New parasitological findings for pre-Hispanic camelids

V. TAGLIORETTI[1,2]*, M. H. FUGASSA[2], D. RINDEL[3] and N. H. SARDELLA[1]

[1] *Paleoparasitología y Arqueología Contextual, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Juan B. Justo 2550, 7600 Mar del Plata, Buenos Aires, Argentina*
[2] *Laboratorio de Paleoparasitología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Funes 3350, 7600 Mar del Plata, Buenos Aires, Argentina*
[3] *Universidad de Buenos Aires, Instituto Nacional de Antropología y Pensamiento Latinoamericano, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina.*

SUMMARY

Paleoparasitological examination provides information of parasite–host associations in the past, shedding light on the geographical origin of some parasites, on the possible dispersal routes and on some of the processes that modelled the parasitic communities. The aim of the present study was to examine parasite remains present in camelid coprolites collected from the archaeological site Alero Destacamento Guardaparque, Patagonia and to discuss the paleoparasitological findings in a biogeographical and paleoecological context. Coprolites were collected from different stratified layers dating from middle to late Holocene, a period covering approximately 7000 years. Paleoparasitological examination revealed the presence of eggs attributed to *Lamanema chavezi* or *Nematodirus lamae*, *Nematodirus spathiger*, *Dictyocaulus* sp., eggs of two unidentified capillariids, *Strongylus*-type eggs and oocysts of *Eimeria macusaniensis*. Enteric parasites of camelids had not changed significantly during the Holocene up to the entry of introduced livestock, although environmental conditions fluctuated greatly throughout this period, indicating the stability of these associations over time. This is the first finding of *N. spathiger* and *Dictyocaulus* sp. in paleoparasitological record and their presence are associated with the interaction of camelids with introduced livestock, which likely allowed parasite host switching. In the present study, the zoonotic importance of parasites of camelids is also discussed.

Key words: paleoparasitology, camelids, introduced livestock, host switching, Holocene.

INTRODUCTION

Paleoparasitological examination provides an important source of information about past parasite–host associations that contribute to the understanding of the features/processes that modelled the structure and composition of parasite communities. The increasing paleoparasitological information recovered from humans and from fauna remains of Patagonia (Fugassa, 2005, 2007; Fugassa *et al*. 2006; Fugassa *et al*. 2007*a*, *b*; Sardella and Fugassa, 2009*a*, *b*; Sardella *et al*. 2010; Taglioretti *et al*. 2015, among others) improved paleoepidemiology inferences and defined the role of caves and shelters in the transmission of zoonoses. Among archaeological fauna, South American camelids are the native artiodactyls of major size in South America (Franklin, 1983). They originated in North America plains about 11–9 million years ago (Wheeler, 1995), and in their diversification and dispersion into South America, they likely carried some parasites and lost

others (Navone and Merino, 1989; Taglioretti, 2015; Taglioretti *et al*. 2015). Previous paleoparasitological examination of coprolites of camelids from Patagonia (Fugassa, 2007; Taglioretti *et al*. 2015) shed light on the importance of this kind of research in order to understand their parasite biogeography and on the necessity to expand the paleoparasitological information of camelids for a better comprehension of the processes that contributed to the establishment of the present camelids–parasites association in the studied area. Moreover, some parasites could remain viable for long time periods and as camelid coprolites were collected from sites occupied by humans, their paleoparasitological examination is an important source of evidence of the potential zoonoses that humans could be exposed in the past (Taglioretti *et al*. 2015).

Alero Destacamento Guardaparque (ADG) is an archaeological site located in Perito Moreno National Park (PMNP; Santa Cruz Province, 47° 57′S 72°05′W, Southern Patagonia) with evidence of camelid occupation since middle (7000 years BF) to late Holocene, including the Historic period (ca. 4000–100 years BP) (Rindel, 2004, 2008). This period is characterized by an increased climate variability including, during the final late Holocene, a trend towards the increase of aridity with a maximum recorded from 1150 to 600 years BP

* Corresponding author: Paleoparasitología y Arqueología Contextual, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Juan B. Justo 2550, 7600 Mar del Plata, Buenos Aires, Argentina, and Laboratorio de Paleoparasitología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Funes 3350, 7600 Mar del Plata, Buenos Aires, Argentina. E-mail: veronicataglioretti@hotmail.com

