Veterinary Parasitology 243 (2017) 24–28

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Short communication

Echinococcus felidis in hippopotamus, South Africa

Ali Halajian[a], Wilmien J. Luus-Powell[a], Francois Roux[b], Minoru Nakao[c], Mizuki Sasaki[c], Antti Lavikainen[d,*]

[a] Department of Biodiversity, University of Limpopo, Sovenga 0727, South Africa
[b] Mpumalanga Tourism and Parks Agency, Lydenburg, Mpumalanga Province, South Africa
[c] Department of Parasitology, Asahikawa Medical University, Asahikawa, Midorigaoka-Higashi 2-1, Hokkaido 078, Japan
[d] Immunobiology Research Program, Department of Bacteriology and Immunology, Faculty of Medicine, P.O. Box 21, FI-00014 University of Helsinki, Finland

ARTICLE INFO

Keywords:
Echinococcosis
Hippopotamus
South Africa
Rostellar hooks

ABSTRACT

Hydatid cysts of *Echinococcus felidis* are described from the hippopotamus (*Hippopotamus amphibius*) from Mpumalanga Province, South Africa. Among six hippopotami investigated, hepatic hydatids were found in three. The identification was based on mitochondrial and nuclear DNA sequences. In addition, the rostellar hook morphology was analysed. This is the first morphological description of the metacestode of *E. felidis*, and the first molecularly confirmed report of the intermediate host of *E. felidis* in South Africa. The definitive host of *E. felidis* in South Africa is the lion (*Panthera leo*).

1. Introduction

Cestodes of the genus *Echinococcus* are parasites of terrestrial mammals. They use carnivores as definitive hosts and herbivorous or omnivorous animals as intermediate hosts, and many species are zoonotic, infecting humans as accidental intermediate hosts. *Echinococcus felidis*, a species endemic to wildlife from Africa, was first described 80 years ago in the lion (*Panthera leo*) from the Northern Transvaal (present Limpopo province), South Africa (Ortlepp, 1937). It was later molecularly characterised based on archive worm material and eggs derived from lion faeces (Hüttner et al., 2008). Based on additional faecal analysis, the spotted hyena (*Crocuta crocuta*) was also confirmed as definitive host for *E. felidis* (Hüttner et al., 2009; Kagendo et al., 2014). Wild herbivores act as intermediate hosts for *E. felidis*, but the host spectrum has not been established due to lack of molecular identification and presence of sympactric species of *Echinococcus* (see e.g. Kagendo et al., 2014; Wassermann et al., 2015) in African wildlife. *Echinococcus felidis* has molecularly been confirmed only once in an intermediate host, namely in a warthog (*Phacochoerus* sp.) in Uganda (Hüttner et al., 2009).

McCully et al. (1967) reported hydatid cysts in hippopotami (*Hippopotamus amphibius*) with a prevalence of 18% in the Kruger National Park, which is located in the provinces of Limpopo and Mpumalanga (former Eastern Transvaal) in South Africa. The specific diagnosis was not established. During the severe drought season in 2016, governmental agencies in South Africa decided to cull selected compromised hippopotami that were most affected in certain nature reserves. Drought and loss of suitable water ponds forced hippopotami to wonder in areas inhabited by humans in the vicinity of the reserves resulting in possible danger to humans as some of these hippopotami became more aggressive. This gave us an opportunity to investigate the health and parasites of hippopotami. In the present study, hydatid cysts in hippopotami from the Mpumalanga Province are described and the causative species is identified as *E. felidis*.

2. Materials and methods

2.1. Origin of hippopotamus specimens

In August and September 2016, because of severe drought in north-eastern South Africa and related health problems of hippopotami, a decision was made to control the population by transporting healthy individuals to game reserves with proper dams and by culling a restricted number of compromised individuals. The nature reserves are located west of the southern or central parts of the Kruger National Park, and wildlife roams freely between these areas. Carcasses of six hippopotami were donated for necropsy. All the animals were adults, two females and four males. None of the females was gravid or lactating.

