WILDLIFE

Parasitism by *Ophidascaris robertsi* with associated pathology findings in a wild koala (*Phascolarctos cinereus*)

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SUMMARY

Five third-stage *Ophidascaris robertsi* larvae, a python parasite, were recovered from a free-ranging mature male koala, *Phascolarctos cinereus*, from South-East Queensland. Most larval nematodes were found obstructing several hepatic blood vessels including the portal vein, causing vascular dilation. Despite the low number of parasitic larvae found, the large size of the larval third stage can lead to circulatory impairment of affected organs. Koalas may acquire *O robertsi* infection possibly by performing geophagy or soil ingestion, contaminated with eggs from python faeces. This is the first report of *O robertsi* in koalas indicating infection and subsequent pathological changes within the vasculature and liver.

BACKGROUND

Ophidascaris robertsi is a python nematode of Australia and Papua New Guinea with an indirect life cycle. To date, this ascarid has been described in small to medium-sized marsupial species, but not koalas. Often, infections of native fauna with endemic parasites such as *O robertsi* result in pathological changes that are self-limiting or cause no disease. However, the size of the infective L3 is considerably large, leading to organ distortion potentially from vascular disturbance. Even if infrequently observed, the description of novel agents is of value, especially in a species with vulnerable conservation status such as the koala in South-East Queensland where disease and trauma have caused catastrophic declines and focal extinctions.

CASE PRESENTATION

A mature wild koala (*Phascolarctos cinereus*) was found on the ground in lateral recumbency in early autumn. Clinical assessment revealed it had lost two claws (right forelimb digit 3 and right hindlimb digit 5) and had four claws broken (right hindlimb digit 5 and right forelimb digit 4) with secondary bacterial infection resulting from a purulent discharge originating at the nail-bed. Numerous ticks were recovered from the koala (not identified), and several small focal granulomatous reactions (eg, tick craters) on the skin at the adult tick bite site were found. The koala also had a markedly low body condition score (2/10). At the hospital, the koala was found to be severely anaemic with a packed cell volume of 10 per cent (reference range mean 37, range 29–44 per cent for free-ranging koalas), hypoproteinaemic with a total protein of 46 g/l (reference range mean 71, range 58–88 for free-ranging koalas)[1] and a slightly low urine specific gravity (1.027). The koala was euthanased on December 4, 2014 due to its deteriorating condition. A preliminary postmortem examination was conducted at Currumbin Wildlife Hospital, the submitting institution in which a heavy burden of tapeworms in the small intestine was reported as well as free round worms within the abdomen. Preliminary postmortem examination dissections are often done on adult koalas at the point of care to collect pap material or for disease investigation. Pap is caecal faeces fed by dams to pouch young to inoculate gut flora, and thus caecal content is harvested from deceased koalas to facilitate hand raising of orphaned young. The specific reason for the preliminary investigation was not recorded on submission history. The carcass was then frozen and sent to the School of Veterinary Science at The University of Queensland for a complete postmortem examination and parasite identification.

The main pathological findings involved the liver and the kidneys. Grossly, the main finding was distortion of the hepatic margins with poorly defined raised areas resulting in a 'dome-like' appearance. On dissection of one of the raised areas, four 8 cm long ascaridoid nematodes were collected from the portal circulatory system (figure 1A). A similar nematode was found encysted next to one of the thoracic vertebrae, 1 cm above the diaphragm (figure 1B). Other findings included a possible distension of the pericardial sac; however, assessment of the pericardial fluid quantity was not possible because the sac was ruptured during the preliminary postmortem examination. As reported by the submitting institution, multiple fragments of anoplocephalid cestodes compatible with *Bertiella obesa* were found within the lumen of the small intestine, considered incidental.

The only relevant histopathological and clinical findings were increased amounts of collagen in both the renal cortical and medullary interstitium, as well as thickening of the Bowman's capsule and proteinuria. Renal, hepatic and nutritional conditions can all lead to hypoproteinaemia; however, given the clinical, gross and histological findings in the kidneys, the hypoproteinaemia in this case

