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# Occurrence of tongue worm, *Linguatula cf. serrata* (Pentastomida: Linguatulidae) in wild canids and livestock in south-eastern Australia

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ABSTRACT

Pentastomids are obligate zoonotic arthropod parasites utilising canids and vulpids as their definitive hosts and several herbivorous species as their intermediate hosts. Reported only 10 times in Australia over the last 150 years as incidental findings, adult Pentastomids referred to as *Linguatula serrata* have been encountered in nasal cavities of domestic and wild dogs, and foxes. Nymphs have been reported in cattle and rabbits. In the present study, a number of potential definitive hosts, including red foxes (*Vulpes vulpes*), wild dogs (*Canis lupus dingo* and *C.l. dingo* x *C. familiaris*) and feral cats (*Felis catus*), and intermediate hosts cattle (*Bos taurus*), sheep (*Ovis aries*), feral pigs (*Sus scrofa*), rabbits (*Oryctolagus cuniculus*), goats (*Capra hircus*) and a European hare (*Lepus europaeus*), from the highlands of south-eastern Australia were examined. Of the animals examined 67.6% of wild dogs (n = 37), 14.5% of red foxes (n = 55) and 4.3% of cattle (n = 164) were found to be infected with Pentastomids, herein identified as *Linguatula cf. serrata*. The common occurrence of the parasite in wild dogs and less frequently in foxes suggests these wild canids have potential to act as a reservoir for infection of livestock, wildlife, domestic dogs and possibly humans. The unexpected high frequency of the parasite in wild dogs and foxes in south-eastern Australia suggests the parasite is more common than previously realised. Of the potential intermediate hosts in the region, only 4.3% of cattle were found to be infected with pentastomid nymphs which suggest the search for the host(s) acting as the main intermediate host in the region should continue. Future studies should investigate transmission patterns, health impacts on hosts and whether the parasite has zoonotic significance in Australia.


## 1. Introduction

Members of the genus *Linguatula,* also known as tongue worms due to their resemblance to the mammalian tongue, are obligate arthropod parasites which inhabit the upper respiratory tract of canids such as domestic dogs, foxes and wolves. After fertilisation, gravid females release millions of eggs during their mature lifetime. These eggs are expelled into the environment in nasal secretions and/or swallowed and passed in faeces (Riley, 1986). Most herbivores, including ruminants such as sheep, cattle and camels may serve as intermediate hosts for *Linguatula* species, becoming infected through accidental consumption of pasture contaminated with eggs resulting in visceral linguatulosis in the herbivore host (Tavassoli et al., 2007). Following ingestion of eggs by an intermediate host, the primary larvae emerge into the lumen of the intestine and penetrate the intestinal wall. Following a phase of migration, the larvae encyst in visceral tissues of the intermediate host such as the liver, lungs and mesenteric lymph nodes where they complete several moults before becoming infective nymphs (Riley, 1986; Paré, 2008). To complete the life cycle, infective nymphs must be consumed by a canid. This usually occurs as a result of predator/prey interaction or scavenging. Following ingestion the nymphs move from the digestive tract, up the oesophagus to the nasal cavity where they develop into mature adults (Riley, 1986; Paré, 2008). Zoonotic cases of infection with *L. serrata* have been reported from several countries (Self and Kuntz, 1967; Riley, 1986; Bowman, 1995; Lazo et al., 1999; Paré, 2008; Koehsler et al., 2011; Bhende et al., 2014; Oluwasina et al., 2014).

In Australia, knowledge of *L. serrata* is poor. The parasite has been reported only 10 times over the past 150 years, almost always as incidental findings. Adult pentastomids, identified as *L. serrata* having been reported in the nasal cavities of dingoes, domestic

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dogs and foxes (Johnson, 1910; Pullar, 1946; Durie and Riek, 1952; Riley et al., 1985) and nymphs have been encountered in cattle and rabbits (Ralph, 1865; Barnard and Park, 1893; Johnston and Cleland, 1910; Johnston, 1911; Pullar, 1936; Durie and Riek, 1952). None of these studies provide a detailed morphological description which makes specific identification of the parasite according to current criteria difficult. Wild dogs predate on livestock (mainly sheep) which in some areas is a major agricultural issue (Allen and Fleming, 2004), but their diet mainly consists of small macropodid marsupials, particularly swamp wallabies (*Wallabia bicolor*) (Newsome et al., 1983; Robertshaw and Harden, 1986). Wild dogs, and to a lesser extent foxes, play a key role in transmission of parasites of veterinary and human health importance in Australia, particularly *Echinococcus granulosus* (Stevenson and Hughes, 1988; Saunders et al., 1995; Jenkins and Morris, 2003; Jenkins et al., 2005). In parts of the world where *Linguatula* spp. occur commonly in canids, prevalence of the parasite in livestock is also high. This transmission cycle between domestic hosts (dogs and livestock) makes these species significant reservoirs for potential zoonotic infection. The lack of reports of *Linguatula* infection in Australian wild or domestic canids suggests the parasite occurs rarely. However, the nasal cavity of canids are rarely examined during post mortem examinations, especially those of wild dogs and foxes and the parasites although present, may simply have been overlooked. The aim of this study was to undertake a preliminary investigation into the occurrence and distribution of adult *Linguatula* spp in wild canids and nymphal stages in domestic livestock in south-eastern Australia.