(Stine and Stine, 1990; Mancini *et al*. 2002; Mancini, 2007). ADG exhibits, in addition, archaeological layers with evidence of the introduction of European livestock to Patagonia (Rindel, 2004, 2008). A combination of both kind of factors, the climate variability and introduction of exotic fauna, which probably represented ecological perturbations to the local faunal assembly are often critical determinants of parasite diversification (Hoberg and Brooks, 2015). As resource specialists, parasites have restricted host ranges, but shifts to relatively unrelated hosts commonly occur during their phylogenetic diversification and can be found in real time. This paradox is explained by Hoberg and Brooks (2015) within the framework of the Stockholm Paradigm. The present research represents an opportunity to add information of the influence of these factors on the diversification of parasite–host assemblages of American fauna.

The aim of the present study was to examine the parasite remains present in camelid coprolites collected from the archaeological site ADG and to discuss the paleoparasitological findings in a biogeographical and paleoecological context. This is the first paleoparasitological examination of camelid coprolites from archaeological layers, which exhibits evidence of the introduction of European livestock to the studied area.

MATERIALS AND METHODS

Studied area

The archaeological site ADG is located in the southern portion of the Cordillera de Los Andes, in PMNP, Santa Cruz Province (47°40′S and 72°30′W), close to (10 km) the archaeological site Cerro Casa de Piedra (CCP) (Fig. 1). ADG is a large shelter (250 m length) situated on a low hill oriented towards southwest. The site consists of nine archaeological layers dated from middle to late Holocene, including layers with evidence of the presence of introduced livestock (Rindel, 2004, 2008).

Late Holocene (ca. 4000–100 years BP) is characterized by increased climate variability in the short term. It has been mainly related to water availability associated with temperature and precipitation (Stine and Stine, 1990; Mancini *et al*. 2002; Mancini, 2007). During the final late Holocene, a trend towards increasing aridity with a maximum record between 1150 and 600 years BP occurred, during the weather phenomenon known as the Medieval Climate Anomaly (MCA) (Stine and Stine, 1990).

Material studied

Twenty pellets assigned to camelid coprolites, collected from six archaeological layers were examined for parasites (Table 1). Samples came from the
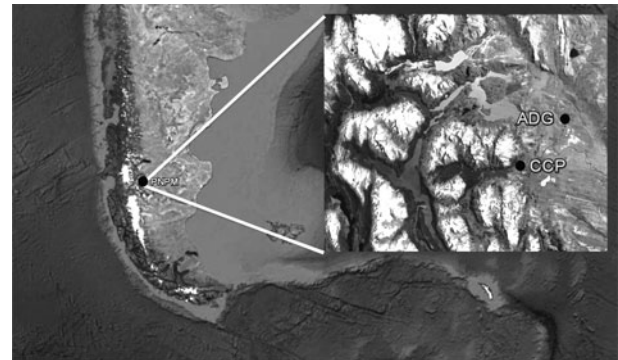

Fig. 1. Archaeological sites located in the PMNP. ADG, Alero Destacamento Guardaparque; CCP, Cerro Casa de Piedra; PMNP, Perito Moreno National Park.

middle and late Holocene, including the historic period, a time span of approximately 7000 years.

Each coprolite was fully processed by rehydration in a 0·5% water solution of tris-sodium phosphate for a week and then two concentration techniques were applied in series: first spontaneous sedimentation (Lutz, 1919) and then flotation-centrifugation with zinc chloride, according to Taglioretti *et al*. (2014*a*). In some cases, sucrose flotation-centrifugation and sodium polytungstate flotation-centrifugation techniques (Taglioretti, 2015) were also applied to recover gastrointestinal parasites (Table 1).

Similarity in the species composition among parasite communities found from different archaeological layers was estimated, since the different archaeological layers represent different time periods. To elucidate if the nearest archaeological layers shared more parasitic species than more distant archaeological layers, a one-way PERMANOVA (considering the factor 'Period' and type I error) and a Non-metric Multi-dimensional Scaling (NMDS) were performed using Jaccard's similarity matrix (Underwood, 1998; Anderson *et al*. 2008).

