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## Camelid Gastrointestinal Parasites from the Archaeological Site of Huanchaquito (Peru): First Results

Matthieu Le Bailly <sup>a</sup>, Nicolas Goepfert <sup>b</sup>, Gabriel Prieto <sup>c</sup>, John Verano<sup>d</sup> and Benjamin Dufour<sup>a</sup>

<sup>a</sup>CNRS UMR 6249 Chrono-environment, University of Bourgogne Franche-Comte, Besancon cedex, France; <sup>b</sup>CNRS-Paris 1, UMR 8096 Archéologie des Amériques, Nanterre, France; <sup>c</sup>Facultad de Ciencias Sociales, Universidad Nacional de Trujillo, Trujillo, La Libertad, 13010, Perú; <sup>d</sup>Department of Anthropology, Tulane University, New Orleans, LA, USA

### ABSTRACT

Palaeoparasitological investigation was conducted on a first set of samples from 13 sacrificed domestic camelids recovered from the pre-Hispanic Chimú culture site of Huanchaquito-Las Llamas, Peru. The aim was to establish the animals' gastrointestinal parasite diversity and enlighten on their health status at the time of their death. To this end, 20 samples of coprolites and intestinal contents were analysed to check for the presence of parasite markers, i.e. preserved eggs and oocysts. Microscopic examinations revealed the presence of five taxa of helminths and protozoans in a majority of the tested animals (61%). Our analysis revealed the presence in some animals of protozoan oocysts belonging to the species *Eimeria macusaniensis* (phylum Apicomplexa). Our study is the first report of the possible presence of a parasite egg attributed to the order Plagiorchiida (family Fasciolidae) in ancient camelids. This preliminary study shows that there is interesting potential for conducting palaeoparasitological analysis at the site and that such analysis is promising for answering questions about the health status of the Huanchaquito-Las Llamas camelids.

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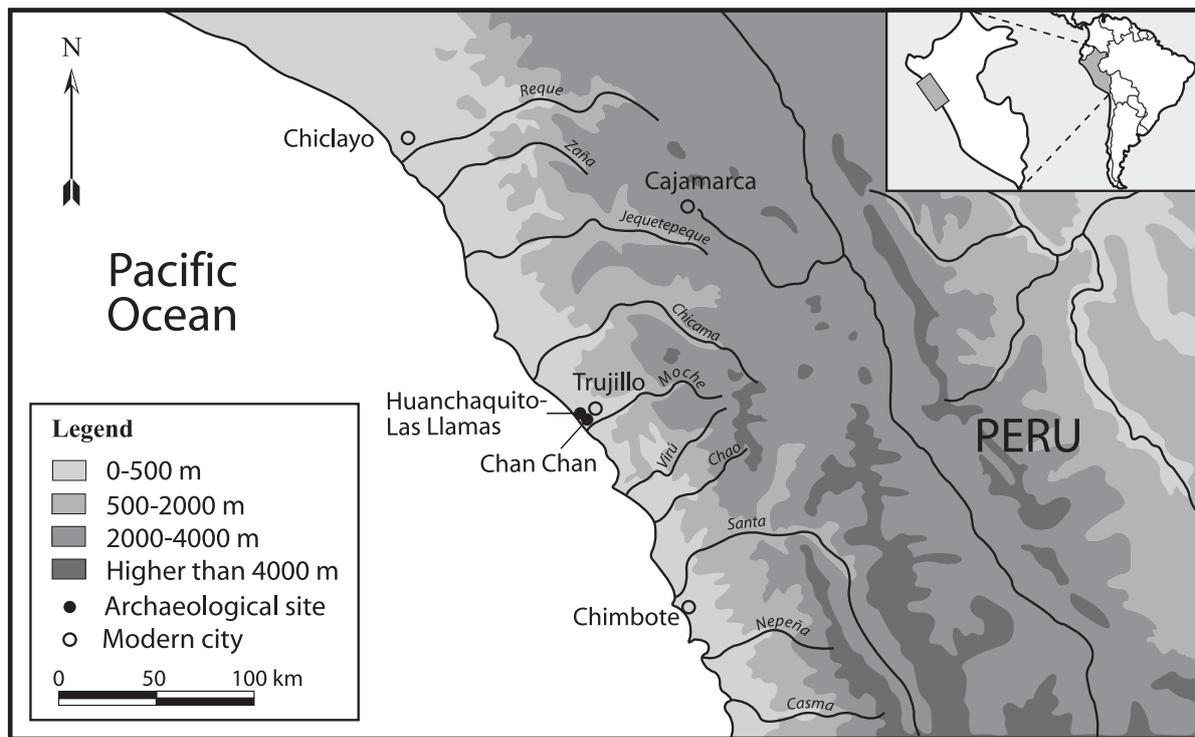
## Introduction

