Red foxes (Vulpes vulpes) and wild dogs (dingoes (Canis lupus dingo) and dingo/domestic dog hybrids), as sylvatic hosts for Australian Taenia hydatigena and Taenia ovis.

Jenkins, David J; Urwin, Nigel A R; Williams, Thomas M; Mitchell, Kate L; Lievaart, Jan J; Armua-Fernandez, Maria Teresa

Abstract: Foxes (n = 499), shot during vertebrate pest control programs, were collected in various sites in the Australian Capital Territory (ACT), New South Wales (NSW) and Western Australia (WA). Wild dogs (dingoes (Canis lupus dingo) and their hybrids with domestic dogs) (n = 52) captured also as part of vertebrate pest control programs were collected from several sites in the ACT and NSW. The intestine from each fox and wild dog was collected, and all Taenia tapeworms identified morphologically were collected and identified to species based on the DNA sequence of the small subunit of the mitochondrial ribosomal RNA (rrnS) gene. Taenia species were recovered from 6.0% of the ACT/NSW foxes, 5.1% of WA foxes and 46.1% of ACT/NSW wild dogs. Taenia ovis was recovered from two foxes, 1/80 from Jugiong, NSW and 1/102 from Katanning, WA. We confirm from rrnS sequences the presence of T. ovis in cysts from hearts and diaphragms and T aenia hydatigena in cysts from livers of sheep in Australia. T. ovis was not recovered from any of the wild dogs examined but T. hydatigena were recovered from 4(8.3%) wild dogs and a single fox. With foxes identified as a definitive host for T. ovis in Australia, new control strategies to stop transmission of T. ovis to sheep need to be adopted.

DOI: 10.1016/j.ijppaw.2014.03.001

International Journal for Parasitology: Parasites and Wildlife 3 (2014) 75–80

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

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

Red foxes (*Vulpes vulpes*) and wild dogs (dingoes (*Canis lupus dingo*) and dingo/domestic dog hybrids), as sylvatic hosts for Australian *Taenia hydatigena* and *Taenia ovis*

David J. Jenkins [a,*], Nigel A.R. Urwin [a], Thomas M. Williams [a], Kate L. Mitchell [a], Jan J. Lievaart [a], Maria Teresa Armua-Fernandez [b]

[a] School of Animal and Veterinary Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia
[b] Institute for Parasitology, Winterthurerstr 266a, University of Zürich, Zürich CH-8057, Switzerland

ARTICLE INFO

Article history:
Received 29 January 2014
Revised 17 March 2014
Accepted 17 March 2014

Keywords:
Taenia ovis
T. hydatigena
Foxes
Dingoes
Australia

ABSTRACT

Foxes (n = 499), shot during vertebrate pest control programs, were collected in various sites in the Australian Capital Territory (ACT), New South Wales (NSW) and Western Australia (WA). Wild dogs (dingoes (*Canis lupus dingo*) and their hybrids with domestic dogs) (n = 52) captured also as part of vertebrate pest control programs were collected from several sites in the ACT and NSW. The intestine from each fox and wild dog was collected, and all *Taenia* tapeworms identified morphologically were collected and identified to species based on the DNA sequence of the small subunit of the mitochondrial ribosomal RNA (*rrnS*) gene. *Taenia* species were recovered from 6.0% of the ACT/NSW foxes, 5.1% of WA foxes and 46.1% of ACT/NSW wild dogs. *Taenia ovis* was recovered from two foxes, 1/80 from Jugiong, NSW and 1/102 from Katanning, WA. We confirm from *rrnS* sequences the presence of *T. ovis* in cysts from hearts and diaphragms and *Taenia hydatigena* in cysts from livers of sheep in Australia. *T. ovis* was not recovered from any of the wild dogs examined but *T. hydatigena* were recovered from 4(8.3%) wild dogs and a single fox. With foxes identified as a definitive host for *T. ovis* in Australia, new control strategies to stop transmission of *T. ovis* to sheep need to be adopted.

1. Introduction

In Australia, *Taenia ovis* and *Taenia hydatigena* are the only species of the genus utilising sheep as their intermediate host. Other *Taenia* species found infecting domestic dogs and wild canids, namely *Taenia pisiformis* and *Taenia serialis* utilise rabbits as their intermediate hosts. Data collected through the Australian National Sheep Health Monitoring Program (AHA, 2011) reported metacestodes of *T. ovis* to be widespread and common in sheep slaughtered in mainland Australia, but less common in Tasmania. Sheep infected with metacestodes of *T. hydatigena* are also found commonly in slaughtered sheep from all sheep rearing areas of mainland Australia but less commonly in Tasmania (Animal Health Australia, unpublished data).

Adult *T. ovis* and *T. hydatigena* tapeworms reside in the small intestine of infected canids, commonly domestic dogs. Tapeworm eggs are passed onto pasture via faeces from infected dogs. Based on experimental studies it has been estimated eggs of *T. ovis* remain viable on pasture for at least 300 days (Arundel, 1979) where they are distributed further by agents such as wind and rain and mechanical vectors such as coprophagous flies (Gemmell and Lawson, 1986). Sheep become infected through accidentally ingesting *T. ovis* eggs whilst grazing. *T. ovis* has never been recorded in Australian native herbivores.