Integration with previous findings

To contribute to the knowledge of some of the patterns that could model the parasitic communities of Patagonian camelids in the past, it was important to integrate the paleoparasitological findings in the region. For this purpose, previous information of parasite that infected pre-Hispanic camelids from the archaeological site CCP located in PMNP (Taglioretti *et al*. 2015), close to (10 km) the archaeological site ADG, was included in the analysis. Paleoparasitological records obtain from CCP7 (Taglioretti *et al*. 2015) dated from Pleistocene-Holocene transition (9640 ± 190 years 14C BP) to late Holocene (3920 ± 80 years 14C BP).

For this purpose, the similarity in species composition was estimated between parasite communities of

Table 1. Radiocarbon dating and archaeological layers of ADG from which coprolites were recovered and concentration techniques applied

Sample	Archaeological layer	Dated years BP	No coprolites	Concentration technique
537	2	–	2	SSed – FZn
532	3	–	4	SSed – Fsac – FZn
573	4	–	4	SSed – Fsac – FZn – FPol
569	5	3440 ± 70	3	SSed – FZn
541	6	4900 ± 70	3	SSed – FZn
556	7	6700 ± 70	4	SSed – Fsac – FZn

ADG, Alero Destacamento Guardaparque; SSed, spontaneous sedimentation; FZn, flotation-centrifugation with zinc chloride; Fsac, sucrose flotation-centrifugation; FPol, sodium polytungstate flotation-centrifugation techniques.

Table 2. Parasitic distribution in archaeological samples from ADG

S	L	*Trichuris* sp.	Capillariid I	Capillariid III	*Nematodirus* sp.	*Nematodirus spathiger*	*Eimeria macusaniensis*	*Dictyocaulus* sp.
537	2	–	+	–	–	–	–	–
532	3	+	–	–	+	+	–	+
573	4	+	+	+	–	–	+	–
569	5	–	+	–	–	–	–	–
541	6	+	+	+	–	–	+	–
556	7	–	–	–	+	–	–	–

Archaeological layer (L), archaeological sample (S).

the archaeological site CCP7 (Taglioretti *et al.* 2015) and ADG (present study), using Jaccard's index. One-way PERMANOVA (considering the factor 'Site' and type I error) and a NMDS analysis were performed using Jaccard's similarity matrix (Underwood, 1998; Anderson *et al.* 2008).

RESULTS

Paleoparasitological examination of ADG

Three representatives of the superfamily Trichostrongyloidea were found in samples of ADG (Table 2), including large eggs [$n = 8$, 175·6 ± 12·7 μm in length (155–190) and 80·4 ± 8·8 (67·5–90) μm in width] compatible with *Nematodirus lamae* or *Lamanema chavezi* (Fig. 2A), three eggs with slightly sharp ends (205 μm in length and 92 μm in width) with third larvae stage (L3) inside (Fig. 2B) attributed to *Nematodirus spathiger* and seven oval eggs [155 ± 5·7 (145–160) μm in length by 72·5 ± 4·2 (67·5–77·5) μm in width] thin-walled (about 3 μm of thick) with larvae of the first stage of development (L1) inside. First larvae stage with cephalic end-rounded and sharp caudal end, forming a slightly conical tail with rounded end, had dark granules inside and measured approximately 559 ± 26·6 μm in length and 20·5 μm in width (Fig. 2C). These Trichostrongylidae eggs are compatible with eggs of the genus *Dictyocaulus*, possibly *D. filaria* (superfamily Trichostrongyloidea, family Dictyocaulidae,

subfamily Dictyocaulinae, genus *Dictyocaulus*). Eggs of *Dictyocaulus* sp. and those compatible with *N. spathiger* were only recorded in sample 532 (Table 2).

Among representatives of the superfamily Trichinelloidea, eggs of *Trichuris* sp. (Fig. 2D) of 63·25 (60–67·5) μm in length and 34 (33·75–35) μm in width, were found in 3/6 samples examined (Table 2). Two unidentified representatives of the family Capillariidae including 116 eggs similar to *Calodium* sp. [66·62 ± 3·4 (57·5–77·5) μm in length by 38·42 ± 2·57 (32·5–50) μm in width], named capillariid morphotype I (Fig. 2E) and 10 eggs of another unidentified capillariid [64·95 ± 2·3 (61·25–67·5) μm in length by 42·25 ± 3·2 (37·5–47·5) μm in width] with globular appearance and thicker walls than capillariid type I, named capillariid morphotype III, were found in 5/6 and 2/6 samples, respectively (Fig. 2F, Table 2). Capillariid type I was the parasite most represented and abundant in the paleoparasitological record of ADG (Table 2).

