Zoonotic parasites associated with felines from the Patagonian Holocene

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Feline coprolites were examined for parasites with the aim of studying ancient infections that occurred in the Patagonian region during the Holocene period. Eggs compatible to Trichuris *sp.,* Calodium *sp.,* Eucoleus *sp.,* Nematodirus *sp.,* Oesophagostomum *sp. (Nematoda),* Monoecocestus *sp. (Cestoda) and* Eimeria macusaniensis *(Coccidia) were recovered from faecal samples. The results obtained from the analysis provide evidence of consumption by felids of the viscera of both rodents and camelids. This knowledge allows for improved explanations as to the distribution of parasitism and its significance to the health of humans and animals inhabiting the area under study during the Middle Holocene.*

Key words: paleoparasitology - feline coprolites - archaeological site - ancient parasitism - Middle Holocene

Paleoparasitology can be defined as the study of parasitic remains collected from both archaeological and paleontological materials (Ferreira et al. 1979). The examination of these historic parasites can provide valuable information related to the antiquity of human-parasite relationships, parasite dispersion, human migrations and the environment and culture of extinct populations. In addition, they provide knowledge of the major conditions of health and illness related to the presence of parasites in the past (Reinhard 1992, Bouchet et al. 2003). Coprolites, mixed with soil sediments, are usually found in archaeological sites. The remains of coprolites are mainly cysts, eggs and larvae and they can often be identified after the rehydration of faecal samples.

Paleoparasitological antecedents from Southern Patagonia were conducted beginning in the year 2000 (Fugassa et al. 2006a, b, 2008, Sardella & Fugassa 2009). In Perito Moreno National Park, Santa Cruz Province, Argentina, several archaeological sites were discovered. These sites represent an important source of evidence for pre aboriginal-European contact (approximately 10,500-3,000 years BP). From these sites, coprolites from human remains and other fauna (camelids, canids, felines, rodents and others) were collected. The paleoparasitological evidence allowed us to gain insight into the bioanthropological (ancient zoonosis, diet etc.), paleoenvironmental and biogeographical conditions prevalent in the area during the Holocene.

The aim of the present study was to examine the parasite fauna found in coprolites of felids collected from an archaeological site within Southern Patagonia. In addition, we evaluated their zoonotic potential to humans inhabiting the area in the past.

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The coprolites examined were recovered from the Cerro Casa de Piedra (CCP) archaeological site. The site is at a longitude and latitude of 47°40'S, 72°30'W, respectively, and located in a Patagonian forest-steppe ecotone in the valley of Burmeister Lake, Perito Moreno National Park. CCP is composed of a hill of volcanic origin, with caves and rock shelters facing the north. In cave 5 (CCP5), human occupations were dated from *ca*. 6,780-6,540, *ca*. 5,170-4,330 and *ca*. 2,740-2,550 years before present (BP) (Civalero & Aschero 2003). Three felid coprolite samples (numbers 40, 43 and 46) collected from layer IV (6540 ± 110 BP) of CCP 5 were examined for parasitic remains.

Coprolites were inventoried and processed individually as described by Fugassa et al. (2006b). The examination consisted of an external observation of faeces (colour, texture, inclusions and measurements) conducted according to Chame (2003). Internal and external samples of each coprolite (0.5 g) were rehydrated in 4 mL of a 5% formaline acetic solution in 0.5% tris-sodium phosphate for one week (Callen & Cameron 1960). Next, samples were homogenised and allowed to spontaneously sediment (Bouchet et al. 2003). The external sediment was separated by excision with a sterile scalpel. The material that was sedimented into the tube was recovered with a pipette and a total of 20 slides for each sample were observed. All slide observations were conducted with the addition of one drop of glycerine. Eggs of the parasites were measured and photographed at a magnification of 400X. The measurements were all taken from well-preserved eggs.

Coprolites exhibited whitish coloration on the surfaces as well as in the interior (Fig. 1). The surfaces were compact and hard, with flat aspects. The larger diameters of the coprolites ranged from 32-36 mm. After rehydration, they exhibited the intense and the typical smell of carnivore faecal material.