T. ovis and *T. hydatigena* infections in sheep are of no apparent veterinary importance and in contrast to the taeniid cestode *Echinococcus granulosus*, dogs infected with adult *T. ovis* or *T. hydatigena* pose no risk to human health. The importance of *T. ovis* and *T. hydatigena* to the Australian meat industry is infection with the intermediate stages of these cestodes leading to important financial losses for the Australian sheep meat industry. Losses due to *T. ovis* occur through downgrading and condemnation of meat and hearts and *T. ovis* infection is also a potential impediment to international trade of sheep meat. The financial impact of *T. hydatigena* is less important but during their time in the liver, larval stages of *T. hydatigena* may cause tissue damage leading to condemnation of infected organs.

Infection of sheep with the metacestodes of *T. ovis* is commonly referred to as "sheep measles" due to the "spotty" appearance of

* Corresponding author. Tel.: +61 2 6933 4179.
E-mail address: djjenkins@csu.edu.au (D.J. Jenkins).

http://dx.doi.org/10.1016/j.ijppaw.2014.03.001
2213-2244/

meat containing the pale cystic lesions. Cysts in sheep muscles are small (approximately 4–6 mm), blister-like lesions, each containing a single cestode scolex. Cysts occur most commonly, in cardiac and diaphragmatic muscle. However, in heavy infections cysts can be found throughout the skeletal musculature of the animal. From the time of infection it takes about two months of development before cysts in sheep are infective to dogs (Ransom, 1913). Cysts remain infective to dogs for only about 1–2 months (Ransom, 1913) and if ingested by dogs during this time give rise to infection with *T. ovis* tapeworms in their small intestine. However, as time passes the immune system of the sheep kills the cysts which form into pus-filled lesions. These lesions eventually become mineralized transforming from gritty masses to hard calcified nodules. From a consumer's perspective, any manifestation of these lesions in meat for human consumption is unacceptable.

Infection of sheep with the intermediate stage of *T. ovis* was first reported from an Australian domestic abattoir (Homebush, New South Wales) in 1930 (Ryan and Croft, 1973). The commercial importance of *T. ovis* came to prominence in Australia in 1967 when the United States Department of Agriculture (USDA) introduced statistically programmed sampling of all consignments of imported boneless mutton. In 1969, this programmed sampling led to 82,000 cartons of boned Australian mutton (worth over $1.5 million at that time) being rejected by the USA following the discovery of sheep measles (Arundel, 1972).

Although domestic dogs are considered the main definitive host of *T. ovis* and *T. hydatigena*, a recent study of over 1400 rural domestic dogs (Jenkins unpublished data) failed to identify a single dog infected with *T. ovis*, strongly suggesting this may not be the case. Therefore, the only other potential candidates are wildlife carnivores, namely red foxes (*Vulpes vulpes*) and wild dogs (dingoes (*Canis lupus dingo*) and dingo/domestic dog hybrids).

The status of foxes as definitive hosts for *T. ovis* and *T. hydatigena* remains controversial. In two major surveys (one each in New South Wales (NSW) and Victoria (Vic)) the intestines of over 2000 foxes were examined for helminths, although several species of taeniid cestode were recovered *T. ovis* and *T. hydatigena* were never recorded (Coman, 1973; Ryan, 1976). Despite an old report of *T. ovis* in foxes (Pullar, 1946) and a more recent report of *T. hydatigena* in two foxes (Dent and Kelly, 1974), Coman and Ryan (1974) concluded foxes did not act as hosts for *T. ovis* or *T. hydatigena*. More recently, Howkins (1986) reported *T. ovis* in foxes from the Australian Capital Territory (ACT) and adjacent areas in NSW and Dybing et al. (2013) reported *T. hydatigena* in a fox from Western Australia (WA). Tapeworm identification, in all cases, was based on rostellar hook measurement, a diagnostic method considered unreliable due to the considerable overlap in hook length between species (Coman, 1973; Beveridge and Gregory, 1976; Edwards and Herbert, 1981).

The situation regarding the role of wild dogs as definitive hosts for *T. ovis* is also unclear. As with rural domestic dogs, past studies concerning intestinal helminth infections of Australian wild dogs commonly focused only on *E. granulosus* (reviewed by Jenkins, 2006). However, none of the authors who reported on the presence of other helminths in the wild dogs, ever reported *T. ovis* (Durie and Reik, 1952; Coman, 1972; Reichel and Gasser, 1994; Brown and Copeman, 2003; Jenkins et al., 2008), but the presence of *T. hydatigena* was mentioned periodically, again with identification based on tapeworm morphology and/or rostellar hook lengths.