Two *Strongylus*-type eggs were also found in one sample (Fig. 2G, Table 2).

Regarding protozoa, oocysts of *Eimeria macusaniensis* [86·75 (82·5–92·5) × 62·8 (58·75–67·5) μm] were found in 2/6 samples examined (Fig. 2H, Table 2).

There were no differences in parasitic species composition between different time periods ($P = 0·8$), as it can be observed in Fig. 3. Archaeological layers of the same time period were not necessarily grouped closely in the NMDS plot (Fig. 3).
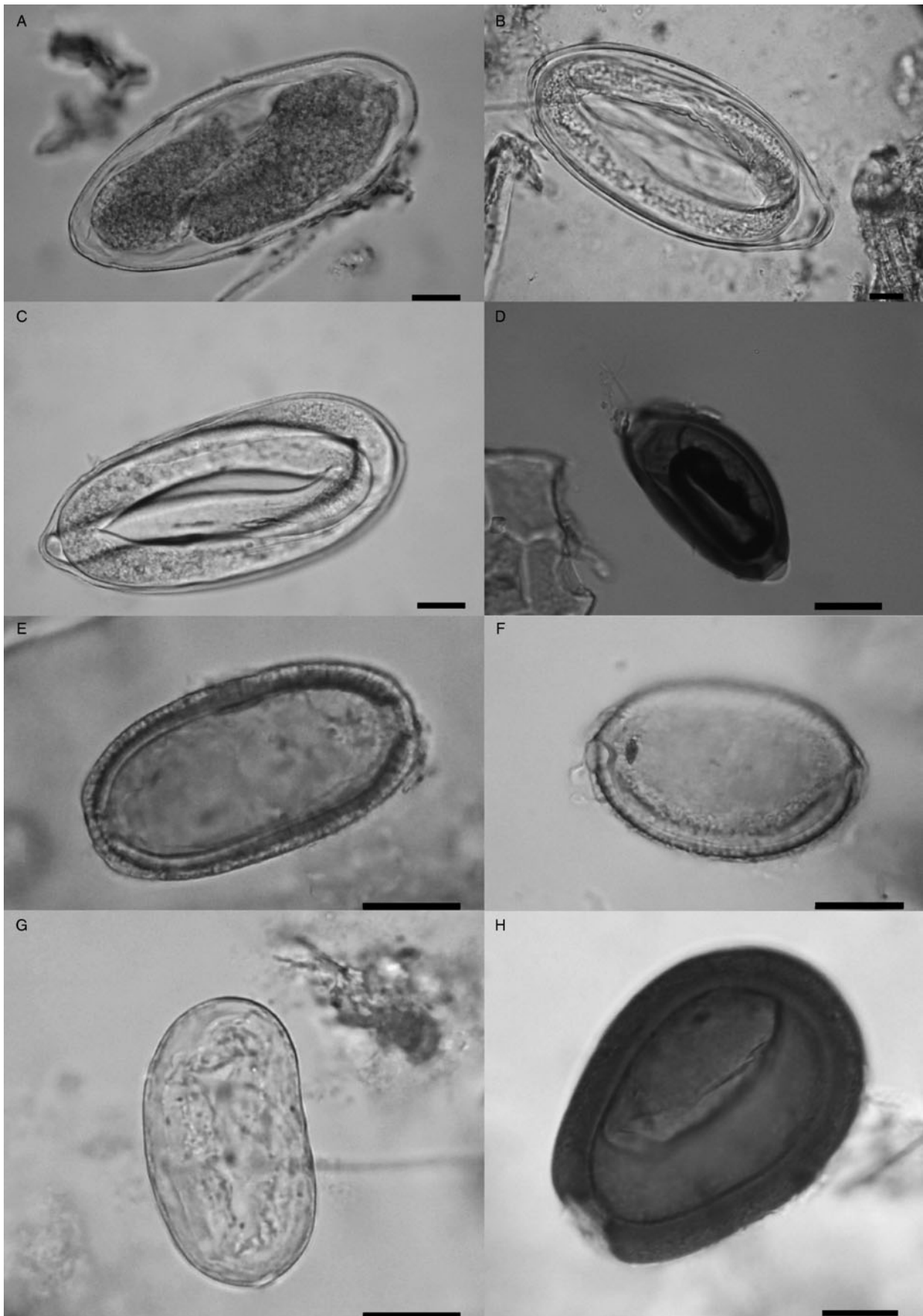
Fig. 2. (A) Egg of Trichostrongylidae, compatible with *Nematodirus Lamae* or *Lamanema chavezi*. (B) Egg attributed to *Nematodirus spathiger*. (C) Eggs compatible with *Dictyocaulus* sp. (D) *Trichuris* sp. egg. (E) Egg of unidentified capillariids, morphotype I. (F) Egg of unidentified capillariid, morphotype III. (G) *Strongylus*-type egg. (H) Oocyst of *Eimeria macusaniensis*. Scale bars: 20 μm.
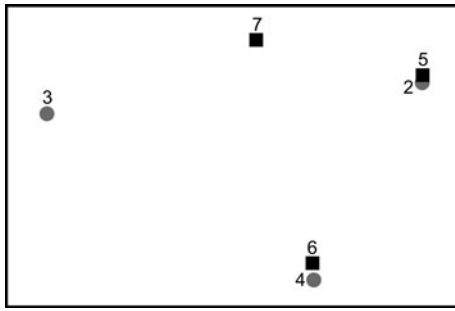
Fig. 3. NMDS ordination diagram of samples based on Jaccard's similarity matrix (stress = 0·0). Numbers identify archaeological layers and symbols represent different time periods. Black square: middle Holocene. Grey circles: late Holocene. Closeness of archaeological layers in the bidimentional space indicates that they have similar parasite communities. NMDS, Non-metric Multi-dimensional Scaling.
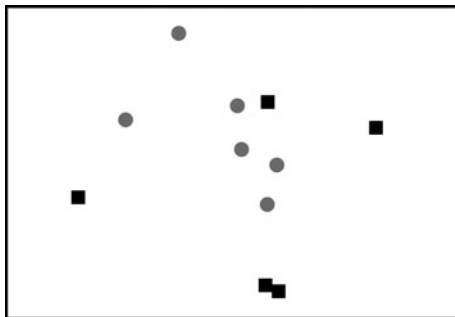