The macroscopic observation of coprolite number 40 showed hairs of 10-25 mm in length that were dark to brown in colour. The observation also showed plant fibres on the surface (probably of foreign origin), remains

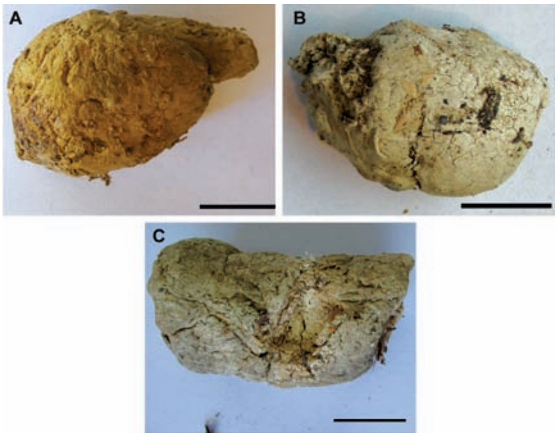

Fig. 1: coprolites examined collected from Cerro Casa de Piedra 5. A: coprolite 40: B: coprolite 43; C: coprolite 46. Bar = 20 mm.

of charcoal, bones of different animals and concretions constituted by bones partially digested in the interior. In the samples from the surface of the coprolite, 273 eggs of Nematoda, attributable to Order Enoplida, Genus *Calodium* Dujardin, 1845, likely *Calodium hepaticum* (Bancroft, 1893) Moravec 1982 (= *Capillaria hepatica* Bancroft, 1893) were found (Fig. 2A). These eggs measured 57.5-75.0 µm (66.48 ± 3.8; n =179) in length and 35.0-45.0 µm (39.72 ± 1.97; n = 179) in width. Capillariids eggs (n = 3), likely from *Eucoleus* sp. were also observed. These eggs had measurements of 67.5 µm in length by 35.75 µm in width. From the interior of the coprolite, 290 eggs of *C. hepaticum* and one egg of *Eucoleus* sp. were recovered.

The macroscopic examination of coprolite number 43 allowed for us to recognise the presence of bones and hairs of brown ochre colour in their distal extremes. These hairs were 40 µm in diameter and 15 mm in length. The medulla was blocked, and compatible with those of small marsupials or rodents. A fragment of hair that was 100 µm in diameter and 12 mm in length with yellowish-brown colour with distal extreme and brown and yellowish-brown apex was also registered. The medulla (triseriate to tetraseriate) and cuticle scale patterns corresponded to the normal mosaic type in the hairs compatible with a rodent, likely belonging to the genera of *Akodon* or *Octodon*. One egg (71.25 by 115.0 µm) identified to likely be *Oesophagostomum* Rudolphi, 1803 (Nematoda: Strongylida) was found in the external sample of the coprolite. From the interior of the faecal material, one egg (68.75 by 43.75 µm) was identified as *Trichuris* Roederer, 1761 (Nematoda: Enoplida) (Fig. 2B) and one egg (32.5 by 30.0 µm) was identified to likely be *Monoecocestus* Beddard, 1914 (Cestoda: Anoplocephalidae) (Fig. 2C). Additionally, one embryonated egg (113.5 by 55.0 µm) of *Oesophagostomum* (Fig. 2D) and one egg of *Calodium*, probably *C. hepaticum* were also registered.

In coprolite number 46, the macroscopic observation allowed us to identify short hairs and fragments of partially digested bones. The microscopic examination registered abundant fragments of plants and several large particles of charcoal. The surface sample was characterised by an intense colloid that was difficult to clearly observe. Both the external and internal samples showed eggs of 60.0-70.0 µm length (65.16 ± 3.3; n = 8) by 35.0-41.25 µm width (39.28 ± 2.14; n = 8), attributable to *C. hepaticum*. From the internal sample, two eggs of 103.75 by 135.0 µm, compatible with *Nematodirus* Ransom, 1907 (Nematoda: Trichostrongylidae) (Fig. 2E) and one oocyst of 112.5 by 80.0 µm (Apicomplexa: Coccidia: Eimeriidae), likely *Eimeria macusaniensis* Guerrero, Hernández, Bazalar et Alva, 1971 were also found.

All coprolites showed similar morphological features. Their shape, colouration and sizes were similar to those of *Puma concolor* (Chame 2003). The macroscopic remains that were found also indicate a strictly carnivorous diet, which rules out a possible human or other omnivorous origin, such as canids. The smell of the rehydrated material without fixation was distinguishable from the smell of canid faecal material (Fugassa et al. 2006b).