For more accurate identification Trachsel et al. (2007) developed a multiplex PCR assay to identify a variety of cestodes based on amplification of mitochondrial DNA sequences from eggs or worm tissue. Identification was based either on amplicon size or in the case of *Taenia* species restriction fragment length polymorphism or direct sequencing. The focus of our study, through the use of molecular methods, was a survey of taeniid cestodes in foxes and wild dogs for infection with *T. ovis*. However, we also report on infection with *T. hydatigena*, *T. pisiformis* and *T. serialis*. In addition, we examined faeces from rural domestic dogs for the presence of taeniid eggs, identified to species with this method. Dietary analysis of foxes collected in one area where a *T. ovis*-infected fox was recovered was undertaken to obtain detailed data on the frequency foxes consume sheep/lambs.

2. Materials and methods

2.1. Collection of foxes and wild dogs

Many of the wild dogs and foxes were collected in the same areas in NSW and the ACT. They were obtained from professional vertebrate pest control officers who trapped or shot the animals during the normal course of their duties. Other foxes were provided by recreational shooters or farmers who were removing potential predators from the vicinity of sheep flocks. Foxes from Western Australia (WA) were shot by farmers and recreational hunters during an official state-wide fox control program (Red Card for Cats, Rabbits and Foxes – http://www.redcard.net.au), focusing on sheep-rearing areas of Western Australia (WA). Time constraints and logistics prevented wild dogs being sampled in WA for this study. As soon after death as possible, intestines were removed from the animals and kept on ice until examined, sometimes up to 48 h later. Some intestines were frozen prior to tapeworm collection.

2.2. Collection of tapeworms and metacestodes

Intestines of foxes and wild dogs, from about 10–20 mm below the stomach to the caecum, were separated from the omentum and retained in a labelled clip-lock plastic bag on ice or frozen. Each intestine was slit longitudinally, using gut scissors, and the contents washed out into a plastic tray with water. The intestine was discarded and the intestinal contents washed through a sieve (350μ mesh) with running tap water. The contents of the sieve were back-washed into a black tray with tap water. All tapeworms present were identified to genus using morphological criteria, *Taenia* species were collected, blotted dry on absorbent paper and placed in 80% ethanol for later identification using molecular methods. Samples were stored at −20 °C prior to DNA extraction. *Taenia* species metacestodes were dissected free of host tissue and preserved similarly to the tapeworms.

2.3. Collection of rural domestic dog faeces

All farmers in NSW, Tasmania and WA who agreed to complete a questionnaire were sent a faeces collection kit and requested to collect a sample from each of their dogs into the containers provided and return them to Charles Sturt University by express post in the envelope provided. On receipt, envelopes containing samples were placed in a refrigerator at 4 °C until "logged-in" and tested by flotation (not more than 24 h).

2.4. Egg floats

Helminth eggs in faeces were visualized using standard flotation methodology utilising saturated sodium nitrate as the flotation solution in Fecalizer® egg flotation devices. Eggs of all species, except taeniid and hookworm eggs, were identified to species microscopically using morphological criteria. Taeniid eggs for extraction of DNA were collected from faeces according to the method of Mathis et al. (1996).

2.5. Extraction of DNA from tapeworm eggs, tapeworms and metacestodes

Genomic DNA was isolated from approximately 20–100 mg of worm tissue or whole metacestodes using a DNeasy® blood and tissue mini kit, (Qiagen, Melbourne, Australia), according to the manufacturer's instructions except tissue samples were homogenized with 400 µl ATL buffer using a 2 ml ground glass "Potter" homogenizer. DNA was purified from an aliquot (180 µl) of this solution. DNA concentration was determined spectrophotometrically. DNA extraction from eggs was performed according to the method of Štefanić et al. (2004).

2.6. PCR and sequencing

The gene encoding the small subunit of the ribosomal RNA, (*rrn*S) from mitochondria was amplified using the multiplex PCR method described by Trachsel et al. (2007). The reaction mix included all five primers however only primers Cest3 and Cest5 were required for amplication from *Taenia* species. All were used because the primer mix was used for amplication of other genera. PCR products were purified using a Wizard® SV gel and PCR clean-up system, (Promega, Sydney, Australia).

Purified PCR products from worms and metacestodes were directly sequenced with primer Cest5seq (Trachsel et al., 2007) at the Australian Genome Research Facility, (Sydney, Australia). PCR products from eggs were directly sequenced with the same primer at Synergene Biotech (Zurich, Switzerland). The sequences obtained were compared with those available in the GenBank nucleotide database by BLAST search (http://www.blast.ncbi.nlm.nih.gov). The species was defined if the sequence identity was 99% or greater i.e. no more than 1–2 bp difference with sequences in GenBank.

2.7. Identification of fox dietary items

Scats and stomach contents were collected into paper bags labelled with the fox number and frozen. They were then allowed to thaw, heated to 100 °C and maintained at that temperature overnight to eliminate the risk of hydatid disease (*Echinococcus granulosus*) transmission via eggs present in scats. After heating, stomach and intestinal contents from each animal were placed in labelled calico bags and washed in a washing machine. Dietary components were identified macroscopically and microscopically according to Watts and Aslin (1981) and Brunner and Coman (1974).

3. Results

3.1. Foxes and wild dogs

A total of 499 foxes (ACT 13; NSW 231; WA 255) and 52 wild dogs were examined during the study. The sample size of foxes from the various collection sites ranged between 3 and 102 animals and the sample size of wild dogs ranged between 1 and 20 animals per site.

