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Short communication

Echinococcus felidis in hippopotamus, South Africa

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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

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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

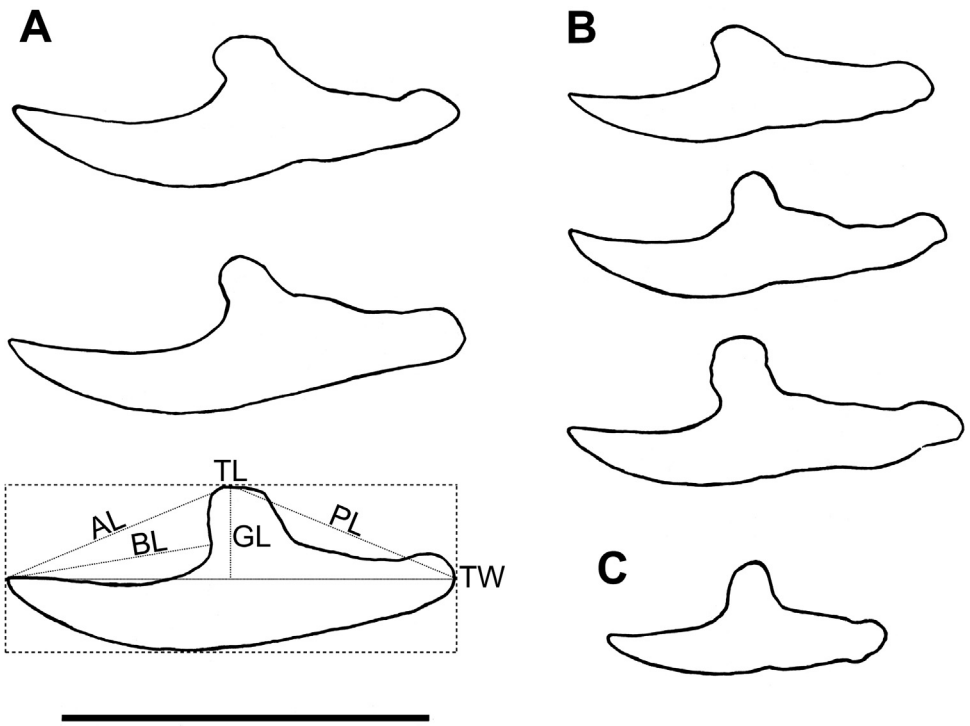

Fig. 1. Outline drawings of metacestode rostellar hooks of *Echinococcus felidis* from two hippopotami. A, large hooks; B, small hooks; C, a small accessory hook. Hook measurements marked with abbreviations: TL, total length; TW total width; AL, anterior length; PL, posterior length; GL, guard length;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

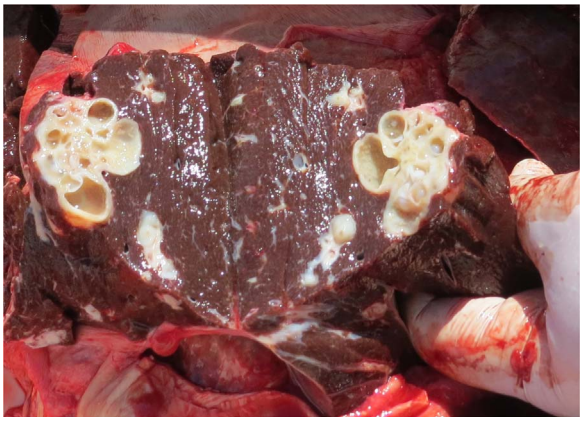

Fig. 2. Polycystic metacestode of *Echinococcus felidis* in the liver of a hippopotamus.

animals. The specimen of one male hippo was unfortunately lost, and thus we were able to investigate only specimens of two animals. Sequences of *cox1* (778 bp) and *nad1* (488 bp) showed respectively 99.6–99.7% and 100% sequence identities with reference sequences derived from the published mitochondrial genome of *E. felidis* (NC_021144). Nuclear sequencing confirmed the identification with 100% and 99.9% sequence identities in *pold* (1930 bp) and *pepck* (1549 bp), respectively, with published sequences of *E. felidis* (FN568360 and FN567989).

Sequence data of the present study are available in DDBJ/EMBL/GenBank databases under the accession numbers KY794644–KY794648.