The paleoparasitology examination showed variability in the parasitic contents. Therefore, it could be suggested that, despite exhibiting a common zoological origin, they corresponded to separate events. However, it is unknown if the samples came from the same individual. Samples 40 and 46 were found in nearby squares, while the sample 43 was obtained from a distant grid within the archaeological site. The three samples shared only the presence of numerous eggs of *Calodium* sp., with morphology similar to *C. hepaticum*. This nematode parasitises the liver of the rodent host. The eggs are deposited in the liver and they are then released when the host dies or when a predator ingests the host, passing to the environment through the faeces of the last host. The presence of numerous eggs of this nematode indicates environmental contamination in addition to the exposure of hunter-gatherers to infection and thus eventual illness. In humans, the infections of *C. hepaticum* can cause hepatitis, splenomegaly, ascites and eosinophilia, diarrhea or constipation and abdominal distension (Acha & Szyfres 1989).

Nematodes from the genera *Nematodirus* sp. and *Oesophagostomum* sp. typically infect herbivores. The eggs are released in the faeces and the larvae then hatch in the ground. In the ground, hosts eat the larvae during ingestion of vegetables or other herbivore food sources, repeating the infectious cycle (Leguía 1991). Thus, the presence of coprolites in carnivores may correspond to their consumption of herbivores.

Therefore, their presence in sample 46 most likely represents a pseudo-parasitism. The eggs are liberated with the faeces and the larvae then hatch in the soil where they are ingested by another host, repeating the infectious cycle (Leguía 1991).

In Patagonia, different species of nematodes exhibit distinctive strategies for dispersal in the rigorous environment. A group of them produced thick-walled eggs throughout the year that can suffer hypobiosis (i.e., *Nematodirus*), while others are reduced in the production of eggs during the winter (i.e., *Oesophagostomum*). In this sense, coprolite 43 would indicate that it was deposited in the summer season. The palynological studies in progress may help to elucidate and potentially confirm this hypothesis.

The eggs of *Trichuris* sp. found were morphologically compatible with *T. campanula*, a parasite of felids. They are also similar to those from another species of *Trichuris* sp., which are known to parasitise rodents and camelids. It should be noted that the discovery of only one egg is insufficient to speculate on its zoological origin. Therefore, it could be a true infection or conversely a result of the consumption of viscera of some prey. In previous investigations conducted at several archaeological sites in the region, *Trichuris* sp. was reported from samples of human origin, but measurements did not correspond with those of the species that currently parasitise humans (*Trichuris trichiura*) (Fugassa et at. 2006a). Consequently, it is suggested that infestations of trichurids could have existed in hunter-gatherers proceeding from other mammals. In this sense, human cases of *Trichuris vulpis* infections, a canid parasite, have been reported several times (Singh et al. 1993, Dunn et al. 2002).

As for the presence of a single anoplocephalid cestode egg, the life cycle involves an oribatid mite that ingests the egg and develops cysticercoid larvae. The definitive host (herbivore or omnivorous) becomes infested when it eats the acari (Beveridge 1994). The egg found in the coprolite most likely came from the intestinal content of a prey animal. Beldoménico et al. (2005) found anoplocephalids in wild felines of Patagonia. In sample 43, there were numerous rodent hairs present, attributable to *Akodon* sp. or *Octodon* sp., both of which are potential hosts for this parasite. The environmental contamination with cestode eggs does not imply a direct zoonotic risk because the human only is infected if it ingests mites that previously have consumed such eggs.

E. macusaniensis is a specific coccidian of South American camelids (Guerrero et al. 1971). For these reasons, the presence of this parasite in the present study would be consequence of the consumption of camelids. Because these coccidians exhibit high host specificity, no risk of infection would be observed for the populations of felines or humans. Their presence, along with the finding of trichostrongylids in the samples examined, reinforces the hypothesis of an alimentary origin via consumption of parts of the digestive organs of prey animals.

There are a growing number of paleoparasitological records from human remains. These records could be used to indicate the frequency of zoonoses during the Holocene in different ancient populations (Sianto et al. 2009). The presence of capillariids, trichurids and trichostrongylids in the coprolites would have represented a potential risk of zoonoses. As previously mentioned, there are reports of human infections by parasites belonging to these wild species (Hall & Sonnenberg 1956, Morishita & Tsuneo 1960, Singh et al. 1993, Taylor et al. 2001, Dunn et al. 2002).