3.2. Tapeworm collection and identification

Data on *Taenia* species recovery from the foxes and wild dogs are presented in Tables 1 and 2. Few of the foxes collected were infected with *Taenia* species (NSW 6.0%, WA 5.1%), except in one area in of NSW (Brindabella/Wee Jasper) where 26% were infected. The prevalence of *Taenia* species recovered from wild dogs was higher (ACT 50%, NSW 48.0%). Three wild dogs were infected with both *T. pisiformis* and *T. serialis* and a single fox was found to be infected with both *T. hydatigena* and *T. pisiformis* The cestodes *Spirometra erinacei* and *Dipylidium caninum* were also found commonly in foxes and wild dogs, often co-habiting with *Taenia* species.

3.3. Rural domestic dog faecal samples

A total of 245 faecal samples from rural dogs were received from owners and tested (NSW 125; Tasmania 101; WA 19).

3.4. Rural domestic dog faecal flotation data

Overall about one third of domestic rural dogs were infected with intestinal helminths, the highest incidence was in NSW (38.4%) and the lowest in WA (16.0%). Only one dog was found infected with a taeniid cestode. This dog lived in Tasmania and was infected with *T. ovis*. The most commonly found intestinal helminth infection in dogs from all states was hookworm. The hookworm species were not identified. Eggs of other tapeworm species identified were *Spirometra erinacei* and *D. caninum* and were only found in dogs from NSW.

3.5. DNA sequences analysis

Sequences of the mitochondrial *rrn*S gene of approximately 220 bp in length, dependent on the species, were obtained by direct sequencing of PCR products obtained from tapeworms, metacestodes and eggs. BLAST searches were used to determine the species. *T. ovis* was identified in two foxes but not in any wild dogs. One fox was from Jugiong, NSW and the other was from Katanning, WA. The sequences from both worms were identical and this sequence was submitted to GenBank KJ591567. The sequence was near identical between bases 12,267 and 12,485 to the *T. ovis* sequence from New Zealand in GenBank AB731675.1 (Nakao et al., 2013) except for a single base substitution. Eggs were recovered from faeces of the Jugiong fox and the sequence of the *rrn*S gene amplified from the eggs was identical to that of the worm present, demonstrating patent infection. An additional egg sample recovered from the faeces of a Tasmanian dog was also demonstrated to contain *T. ovis* DNA. This sequence was identical to that from the *T. ovis* infecting the foxes (GenBank KJ591567).

To confirm cysts in sheep contained metacestodes of *T. ovis* and to look for sequence diversity we obtained cysts from hearts and diaphragms of abattoir material from sheep in three states. We obtained 7 from an abattoir in NSW, 12 from one in Tasmania and 13 from one in WA. All metacestodes were confirmed to be *T. ovis* on sequence data and all contained either our previously identified sequence (GenBank KJ591567) or a sequence identical to the New Zealand sequence, GenBank ID AB731675.1 (Nakao et al., 2013). This second sequence has been submitted to Genbank (ID GenBank KJ591568). Both genotypes were found in metacestodes collected in each state.

T. hydatigena was identified in a single fox and 4 wild dogs. Three genotypes were observed, one being identical to GenBank GQ228819.1 between bases 9951 and 10,168, which was obtained from a abdominal cyst of a sheep in Qinghai Province, China (Jia et al., 2010). The fox worm sequence varied by a single base substitution. The additional genotype found in a dog also varied from the Chinese sequence by single different base substitution to that found in the fox worm. Our three sequences are GenBank KJ591569, GenBank KJ591570 and GenBank KJ591571 respectively. In addition, the sequence was determined at this locus for 3 metacestodes recovered from 3 hepatic cysts obtained from an abbatoir in NSW. All three sequences were identical to the Chinese sequence and our own (GenBank KJ591569) identified in a worm.

Table 1
Taenia species recovery data from foxes collected at various sites in the Australian Capital Territory, New South Wales and Western Australia.

Foxes	ACT	NSW				NSW totals	WA			WA totals
Location	Baroomba/ Stromilo	Brindabella/ Wee Jasper	Bathurst/ Taralga	NSW various sites	Jugiong/ Tarcutta		Quirading	Kataning	Boddington/ Niabing/Williams	
n examined	13	27	79	32	80	231	87	102	66	255
n (%) infected with *Taenia spp*	0	7(26.0)	4(5.0)	0	5(6.25)	14(6.0)	3(3.4)	7(6.9)	3(4.5)	13 (5.1)
n (%) *T. ovis*	0	0	0	0	1(1.2)	1(0.4)	0	1(1.0)	0	1(0.4)
n (%) *T. hydatigena*	0	0	0	0	0	0	0	1(1.0)	0	2(0.8)
Sheep wool in intestine (*n* %)	0	0	7(8.9)	0	1(1.2)	8(3.5)	16(18.4)	28(27.4)	13(19.7)	57(22.3)
n (%) *T. pisiformis*	0	1(3.7)	1(1.3)	0	0	2(0.9)	0	0	0	0
n (%) *T. serialis*	0	5(18.5)	1(1.3)	0	4(5.0)	10(4.4)	3(3.4)	5(4.9)	3(4,5)	11(4.3)

Table 2
Taenia species recovery data from wild dogs collected at various sites in the Australian Capital Territory and New South Wales.

