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SL1 RNA gene recovery from *Enterobius vermicularis* ancient DNA in pre-Columbian human coprolites ☆

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Abstract

Enterobius vermicularis, pinworm, is one of the most common helminths worldwide, infecting nearly a billion people at all socio-economic levels. In prehistoric populations the paleoparasitological findings show a pinworm homogeneous distribution among hunter-gatherers in North America, intensified with the advent of agriculture. This same increase also occurred in the transition from nomad hunter-gatherers to sedentary farmers in South America, although *E. vermicularis* infection encompasses only the ancient Andean peoples, with no record among the pre-Colombian populations in the South American lowlands. However, the outline of pinworm paleoepidemiology has been supported by microscopic finding of eggs recovered from coprolites. Since molecular techniques are precise and sensitive in detecting pathogen ancient DNA (aDNA), and also could provide insights into the parasite evolutionary history, in this work we have performed a molecular paleoparasitological study of *E. vermicularis*. aDNA was recovered and pinworm 5S rRNA spacer sequences were determined from pre-Columbian coprolites (4110 BC–AD 900) from four different North and South American archaeological sites. The sequence analysis confirmed *E. vermicularis* identity and revealed a similarity among ancient and modern sequences. Moreover, polymorphisms were identified at the relative positions 160, 173 and 180, in independent coprolite samples from Tulán, San Pedro de Atacama, Chile (1080–950 BC). We also verified the presence of peculiarities (Splicing leader (SL1) RNA sequence, spliced donor site, the Sm antigen biding site, and RNA secondary structure) which characterise the SL1 RNA gene. The analysis shows that the SL1 RNA gene of contemporary pinworms was present in pre-Columbian *E. vermicularis* by 6110 years ago. We were successful in detecting *E. vermicularis* aDNA even in coprolites without direct microscopic evidence of the eggs, improving the diagnosis of helminth infections in the past and further pinworm paleoepidemiological studies.

Keywords: Ancient DNA; *Enterobius vermicularis*; Coprolites; 5S rRNA spacer; Splicing leader RNA gene; Paleoparasitology; Polymerase chain reaction

1. Introduction

For the past two decades molecular biology techniques have been used to study DNA recovered from archaeological remains or preserved biological material, establishing the research in ancient DNA (aDNA) (Higuchi et al., 1984; Brown and Brown, 1994; Herrmann and Hummel, 1994; Hofreiter et al., 2001a; Marota and Rollo, 2002; Pääbo et al., 2004). The aDNA research introduced one more possibility of studying human and infectious diseases within an evolutionary perspective. According to Drancourt and Raoult (2005) reliable diagnoses were done through aDNA analysis focusing on pathogens such as the bacterias *Mycobacterium tuberculosis* (Crubézy et al., 1998; Rothschild et al., 2001; Zink et al., 2003), *Mycobacterium leprae* (Haas et al., 2000; Montiel et al., 2003; Donoghue et al., 2001), *Treponema pallidum* (Kolman et al., 1999)

☆ Nucleotide sequences reported in this paper are available in the GenBank™ database under accession numbers: AY234771–AY234784.

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and *Yersinia pestis* (Raoult et al., 2000; Drancourt et al., 2004); the parasitic protozoan *Plasmodium* sp. (Sallares and Gomzi, 2001) and more recently *Trypanosoma cruzi* (Guhl et al., 1999; Aufderheide et al., 2004); and the parasitic nematode *Enterobius vermicularis* (Iniguez et al., 2003a).

Enterobius vermicularis, pinworm, is one of the most common helminths worldwide, infecting nearly a billion people at all socio-economic levels and is known to have a major impact on the well-being of infants (Lukes et al., 2005). Parasite transmission has no environmental restrictions and the parasite can be transmitted from host to host without an obligatory stage in soil or intermediary hosts. It is considered that the human–*E. vermicularis* relationship originated in pre-hominid times, having evolved in Africa and dispersed to other continents through pre-historic human migrations (Ferreira et al., 1997; Hugot et al., 1999). Paleoparasitological findings and the parasite biological cycle suggest that pinworms crossed the Bering Land Bridge with human hosts during the first migratory movements into the Americas. However, transpacific routes have also been postulated (Ferreira et al., 1997).

