 to 11 million years ago and during the Pliocene-Pleistocene transition. Some extinct species of the genus *Hemiauchenia* migrated to South America, via the Isthmus of Panama, where they had diversified (Wheeler 1995). In the course of their dispersion into South America, camelids probably carried parasites similar to those found in the present study. Leguía et al. (1995) found similar parasite diversity in coprolites of prehispanic (900–1000 years of antiquity) llamas and alpacas of the archaeological site “El Yaral”, Peru. The author reported the presence of *E. macusaniensis* and *Eimeria invivaensis* oocysts as well as *N. lamae*, *L. chavezii*, *Capillaria* sp. and eggs of *Trichuris* sp.

The presence of similar parasite fauna in prehispanic camelids from Peru (Leguía et al. 1995) and from Patagonia (present research) is indicative of both the strength and stability of the co-phylogenetic host-parasite assemblages across the geographical and environmental distribution. It also reinforces the hypothesis that camelids carried most of the parasites found in the present research during their dispersion into South America.

The knowledge of parasite fauna that affected wild prehispanic camelids provided information on the geographical origin of some parasites, on possible dispersal routes of some parasite diseases that affect current wild and domestic camelids and on issues related to the evolution of the host-parasite relationship.

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