Palaeoparasitology is the study of ancient parasites from archaeological and palaeontological contexts (Dittmar, Aduato, and Reinhard 2012; Le Bailly and Araujo 2016). It aims to provide information about the health status, hygiene and lifestyle of ancient populations, be they human or animal. Anthropocentric contexts, such as human skeletons, human mummified bodies and latrines, are frequently analysed, and our knowledge of the history of past human parasites, though far from complete, is becoming more and more precise. However, while animal parasites are often retrieved and analysed from human contexts, analyses performed on parasites retrieved from animals themselves are scarcer (Bouchet, Harter, and Le Bailly 2003; Gonçalves, Araujo, and Ferreira 2003; Sianto et al. 2009). Just like studies performed on humans, veterinary palaeoparasitological studies yield information about the health status of individuals and highlight anthrozooses, i.e. diseases common to both humans and animals (Dittmar and Teegen 2003; Le Bailly, Leuzinger, and Bouchet 2003, 2008, 2014). Moreover, the increasing number of studies in veterinary contexts also appears to be a way to understand the evolution of the host-parasite relationship throughout history and to identify possible changes in parasite lifecycles, thereby overcoming the limitations inherent in the use of the modern-day parasitological literature.

In the present work, the studied materials were collected from the abdominal cavity and the coxal area of camelids recovered from a pre-Hispanic site known as Huanchaquito-Las Llamas (hereafter Huanchaquito), located on the northern coast of Peru. The initial aim was to establish the diversity of gastrointestinal parasites in each animal, thereby enlightening us on their health status and, in a general way, yielding information about camelid breeding in ancient Peru.

## Materials and method

The site of Huanchaquito is located in the modern-day fishing town of the same name, 1.5 km northwest of the ancient city of Chan Chan, the capital of the Chimú Empire (Figure 1). During three seasons of excavation (2011, 2014 and 2016), 140 children and 206 camelids were found that had been deposited together during one major event around cal. AD 1400–1450 (Goepfert et al. 2018; Goepfert and Prieto 2016; Prieto, Goepfert, and Vallares 2015, 2017, forthcoming). The site of Huanchaquito represents the biggest child and animal mass sacrifice known in the central Andean area and, indeed, in the Americas. The arid climatic conditions of the desert coast of Peru have led to the preservation of organic remains at this site, including not only the coat, but also the intestinal contents and faeces.

