

**THE ZOONOTIC POTENTIAL  
OF  
TASMANIAN WILDLIFE**

**NORMAN DAVIES**

Submitted in fulfilment of the requirements for the  
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Department of Pathology  
University of Tasmania

Hobart

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### Summary:

Approximately eighty percent of all infectious diseases in humans are shared in nature by other animals. These diseases are termed as "zoonoses". Most newly recognised human infections over the last fifty years have been zoonoses.

As the continent of Australia is so isolated, few diseases probably infected the Aborigines and most of the diseases that did infect them were in all likelihood zoonotic. Early explorers noted that Tasmanian Aborigines, being even more isolated, appeared to be relatively free from disease.

With the arrival of the Europeans, a whole new range of diseases affected the Aborigines, including not only human diseases such as measles, but also diseases of the domestic animals they brought with them. In Tasmania over the past two hundred years, most of these imported diseases have either been eradicated, or at least controlled. Examples are brucellosis and hydatid disease. With human activities moving into previously uninhabited areas, we must therefore now look to the native animals as a potential source of human disease.

A number of newly recognised zoonoses, and potential zoonoses have emerged in recent times. Evidence is emerging of newly recognised parasitic diseases, or diseases such as giardiasis, previously thought to be specific to humans, being actually zoonotic.

This research project set out to examine what risk is posed by Tasmanian native animals in the spread disease to the human population.

A survey of animals was carried out to determine what pathogenic bacteria and parasites were present. A number of *Salmonella* spp.(4) were recorded, along with 4 species of protozoa, 2 species of nematode and 1 trematode.

Due to reports of diarrhoea in bushwalkers, a survey using an enzyme linked immunosorbent assay for *Giardia* species was carried out, with surprising results, antigens to *Giardia* sp. being detected in 5.5% of native animals.

An indirect fluorescent antibody test was developed to survey the human population in an attempt to establish whether evidence existed to any widespread human infection with *Trichinella pseudospiralis*. The first human case of infection with this parasite, thought to have originated in Tasmania, having just been described. A number of positive samples were detected.

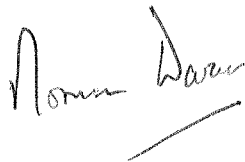
The project has shown that although the risk is relatively low, native animals do have the potential to serve as reservoirs for diseases of humans in Tasmania. This is especially so where humans eat meat from native animals, or venture into more remote areas where water supplies may be contaminated.

## STATEMENT

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Signed:

A handwritten signature in cursive script, reading "Norman Davies". The signature is written in dark ink and is positioned above a horizontal line.

Norman Davies     June 1995



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KEY TO ORIGINS OF PARASITES DESCRIBED IN FIGURES	
Figure #	Species of Animal
Fig 2	Possum
Fig 3	Wombat (top), Wallaby (bottom)
Fig 4	Quoll
Fig 5	Wallaby
Fig 6	Possum
Fig 7	Dasyurid
Fig 8	Wallaby
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**CHAPTER 1**  
**INTRODUCTION**

The agents of approximately four-fifths of all described infections in humans are shared in nature by other vertebrate animals (Schwabe 1984).

Humans are exposed to a wide range of animal diseases through their contact with meat-producing animals and their products, domestic pets, and wild life. These shared infections are termed "zoonoses", and are defined by The World Health Organisation as being "those diseases and infections which are transmitted between vertebrate animals and man" (Stevenson and Hughes 1988).

Zoonoses include many of the most important diseases globally, and most newly recognised human infections over the past fifty years have been zoonoses (Schwabe 1984).

Schwabe (1984) has also noted that, of the 45 diseases about which the World Health Organisation provides advice to international travellers, about half are zoonotic, and that in these diseases, other vertebrate species are the sole source of human infection.

We can thus see that zoonoses comprise a major health problem to humans and such, must be regarded as a significant threat to human health.

The present study has been undertaken to examine the zoonitic potential of Tasmania's wildlife. The experiments and surveys have been conducted in a small routine diagnostic laboratory, which has to some extent limited the scope of work performed. It has not been possible for instance, to use techniques such as "Random Amplified Polymerase DNA PCR" to examine strains of *Giardia* spp.

**CHAPTER 2**  
**LITERATURE REVIEW**

## **2.1 CLASSIFICATION OF ZOONOSES**

Zoonoses have been classified by Schwabe (1984) according to their maintenance cycles in nature as follows:

- i) *Direct Zoonoses* (e.g. rabies or brucellosis), which may be perpetuated in nature by a single species of vertebrate,
- ii) *Cyclozoonoses* (e.g. taeniasis or hydatid disease), in which maintenance cycles require more than one vertebrate species,
- iii) *Metazoonoses* (e.g. arbovirus infections), in which cycles require both vertebrate and invertebrate species, and,
- iv) *Saprozoonoses* (e.g. visceral larva migrans), where the infectious agents depend upon inanimate reservoirs or development sites as well as upon vertebrate hosts.