Wild Dogs	ACT	NSW
n examined	4	48
n (%) with *Taenia* spp	1(25.0)	23(48.0)
n (%) with *T. ovis*	0	0
n (%) with *T. hydatigena*	0	4(8.3)
n (%) with *T. pisiformis*	1(25.0)	15(31.2)
n (%) with *T. serialis*	0	4(8.3)
n (%) with sheep wool in intestine	0	0

T. pisiformis was identified in 2 foxes and 15 wild dogs. Only two genotypes were observed. One had a single base substitution from the GenBank sequence GU569096.1, (bases 9919–10,138) of a Chinese isolate (Jia et al., 2010). The other genotype varied by 2 bp from the Chinese isolate with an additional single base substitution. Our sequences were GenBank KJ591572 and KJ591573 respectively. One example of this latter genotype was found and both genotypes were found within one wild dog.

T. serialis was identified in 21 foxes and 4 wild dogs and was the most prevalent species. The sequences of all worms were identical to an Australian isolate GenBank AB731674.1 between bases 12247and 12,468, (Nakao et al., 2013) and our sequence was deposited as GenBank KJ591574.

3.6. Dietary items identified

Dietary items consumed by 36 NSW foxes are listed in Table 3. The most commonly identified non-plant items were insects followed by sheep remains (22.2%).

Other items included remains of fox (fur attached to skin, bone fragments and claws, most likely scavenged from road kills or culled foxes left in paddocks), birds, mice, cows (scavenged carcass), rabbits, insect larvae and deer (scavenged carcass). Plant material recovered included grass, fruit and seeds.

4. Discussion

Data collected in this study confirm, unequivocally, that a sylvatic transmission pathway for *T. ovis*, utilising foxes as definitive hosts, and for *T. hydatigena* utilizing both foxes and wild dogs as definitive hosts are operating in Australia. This is the first time confirmatory molecular methods have been used to identify *T. ovis* and *T. hydatigena* recovered from foxes and wild dogs in Australia.

The role of domestic dogs in the transmission of *T. ovis* in Australia has not been investigated for over 40 years, during which time palatable and nutritionally balanced dry dog foods have been developed and the highly efficient cestocidal drug, praziquantel, has become widely available, being included in many brands of commercial dog de-worming products. These developments alone have had a profound impact on the prevalence of intestinal helminths in Australian dogs. During the current study 245 rural domestic dog faecal samples were examined for eggs of intestinal helminths and only about one third of the samples contained eggs of any species of intestinal helminth. Eggs of *Taenia* species tapeworm were found only in one faecal sample from a Tasmanian dog. These eggs were shown to be *T. ovis*. The almost complete absence of *Taenia* of any species in dogs sampled in all jurisdictions is of interest, suggesting the role of domestic dogs in the transmission of *T. ovis*, and also *T. hydatigena*, is currently less important than in previous decades. These data are corroborated by other recently obtained data (Jenkins, unpublished data) from a study of 1425 rural domestic dogs from all states of eastern Australia (1119 from mainland Australia and 306 from Tasmania). Only 11 dogs were found infected with *Taenia* species, and of these, four were infected with *T. hydatigena* and none was infected with *T. ovis*.

A recent publication on *T. ovis* in Western Australia (Palmer et al., 2013) dismissed the role of foxes in transmission, nominating domestic dogs as the primary definitive host for this parasite. Based on the dog faecal flotation data from this study ($n = 245$) and those of 1425 dog faeces samples examined separately (Jenkins et al. unpublished data), eggs of *Taenia* species tapeworms were uncommon and those of *T. ovis*, rare. Unpublished survey data regarding the diets fed to rural domestic dogs revealed many owners periodically feed raw sheep meat and/or ovine hearts to their dogs. Through such behaviour, there is little doubt some rural domestic dogs may periodically become infected with *T. ovis*. However, most owners also de-worm their dogs several times per year with an all-wormer containing praziquantel (most commonly two monthly or four monthly), therefore infections of *T. ovis* in rural dogs may be removed before reaching patency or only remain patent in the dog for a limited time. However, it could be argued that since participation in this study was voluntary, farmers who completed our questionnaire were more motivated and hence, more likely to de-worm their dogs, compared to the rest of the farming community. The farmers surveyed in this study with *T. ovis* in their sheep and a second group without infection in their sheep were selected randomly from data collected by the National Sheep Health Monitoring Program. Therefore, we feel the de-worming data presented generally represents what is going on farms in Tasmania, NSW and WA. Based on our findings and those of Jenkins (unpublished data), we have a contrary view to that of Palmer et al. (2013) who suggest that rural domestic dogs are the main definitive host for Australian *T. ovis* and foxes unimportant in transmission. It is also of interest that in Tasmania, where foxes and wild dogs are

absent, domestic dogs are the only available definitive host for *T. ovis*. However, Tasmania has the lowest occurrence of lines of sheep with *T. ovis* infection reported in the National Sheep Health Monitoring Survey (AHA, 2011).