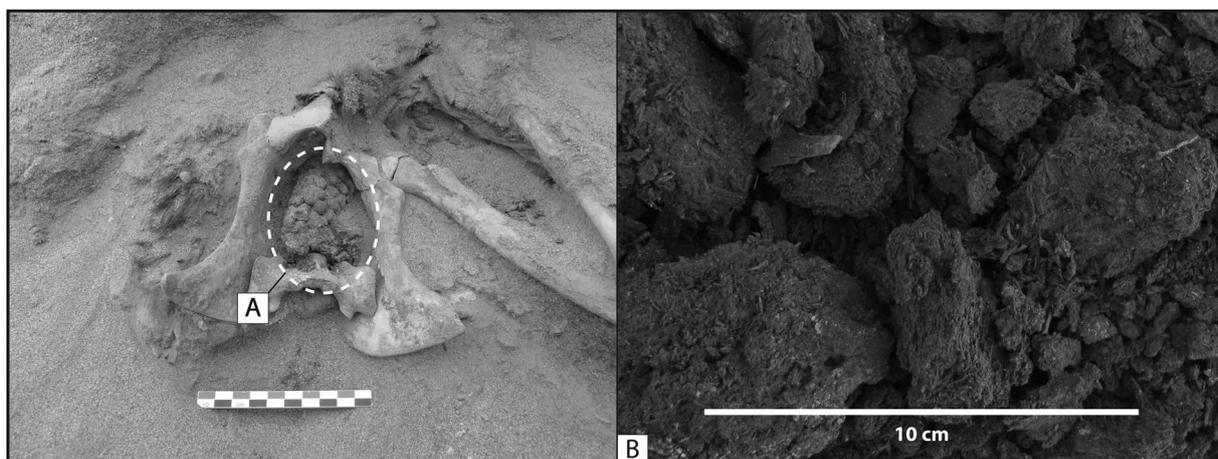


**Figure 1.** Location of the sacrificial site of Huanchaquito-Las Llamas, Peru.

As a preliminary study to evaluate the palaeoparasitological potential of the site, a first set of 20 samples was taken from the abdominal cavity and the coxal region of 13 animals (Figure 2) and exported under the permissions obtained from the Ministerio de Cultura of Peru (N°050-2016-VMPCIC-MC). Compact material recovered around the coxal region was classified under faeces (7 out of 20 samples), and powdered material recovered in the abdominal cavity was classified under intestinal contents (13 out of 20 samples). Table 1 presents an overview of the studied material.

All the samples were prepared following the standard protocol used by the Besancon-France palaeoparasitology research team. This protocol consists of three

steps: rehydration, homogenisation and microsieving (Dufour and Le Bailly 2013). During the rehydration step, 5 gr of each sample is placed in a solution composed of 50 ml 0.5% tri-sodium phosphate and 50 ml 5% glycerinated water for one week. Then the samples are homogenised in a mortar, after which they are submitted for 1 min in an ultrasonic bath. This second step aims to separate the possible parasite markers from the other sample elements. Finally, the samples are filtered in a sieving column composed of four levels, with meshes of 315, 160, 50 and 25  $\mu\text{m}$ . Because of the size of the parasite dissemination forms, measuring 30 to 160  $\mu\text{m}$  in length and 15 to 90  $\mu\text{m}$  in width, only the sieve contents from the bottom two levels, 50 and 25  $\mu\text{m}$ , are analysed, under light microscopy



**Figure 2.** Example of samples recovered in Huanchaquito camelids. (A) Faeces in the coxal region. (B) Intestinal contents of the abdominal cavity (Photography N. Goepfert).

**Table 1.** Results of the preliminary palaeoparasitological analysis of the Huanchaquito camelid samples. Values correspond to the average number of eggs per slide. Rows highlighted in grey correspond to negative samples.

Individual #	Age	Sample Type	Order Trichocephalida			Order Plagiorchiida	Order Eucoccidiorida
			<i>Trichuris</i> sp.	<i>Trichuris</i> type with ornamentation	Rounded, lemon-shaped egg	Fasciolidae	Eimeriidae
CA-01	1 year	Intestinal contents					
CA-03	9 months	Faeces					3.25
		Intestinal contents					
CA-05	1 year 3 months– 1 year 6 months	Faeces		4.5	1		0.25
		Intestinal contents	0.25				
CA-11	6 months	Faeces	0.5				
		Intestinal contents					
CA-16	1 year	Intestinal contents	3.25				0.75
CA-17	6 months	Intestinal contents					
CA-25	1 year 3 months	Faeces					145.5
		Intestinal contents	1				400
CA-32	9 months	Intestinal contents					
CA-43	9 months–1 year	Intestinal contents					
CA-50	Indeterminate	Faeces	1.5			0.25	
		Intestinal contents					
CA-62	1 year	Faeces					269.5
		Intestinal contents	0.25				338
CA-63	1 year	Faeces					
		Intestinal contents					
CA-65	6 months–9 months	Intestinal contents	3				8

(Olympus BX-51). Photos and measurements are taken with the Saisam® software commercialised by Microvision Instruments.