3.2. Metacestode morphology

The metacestodes (ca. 1–3 cm in diameter) were located in the liver with one or two hydatids per animal. Both unilocular cysts and polycystic metacestodes with multiple vesicles were found (Fig. 2). Some unilocular cysts contained many daughter cysts. The cyst wall consisted of three typical layers (Fig. 3): outermost thick fibrous pericyst, middle acellular laminated membrane and inner germinal cell layer. The laminated layer was ca. 200–400 µm thick in hematoxylin-eosin stained microscope slides. The cysts were fertile containing brood capsules and numerous protoscoleces (Fig. 4).

3.3. Hook morphology

Protoscoleces were invaginated and on average 160 × 130 µm in size. The number of rostellar hooks was 34–40 (mean = 37, n = 16).

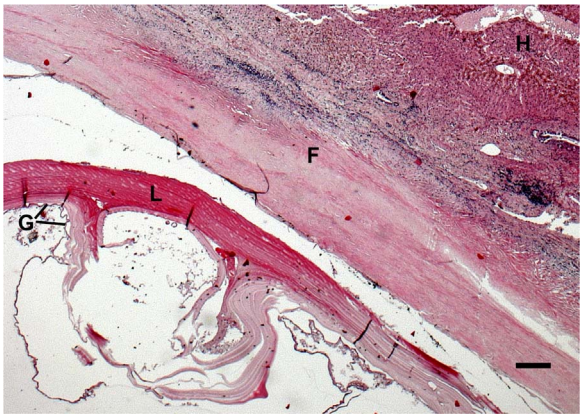

Fig. 3. Histopathological section through the cyst wall of *Echinococcus felidis* from a hippopotamus. G, germinal layer; L, laminated membrane; F, fibrotic pericyst; H, hepatic parenchyma. Hematoxylin-eosin stain. Scale bar: 200 µm.

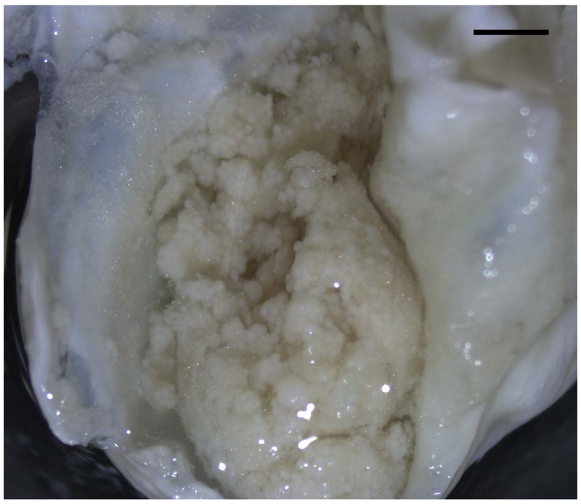

Fig. 4. Protoscolex mass in a metacestode of *Echinococcus felidis* from a hippopotamus. Ethanol preserved specimen. Scale bar: 1 mm.

The hooks were typically in two rows, but an incomplete third row with small accessory hooks (not included in the hook numbers above) was seen in two protoscoleces among dozens screened. Hook drawings are presented in Fig. 1, and measurements are given in Table 1. The total length of the large hooks was 30.0–31.9 µm (mean = 30.9 µm, n = 13) and that of the small hooks 22.8–28.6 µm (mean = 26.2 µm, n = 13). The guard was located in the middle or slightly posteriorly in the large hooks (anterior length, AL ≥ posterior length, PL), but in the small hooks the blade was shorter than the handle (AL < PL).

The adult hooks from the specimens from a lion (Fig. 5) were clearly larger than the protoscolex hooks (Table 1) due to larger handle and guard. The shape and size of the blade were identical. During the development of the adult hooks, extra material is laid on the handle and guard, and the embedded larval hook can be observed within the adult hook (Hobbs et al., 1980). In the present specimens, the adult hooks were covered by crushed strobilae (mainly eggs), and in most cases only the outline of the adult hook could be seen. We were able to measure embedded larval hooks within three large and two small adult hooks only (Table 1). The embedded hooks corresponded in size, and in general shape, to the protoscolex hooks from hippopotamus, but in two embedded large hooks the guard was more anterior (AL < PL).

