

Parasitological study of mountain viscacha fecal pellets from patagonia over the last 1200 years ('Cueva Peligro', Chubut province, Argentina)

Research Article

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Abstract

The aim of the present study was to examine the parasite fauna present in mountain viscacha *Lagidium viscacia* (Caviomorpha, Chinchillidae) fecal pellets collected from 'Cueva Peligro', a cave located in Chubut province, Patagonia, Argentina, throughout the last 1200 years. A total of 84 samples were examined for parasites. Each pellet was whole processed: rehydrated, homogenized, sediment and examined using light microscopy. The samples and eggs of parasites present were described, measured and photographed. Thirty-eight samples tested positive for the nematodes *Heteroxytnema* (*Cavioxyura*) *viscaciae* Sutton & Hugot, 1989, *Helminthoxys effilatus* Schuurmans-Stekhoven, 1951 (Oxyurida: Oxyuridae), *Trichuris* sp. Roederer, 1761 (Trichinellida: Trichuridae) and one anoplocephalid species (Cestoda: Anoplocephalidae). This is the first time that *H. effilatus* is reported from ancient times. Significant differences of parasite occurrences through this temporal period were recorded. Parasitic life cycles and their presence along the studied period are so discussed.

Introduction

Mountain viscachas are large caviomorph rodents that inhabit arid regions of western and southern South America, from the highlands of Ecuador through the Andes of Peru and Bolivia to the coastal mountains of Chile and Patagonian steppe of Argentina. This rodent is found in rocky outcrops and is highly gregarious, living in colonies that may range widely in size. The presence of viscachas is readily detected by distinctive fecal pellets that can be found throughout the colony, on top of rocks or in sheltered crannies (Spotorno and Patton, 2015).

In the last years, several studies have been carried out in different areas of Patagonia involving micromammals fossil remains. The available data indicate that these communities had minor changes during the last 10 000 years. This holocene stability, despite the occurrence of climatic pulses, contrasts with the current structure of the assemblages, which shows a remarkable loss of diversity and even local or regional extinctions (e.g. Pardiñas *et al.*, 2000, 2011, 2012; Udrizar Sauthier, 2009; Pardiñas and Teta, 2013; Teta *et al.*, 2014). It has been suggested that these recent changes have been unleashed by anthropogenic activities, such as overgrazing, deforestation or the implantation of agroecosystems (Teta *et al.*, 2014 and references cited therein).

Paleoparasitology is the study of parasites found in the archaeological or paleontological material. In a broad sense, paleoparasitologists are interested in the evolution of parasite–host–environment relationships, as well as in the origin and the evolution of infectious diseases within a paleoepidemiological perspective (Araújo *et al.*, 2003). Beltrame *et al.* (2012, 2014, 2016) performed paleoparasitological studies on mountain viscacha coprolites from Patagonia, where nematode and cestode eggs were reported. The species found were also recorded in studies with modern mountain viscachas (Hugot and Sutton, 1989a; Denegri *et al.*, 2003; Tantaleán *et al.*, 2013). The environmental changes occurred in the last 100 years in Patagonia reflected in diversity changes of micromammals, could also be reflected in the evolution of parasite–mountain viscacha–environment relationships throughout time.

The aim of the present study was to examine the parasite remains from mountain viscacha fecal pellets from the site 'Cueva Peligro', Patagonia, through the last 1200 years and to discuss the paleoparasitological findings in temporal and paleoecological contexts. This is the first paleoparasitological examination of pellets of mountain viscachas throughout time, which include samples from modern, recent and ancient times. This study is part of an ongoing project in the area, where the impact of anthropic activities on micromammal community is under study.