## Results

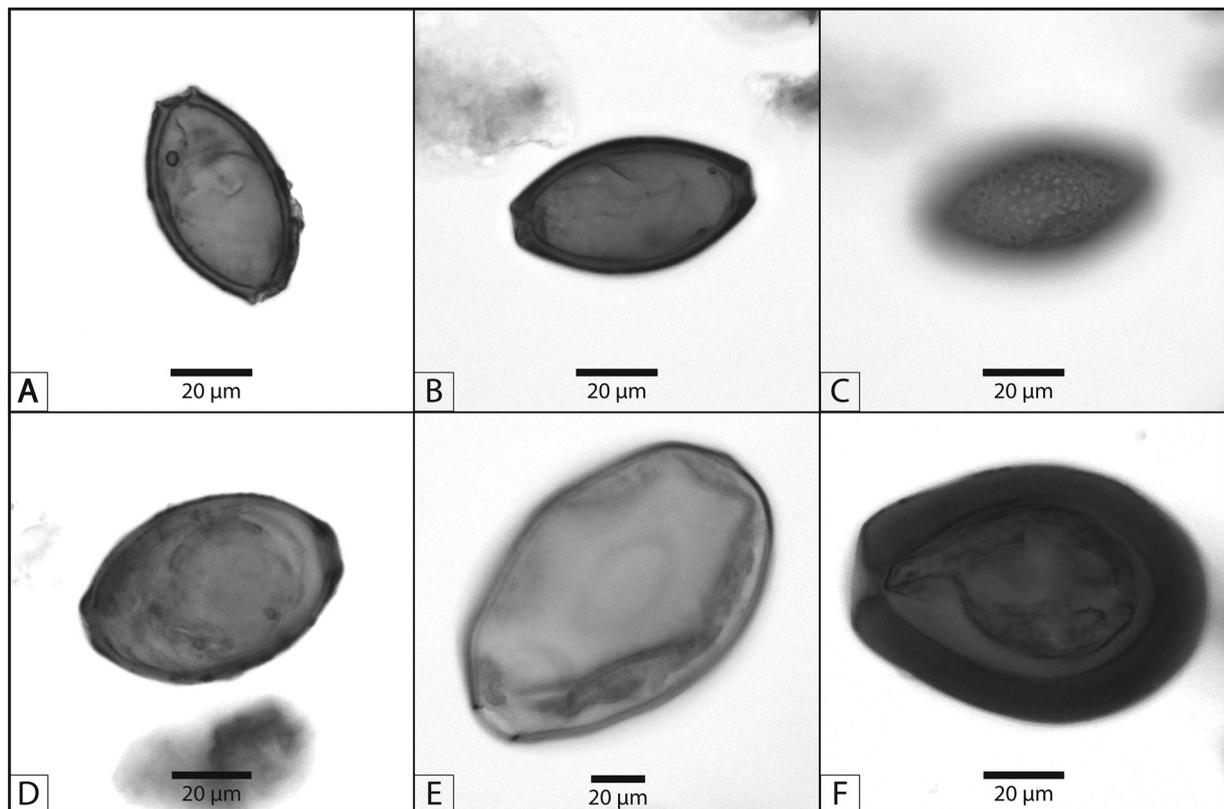
A total of 55% of the studied samples (i.e. 11 out of 20) and 61% of the tested animals (i.e. 8 out of 13) were positive for the presence of at least one taxon of intestinal parasite. Five taxa of parasites were observed, and these were identified at different levels of taxonomic precision. Four of the five taxa belong to one of two phyla, namely, Nematoda and Platyhelminthes. One of the five taxa corresponds to a protozoan in the genus *Eimeria*. No eggs of tapeworm (class Cestoda) were recovered in this preliminary study (Table 1).

The class Nematoda is represented by three taxa, identified as members of the order Trichocephalida. The first corresponds to the genus *Trichuris*, the whipworm, and it infected 46% of the studied animals. Eggs of the genus *Trichuris* present two polar plugs, which gives them a characteristic lemon shape (Figure 3(A)). The average size of the eggs in our samples is about  $59.6 \pm 4.6 \mu\text{m}$  in length and  $33.8 \pm 2.8 \mu\text{m}$  in width ( $n = 22$ ), and this matches with the whipworm species known to be herbivorous, such as, for example, *Trichuris tenuis*, *T. ovis*, *T. discolor* and *T. globulosa* (Dufour 2015; Léger et al. 1991; Rickard and Bishop 1991; Soulsby 1982). A second taxon of nematode of the order Trichocephalida was observed in a single individual (individual CA-05) and thus far remains unidentified. Eggs of this second taxon share some characteristics with those of the genus *Trichuris*: they are lemon-shaped and present an average size of

about  $59.1 \pm 1.9 \times 33.4 \pm 1.5 \mu\text{m}$  ( $n = 8$ ) (Figure 3(B)). However, the external eggshell displays a network-like ornamentation that is not described in the literature (Figure 3(C)). The regularity and the density of the pattern indicate that this ornamentation does not relate to a taphonomic process. The third and last identified nematode, also recovered from CA-05, is represented by lemon-shaped eggs that are more rounded than the first unidentified taxon, discussed above, and without ornamentation (Figure 3(D)). The average size of these eggs is about  $65 \pm 0.59 \times 43 \pm 1.57 \mu\text{m}$  ( $n = 3$ ). Characteristics of the eggs would suggest the genera *Anatrichosoma* or *Trichosomoides*, which are parasites of primates and rodents, respectively (Kassai 1999; Soulsby 1982). This second unidentified taxon could also correspond to a member of the family Capillaridae, but precise identification remains impossible because of the lack of consensus and knowledge about this family, mainly with respect to egg morphology.