Our data regarding *T. ovis* infection in foxes (n = 486) revealed *T. ovis* infection in 2 animals, one from NSW and one from WA. The single worm present in each fox was mature with gravid segments; one infection was confirmed to be patent with eggs present in the host faeces. Faeces were not collected from the other fox. Foxes were collected from multiple sites in both NSW and WA but the sample sizes from the locations in each jurisdiction varied widely. In each jurisdiction, the *T. ovis*- infected fox was found in the location where the largest sample was collected, giving a prevalence in each location of approximately one percent.

From DNA sequence data genetic diversity, at least for the locus studied, appears low in *Taenia* species in Australia. Both *T. ovis* worms had identical sequences and only one additional genotype was detected in 32 metacestodes excised from sheep hearts and diaphragms. The two genotypes were found in NSW, Tasmania and WA. Similarly three metacestodes from liver were identified as *T. hydatigena* and from worms and cysts only three genotypes were found. Only two genotypes were observed for *T. pisiformis* and one for *T. serialis*.

The presence of sheep or lamb's wool in fox intestines was identified macroscopically in 1.2% of intestines of the Jugiong foxes confirming these foxes had predated on lambs and/or scavenged carcasses of sheep or lambs recently (Table 1). However, based on a detailed microscopic analysis of intestinal and stomach contents of 36 of the same foxes from Jugiong it was found the foxes had been consuming sheep/lambs even more commonly. Sheep remains were seen in 22.2% (Table 3) of the 36 intestinal contents examined microscopically, compared to the 1.2% seen macroscopically (Table 1). However, predated or scavenged lambs less than 2 months old are unlikely to be important in the transmission of *T. ovis* because the parasite will not have developed sufficiently to be infective to a definitive host. However, predating or scavenging of older lambs would be a likely scenario for exposing foxes to infection with *T. ovis*. *T. ovis* metacestodes in carcasses of older adult sheep are more likely to be non-infective having been killed by the immune system of the sheep, however, metacestodes of *T. hydatigena* are likely to still be infective.

Fox density is highly variable across the various climatic zones of Australia, mainly influenced by availability of food and water. In the temperate grazing areas of NSW, fox density can be as high as 4.6 to 7.2/km^2 whilst in the semi arid grazing areas of south western WA density is lower at between 0.6 and 0.9/km^2 (Saunders et al., 1995). Therefore, despite the presence of *T. ovis* in only about one percent of a regional fox population this is likely to represent numerous infected animals. Considering foxes may travel between 4.2 and 16 km per night (Carter et al., 2012), eggs passed by an infected animal have the potential to be spread widely over pasture, assisted by agents such as wind, rain and mechanical vectors such as coprophageous flies (Gemmell and Lawson, 1986). This, combined with the capacity for eggs of *T. ovis* to remain viable for at least 300 days (Arundel, 1972), only a few infected foxes need be present in a given geographical area to ensure regular exposure of local sheep to eggs of *T. ovis*.

Table 3
Percentage occurrence of food items identified in the stomach and intestine of 36 foxes collected in Jugiong, NSW.

Dietary item	% Occurrence (n)
Beetles (not identified)	100 (36)
Grasshoppers (not identified)	72.2 (26)
Grass (not identified)	50(18)
Sheep (*Ovis aries*)	22.2 (8)
Fox (*Vulpes vulpes*)	16.7(6)
Birds (not identified)	16.7 (6)
Seeds/fruit (not identified)	13.9(5)
Mice (*Mus muscularis*)	13.9(5)
Cow (*Bos Taurus*)	5.5 (2)
Rabbits (*Oryctolagus cuniculus*)	5.5 (2)
Insect larvae (not identified)	2.8 (1)
Fallow Deer (*Dama dama*)	2.8(1)

During the last 100 years there have been several attempted introductions of foxes into Tasmania but it was not until sometime between 1998 and 2001 that a successful, illegal, release occurred (Sarre et al., 2012). There followed an intense eradication program by the Tasmanian Department of Primary Industry Parks Water and the Environment (DIPWA) using baits containing 1080 (sodium monofluroacetate) a highly effective toxicant for canids. However, the elusive nature of foxes and the small number of animals likely to be present in the environment, spread over a wide area, has made assessing the effectiveness of this eradication campaign difficult to determine. However, through DNA genotyping of DNA extracted from fox faeces, Sarre et al. (2012) established a minimum of 18 individuals were present in Tasmania during the last 4 years. Despite the presence of a few, widely dispersed, foxes in Tasmania, from a *T. ovis* transmission perspective, they are, for the time being, irrelevant. In the absence of wild dogs, rural domestic dogs remain the only widely available, potential definitive host for *T. ovis* in Tasmania in contrast to mainland Australia.