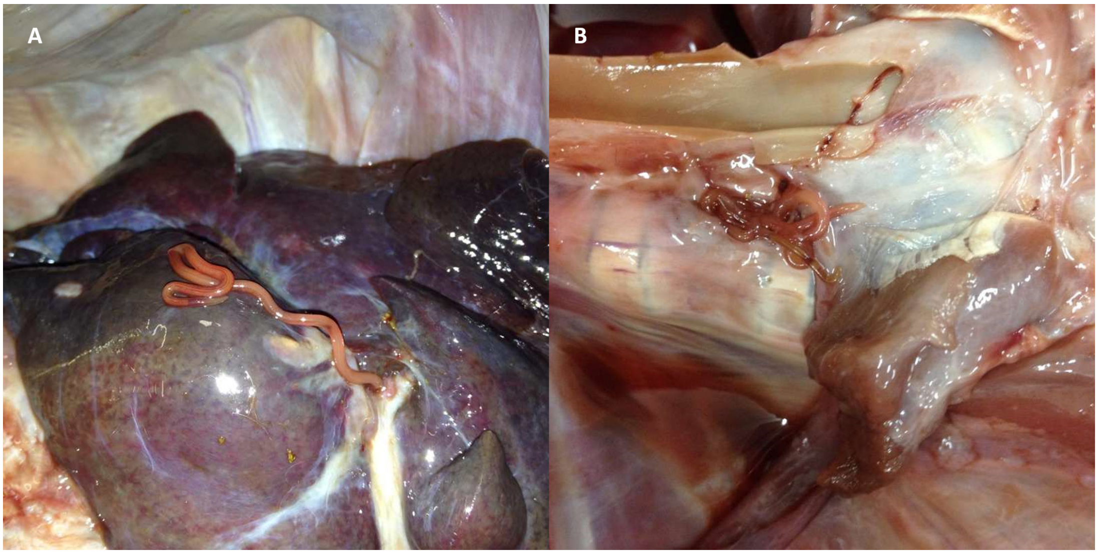

Figure 1 *Ophidascaris robertsi* (A) infecting the portal vein in a koala, and (B) parasitising the paravertebral thoracic tissue in a koala.

was presumed to be renal related. Unfortunately, the liver was autolysed which hampered histological assessment. Although autolysis and freezing are known to alter tissue morphology, changes such as hypercellularity (eg, inflammation, neoplasia) and fibrosis can still be observed in instances where there is poor differential staining. It was presumed that infection by *O robertsi* caused local tissue scarring and vascular changes in the liver. However, this was likely unrelated to the debilitation and anaemia causing the euthanasia of the koala, which was caused by chronic renal disease.

INVESTIGATIONS

Parasitological keys

Primary *O robertsi* morphological features (figure 2) include clearly defined body cavity, excretory cell, oesophagus and a rectum adjacent to the intestine.(figure 2) The nematode's mouth sits in a concavity circumscribed by a cuticular rim with three lips and a lateral papilla(Figure 3) . Lateral chords are found extending the whole length of the body, but no lateral alae was observed in L3; however, occasionally rudimentary alae can be seen. The tail is directed ventrally and terminates in a needle-like spike. Lastly, a cuticular annulation is evident behind the lips as the parasite develops.[2]

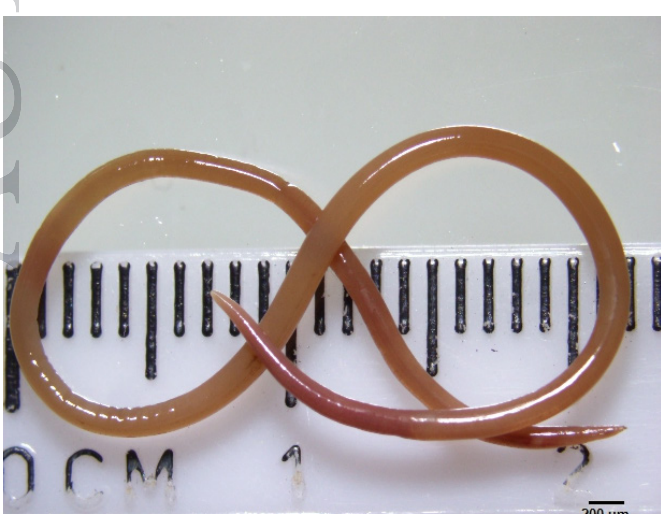

Figure 2 Larvae stage 3 from *Ophidascaris robertsi* extracted from the liver.

DISCUSSION

This case represents the first report of parasitism by *O robertsi* in koalas, despite being found in most small to medium-sized marsupials in Papua New Guinea and Australia.[3]

O robertsi[4] has pythons as definitive hosts and has an indirect life cycle.[2 4] The adult parasites occur in the oesophagus and stomach of carpet pythons forming nodular masses, inserting a small anterior portion of the body into the nodule protruding from the digestive mucosa. The L3 is typically found in the liver of the intermediate host (small rodents, marsupials) that have ingested embryonated eggs. Eggs are shed in faeces which undergo multiple development stages until becoming motile larvae (0.4–0.5 mm) in days 10–12. Eggs containing L2 (0.4–0.6 mm) are apparently more resistant to harsh environmental conditions (desiccation), and only require a moisture cue to trigger a signal to hatch. The larval form of *O robertsi* displays little host specificity, being commonly found in small marsupials and rodents in forested areas where the cycle is completed once the intermediate host is consumed by a python, the definitive host. Then, the L3 larvae emerge following the second moult in the liver of the infected host, measuring up to 8 cm long, which corresponds to what was found in this koala. Infections