Fig. 4. NMDS ordination plot considering samples from both archaeological sites (CCP7 and ADG) based on Jaccard's similarity matrix (stress = 0·09). Grey circles: CCP7. Black square: ADG. ADG, Alero Destacamento Guardaparque; CCP, Cerro Casa de Piedra; NMDS, Non-metric Multi-dimensional Scaling.

Integrating paleoparasitological results of camelids from the PNPM – Southern Patagonia

Results indicated that there were no significant differences in parasite species composition among different time periods or archaeological sites ($P = 0.13$). Samples from both archaeological sites were arbitrarily mixed in the NMDS ordination plot (Fig. 4).

DISCUSSION

The parasite richness registered in ADG (eight species) constitutes the highest found for camelids coprolites at present. This richness compared with those documented in the archaeological site CCP7 (five species) (Taglioretti *et al.* 2015) was due to the presence of *N. spathiger* and *Dictyocaulus* sp. in the sample 532, parasites that had not been previously found in the paleoparasitological record. Sample 532 was collected from the stratigraphic layer 3, corresponding to the late Holocene (post-1000 years BP) (Rindel, personal communication).

The presence of *N. spathiger* and *Dictyocaulus* sp. in the present guanacos from Patagonia was associated with their contact with the introduced livestock (Beldoménico *et al.* 2003). This is the first record of *Dictyocaulus* sp. and *N. spathiger* in archaeological samples. Rindel (2004, 2008) reported the presence of sheep's remains in the upper archaeological layer (layer 2); so the presence of such parasites in layer 3 was therefore associated with the entry of European livestock into Patagonia and to the interaction with camelids, potentially facilitating host switching. Capillariids, mainly capillariid morphotype I, are commonly found in Patagonian archaeological sites (Fugassa *et al.* 2008; Taglioretti *et al.* 2014b). The finding of the capillariid morphotype III in the present research increases the capillariid biodiversity for camelids and also for the paleoparasitological records of Patagonia. Most capillariids are considered zoonotic. Their presence in camelid coprolites collected from archaeological sites brings information of the potential zoonotic infections that humans in the past may have been exposed by the ingestion of contaminated food or water with camelid dungs.