Theoretically, *T. ovis* transmission from foxes could be controlled through the distribution of praziquantel-medicated baits. It has been shown in a study in Germany (Schelling et al., 1979) taeniid helminthes (*Echinococcus multilocularis*) can be controlled in wild fox populations, but bait distribution has to be regular and at high density to maintain effective control. This is a completely impractical scenario in Australia due mainly to the size of the country, but also foxes are an introduced pest and eradicating the animals remains the main focus. In view of our findings in respect of *T. ovis* in foxes, the most practical parasite control strategy, would be maintaining conventional control for domestic dogs, but in addition, to also provide protection for sheep through vaccination.

In 1989 data were published on a recombinant vaccine for sheep against *T. ovis* (Johnson et al., 1989). This highly efficient vaccine was registered for use in New Zealand but never in Australia. The vaccine is no longer available in New Zealand, not because of a lack of efficacy but because of a marketing oversight in that the vaccine was manufactured as a "stand alone" vaccine. Since most of the financial loss due to sheep measles in New Zealand was borne by the processors and not the farmers, farmers were not motivated to buy the vaccine. Had the vaccine against *T. ovis* been included with a vaccine producers were obliged to use to maintain the wellbeing of their sheep, the vaccine would have been used widely and solved the infection issues with *T. ovis* in New Zealand sheep. Since there are no potential wildlife definitive hosts in New Zealand to perpetuate transmission, over time, there is a realistic possibility *T. ovis* could have been eradicated. In the Australian environment, there is little doubt if the strategy of vaccinating sheep was adopted by producers and particularly if abattoirs also paid a premium for sheep vaccinated against *T. ovis*, infection, levels of infection in Australian sheep would fall rapidly. Despite the presence of sylvatic definitive hosts perpetuating transmission, with wide uptake of the vaccine it is realistic to anticipate, in time, *T. ovis* could also be eradicated in Australia.

Acknowledgements

Funding for this study was provided by Meat and Livestock Australia.

Additional funding for the input of K. Mitchell was from a Summer Scholarship and for T. Williams from a New Initiative Grant, both provided by the Graham Centre for Agricultural Innovation, an alliance between Charles Sturt University (CSU) & New South Wales Department of Primary Industries. Williams also received a CSU Faculty of Science Honours Project Operating Grant.

The authors acknowledge the contribution of data from the Sheep Health Monitoring Survey co-ordinated by Animal Health Australia on behalf of Wool Producers Australia and the Sheep Meat Council of Australia. The authors thank Dr Georgeanna Story (www.scatsabout.com.au) for undertaking the fox diet analysis.

The authors also thank all the producers involved in this study for their support and assistance. The authors are indebted to the Vertebrate Pest Control Officers of the LHPA Yass (Bill Morris and Andrew McDougall), the ACT Parks & Conservation ACT (Mick Clark), Forests NSW (Mark Goldspink), officers of the LHPA Bathurst (District Veterinarian Dr Bruce Watt and Research Officer Jeff Epplestone) and shooters Mat Simpson, Mat Priestly, Adam Kuncio, Geoff Brill and Rob Ballard for providing the canid carcasses or intestines used in this study.

References

AHA (2011) <http://www.animalhealthaustralia.com.au/programs/disease-surveillance/the-national-sheep-health-monitoring-project/>.

Arundel, J.H., 1972. A review of cysticercosis of sheep and cattle in Australia. Aust. Vet. J. 48, 140–155.

Arundel, J.H. 1979 Cysticercosis: Epidemiology and Immunology. Australian Meat Research Committee Review, number 37: 1–11.

Beveridge, I., Gregory, G.G., 1976. The identificaton of *Taenia* species from Australian carnoivores. Aust. Vet. J. 52, 369–373.

Brown, B., Copeman, D.B., 2003. Zoonotic importance of parasites in wild dogs caught in the vicinity of Townsville. Aust. Vet. J. 81, 700–702.

Brunner, H., Coman, B.J., 1974. The Identification of Mammal Hair. Inkata Press, Melbourne.

Carter, A., Luck, G.W., McDonald, S.P., 2012. Ecology of the red fox (*Vulpes vulpes*) in an agricultural landscape. 2. Home range and movements. Aust. Mammal. 34 (2), 175–187.

Coman, B.J., 1972. Helminth parasites of the dingo and feral dog in Victoria with some notes on the diet of the host. Aust. Vet. J. 48, 456–461.

Coman, B.J., 1973. Helminth parasites of the fox (*Vulpes vulpes*) in Victoria. Aust. Vet. J. 49, 378–384.

Coman, B.J., Ryan, G., 1974. The role of the fox as a host for *Taenia ovis* and *Taenia hydatidena* in Australia. Aust. Vet. J. 50, 577–578.

Dent, C.H., Kelly, J.D., 1974. Cestode parasites of the fox in the central tablelands of New South Wales. Aust. Vet. J. 50, 176–177.

Durie, P.H., Reik, R.F., 1952. The role of the dingo and wallaby in the infestation of cattle with hydatids (*Echinococcus granulosus*) (Batch 1786) (Rudolphi, 1805) in Queensland. Aust. Vet. J. 28, 249–254.