The sole taxon belonging to the class Trematoda is represented by a single egg with the characteristics of a fluke (order Plagiorchiida). The egg is ovoid, with an operculum and a knob at the abopercular side (Figure 3(E)), both characteristics typical of parasite eggs belonging to the family Fasciolidae. The recovered egg is around  $130 \mu\text{m}$  long and  $85.7 \mu\text{m}$  wide, which could correspond to the genus *Fasciola*, the liver fluke.

The fifth recovered taxon is a protozoan belonging to the phylum Apicomplexa, order Eucoccidiorida. The recovered markers correspond to oocysts. These oocysts are ovoid to pyriform and present a thick, brown wall with a micropyle (Figure 3(F)). These characteristics are consistent with the family



**Figure 3.** (A) Egg of *Trichuris* sp. ( $55.69 \times 31.42 \mu\text{m}$ , CA-16, intestinal contents). (B and C) Egg of unidentified *Trichuris* sp. reticulated morphotype, in cross-section (B) and in plan view showing the surface texture (C) ( $60.13 \times 33.04 \mu\text{m}$ , CA-05, faeces). (D) Egg of unidentified Trichocephalida ( $67.06 \times 44.99 \mu\text{m}$ , CA-05, faeces). (E) Egg of Fasciolidae ( $130.00 \times 85.70 \mu\text{m}$ , CA-50, faeces). (F) Oocyst of *Eimeria* sp. ( $88.56 \times 64.37 \mu\text{m}$ , CA-62, intestinal contents).

Eimeriidae, genus *Eimeria* (Taylor, Coop, and Wall 2007). Five species in the genus *Eimeria* are reported for South American camelids (SACs) in the modern parasitology literature: *E. alpaca*, *E. punonensis*, *E. lamae*, *E. ivitaensis* and *E. macusaniensis*. The morphology and the main measurements of the recovered oocysts,  $79.4 \pm 10.2 \mu\text{m}$  in length and  $56.5 \pm 8 \mu\text{m}$  in width ( $n = 30$ ), are consistent with the species *E. macusaniensis* (Guerrero et al. 1971; Leguía 1991; Leguía and Casas 1998; McKenna 2006).

## Discussion

Palaeoparasitological data for ancient South American camelids are scarce. Only a few references are available in the literature, and these allow only partial comparison with the current results. Leguía, Casas, and Wheeler (1995) were the first to report parasitological data relating to ancient SACs, from samples of mummified llamas from the pre-Hispanic site of El Yaral, Peru, dating to around 900 to 1000 ybp. Analysis revealed the presence of six taxa, including nematodes belonging to the order Strongylida (family Trichostrongylidae) and the order Trichocephalida (genus *Capillaria* and genus *Trichuris*). In addition, protozoan cysts were identified belonging to the Apicomplexa, order Eucoccidiorida (*Sarcocystis* sp., *Eimeria macusaniensis* and *E. ivitaensis*) (Leguía, Casas, and Wheeler