Material and Methods

The paleontological site 'Cueva Peligro' (CP; 43°40'18" S, 66°24'52" W) is located near the southern margin of the Chubut River, about 6 km downstream of the Villa Dique Florentino Ameghino, Chubut Province, Argentina (Fig. 1). The site is a cave emplaced on a rocky front of approximately 35 m of height (Marifil Formation, Jurassic), with a single entrance (4.57 m wide) and a single long gallery (ca.30 m). The general dip of the floor of the cave is towards the outside, with some sectors that can reach 45° of inclination and others that approach 0°. From a phytogeographic point of view, CP is located in the Monte Phytogeographic Province (León *et al.*, 1998). A 1 × 1 m² square grid was defined and the sequence was excavated by artificial layers of 3 cm thick until reaching the basement rock at a depth of 0.42 m. The extracted sedimentary material was sieved through a 5 mm mesh.

Feces samples from three layers were processed at the Laboratorio de Tritio y Radiocarbono (LATYR), Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata (LP), Argentina, to obtain radiocarbon dates. Calibration for the Southern Hemisphere was made through SHCal13 curve (Hogg *et al.*, 2013) using OxCal 4.2 software (Bronk Ramsey, 2009) and weighted average was used as age estimator (Telford *et al.*, 2004; Table 1). All the sequence was dated from the Late Holocene, including the historic period and actual times, approximately 1200 years throughout time. The radiocarbon dates of these layers range between 1220 ± 70 ¹⁴C years BP to modern years (Table 1).

The area has been under a progressive transformation of natural environments, mainly due to extensive sheep farming. The beginning of this activity corresponds to the 1880–1885 AD period. Based on the number of sheep feces found in each level, three time periods were defined: modern (1950–2015 AD, high sheep feces content), recent (1885–1950 AD, medium sheep feces content) and ancient (previous to 1885 AD, low/null sheep feces content). Taking into account that: low/null: 0–15 sheep feces units 1.200 cm⁻³ of sediment, medium: 15–80 sheep feces units 1.200 cm⁻³ of sediment, high: >80 sheep feces units 1.200 cm⁻³ of sediment.

Six fecal pellets assigned to mountain viscachas from each layer were examined for parasites. A total of 84 samples were observed. Samples were inventoried and processed individually. Each sample was fully processed by rehydration in a 0.5% water solution of tris-sodium phosphate in a glass tube for at least 3 days, followed by homogenization, processed by spontaneous sedimentation (Lutz, 1919) and preserved in 70% ethanol. Twenty slides were prepared from each sample, along with the addition of one drop of glycerin to each slide and were examined using light microscopy. Eggs of parasites were measured and photographed at 400 × magnification.

Differences in parasite assemblages composition among modern, recent and ancient pellets were tested using permutational multivariate analyses of variance (PERMANOVA, Anderson, 2001). This is a non-parametric technique that uses label permutation to estimate the distribution of the test statistics under the hypothesis that within-group distances are not significantly different from between-group distances. The Sørensen similarity index was used as the distance measure for occurrence data. The Sørensen index of similarity was calculated on the basis of presence/absence between samples, according to the formula: $S = 2C/(A + B)$, where S = index of similarity, A = number of species in one sample, B = number of species in the other sample and C = number of species common to both samples. A test of homogeneity of dispersions (PERMDISP, Anderson, 2006) was done in order to test differences in dispersions among groups because

PERMANOVA is sensitive to differences in dispersions. A one-way PERMANOVA was performed using 'Period' as a factor (with three levels: modern, recent and ancient). Type III error and unrestricted permutation of raw data were used for unbalanced designs. Besides, a non-metric multidimensional scaling (NMDS, Clarke and Warwick, 2001) was used to ordinate samples in an ordination space. NMDS was used to avoid assumptions of linearity. Multivariate analyses were performed using the software PERMANOVA + for PRIMER (Plymouth Routines In Marine Ecological Research) (v. 5.2) (Anderson *et al.*, 2008).

Results

Fecal pellets were dark brown and cylindrical, with smooth surfaces and a flat end and the other point (Fig. 2). Average measurements of feces (N = 84) were 13.77 ± 2.38 mm long by 4.97 ± 0.49 mm wide. All layers examined contained parasite eggs (Table 1). Thirty-eight pellets were positive for parasites. Eggs of the parasite found were assigned to the nematodes *Heteroxyema (Cavioxyura) viscaciae* Sutton and Hugot, 1989, *Helminthoxys effilatus* Schuurmans-Stekhoven, 1951 (Oxyurida: Oxyuridae), *Trichuris* sp. Roederer, 1761 (Trichinellida: Trichuridae) and one anoplocephalid (Cestoda: Anoplocephalidae).