## 2. Materials and methods

### 2.1. Wildlife definitive hosts

Wild dogs (*Canis lupus dingo* and *C.l. dingo* x *Canis familiaris*), foxes (*Vulpes vulpes*) and feral cats (*Felis catus*) were obtained from professional vertebrate pest control officers of the Australian Capital Territory (ACT) Parks and Conservation Service, New South Wales (NSW) Forests, NSW Local Lands Services and the Victorian Department of Environment, Land, Water and Planning. These animals were trapped and shot by these officers during the normal course of their duties. The heads of the animals were removed, packed in labelled plastic bags and kept frozen until examined.

### 2.2. Collection of mesenteric lymph nodes from cattle

Mesenteric lymph nodes from cattle (*Bos taurus*) were collected by meat inspectors in a local abattoir. Since there were no recent data on the occurrence and prevalence of *Linguatula* spp in cattle in south eastern Australia, collection of lymph nodes was restricted to cattle that were most likely to have been grazing in rough bush pasture, areas most likely also to be inhabited by wild dogs. Between 1 and 7 mesenteric lymph nodes were collected from each animal. All lymph nodes collected from a single animal were placed into labelled plastic bag and stored at 4 °C until examined.

### 2.3. Other potential intermediate hosts examined

Mesenteric lymph nodes from small numbers of a range of other potential intermediate hosts were also examined. These animals were collected opportunistically or donated from several sources. Mesenteric lymph nodes from sheep (*Ovis aries*) were collected in a local abattoir. The sheep were from a property near Dubbo and one near Holbrook, both locations in NSW. The rabbits (*Oryctolagus cuniculus*) were provided by the NSW forests vertebrate pest control officer, Tumbarumba. The hare (*Lepus europaeus*) was found as road kill on the Charles Sturt University Campus, Wagga Wagga. The two feral pigs (*Sus scrofa*) were found dumped on the side of the road between Wagga Wagga and Coolamon, NSW, but their origin was unknown. The two feral goats (*Capra hircus*) were shot on a property at Mangoplah, NSW and donated by the property owner.

### 2.4. Parasite collection

The skulls of dogs, cats and foxes were split into two halves using a hatchet and a hammer. This unsophisticated procedure enabled us to cleave the skull whilst not damaging tongue worms that may be been present. It also enabled us to obtain a clear view into the right and left sides of the nasal cavity and to see any tongue worms that were present (Fig. 1). Each side of the nasal cavity was extensively searched macroscopically for adult tongue worms by carefully removing any tissue or structures such as the conchae with forceps (Fig. 1). After removal of the tissue and any clearly visible tongue worms the nasal cavities were irrigated with running water into a 300μ sieve and all additional tongue worms dislodged (usually the small males) were backwashed from the sieve into a dish and collected (Fig. 2). All tongue worms collected were rinsed in distilled water before being preserved in ethanol (70%) or 10% formalin solution. Mesenteric lymph nodes from cattle, sheep, pigs, hare, rabbit and goat were cut longitudinally using a scalpel. Nymphs contained in their capsules could be easily observed macroscopically as distinct white round masses about 2–3 mm in diameter (Fig. 1). Nymphs were released from the capsule tissue surrounding them and viewed microscopically and then preserved in 70% ethanol or 10% formalin. Parasite data, including number of parasites, developmental stages, geographic location, host, host age and location in the host were recorded in an Excel spreadsheet. Fisher's exact test was employed to test the correlation between prevalence of infection between dogs and foxes.

### 2.5. Faecal egg count

Faeces from a Tumbarumba wild dog infected with one male and two female tongue worms were examined. Four flotations were prepared from the faecal sample but only 1 to 3 eggs were recovered from each flotation. No faeces from foxes were examined. One gram of faeces was placed in the base container of a Faecalizer® (EVSCO Pharmaceuticals, NJ, USA) with approximately 2 ml of saturated sodium nitrate flotation solution (SG 1.25) and mixed well. The green sieve insert was placed firmly into the Faecalizer® before it was filled with saturated sodium nitrate flotation solution until a meniscus was achieved. A glass cover slip was floated on the meniscus and allowed to stand for 10 min. After 10 min the cover slip was carefully lifted off and placed on a slide. The slide was scanned microscopically for parasite eggs under ×4 and ×10 magnifications.

## 3. Results

### 3.1. Parasite identification

Adult specimens in the present study were placed in the family Linguatulidae based on their general morphology, including a fluke-like flattened body, and the location in which they were found (nasal cavity). In the present study parasite specimens are referred to as *Linguatula cf. serrata* until detailed morphological and molecular studies are done.

### 3.2. Prevalence in potential definitive hosts

A total of 37 wild dogs, 55 foxes and 5 feral cats were examined. A Fisher's exact test showed that there was a significant difference