2.2. Hydatid specimens

The hippopotami were dissected in the field and their parasites were collected for subsequent investigations. Hydatid cysts were preserved in

* Corresponding author.
E-mail address: antti.lavikainen@helsinki.fi (A. Lavikainen).

http://dx.doi.org/10.1016/j.vetpar.2017.06.001
Received 26 March 2017; Received in revised form 29 May 2017; Accepted 1 June 2017

Table 1
Variation in measurements (μm) of the metacestode (hippo) and adult (lion) rostellar hooks in *Echinococcus felidis*. Figures show the range with the mean in parentheses. L, large hooks; S, small hooks; TL, total length; TW total width; AL, anterior length; PL, posterior length; GL, guard length; BL, blade length (see Fig. 1).

Host, hooks	TL	TW	AL	PL	GL	BL
Hippo, L (n = 13)	30.0–31.9 (30.9)	9.9–12.1 (10.8)	16.4–17.8 (16.8)	14.9–16.9 (16.2)	4.6–7.3 (5.8)	14.0–15.8 (14.9)
Hippo, S (n = 13)	22.8–28.6 (26.2)	7.9–10.4 (9.0)	11.9–13.5 (12.8)	13.0–17.7 (15.4)	4.2–6.5 (5.2)	9.3–11.4 (10.8)
Lion, L embedded[a,b] (n = 3)	28.3–30.6	10.4–13.2	15.7–16.2	15.1–19.1	6.6–9.3	13.0–14.4
Lion, S embedded[a,b] (n = 2)	24.3–24.4	9.3–9.9	11.5–12.9	14.5–16.1	6.1–6.5	10.3–10.5
Lion, L[b] (n = 14)	44.0–51.9 (47.5)	15.4–20.6 (18.6)	17.0–21.8 (19.7)	31.6–41.3 (37.8)	11.0–16.8 (14.8)	12.8–15.7 (14.4)
Lion, S[b] (n = 6)	29.5–39.1 (33.9)	12.2–15.0 (13.5)	13.9–15.1 (14.3)	21.9–32.0 (26.7)	9.9–11.6 (10.6)	9.7–11.3 (10.3)
Lion, L[c] (n = 4)	38.2–41.5 (39.6)	13.8–18.2 (16.7)	15.6–19.0 (17.5)	25.0–28.6 (27.0)	7.7–11.7 (9.8)	11.1–14.6 (13.1)
Lion, S[c] (n = 4)	30.4–34.3 (31.7)	12.2–15.1 (13.8)	11–13.3 (12.3)	23.0–25.5 (23.8)	5.6–9.2 (7.9)	8.2–10.1 (9.3)

[a] Larval hooks, which were visible within the adult hooks.
[b] Hooks from specimens identified to species by Anna Verster.
[c] Hooks measured from drawings in Ortlepp (1937).

pure ethanol.

As our novel innovation, protoscoleces were digested with a proteinase K lysis buffer (a reagent of DNeasy blood and tissue kit, Qiagen) on microscope slides for examining hook morphology. Enzymatic lysis was used because it yielded, without affecting the hook measurements, a clearer view and higher number of well-aligned hooks than e.g. mounting with Berlese's medium. Hooks were photographed and measured with ImageJ (https://imageJ.nih.gov). Six linear measurements (Hobbs et al., 1980; Gubányi, 1995; Haukisalmi et al., 2016) were taken from hooks aligned well in the horizontal plane (see Table 1, Fig. 1). The measured hooks were from 15 different protoscoleces from two hosts. Hook numbers were counted from intact crowns. Histology of the cyst wall was examined with hematoxylin and eosin stained slides.