The large adult hooks examined in this study were longer than in the description of *E. felidis* by Ortlepp (1937) (Table 1, Fig. 5). This is due to the longer handle. The size and shape of the small hooks were more similar.

The complete set of *E. felidis* hook drawings and measurements is available at Mendeley data (http://dx.doi.org/10.17632/7xjymk48wg.1).

4. Discussion

The present study shows the infectivity of *E. felidis* in the hippopotamus. Metacestodes were fertile, indicating that hippopotami can participate as intermediate hosts in the life cycle of the parasite. According to a literature survey (Hayward and Kerley, 2005) hippopotamus comprised almost 6% of the reported total kills by lion in Africa. This might be an overestimate, since hippopotamus is not an optimal lion prey due to its large size, strength and aggressive nature. However, lions not only prey on, as collective hunters, but also scavenge hippopotami (Hayward and Kerley, 2005).

The hippopotamus is widespread in Sub-Saharan Africa, but populations are decreasing in many countries (Lewison and Oliver, 2008). Available population estimates range between 125,000 and 148,000, and currently the hippopotamus is classified as a vulnerable species by

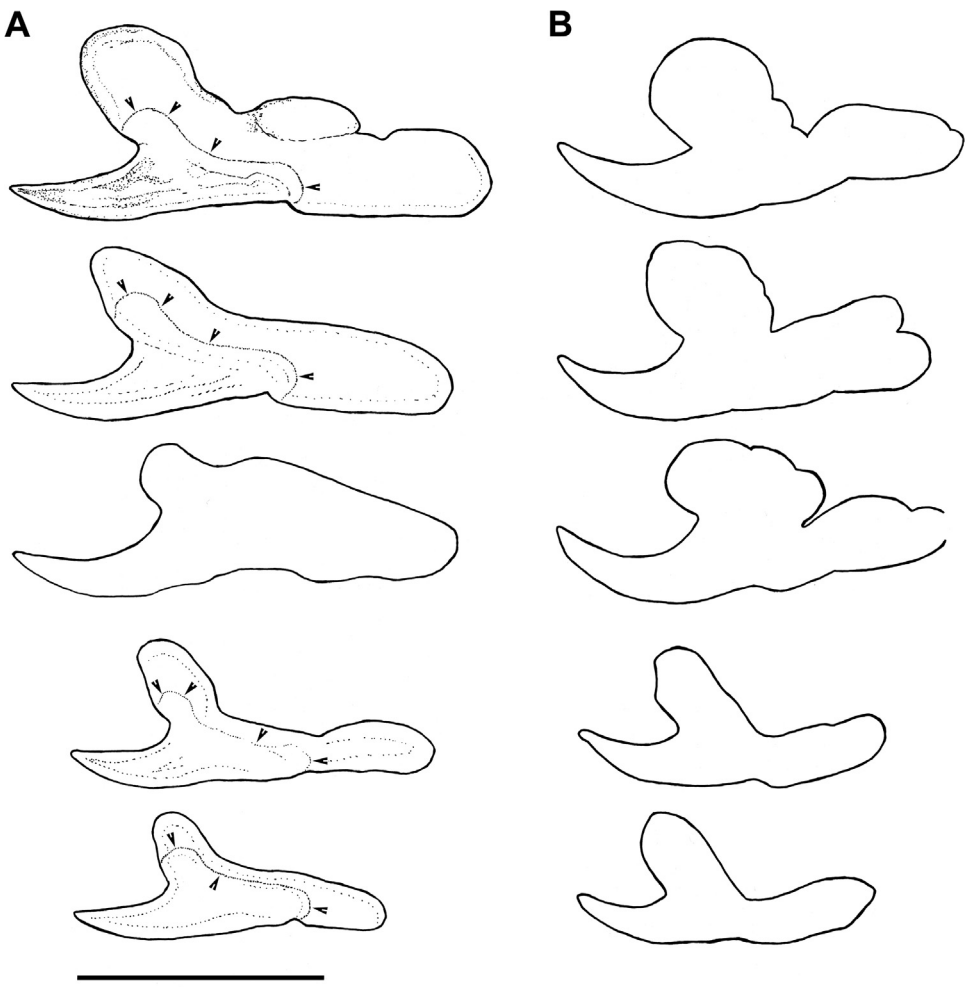

Fig. 5. Drawings of adult rostellar hooks of *Echinococcus felidis* from lions. A, large and small hooks from specimens examined in the present study. Outline of embedded metacestode hooks shown with arrows. B, large and small hooks redrawn from Ortlepp (1937). Scale bar: 25 µm.

