Veterinary Parasitology 235 (2017) 83–85

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

First paleoparasitological record of digenean eggs from a native deer from Patagonia Argentina (Cueva Parque Diana archaeological site)

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ARTICLE INFO

Article history:
Received 1 September 2016
Received in revised form 11 January 2017
Accepted 12 January 2017

Keywords:
Paleoparasitology
Coprolites
Trematode
Argentina
Ruminant

ABSTRACT

Eggs representative of a digenean species were found in coprolites belonged to an endemic deer from Patagonia. Samples were collected from the archaeological site named "Cueva Parque Diana". This site is a cave located at the Lanín National Park, Neuquén Province, Argentina. The coprolites were dated from 2370 ± 70 to 580 ± 60 years B.P. The eggs were ellipsoidal, operculated, yellowish and thin-shelled. Measurements (n = 65) ranged from 120.0 to 142.5 (133.2 ± 6.53) μm long and 62.5 to 87.5 (72.6 ± 6.15) μm wide. Eggs were well-preserved and were identified as belonged to Class Trematoda, Subclass Digenea, similar to those of *Fasciola hepatica* or with another species not identified at present from Patagonia. This is the first report of digenean eggs from ancient deer worldwide. The present study confirms the presence of representatives of digenean species in endemic deer from Patagonia in ancient times and the presence of a trematode disease prior to the arrival of European cattle.

1. Introduction

Trematodioses are widespread helminthoses in domestic and wild ruminants causing a serious health problem over the world. In Argentina, digenean parasitism has been considered as one of the major constraints of livestock production. Fascioliosis and paramphistomosis are considered the most important trematodioses in domestic ruminants (Suárez et al., 2007; Sanabria and Romero, 2008).

The southern pudú (*Pudu puda*) and the huemul (*Hippocamelus bisulcus*) are the two endemic species of cervids inhabiting the narrow Andean-Patagonian temperate forest strip. Actually, the southern pudú is considered as near threatened species and the huemul as endangered species (IUCN, 2016). Both species are declined and some of the possible reasons are the susceptibility to livestock diseases and the introduction of exotic species (Simonetti, 1995; Flueck and Smith-Flueck, 2012).However, there are few studies on parasitoses and other diseases that can affect to decline populations of both species.

The paleoparasitology is the study of parasites in the antiquity. Only two paleoparasitological studies were done on deer from South America. Ferreira et al. (1992) found *Eimeria* oocysts in deer coprolites from 9000 years B.P. from the site Perna I, São Raimundo Nonato, northeast Brazil and Sianto et al. (2012) found *Trichuris* sp. eggs from 1040 ± 50 year-old Cervidae coprolites from the archaeological site Furna do Estrago, Pernambuco, Brazil.

In the present paper, eggs representative of a digenean species are reported. This finding is part of the study of 33 deer coprolites examined for paleoparasitological purposes from the "Cueva Parque Diana" (CPD) archaeological site. This site is located at the Lanín National Park, Neuquén Province, Patagonia, Argentina (40°19′93′íS, 71° 20′74′íW). The site is a rock shelter part of the archaeological locality named Meliquina. It is located at 964 m.a.s.l. and 50 m close to the Hermoso River. The archaeological sequence was divided in three components representing different hunter-

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http://dx.doi.org/10.1016/j.vetpar.2017.01.011
0304-4017/