Eggs of *H. viscaciae* (Fig. 3a), with single thick walls and with a rounded pole and the other sharp, without operculum, were found in five pellets from modern, recent and ancient times. Average egg measurements (N = 31) were 135.5–142.5 (135.2 ± 7.18) µm long by 57.5–70.0 (64.75 ± 3.59) µm wide.

Twelve pellets contained eggs of nematode attributed to *H. effilatus*. The samples belong to recent and ancient times. This nematode was not found from modern samples. The eggs were oblong, brown, with the striated and thick wall. One rounded pole and the other sharp, with a subterminal and notorious operculum (Fig. 3b), and some of them were embryonated. Average egg measurements (N = 29) were as follows: 110.0–122.5 (119.57 ± 4.77) µm long by 62.5–75.0 (67.74 ± 3.64) µm wide.

Eggs of *Trichuris* sp. (Fig. 3c) were observed in one pellet from modern times. These eggs were lemon-shaped, with a smooth surface and polar plugs. Measurements of eggs (N = 4) were 70.0–75.0 (71.63 ± 2.88) µm long by 37.5–42.5 (39.95 ± 2.5) µm wide.

Twenty-one samples were positive for anoplocephalids (Fig. 3d). Samples belong to modern, recent and ancient period. The eggs presented square or circular shape, with a piriform apparatus. The size ranges (means) of the eggs that were measured (N = 81) were 62.5–82.5 (69.53 ± 6.75) µm long by 62.5–80.5 (56.7 ± 1.5) µm wide.

Multivariate analysis performed on parasite occurrences in coprolites showed no significant differences in dispersions between groups (PERMDISP, $P = 0.156$), but significant differences between periods (PERMANOVA, $P \leq 0.01$, Table 2). The Pair-wise result showed a significant difference between modern and ancient periods ($P \leq 0.01$, Table 1). NMDS plot evidenced these differences with a stress value of 0.01 (Fig. 4). This difference between modern and ancient periods could be explained by the presence of *Trichuris* sp. and the absence of *H. effilatus* in actual coprolites in comparison with ancient samples.

Discussion

This is the first paleoparasitological study of mountain viscacha fecal pellets that includes a temporal period through the last 1.200 years to modern times. Previously, Beltrame *et al.* (2012) studied mountain viscacha coprolites collected from Cueva Huenul 1, northern Neuquén (Patagonia, Argentina), an



Fig. 1. Geographic location of the paleontological site 'Cueva Peligro', Chubut Province, Patagonia Argentina.

archaeological site that provides stratified sequences of archaeological and palaeontological remains, dated from Late Pleistocene/Early Holocene Transition to Late Holocene period ($13\,844 \pm 75$ – $14\,16 \pm 37$ years BP). The feces were positive for *Viscachataenia quadrata* and *Monoecocestus* sp. (Cestoda: Anoplocephalidae), and for *H. viscaciae*. Furthermore, coprolites from fossil rodent middens (dated among $9\,240 \pm 130$ and $2\,540 \pm 80$ ^{14}C years B.P.) attributed to mountain viscachas were studied. Parasite eggs assigned to *H. viscaciae*, *Helminthoxys* sp., *Trichuris* sp. and one unidentified nematode (Beltrame *et al.*, 2016) were found. Mountain viscacha coprolites (dated at $2\,210 \pm 70$ BP to present) from the paleontological site 'Los Altares Profile', Chubut Province were also studied and the samples were positive for eggs of *H. viscaciae* and for two morphotypes of anoplocephalids (Beltrame *et al.*, 2014).

In the present study, the parasitic species *H. viscaciae* and an anoplocephalid, were registered in modern, recent and ancient pellets. However, *H. effilatus* was just observed from recent and ancient samples. *Trichuris* sp. was only recorded in one sample from the modern period.