Enteric parasites of camelids had not changed since the Pleistocene-Holocene transition to late Holocene, up to the entry of introduced livestock in the PMNP, despite the climate fluctuations that were affecting Patagonia during this period (Mancini *et al.* 2002; Mancini, 2007), indicating the high stability of camelids-parasite assemblages through time. The increase in parasite richness and also the differences in the composition of parasite communities observed up to the historic period (layer 3) could be interpreted as a result of the introduction of new hosts (as red deer or sheep) with similar physiological and ecological features that could facilitate the transmission of 'new' parasite species to native camelids. Moreover, as it is suggested by Hoberg and Brooks (2015) within the framework of the Stockholm Paradigm, under the concept of 'ecological fitting', phenotypic flexibility of these parasites could provide opportunities for rapid host switching in changing environments, a scenario that had probably occurred in Patagonia during the introduction of European livestock. These findings provide the first paleoparasitological evidence supporting the hypothesis by Beldoménico *et al.* (2003) on the presence of *N. spathiger* and *Dictyocaulus* sp. in the present guanacos from Patagonia.

Similarity between parasitic compositions of both archaeological sites (CCP7 and ADG) could be explained by the interaction between camelid populations that had inhabited these sites. As archaeological sites are in the home range of the guanaco (up to 9 km) (Burgi, 2007), it is suggested that guanaco populations of ADG and CCP7 were in contact during the Holocene, enabling parasites transmission.

The present study provides information on the dispersion of some parasites in space and on the potential parasite host switching between sympatric species of herbivores that occupied the studied area. It also reinforces the hypothesis of the acquisition by the guanacos of new parasite species by the introduction of European livestock.

REFERENCES

Anderson, M. J., Gorley, R. N. and Clarke, K. R. (2008). *PERMANOVA + for PRIMER: Guide to Software and Statistical Methods*. PRIMER-E, Plymouth.

Beldoménico, P. M., Uhart, M., Bono, M. F., Marull, C., Baldi, R. and Peralta, J. L. (2003). Internal parasites of free-ranging guanacos from Patagonia. *Veterinary Parasitology* **118**, 71–77.

Burgi, M. E. (2007). Radio de acción del guanaco (*Lama guanicoe*) en el NE de Chubut. *Mastozoología Neotropical* **14**, 285.

Franklin, W. L. (1983). Contrasting Socioecologies of South American Wild Camelids: the Vicuña and the Guanaco. In *Advances in the Study of Mammalian Behavior* (ed. Eisenberg, J. F. and Kleiman, D. G.), pp. 573–629. American Society of Mammalogists, Special Publication 7.

Fugassa, M. H. (2005). New paleoparasitological analyses of human coprolites from Southern Patagonia. *Paleopathology Newsletter* **132**, 25.

Fugassa, M. H. (2007). Camélidos, parásitos y ocupaciones humanas: registros paleoparasitológicos en CCP7 (P. N. Perito Moreno, Santa Cruz, Argentina). *Intersecciones en Antropología* **8**, 265–269.

Fugassa, M. H., Denegri, G. M., Sardella, N. H., Araújo, A., Guichón, R. A., Martinez, P. A., Civalero, N. T. and Aschero, C. (2006). Paleoparasitological records in a canid coprolite from Patagonia, Argentina. *Journal of Parasitology* **92**, 1110–1111.

Fugassa, M. H., Bayer, M. S. and Sardella, N. H. (2007*a*). Examen paleoparasitológico de coprolitos de felinos: zoonosis durante el Holoceno Patagónico. *Revista Argentina de Antropología Biológica* **9**, 156.

Fugassa, M. H., Sardella, N. H. and Denegri, G. M. (2007*b*). Paleoparasitological analysis of a raptor pellet from Southern Patagonia. *Journal of Parasitology* **93**, 421–422.