IUCN (the International Union for Conservation of Nature) Red List categories (Lewison and Oliver, 2008). The population decline can be attributed to habitat loss, exploitation, drought and poaching, although diseases may also be a contributing factor. Because the animals of this study were chosen from those looking unhealthy and in poor condition, and due to the fact that four of them also had fascioliasis in liver, we cannot draw a conclusion regarding the pathogenicity of *E. felidis* in hippopotami.

The nature reserves of this study are adjacent to the Kruger National Park with fenceless borders, indicating that the life cycle of *E. felidis* involving lions and hippopotami may occur widely in the area. Warthogs probably serve together with hippopotami as intermediate hosts, because it is known that *E. felidis* is infective to warthogs (Hüttner et al., 2009) and hydatid cysts have previously been reported in warthogs in South Africa in different localities (Horak et al., 1988; Boomker et al., 1991; Van Wyk and Boomker, 2011).

The nature reserves were established as 'big five game reserves' i.e. for the lion, leopard (*Panthera pardus*), elephant (*Loxodonta africana*), rhinoceroses (*Diceros bicornis* and *Ceratotherium simum*) and Cape buffalo (*Syncerus caffer*). Carnivore species living in these reserves, and previously reported as definitive hosts of *Echinococcus* spp. in Africa (Hüttner and Romig, 2009), include the lion, spotted hyena, African wild dog (*Lycaon pictus*), black-backed jackal (*Canis mesomelas*) and African wild cat (*Felis silvestris lybica*). The latter is probably an accidental host in the transmission of cystic echinococcosis, as it mainly eats small animals. Other potential definitive hosts for *Echinococcus* spp. in this area, due to their role as predators or scavengers, include the leopard, cheetah (*Acinonyx jubatus*) and side-striped jackal (*Canis adustus*). Regarding potential intermediate hosts, there are no domestic animals in these reserves but wild herbivores like common South African antelopes, giraffe (*Giraffa giraffa*), zebra (*Equus quaqqa burchellii*), bushpig (*Potamochoerus larvatus*), warthog and others occur widely.

Considering the diversity of available potential hosts, the life cycle of *E. felidis* may resemble a network related to predator-prey relationships. In addition, interactions with the surrounding pastoral areas and evidence of different species of *Echinococcus* in African wildlife (e.g. *Echinococcus equinus* in lion/jackal-zebra cycle in Namibia, *Echinococcus ortleppi* in the oryx, *Oryx* sp., in Namibia, and *Echinococcus granulosus* sensu stricto in lions in Kenya and a warthog in Uganda) (Hüttner et al., 2009; Kagendo et al., 2014; Wassermann et al., 2015; Addy et al., 2017) suggest that the epizootology of echinococcosis is complex. Further studies are needed to establish all hosts of *E. felidis*, its prevalence in different species, as well as the public health importance of the parasite. Veterinary services must be aware of the need of inspection of wildlife meat, as carcasses are often given to local communities during culling events. Dogs are typically the source of human echinococcosis, but in the case of *E. felidis*, domestic cats should also be taken into account.

McCully et al. (1967) reported unilocular hydatid cysts in hippopotami (in 17 out of 97 animals examined) from the same biogeographic area as our study area. We found not only unilocular cysts but also polycystic lesions. Polycystic metacestodes are typical of Neotropic *Echinococcus vogeli* but have also been reported in *E. granulosus* sensu lato in cattle (Rausch, 1995; D'Alessandro and Rausch, 2008). Verster (1965) redescribed the strobilar morphology of *E. felidis*, but she did not examine the rostellar hooks. We noted differences in the length and shape of large hooks between specimens identified by Verster and the original description by Ortlepp (1937). We used, however, Ortlepp & s

(1937) rather rough drawings, not the type specimens, for comparison. In addition, different age of worms may affect the hook morphology (Hobbs et al., 1980). Ortlepp & s (1937) written description matches better than his drawings with the specimens we examined, as he described rugged handle and guard with gnarled appearance and not strongly curved blade. The variation in strobilar morphology including rostellar hooks, as well as metacestode characteristics in different hosts, remain topics for further research.

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