Species of oxyurid nematodes are monoxenic parasites that live in the posterior third of the digestive tract of various vertebrates and arthropods. Eggs are dispersed to the environment, where with favorable conditions of humidity and temperature can become a continual source of eggs for oral infection (Anderson, 2000). Oxyuroidea from vertebrates can be grouped into three families: Pharyngodonidae, Oxyuridae and Heteroxynematidae (Petter and Quentin, 2009). Heteroxynematidae includes nematodes that evolved in sciuriform, caviomorph and miomorph mammals. *Heteroxynema viscaciae* (Heteroxynematidae) is a parasite found in the caecum and large intestine from *L. viscacia* from Chubut Province, Argentina, first described by Hugot and Sutton, 1989a. It was also observed from mountain viscachas

and wild viscachas (*Lagostomus maximus*) from other areas from Argentina and Perú (Foster *et al.*, 2002; Ferreira *et al.*, 2007; Tantaleán *et al.*, 2013).

Helminthoxys sp. (Oxyuridae: Syphaciinae) is a parasite of neotropical caviomorph rodents. The genus *Helminthoxys* comprises seven species: *Helminthoxys caudatus* (syn. *Helminthoxys pujoli*), *Helminthoxys effilatus* (syn. *Helminthoxys velizi*), *Helminthoxys freitasi*, *Helminthoxys tiflophila*, *Helminthoxys quentini*, *Helminthoxys urichi*, *Helminthoxys gigantea* and *Helminthoxys abrocomae* (Hugot and Sutton, 1989b). Several studies have demonstrated the high host-specificity among Oxyuridae (Hugot, 2003). In particular, these studies showed that the distribution of Syphaciinae belonging to the same family of rodent hosts has a close parallel phylogeny with them. *Helminthoxys effilatus* is a parasite of the mountain viscacha (Hugot and Sutton, 1989b). Eggs of *Helminthoxys* spp. are similar. Nevertheless, due to the specificity of this genus, we can hypothesize that the eggs found in the studied samples belong to the species *H. effilatus*. This would be the first time that *H. effilatus* is reported from ancient times. This oxyurid was also registered in actual samples of mountain viscacha from Argentina and Perú (Hugot and Sutton, 1989b; Tantaleán *et al.*, 2013). Nevertheless, in the present study, it was not found in samples from modern times. Due to the favourable conditions of humidity and temperature that these eggs need to continue the biological cycle, the environmental changes along the last period could be responsible for the absence/presence of this species in the samples under study.

Trichuris spp. include intestinal parasites of the caecum and colon of mammals, mainly humans, primates, pigs, ovines, goats, cervids, rodents and canids, with eggs that mature in the soil. They hatch in the small intestine of the definitive hosts and the larvae migrate to the large intestine, where they penetrate the intestinal mucosa and develop through four molts before reaching the adult stage (Anderson, 2000). To date, a total of 24 species of *Trichuris* have been described from 10 families of American rodents (Robles and Navone, 2014). *Trichuris* spp. in South American rodents from ancient material were cited (Beltrame *et al.*, 2014). Moreover, there is a previous record of two *Trichuris* species in coprolites of *L. viscacia* from fossil rodent middens of Cueva Huenul 1 archaeological site, northwest Patagonia, dated at $2\,540 \pm 80$, $5\,730 \pm 70$ and $8\,438 \pm 37$ years B.P. (Beltrame *et al.*, 2016). Species of *Trichuris* have been infrequently recorded in high latitudes. This infrequent recording is probably related to the behavior of host species, characteristics of the environments where eggs are deposited, and/or lower degree of sampling effort in the area with respect to other areas of the country (Robles and Navone, 2014).

The anoplocephaline cestodes (Cyclophyllidae: Anoplocephalidae) represent a diverse group of parasites infecting both terrestrial mammals (placentals and marsupials) and birds. Based on the number of genera present in these hosts, the most important radiation of anoplocephalines has been in rodents and lagomorphs (Beveridge, 1994; Wickström *et al.*, 2005). The intermediate hosts of these cestodes are oribatid mites, which are ingested by their herbivorous definitive hosts (Beveridge, 1994). Anoplocephalids are parasites of zoonotic importance for animals and humans (Denegri *et al.*, 1998). As previously mentioned, *V. quadrata* and *Monoecocestus* spp. were found in modern and ancient samples. In the present study, only one anoplocephalid morphotype egg was found with similar characteristics to those found from Los Altares and Cueva Huenul (Beltrame *et al.*, 2013).