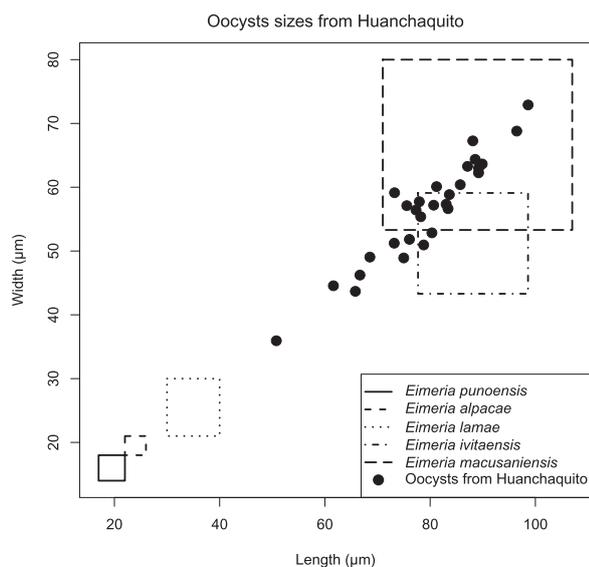
1995). Fugassa (2007) analysed camelid coprolites from the Argentinean site of Cerro Casa de Piedra 7 (CCP7), radiocarbon dated to around 8000 ybp, and found eggs of nematodes (*Capillaria* sp. and *Nematodirus* sp.) as well as oocysts of *E. macusaniensis*. Taglioretti, Fugassa, and Sardella (2015) added to these data by studying additional 24 samples from CCP7 dated to between  $9700 \pm 100$  and  $3600 \pm 60$  ybp. During the latter analysis, remains of six taxa were recovered, namely, eggs of *Strongylus* type, eggs of the family Trichostrongylidae that are compatible with *Nematodirus* sp. or *Lamanema* sp., eggs of three different morphotypes of *Capillaria* sp., and oocysts of *E. macusaniensis*. Finally, in 2017, the same research group analysed 20 samples from the Argentinean archaeological site of Alero Destacamento Guardaparque (ADG), located in Perito Moreno National Park, 10 km from CCP7. Microscopic examinations were performed on camelid coprolites sampled from archaeological layers dating from the middle to late Holocene (7000–100 ybp). This work revealed the presence of eggs of the family Trichostrongylidae (namely, *Nematodirus* sp., *N. spathiger* and *Dictyocaulus* sp.), *Strongylus* type eggs, eggs of *Trichuris* sp., eggs of two morphotypes of *Capillaria* sp., and oocyst of *E. macusaniensis* (Taglioretti et al. 2017). Table 2 synthesises the previous records of intestinal parasites in ancient SACs.

Previous palaeoparasitological analyses performed on ancient SACs have revealed an intestinal parasite diversity of between three and seven taxa on average. The results from the site of El Yaral are the most comparable to our study archaeologically, geographically and chronologically. At El Yaral, six to seven gastrointestinal parasite taxa were identified (Leguia, Casas, and Wheeler 1995). This is also the case with the samples from Huanchaquito. The variation in biodiversity among the studies synthesised in Table 2 is often due to the limitations, when using microscopy, for the identification of eggs in the order Strongylida (i.e. family Strongylidae and family Trichostrongylidae), which almost all present a similar morphology. No eggs of the order Strongylida were recovered during this preliminary study from Huanchaquito.

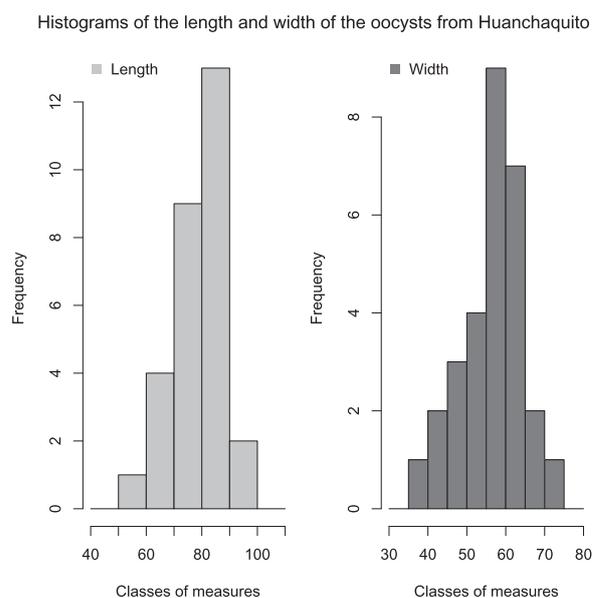
As noted above, one sample contained a single egg attributed to the family Fasciolidae. The modern parasitological literature indicates that liver flukes of the genus *Fasciola* are present in SACs today with prevalence ranging from 8% to 73.8% and that mortality is up to 100% (Camareno et al. 2016; Flores et al. 2014; Leguia 1991). Our results would constitute the first mention of fluke in ancient SACs, but due to the recovery of only a single egg, this will have to be confirmed by analysis of additional samples.