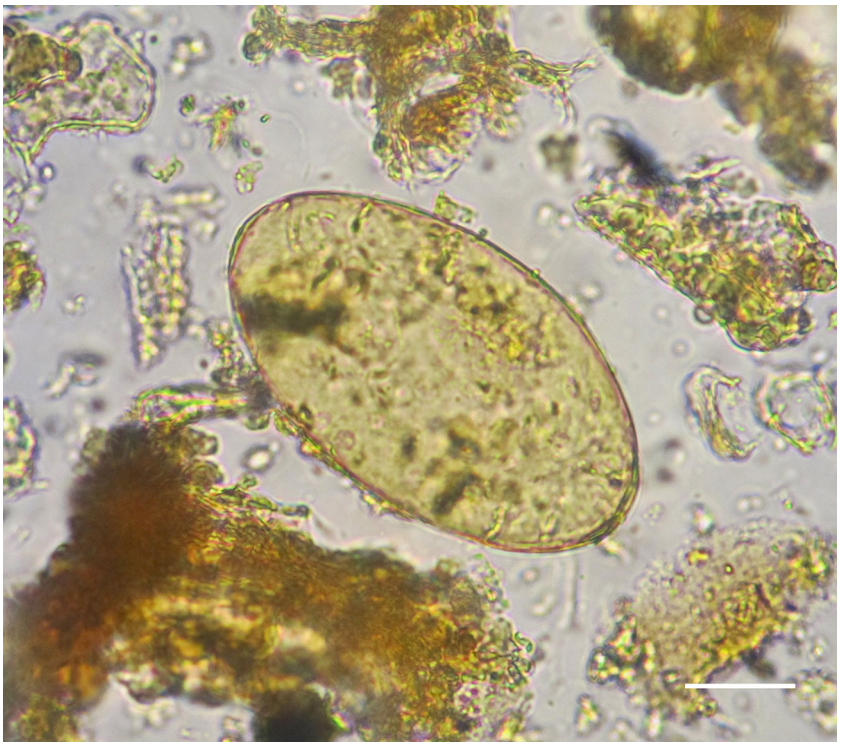

Fig. 1. Digenean eggs found in deer coprolites from the archaeological site "Cueva Parque Diana", Patagonia, Argentina. Bar: 30 μm.

gatherer occupation processes. The Upper Component was dated between 760 ± 60 to 580 ± 60 14C years B.P. (vegetal charcoal), the Middle Component was dated between 990 ± 60 to 900 ± ^{14}C years B.P. (vegetal charcoal) and the Lower Component was dated at 2370 ± 70 ^{14}C years B.P. (vegetal charcoal). The site was occupied by hunters-gatherers and fishermen along the late Holocene, incorporating pottery from the Middle Component and possibly the production of plant foods from the Upper Component (Pérez, 2010; Pérez et al., 2015). The weather in the area is cold and wet, with annual precipitations around 1500 – 2000 mm. The environment is mainly composed by colihue bamboo (*Chusquea culeou*), radal trees (*Lomatiahirsuta*) and coihues (*Nothofagus dombeyi*) and is habited by 166 native vertebrate species.

Coprolites recovered from the site were whole processed by rehydration in a 0.5% water solution of trisodium phosphate (TSP) in a glass tube for at least 72 h (Callen and Cameron, 1960), followed by homogenization. After, the samples were sieved through thrice-folded gauze for spontaneous sedimentation (Lutz, 1919) and preserved in ethanol 70%. Up to 20 slides were prepared, along with the addition of one drop of glycerin, and examined at 10 and 40 × using a light microscopy. Eggs of parasites were measured and photographed at 40 × magnifications. Egg dimensions and morphologies were compared with data from the literature in order to identify the parasites at the lowest taxonomic level. The macroscopic remains were separated and dried to room temperature for diet analysis.

Coprolites were identified by morphometry, parasite findings and biogeographic locality as belonging to huemul or southern pudú. Macroscopical observations showed vegetal remains. Sixteen of the 33 samples were positive for digenean eggs. The eggs were ellipsoidal, operculated, yellowish and thin-shelled (Fig. 1). A total of 84 eggs were counted in all samples. Measurements (n = 65) ranged from 120.0 to 142.5 (133.2 ± 6.53) μm long and 62.5 to 87.5 (72.6 ± 6.15) μm wide. Eggs were well-preserved and were identified as belonged to Class Trematoda, Subclass Digenea. The measurements and color of eggs found in this study are similar to those of *Fasciola hepatica* or with another species not identified at present from Patagonia.