The difference between modern, recent and ancient periods could be explained by the presence of *Trichuris* sp. and the absence of *H. effilatus* in actual coprolites in comparison with recent and ancient samples. The fact that two of the recorded

Table 1. Depth, time period, radiocarbon dates, calibrated age, other fecal pellets present and parasite egg found from all studied layers from 'Cueva Peligro'

Temporal period	Layer	Depth (cm)	Other fecal pellets found	Dating (¹⁴ C BP)	Calibrated age (cal AD; <i>weighted average</i>)	Pellet N°	Parasites found		
Modern	1	0	Micromammals and sheep			1.1	Negative		
						1.2	Anoplocephalid		
						1.3	Anoplocephalid		
						1.4	Negative		
						1.5	Anoplocephalid		
						1.6	Negative		
	2	0–3	Micromammals and sheep				2.1	Anoplocephalid	
							2.2	Anoplocephalid	
							2.3	Negative	
							2.4	Negative	
							2.5	Anoplocephalid	
							2.6	<i>Heteroxynema viscaciae</i>	
	3	3–6	Micromammals and sheep				3.1	Negative	
							3.2	Negative	
							3.3	<i>Trichuris</i> sp.	
							3.4	Negative	
							3.5	Anoplocephalid	
							3.6	Negative	
Recent	4	6–9	Micromammals and sheep				4.1	Negative	
							4.2	Negative	
							4.3	<i>Heteroxynema viscaciae</i>	
							4.4	Negative	
							4.5	Negative	
							4.6	Anoplocephalid	
	5	9–12	Micromammals					5.1	Negative
								5.2	Negative
								5.3	Anoplocephalid
								5.4	Negative
								5.5	Negative
								5.6	Negative
	6	12–15	Micromammals					6.1	<i>Heteroxynema viscaciae</i>
								6.2	Negative
								6.3	Negative
								6.4	Anoplocephalid
								6.5	Anoplocephalid
								6.6	Anoplocephalid
	7	15–18	Micromammals					7.1	Anoplocephalid
								7.2	Negative
								7.3	Negative
								7.4	<i>Helminthoxys effilatus</i> Anoplocephalid
								7.5	Negative
								7.6	Negative
8	18–21	Micromammals		Modern (LP-3076)		8	Without samples		
Ancient	9	21–24	Micromammals			9.1	Negative		

(Continued)

Table 1. (Continued.)

Temporal period	Layer	Depth (cm)	Other fecal pellets found	Dating (^{14}C BP)	Calibrated age (cal AD; weighted average)	Pellet N°	Parasites found
						9.2	Negative
						9.3	<i>Helminthoxys effilatus</i>
						9.4	Negative
						9.5	<i>Helminthoxys effilatus</i>
						9.6	Negative
	10	24–27	Micromammals	880 ± 70 (LP-3153)	1183	10.1	Anoplocephalid
						10.2	Anoplocephalid
						10.3	<i>Helminthoxys effilatus</i>
						10.4	<i>Helminthoxys effilatus</i>
						10.5	Negative
						10.6	<i>Heteroxynema viscaciae</i>
	11	27–30	Micromammals			11.1	Negative
						11.2	Anoplocephalid
						11.3	Negative
						11.4	<i>Helminthoxys effilatus</i>
						11.5	<i>Helminthoxys effilatus</i>
						11.6	Negative
	12	30–33	Micromammals			12.1	Negative
						12.2	<i>Helminthoxys effilatus</i>
						12.3	<i>Helminthoxys effilatus</i>
						12.4	Negative
						12.5	<i>Helminthoxys effilatus</i>
						12.6	Negative
	13	33–36	Micromammals			13.1	Negative
						13.2	Negative
						13.3	Anoplocephalid
						13.4	<i>Helminthoxys effilatus</i>
						13.5	Negative
						13.6	Negative
	14	36–39	Micromammals	1220 ± 70 (LP-3070)	856	14.1	Negative
						14.2	Negative
						14.3	<i>Heteroxynema viscaciae</i>
						14.4	Anoplocephalid
						14.5	Anoplocephalid
						14.6	Negative
	15	39–42	Micromammals			15.1	Negative
						15.2	Negative
						15.3	Anoplocephalid
						15.4	Negative
						15.5	Negative
						15.6	<i>Helminthoxys effilatus</i>