The presence of *Eimeria macusaniensis* has been mentioned once previously for ancient SACs (see table 2). Adding to this previous observation, our results allow for the possibility that the parasite was present in SACs continuously from 10,000 years ago. The average size of the *Eimeria* oocysts retrieved

from the Huanchaquito camelids shows large variation, with a significant standard deviation of more than 5  $\mu\text{m}$  (Figure 4). However, histograms of the length and width of the oocysts demonstrate a regular distribution and suggest the presence of a single species (Figure 5). Polymorphism is known in coccidians; it can be due to many factors, such as the host's nutritional plane, state of immunity, level of stress, or parasite load (Berto, McIntosh, and Lopes 2014). In addition, the effects of taphonomic processes can slightly modify the size of the markers, as can the nature of the sample, i.e. whether from the intestines or from faeces (Jarvinen 2008). In an ancient context, Fugassa et al. (2008) observed and discussed the size variation among *Eimeria* sp. oocysts recovered during the analysis of Argentinean archaeological samples, having used statistics to show a relationship among oocyst size, environmental changes during the Holocene, and the antiquity of the samples. In our study, the animals were all sacrificed during the same short event, so unlike was the case in the Fugassa et al. study, varying antiquity of the material cannot explain the observed size variation. Because all the animals were recovered from the same environmental context, we can hypothesise that taphonomic factors affected the palaeoparasitological markers uniformly and that therefore taphonomy cannot explain it either. Under these circumstances, we can hypothesise that the oocyst size variation is due to differences among the animals themselves, including (1) health status, (2) size, (3) age and (4) origin (breeding or geography). The two last hypotheses have been challenged, however, by the archaeozoological and the stable isotopic analyses performed on the same individuals. The age of the animals was estimated using existing references for tooth



**Figure 4.** Comparison of the size of the *Eimeria* oocysts from Huanchaquito (black points) and the size range of oocysts known from modern camelids: *Eimeria punoensis* (solid line), *E. alpaca* (dashed line), *E. lamae* (dotted line), *E. ivitaensis* (dot-dash line), and *E. macusaniensis* (long-dash line) (size ranges after Fugassa et al. 2008; Guerrero 1967; Guerrero et al. 1971; Leguia and Casas 1998; Twomey et al. 2010).



**Figure 5.** Histograms of length (at left) and width (at right) of the *Eimeria* oocysts from Huanchaquito. Y axis: frequencies, X axis: classes of measures.

**Table 2.** Synthesis of previously published palaeoparasitological data on ancient South American camelids (N/A: Not Available).

Country	Site	Date	Sample type	Sample number	Taxon		Reference(s)
					Helminths	Protozoans	
Argentina	Cerro Casa de Piedra 7	9700–3600 BP	Camelid coprolites from archaeological layers	24	Trichostrongylidae <i>Strongylus</i> type <i>Capillaria</i> sp. (three morphotypes)	<i>E. macusianensis</i>	Taglioretti, Fugassa, and Sardella 2015
	Cerro Casa de Piedra 7	8000 BP	Camelid coprolites from archaeological layers	N/A	Trichostrongylidae <i>Capillaria</i> sp.	<i>E. macusianensis</i>	Fugassa 2007
	Alero Destacamento Guardaparque	7000–100 BP	Camelid coprolites from archaeological layers	20	Trichostrongylidae ( <i>Nematodirus</i> sp., <i>N. spathiger</i> and <i>Dictyoaulus</i> sp.) <i>Strongylus</i> type <i>Trichuris</i> sp. <i>Capillaria</i> sp.	<i>E. macusianensis</i>	Taglioretti et al. 2017
Peru	El Yaral	1000–900 BP	Samples from camelid mummies	N/A	Trichostrongylidae <i>Capillaria</i> sp. <i>Trichuris</i> sp.	<i>Sarcocystis</i> sp. <i>E. macusianensis</i> <i>E. ivitaensis</i>	Leguia, Casas, and Wheeler 1995

eruption and wear available for domestic camelids (Wheeler 1982) (Table 1). It revealed that among the 20 camelids studied here, only young animals were sacrificed aged between 6 months for the younger and 1 year and 6 months for the oldest (Goepfert et al. 2018). The stable isotopic analyses showed a coastal and/or middle valley origin for all of the sacrificed camelids (Dufour et al. 2018).