The most parsimonious hypothesis is that the small number of parasitological studies carried out in ancient and present populations generates an underestimation of the potential zoonoses in the past. The utilization of the caves inhabited by big felines and by other mammals was probably not simultaneous with the human occupations, but it could be contemporary and alternative. The dispersal stages of many parasites (eggs, oocysts) are highly resistant

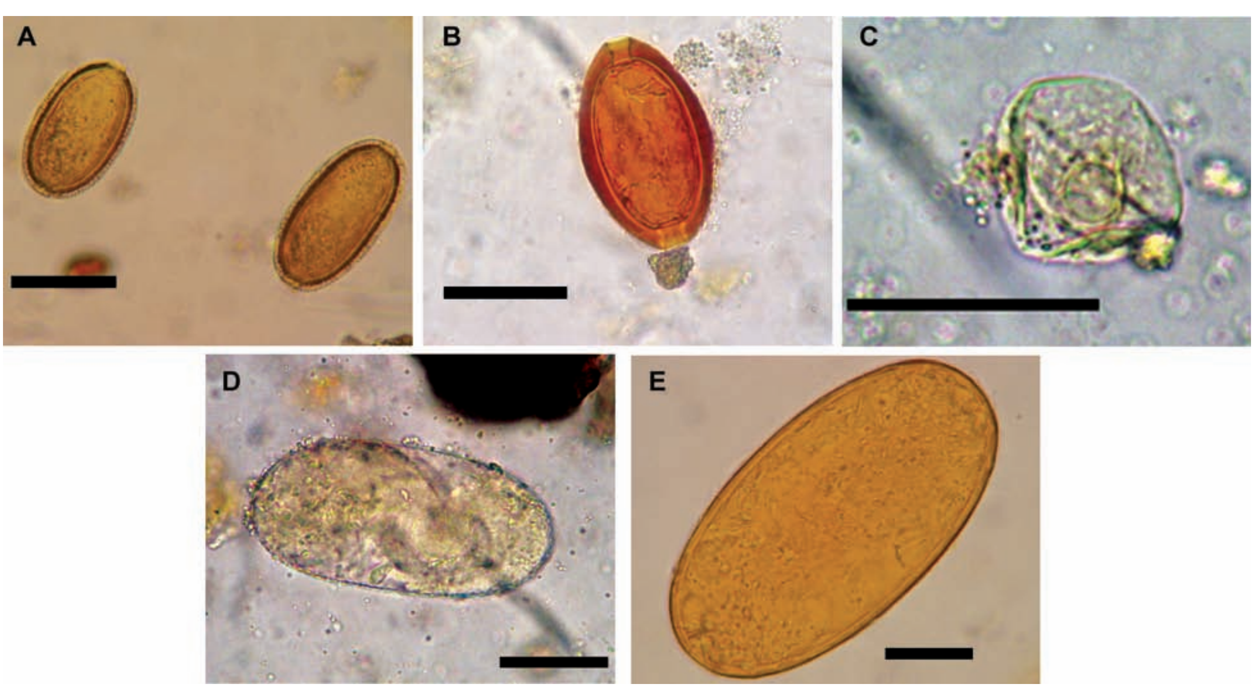

Fig. 2: parasites recovered from samples. A: *Calodium hepaticum*; B: *Trichuris* sp.; C: *Monoecocestus* sp.; D: embryonated egg of *Oesophagostomum*; E: *Nematodirus* sp. Bar = 40 µm.

and stable under varying ranges of humidity and temperature. This is particularly true when they are away from UV rays, such as those kept in rock shelters and caves, where the egg and cyst viability could be favoured.

This report increases the body of results regarding the occurrence of contributing species to ancient parasite distributions and to the paleoecological/biogeography analysis of specific parasites that are commonly associated with the colonization of domestic fauna along with the European colonization. Likewise, the results of the present study are an addition to other studies and they allow us to propose that the caves and rocky shelters of Patagonia, with intensive use by humans, have shown a parasitic contamination, meaning that they are potential centres for the dispersion of some potentially zoonotic parasites, both for humans and other animals.

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REFERENCES

Acha PN, Szyfres B 1989. *Zoonosis y enfermedades transmisibles comunes al humano y a los animales*, 2nd ed., Organización Panamericana de la Salud, Washington DC, 989 pp.