DNA was extracted from protoscoleces using the DNeasy blood and tissue kit. Previously published primers (Bowles and McManus, 1993; Nakao et al., 2000; Knapp et al., 2011) were used to amplify partial sequences of two mitochondrial genes (*cox1*, cytochrome *c* oxidase subunit 1; *nad1*, NADH dehydrogenase subunit 1) and two nuclear genes (*pepck*, phosphoenolpyruvate carboxykinase; *pold*, DNA polymerase delta). PCR and sequencing were performed as previously described (Knapp et al., 2011; Hailemariam et al., 2012).

2.3. Morphology of adult Echinococcus felidis hooks

Morphology of adult rostellar hooks of *E. felidis* was studied for comparison. The adult worm specimens were from an archived piece of small intestine taken from a lion in South Africa. The parasite species had been morphologically identified as *E. felidis* by Anna Verster in the 1960s, and subsequently molecularly characterised by Hüttner et al. (2008). The specimens available to us were surplus from the study by Hüttner et al. (2008). The worms had been crushed with a mortar for DNA extractions, and stored in a freezer. Therefore, we could not examine other characteristics than hook morphology. Because the intestinal sample had originally been fixed in formalin, a digestion with proteinase K could not be applied, and we cleared and mounted the residue in Berlese's medium for observing adult hooks. The same measurements were taken as for larval hooks.

3. Results

3.1. Species identification

Hydatid cysts were found in the liver of one female and two male hippopotami. No cysts were found in any other organs of the examined

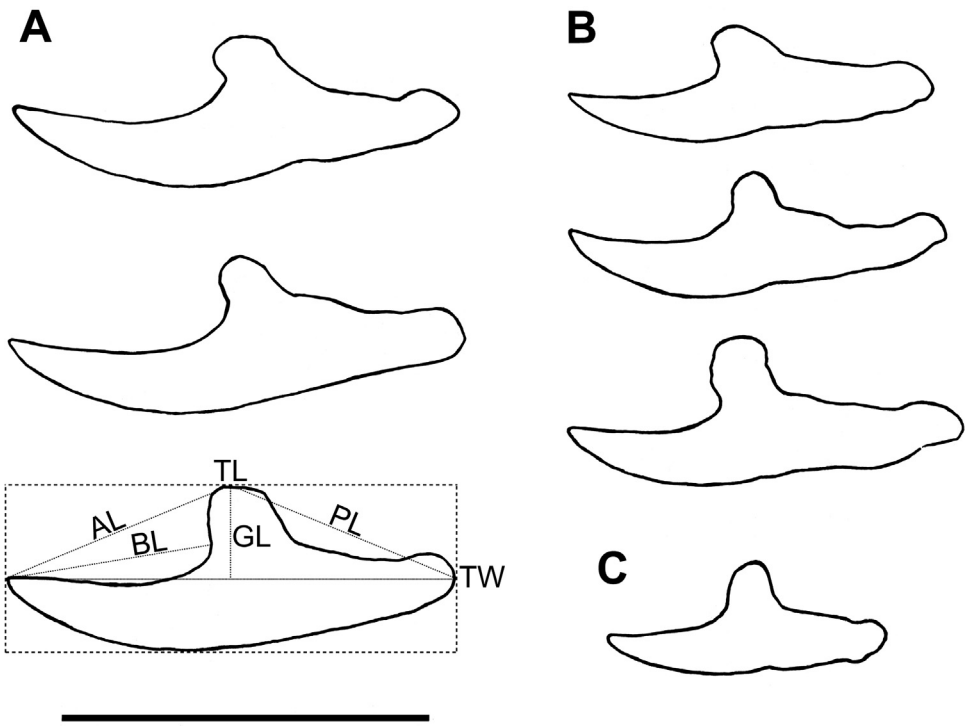

Fig. 1. Outline drawings of metacestode rostellar hooks of *Echinococcus felidis* from two hippopotami. A, large hooks; B, small hooks; C, a small accessory hook. Hook measurements marked with abbreviations: TL, total length; TW total width; AL, anterior length; PL, posterior length; GL, guard length; BL, blade length. Scale bar: 25 μm.