The preliminary palaeoparasitological analysis carried out on the camelids from the pre-Hispanic site of Huanchaquito informs us that some animals were infected with several gastro-intestinal parasites. Some of these parasites including *Eimeria macusaniensis* and possibly the liver fluke *Fasciola*, both of which are known to be responsible for severe symptoms and sometimes high mortality in modern camelid herds, especially in cases of co-infestations with other pathogens (Cafrune et al. 2009; Leguia 1991). Even though questions remain about the pathogenicity of ancient parasites, it is possible to hypothesise that some of the sacrificed animals were not in good health and may have presented visual symptoms of infection. While building on the current analysis in the future, we will have to differentiate between funerary deposits, which could occur without sacrifice, composed with animals that had died naturally or because of infections, and sacrificial deposits (such as the one at Huanchaquito), where the deaths were intentional. Ethnohistorical and ethnographic data concur that the selection for sacrifice targeted camelids which are considered as the best animals (Flores Ochoa, MacQuarrie, and Portus 1994). Under these circumstances, two hypotheses can be formulated for the sacrifice at Huanchaquito. (1) The animals, even those infected by many parasites, did not present any visual symptoms of parasite infection while alive. (2) In some cases of mass sacrifice, as at Huanchaquito, the sacrifice was partly opportunistic, involving the elimination of sick animals. If hypothesis (2) was proven to be valid, this would add nuance to the paradigm of sacrifice.

## Conclusion

This preliminary study indicates good preservation of the parasite markers in Huanchaquito and excellent potential for additional analyses. Our results also provide new evidence of the presence of intestinal parasites in ancient South American camelids. This is only the second such study performed on ancient Peruvian camelids and the first concerning the Chimú culture. Our results are promising for reaching the initial goal, i.e. to enlighten on the health status of the Huanchaquito camelids. From a strictly palaeoparasitological point of view, this study may indicate the presence of liver fluke in SACs for the first time and thereby adds to the existing data about the range of parasites present in pre-Hispanic animals. Some of the studied camelids appear to be more infected than others. In future, we plan to analyse additional samples and contrast the parasitological data with the archaeozoological and stable isotopic data. This could allow us to identify correlations between the life trajectory of individual animals and their parasite load.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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Geographic Society, Beca de Repatriación de Investigadores Peruanos, INNOVATE PERU, and Universidad Nacional de Trujillo.

## Notes on contributors

**Matthieu Le Bailly** is a Permanent lecturer/researcher in CNRS UMR 6249 Chrono-environment (Besancon, Faculty of Sciences and Techniques).

**Nicolas Goepfert** is an investigator at the CNRS (the French national centre for scientific research). He is an archaeologist and zooarchaeologist specializing in the northern coast of Peru. His research deals with funerary and sacrificial practices linked to animals, especially South American camelids, and the adaptation of human and animals to the desert Pacific coast of Peru. He has published several articles on the topic, and he is co-editor, with S. Vásquez, C. Clément and A. Christol, of *Las sociedades andinas frente a los cambios pasados y actuales: dinámicas territoriales, crisis, fronteras y moviidades* (IFEA-LabEx DynamiTe-UNT, 2016).

**Gabriel Prieto** is a professor in archaeology at the National University of Trujillo. Since 2010 he has been working on the northern coast of Peru, specifically at Huanchaco, excavating fishing settlements from different periods. His projects focus on understanding the social dynamics and social interactions of ancient maritime communities. Prieto has published articles and book chapters in different peer-reviewed journals and books. Currently, he is co-editing, with Daniel Sandweiss, a book on ancient maritime adaptations on the South American Pacific coast.

**John Verano** is a biological anthropologist who specialises in human osteology, palaeopathology, bioarchaeology and forensic anthropology. Professor Verano's primary research area over the past 30 years has been Andean South America, with a focus on prehistoric populations of coastal and highland Peru. His research interests include the study of disease in skeletal and mummified remains, trepanation and other ancient surgery, warfare, human sacrifice, and mortuary practices. He is co-editor, with Douglas Ubelaker, of *Disease and Demography in the Americas* (Smithsonian Press, 1992) and, with Andrew Scherer, of *Embattled Bodies, Embattled Places: War in Pre-Columbian Mesoamerica and the Andes* (Dumbarton Oaks, 2014). His most recent book is *Holes in the Head: The Art and Archaeology of Trepanation in Ancient Peru* (Dumbarton Oaks, 2016).

**Dr Benjamin Dufour** is a paleoparasitologist working in Besancon university since 7 years.

## ORCID

Matthieu Le Bailly  <http://orcid.org/0000-0001-6320-6741>

Nicolas Goepfert  <http://orcid.org/0000-0002-6155-1856>

Gabriel Prieto  <http://orcid.org/0000-0001-6229-986X>

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