Dybing, N.A., Fleming, P.A., Adams, P.J., 2013. Environmental conditions predict helminth prevalence in red foxes in Western Australia. Int. J. Parasitol.: Parasites Wildlife 2, 165–172.

Edwards, G.T., Herbert, I.V., 1981. Some quantitive characters used in the identification of *Taenia hydatigena*, *T ovis*, *T. pisiformis* and *T. multiceps* adult worms and *T multiceps* metacestodes. J. Helminthol. 55, 1–7.

Gemmell, M.A., Lawson, R.J., 1986. Epidemiology and control of hydatid disease. In: Thompson, R.C.A. (Ed.), The Biology *Echinococcus* and Hydatid Disease. George Allen and Unwin, Boston and Sydney, pp. 189–216.

Howkins, A.B., 1986. The significance of the red fox on the distribution of hydatid disease in Eastern New South Wales. Proceedings of the Fifth conference on Science and Technology "Technology Today and Tomorrow", pp. 152–164.

Jenkins, D.J., 2006. *Echinococcus granulosus* in Australia, widespread and doing well. Parasitol. Int. 35, S203–S206.

Jenkins, D.J., Allen, L., Goullet, M., 2008. Encroachment of *Echinococcus granulosus* into urban areas of eastern Queensland. Aust. Vet. J. 86, 294–300.

Jia, W.Z., Yan, H.B., Guo, A.J., Zhu, X.Q., Wang, Y.C., Shi, W.G., Chen, H.T., Zhan, F., Zhang, S.H., Fu, B.Q., Littlewood, D.T., Cai, X.P., 2010. Complete mitochondrial genomes of *Taenia multiceps*, *T. hydatigena* and *T. pisiformis*: additional molecular markers for a tapeworm genus of human and animal health significance. BMC Genomics 11, 447.

Johnson, K.S., Harrison, G.B.L., Lightowlers, M.W., O'Hoy, K.L., Cougle, W.G., Dempster, R.P., Lawrence, S.B., Vinton, J.G., Heath, D.D., Rickard, M.D., 1989. Vaccination against ovine cysticercosis using a defined recombinant antigen. Nature 338, 585–587.

Mathis, A., Deplazes, P., Eckert, J., 1996. An improved test system for PCR-based specific detection of *Echinococcus multilocularis* eggs. J. Helminthol. 70, 219–222.

Nakao, M., Lavikainen, A., Haukisalmi, V., Konyaey, S., Oku, Y., Okamoto, M., Ito, A., 2013. Molecular phylogeny of the genus *Taenia* (Cestoda: Taeniidae): Proposals for the resurrection of *Hydatigera*, Lamark, 1816 and the creation of a new genus *Versteria*. Int. J. Parasitol. 43, 427–437.

Palmer, D., Quia, C., Butler, R., 2013. Sheep measles in Western Australia: foxes are unlikely to play a role. Ovine Observer 62, 1–3.

Pullar, E.M., 1946. A suvey of Victorian canine and vulpine parasites. Aust. Vet. J. 22, 12–21.

Ransom, B.H., 1913. *Cysticercus ovis*, the cause of tapeworm cysts in mutton. J. Agric. Res. 1, 15–57.

Reichel, M.P., Gasser, R.B., 1994. Hyperindemic focus of echinococcosis in north eastern Victoria. Med. J. Aust. 160, 499–501.

Ryan, G.E., 1976. Helminth parasites of the fox (*Vulpes vulpes*) in New South Wales. Aust. Vet. J. 52, 126–131.

Ryan, G.E., Croft, D.J., 1973. Survey of Foxes – *Taenia ovis* and Fox Biology. Report Number AI73/1528. New South Wales Department of Agriculture, Veterinary Research Station, Glenfield, NSW.

Sarre, S.D., MacDonald, A.J., Barclay, C., Saunders, G.R., Ramsay, D.S.L., 2012. Foxes are now widespread in Tasmania: DNA detection defines the distribution of this rare but invasive carnivore. J. Appl. Ecol. 50, 459–468.

Saunders, G.R., Coman, B., Kinnear, J.E., Braysher, M., 1995. Managing Vertebrate Pests: Foxes. Australian Government Publishing Service, Canberra.

Schelling, U., Frank, R., Romig, T., Lucius, R., 1979. Chemotherapy with praziquantel has the potential to reduce the prevalence of *Echinococcus multilocularis* in wild foxes (*Vulpes vulpes*). Ann. Trop. Med. Parasitol. 91, 179–186.

Štefanić, S., Shaikenov, B.S., Deplazes, P., Dinkel, A., Torgerson, P.R., Mathis, A., 2004. Polymerase chain reaction for detection of patent infections of *Echinococcus granulosus* ("sheep strain") in naturally infected dogs. Parasitol. Res. 92 (2004), 347–351.

Trachsel, D., Deplazes, P., Mathis, A., 2007. Identification of taeniid eggs in the feces from carnivores based on multiplex PCR using targets in mitochondrial DNA. Parasitology 134, 11–920.

Watts, C.H.S., Aslin, H.J., 1981. The Rodents of Australia. Angus and Robertson, Sydney.