The presence of pinworm eggs was shown in a 10,000-year-old human coprolite from Utah, USA, one of the oldest human coprolites found (Fry and Hall, 1969). Mummies and coprolites from several North American archaeological sites were positive for pinworm infection (Reinhard, 1990; Gonçalves et al., 2003), delineating a homogeneous distribution among hunter-gatherers in North America, intensifying with the advent of agriculture. This same increase also occurred in the transition from nomad hunter-gatherers to sedentary farmers in South America. *Enterobius vermicularis* infection encompasses the ancient Andean peoples, with no record among the pre-Colombian populations in the South American lowlands. Pinworm eggs were observed in coprolites from localities in Chile dating from 4100 BC (Before Christ) to 800 AD (Anno Domini) (Araujo et al., 1985; Ferreira et al., 1989); in Peru dating from 2277 ± 180 BP (Before Present) (Patruco et al., 1983) and in pre-Columbian human remains from Argentina (Zimmerman and Morilla, 1983). Old World data on this subject is curiously scarce (Bouchet et al., 2003). Herrmann (1985) found *E. vermicularis* eggs in Roman latrines and Horne (2002) recorded eggs in an Egyptian mummy.

Microscopic examination is useful in paleoparasitological diagnosis only when the recovered specimens are of good quality. Consequently, the prevalence of pinworm infection in ancient populations may have been underestimated (Reinhard, 1990; Araujo and Ferreira, 2000). Therefore, a molecular biology approach provides a specific and sensitive diagnostic tool and the opportunity to access a parasite's ancient genetic information. The 5S rRNA intergenic spacer was successfully used as a PCR target for *E. vermicularis* diagnosis in Amerindian coprolites (Iniguez et al., 2003a).

In order to verify the retrieval of *E. vermicularis* aDNA sequences directly from human coprolites and as well to investigate genetic diversity of pinworms and the relationship among geographically and temporally distinct populations of human pinworm, we have determined 5S rRNA spacer DNA sequences from 27 pre-Columbian coprolites that range in age from about 1100 to at least 6110 years and originate from four different North and South American archaeological sites. We have also characterised the presence of the SL1 RNA gene in the ancient pinworm populations.

2. Materials and methods

2.1. Coprolites and paleoparasitological analysis

Twenty-seven coprolites from Chilean and United States archaeological sites, previously examined microscopically for helminth eggs and larvae by microscopic techniques (Gonçalves et al., 2003) were used for aDNA extraction. Information about the coprolite samples is found in Table 1. The human origin of coprolites was suggested by archaeological context and confirmed by the finding of parasites specific to humans.

One sample from each of the 27 coprolites was rehydrated by immersion in a 0.5% aqueous solution of trisodium phosphate for 72 h, following the technique of Callen and Cameron (1960). The material was submitted to spontaneous sedimentation following the technique proposed by Lutz (1919). A portion of sediment was used for microscopic examination. The material was placed on a slide

Table 1
Coprolite locality, data, morphological and molecular paleoparasitological diagnosis results from *Enterobius vermicularis*

Archaeological site	Date	References	No.[a]	PA[b] (%)	PCR[c] (%)
Tiliviche, Iquique, Chile	4110–1950 BC	Araujo et al. (1985), Gonçalves et al. (2003)	2	50	50
Tulán, San Pedro de Atacama, Chile	1080–950 BC	Ferreira et al. (1989)	20	55	20
Caserones, Tarapaca Valley, Chile	400 BC–800 AD	Araujo et al. (1985)	2	50	50
Antelope House, Arizona, USA	900 AD	Reinhard (1990)	3	33.3	100
		Iniguez et al. (2003a)	27	51.8	33.3

BC: Before Christ; AD: Anno Domini.
[a] The total number of coprolites analysed.
[b] Paleoparasitological analysis. The percent of *Enterobius vermicularis* positive samples by microscopy assay.
[c] The percent of positive samples by PCR assay.