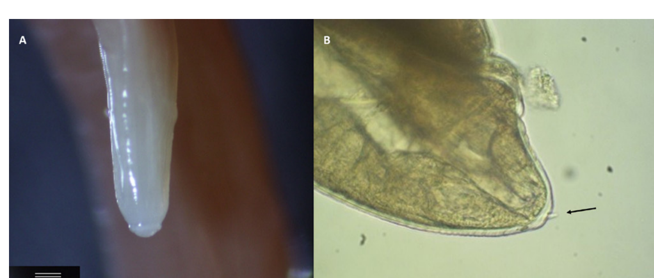

Figure 3 (A) Anterior end of larvae displaying a concave mouth with three lips, (B) posterior end with spine (arrow).

have been reported to reach high densities (up to 200 nematodes in a liver of a short-eared brushtail possum, *Trichosurus caninus*). Besides the present report in koalas, up to 23 species of intermediate hosts had been reported for *O robertsi* belonging to taxonomic marsupial families of Dasyuridae, Macropodidae, Hypsiprymnodontidae, Peramelidae, Petauridae, Phalangeridae, Potoridae, spiny anteaters (Tachyglossidae) and in murid rodents.[2 4–7] A striking feature of the morphology of *O robertsi* is the size of the L3 (8 cm), particularly noticeable during heavy burdens in small 30–40 g marsupials (antechinuses).

It is likely that koalas and *O robertsi* have shared the same geographical distribution judged by that of their definitive and intermediate hosts. The fact that it has not been previously detected in koalas in comparison to other metatherian mammals is plausibly due to the arboreal life of this species. Koalas have a highly specialised diet based primarily in *Eucalyptus* trees, a highly toxic but broadly distributed food source. There is some evidence that koalas practise geophagy or soil ingestion.[8] Many factors can trigger geophagy, including diet supplementation,[9] alleviation of the toxic effects of secondary plant metabolites, as famine food or just to satisfy the senses.[10 11] Through geophagy, koalas could easily ingest soil or gravel contaminated with embryonated eggs excreted by a python. Transmission may also be plausible if this koala consumed browse contaminated with python faecal matter containing *O robertsi* eggs. Intermediate hosts may also become infected via alternative methods, as larvae are extremely active and can penetrate the skin and reach body cavities or come in and out of thoracic and abdominal organs (Spratt, personal communication in ref 12).

Geophagy may also be the route of accidental ingestion of soil mites (Oribatidae) contaminated with eggs of *B obesa*, a common and apathogenic anoplocephalid cestode known to parasitise koalas.[13]

This wild koala was euthanased due to its poor prognosis derived from the anaemia, hypoproteinaemia and extreme low body condition. Renal histopathological findings are compatible with a degenerative nephropathy, which was considered unrelated to the presence of the *Ophidascaris*. The anaemia could have a renal origin, but might also relate to the high tick infestation reported in the clinical history. Although tick identification and life stage were not determined in this case, ectoparasitosis in koalas is vastly represented by hard ticks (Mesostigmata:Ixodida).[14] Exsanguination from marked tick infestation has been documented in koalas, primarily affecting juveniles and occasionally adults.[15–17] This may occur when nature habitat has changed forcing koalas to travel for longer distances through dense scrubby undergrowth.[16] Although histopathological assessment in the liver was hampered due to autolysis and freezing artefact, gross findings were indicative of fibrosis and a potential blockage plausibly causing vascular disturbance, but given the localised effect, it is unlikely it significantly contributed to health impairment in this koala.

This is the first report of infection by *O robertsi* in a wild koala illustrating a potential for vascular disturbance due to the size of the worms; a minor pathological sequela of infection by a native parasite. Despite the numbers of larva found being low, reporting the case is relevant for koala health due to their current vulnerable conservation status, particularly in South-East Queensland. In future koala postmortem examinations, nematodes should be identified so the frequency of infection by *O robertsi* in koalas admitted to hospitals can be monitored, particularly in cases indicating vascular congestion or disturbance.

Contributors VGA conducted the research and drafted the manuscript. LK performed the parasitology identification and helped take figure images. LV helped perform the necropsy, assisted in documenting the gross findings and taking images of the gross findings. JH codirected the koala pathology research project with RA and helped do data analysis on the cases. As the senior diagnostic pathologist, RA reviewed the gross and microscopic findings of the cases, drafted and edited the manuscript and was codirector of the pathology research project.

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