Beldoménico PM, Kinsella JM, Uhart MM, Gutiérrez GL, Pereira J, Ferreira HDV, Marutti C 2005. Helmintos of Geoffroy's cat, *Oncifelis geoffroyi* (Carnivora: Felidae) from the Monte Desert, central Argentina. *Acta Parasitol 50*: 263-2821.

Beveridge I 1994. Family Anoplocephalidae Cholodkovsky, 1902. In LF Khalil, A Jones, RA Bray, *Key to the cestode parasites of vertebrates*, CAB International, Cambridge, p. 315-366.

Bouchet F, Guidon N, Dittmar K, Harter S, Ferreira LF, Chaves SM, Reinhard K, Araújo A 2003. Parasite remains in archaeological sites. *Mem Inst Oswaldo Cruz 98*: 47-52.

Callen EO, Cameron TWM 1960. A prehistoric diet revealed in coprolites. *New Scient 8*: 35-40.

Chame M 2003. Terrestrial mammal feces: a morphometric summary and description. *Mem Inst Oswaldo Cruz 98*: 71-94.

Civalero MT, Aschero CA 2003. Early occupations at Cerro Casa de Piedra 7, Santa Cruz Province, Patagonia Argentina. In L Miotti, M Salemme, N Flegenheimer, *Where the south winds blow: ancient evidences for Paleo South Americans*, Center for the Study of the First Americans, Texas A&M University Press, College Station, Texas, p. 141-147.

Dunn JJ, Columbus ST, Aldeen WE, Davis M, Carroll KC 2002. *Trichuris vulpis* recovered from a patient with chronic diarrhea and five dogs. *J Clin Microbiol 40*: 2703-2704.

Ferreira LF, Araújo A, Confalonieri U 1979. Subsídios para a paleoparasitologia do Brasil: parasitos encontrados em coprólitos no município de Unaí, MG. Anais do V Congresso da Sociedade Brasileira de Parasitologia, Campinas, p. 56.

Fugassa MH, Araújo A, Guichón RA 2006a. Quantitative paleoparasitology applied to archaeological sediments. *Mem Inst Oswaldo Cruz 101*: 29-33.

Fugassa MH, Denegri GM, Sardella NH, Araújo A, Guichón RA, Martínez PA, Civalero MT, Aschero C 2006b. Paleoparasitological records in canid coprolite from Patagonia, Argentina. *J Parasitol 92*: 1110-1111.

Fugassa MH, Taglioretti V, Gonçalves MLC, Araújo A, Sardella NH, Denegri GM 2008. *Capillaria* spp. eggs in Patagonian archaeological sites: statistical analysis of morphometric data. *Mem Inst Oswaldo Cruz 103*: 104-105.

Guerrero C, Hernández J, Bazalar H, Alva J 1971. *Eimeria macusaniensis* (Protozoa: Eimeriidae) of the alpaca *Lama pacos*. *J Protozool 185*: 163-165.

Hall JE, Sonnenberg B 1956. An apparent case of human infection with the whipworm of dogs, *Trichuris vulpis* (Froelich, 1789). *J Parasitol* 42: 197-199.

Leguía GP 1991. The epidemiology and impact of llama parasites. *Parasitol Today 7*: 54-56.

Morishita K, Tsuneo T 1960. A case of *Capillaria* infection causing cutaneous creeping eruption in man. *J Parasitol 46*: 79-83.

Reinhard KJ 1992. Parasitology as an interpretative tool in archaeology. *Am Antiq* 57: 231-245.

Sardella NH, Fugassa MH 2009. Parasites in rodents coprolites from the historical archaeological site Alero Mazquiarán, Chubut Province, Argentina. *Mem Inst Oswaldo Cruz 104*: 37-42.

Sianto L, Chame M, Silva CSP, Gonçalves MLC, Reinhard K, Fugassa MH, Araújo A 2009. Animal helminths in human archaeological remains: a review. *Rev Inst Med Trop Sao Paulo 51*: 119-130.

Singh S, Samantaray JC, Singh N, Das GB, Verma IC 1993. *Trichuris vulpis* infection in an Indian tribal population. *J Parasitol 79*: 457-458.

Taylor LH, Latham SM, Woolhouse MEJ 2001. Risk factors for human disease emergence. *Phil Trans R Soc Lond B 356*: 983-989.