Current knowledge of the digenean species on deer from Patagonia is limited. The unique species reported in wild deer was *F. hepatica*, recorded in southern pudú (Cortés, 2006; Bravo Antilef, 2013) and huemul (Díaz and Smith-Fluek, 2000; Serret, 2001). The same species was also reported in the area from the introduced red deer (*Cervus elaphus*) (Flueck and Smith-Flueck, 2012).

Fascioliosis is a foodborne important disease caused *F. hepatica* and *F. gigantica*. Fasciolid flukes follow a two-host life cycle, including a less specific adult stage which develops in many species of herbivorous mammals and even in a few omnivorous ones and highly specific larval stages which only develops in given freshwater snail species of the family Lymnaeidae (Bargues and Mas-Coma, 2005). Argentina presents a very widely distributed veterinary problem of fascioliosis in livestock (Suárez et al., 2007). Additionally, recent analysis highlights that human fascioliasis in the country may have been overlooked in the past and its real epidemiological situation may currently be underestimated (Mera y Sierra et al., 2011). Different studies on dynamics and potential reservoirs of fascioliosis in the Andean Patagonian valleys, where the disease is endemic, were made (Kleiman et al., 2007; Cuervo et al., 2015 Cuervo et al., 2015) with the final purpose to reduce the incidence of fasciolosis in cattle.

Paramphistomosis is another worldwide parasitic disease affecting cattle and other ruminants, produced by several amphistome species that can cause production losses and even mortality. This disease has shown an increased spread over the last years in Argentina. Up to date, amphistome species causing paramphistomosis in Argentina were classified as *Paramphistomum leydeni* (formerly *Cotylophoron cotylophorum*) and *Balanorchis anastrophus* (Sanabria and Romero, 2008). These rumen flukes require aquatic snails as intermediate hosts and the preparasitic stages of miracidia and stages in snails (sporocyst, redia and cercaria) are similar to those of liver flukes, such as *F. hepatica* (Ma et al., 2015).

In trematodioses shape and size of the fluke eggs shed with feces are crucial diagnostic features because of their typically reduced intraspecific variability (Valero et al., 2009). In this case, eggs of *F. hepatica* resemble those of *Paramphistomum* spp. However, eggs of *Paramphistomum* spp. are similar in shape to those of *F. hepatica* but slightly larger (160–180 μm) and transparent in aspect (Sanabria and Romero, 2008).

Fasciola hepatica is believed to be of European origin, with *Galba truncatula* as the original intermediate host species (Mas-Coma et al., 2009). Human infections with *Fasciola* spp. have been detected in coprolites and ancient latrines sediment after herding begun and until now only in the Old World paleofaeces (Gonçalves et al., 2003; Dittmar and Teegen, 2003). The most recent review of the antiquity of livestock parasites concludes that *F. hepatica* was restricted to the Old World before colonization of the New World (Reinhard et al., 2013). If the presence of *F. hepatica* in endemic deer from Patagonia from prehistoric times is confirmed, the conventional wisdom regarding the paleobiogeography of this species must be reconsidered. Future studies will confirm the diagnoses of the species.

The stratigraphic sequence of CPD displayed valuable faunistic data regarding the time span from 2370 ± 70 years B.P. to present. The study of the malacofaunistics remains recovered in sites of the archaeological locality Meliquina allowed to identify terrestrial (*Zilchogyra michaelseni*), fluvial (*Diplodon chilensis* and *Chilina gibbosa*) and marine (*Choromytilus chorus* and *Homalopoma cunninghami*) species (Pérez and Batres, 2010). Snails and freshwater bodies are crucial in the life cycle of digeneans. Previous studies also shown that autochthonous cervids (huemul and pudú) were exploited by hunter-gatherers from CPD between 2370 and 580 years B.P. (Pérez and Batres, 2008).

The present study confirms the presence of a digenean species in endemic deer from Patagonia in ancient times and the possible existence of a trematode disease prior to the arrival of European cattle.

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