Fugassa, M. H., Taglioretti, V., Gonçalves, M. L. C., Araújo, A., Sardella, N. H. and Denegri, G. M. (2008). *Capillaria* spp. eggs in Patagonian archaeological sites: statistical analysis of morphometric data. *Memórias do Instituto Oswaldo Cruz* **103**, 104–105.

Hoberg, E. P. and Brooks, D. R. (2015). Evolution in action: climate change, biodiversity dynamics and emerging infectious disease. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **370**, 20130553.

Lutz, A. (1919). *Schistosoma mansoni* e a schistosomatose segundo observaçoes feitas no Brasil. *Memórias do Instituto Oswaldo Cruz* **11**, 121–155.

Mancini, M. V. (2007). Cambios paleoambientales en el ecotono bosque-estepa: análisis polínico del Sitio Cerro Casa de Piedra 7, Santa Cruz (Argentina). *Actas de la 6ta Jornada de Arqueología de Patragonia, Punta Arenas, Chile* 89–93 pp.

Mancini, M. V., Paez, M. M. and Prieto, A. R. (2002). Cambios paleoambientales durante los últimos 7·000 14C años en el ecotono bosque-estepa, 47–48°S, Santa Cruz, Argentina. *Ameghiniana* **39**, 151–152.

Navone, G. T. and Merino, M. L. (1989). Contribución al conocimiento de la fauna endoparasitaria de *Lama guanicoe* Muller, 1776, de Península Mitre, Tierra del Fuego, Argentina. *Boletín Chileno de Parasitología* **44**, 46–51.

Rindel, D. D. (2004). Patrones de procesamiento faunístico en el sitio Alero Destacamento Guardaparque durante el Holoceno Medio. In *Contra Viento y Marea. Arqueología de la Patagonia* (ed. Civalero, M. T., Fernández, P. M. and Guraieb, A. G.), pp. 263–276. SAA and AINA, Buenos Aires.

Rindel, D. D. (2008). *Arqueología de Momentos Tardíos en el Noroeste de la Provincia de Santa Cruz (Argentina): una Perspectiva Faunística*. Ph.D. thesis. Universidad de Buenos Aires, Facultad de Filosofía y Letras, Buenos Aires, Argentina, 526 pp.

Sardella, N. H. and Fugassa, M. H. (2009*a*). Parasites in rodent coprolites from the historical archaeological site Alero Mazquiarán, Chubut Province, Argentina. *Memórias do Instituto Oswaldo Cruz* **104**, 37–42.

Sardella, N. H. and Fugassa, M. H. (2009*b*). Paleoparasitological analysis of rodent coprolites in Holocenic samples from Patagonia, Argentina. *Journal of Parasitology* **95**, 646–651.

Sardella, N. H., Fugassa, M. H., Rindel, D. D. and Goñi, R. A. (2010). Paleoparasitological results for rodent coprolites from Santa Cruz Province, Argentina. *Memórias do Instituto Oswaldo Cruz* **105**, 33–40.

Stine, S. and Stine, M. (1990). A record from Lago Cardiel of climate change in Southern South America. *Nature* **345**, 705–708.

Taglioretti, V. (2015). *Estudios paleoparasitológicos en coprolitos de camélidos sudamericanos*. Ph.D. thesis. Mar del Plata National University, Mar del Plata, Argentina, 195 pp.

Taglioretti, V., Sardella, N. H. and Fugassa, M. H. (2014*a*). Effectiveness of coproscopic concentration techniques. *Helminthologia* **51**, 2010–2014.

Taglioretti, V., Fugassa, M. H., Beltrme, M. O. and Sardella, N. H. (2014*b*). Biometric identification of capillariid eggs from archaeological sites in Patagonia. *Journal of Helminthology* **88**, 196–202.

Taglioretti, V., Fugassa, M. H. and Sardella, N. H. (2015). Parasitic diversity found in coprolites of camelids during the Holocene. *Parasitology Research* **14**, 2459–2464.

Underwood, A. J. (1998). *Experiments in Ecology: their Logical and Interpretation using Analysis of Variance*. Cambridge University Press.

Wheeler, J. C. (1995). Evolution and present situation of the South American Camellidae. *Biological Journal of the Linnean Society* **54**, 271–295.