species (i.e. *H. viscaciae* and an anoplocephaline species) along the span of time studied had remained constant, may be due to a different capacity of adapting to a changing environment or/and that the conditions were not different enough to affect them. The record of trichurid eggs in just one sample in the

modern period strengthens the previous discussion that this species is infrequently recorded in high latitudes and very rare in the ancient material. Nonetheless, the pattern presented by *H. effilatus* where the high abundances recorded in the ancient period declined in the recent period, to finally be absent in modern



Fig. 2. Fecal pellets studied from the paleontological site 'Cueva Peligro'.



Fig. 3. Egg found from 'Cueva Peligro' (A) *Heteroxynema viscaciae* (Nematoda: Oxyuridae), (B) *Helminthoxys effilatus* (Nematoda: Oxyuridae), (C) *Trichuris* sp. (Nematoda: Trichuridae), (D) Anoplocephalid (Cyclophyllidea: Anoplocephalidae). Bar: 20 μ m.

Table 2. PERMANOVA and pair-wise PERMANOVA results based on Sorensen similarity coefficient between parasite occurrences in fecal pellets from 'Cueva Peligro' of different periods

Source	df	Pseudo-F	p (perm)
Period	2	4.07	0.0063
Residuals	35		
Total	37		
Pair-wise tests			
Groups	t	p (perm)	
Actual-Recent	0.54	1	
Actual-Ancient	2.36	0.01	
Recent-Ancient	2.08	0.02	

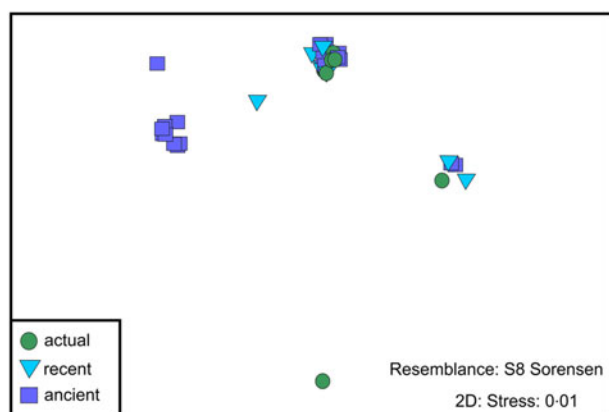


Fig. 4. NMDS plot of presence-absence of parasites in mountain viscacia fecal pellets from 'Cueva Peligro'.

times, is remarkable. This can be a consequence of a higher sensibility of this species to environmental changes. Indeed, oxyurid species are geohelminthes which undergo a period of development in the soil before being ingested. In a greater or lower degree, all these geohelminthes suffer the influence of the alterations of the soil characteristics and climate of the region (Chieffi, 2015). However, evidence showed that eggs of different species vary their resistance to extreme temperatures (Bunday and Cooper, 1989).

Concluding remarks

This is the first study of mountain viscacha parasites along the time. The presence of *H. viscaciae* and anoplocephalids in fecal pellets from mountain viscachas from CP have not changed significantly during the last 1200 years, although environmental conditions fluctuated throughout this period, indicating the stability of these associations over time. *Helminthoxys effilatus* was reported for the first time in ancient samples and was absent in modern fecal pellets studied. New studies are now needed and could contribute with the study of mountain viscacha-parasite-environment relationships throughout time.

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Conflicts of interest. None.

Ethical standards. Not applicable.

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