Other classifications described by Schwabe (1984) have been based on the direction of transmission between humans and lower vertebrate animals:

- i) Reservoir hosts of most zoonoses are various species of domestic and wild vertebrate animals, and this group of animal-to-human infections have been termed *anthropozoonoses*. In many of these diseases, humans are considered as dead end hosts as far as transmission is concerned, and many of these diseases are able to exist in nature independently of humans. Examples of these zoonoses are hydatid disease, visceral larva migrans and brucellosis.
- ii) The group of zoonoses where both humans and animals are equally suitable reservoir hosts, with infection occurring in both directions, are termed *amphixenoses* (e.g. staphylococcal and streptococcal infections).



iii) A minor group of zoonotic infections are those which normally pass from human to human, but which may also infect other vertebrates, examples of which are tuberculosis and diphtheria. This group has been classified as *zooanthroponoses*.

Zoonoses may also be classified as wild animal-human shared infections (ie.sylvatic) and domestic animal-human shared infections.

Of course, zoonoses may also be classified according to other criteria, one of the most common classifications being that of the type of aetiological agent, e.g. parasitic zoonoses, bacterial zoonoses and viral zoonoses (Schwabe 1984), and it is this last, probably simplest classification that has been used in this study.

## **2.2 HISTORICAL BACKGROUND**

A broad hypothesis relating to the development and evolution of the disease spectrum in Australia has been suggested by Goldsmid (1988).

The theory of continental drift suggests that about 200 million years ago, the supercontinent of Pangaea broke up to form the two continents of Laurasia in the north and Gondwanaland in the south, of which present day Australia was a part.

Over the past 150 million years, there have been further separations, the last of which was the disintegration of Antarctica, Australia and India. India drifted northwards to join Asia, whilst the Australia - Tasmania - New Guinea group moved to their present positions resulting in the seven continents that we recognise today.

Land bridges appeared and disappeared with the changes in sea levels associated with the various ice ages, and during the last ice age, some 20,000 years ago, Australia, Tasmania and New Guinea comprised a single continent.

It is likely that flora and fauna colonised Australia through its connections with the southern continent of Gondwanaland, early marsupials probably coming across from South America 80-120 million years ago. As Australia drifted away, the continent became more isolated, thus allowing these animals to multiply without competition from other animals which were becoming dominant in the rest of the world. These marsupials remained isolated for about 60 million years before being supplemented by some modern mammals which were able to reach the continent by air

(e.g. bats) or on floating logs from South East Asia (e.g. some rodent species).

It is suggested that from the original marsupials evolved the carnivorous dasyurids, along with the omnivorous perameloids or bandicoots. Subsequently herbivorous animals such as the wombats, koalas, possums, kangaroos and wallabies evolved.

Humans are believed to have entered the continent via Papua New Guinea about 50,000 years ago during the Pleistocene times (Fenner 1990). Rising sea waters resulted in their isolation from the remainder of the area. Tasmanian Aborigines were further isolated when the sea level rose and the Bass Strait was flooded about 10,000 to 13,000 years ago (Goldsmid 1988).

Because of the relatively small human population (thought to have been about 2000 - 3000 in Tasmania), it is unlikely that acute epidemic human diseases such as measles could have been maintained, but diseases having an animal source (i.e. zoonotic diseases) could have affected the Aboriginal population at that time. Goldsmid (1988) noted that the dingo did not reach Tasmania before the land bridge was flooded, and therefore it is unlikely that Tasmanian Aborigines were exposed to parasites such as *Toxocara canis*, hydatid or *Dirofilaria immitis*, carried by these animals.

A number of zoonotic diseases were probably endemic to Australia in that period, including viral diseases (e.g. Ross River Virus and Murray Valley Encephalitis) and fungal diseases, the latter being reported by early European observers (Goldsmid 1988).

It has also been suggested that *Mycobacterium ulcerans* was present in the precontinental drift era, and therefore the Aborigines could well have been exposed to it. Goldsmid (1988) has suggested that this could perhaps account for the illness known as "spitting of blood" amongst the Aborigines and noted that some marsupials (e.g. the brush - tailed possums) are highly susceptible to mycobacterial infections.

Early explorers noted that the Tasmanian Aborigines appeared to be relatively free from disease (Goldsmid 1988), but it is likely that the Aborigines were exposed to *Salmonella* infections through their diet of small animals, some of which have recently been shown to have *Salmonella* infection rates of up to 90% in Western Australia (Goldsmid 1988). A wide range of serotypes have been identified from many native animals in Tasmania (Goldsmid *et al* 1992).

These humans may also have been exposed to other diseases such as scabies, which can be harboured by animals such as wombats, as well as diseases transmitted by ectoparasites such as lice and ticks and ectoparasites from migrating birds (Goldsmid 1988).

It would seem reasonable to assume that diseases such as psittacosis or "parrot fever" and "Flinders Island Spotted Fever" may have occurred in Australia prior to European settlement. Psittacosis is caused by *Chlamydia psittaci*, and is transmitted to humans by psittacine and other

of bird species usually causing a pneumonia (Stevenson and Hughes 1988). "Flinders Island Spotted Fever" is a form of tick typhus recently described by Stewart (1991). It would also seem probable in view of recent investigations that provide evidence of an association between *Cryptococcus neoformans* and the river red gum tree (*Eucalyptus camaldulensis*) (La Rocco 1992) that *C.neoformans* was present prior to European settlement. *C.neoformans* is an encapsulated yeast causing opportunistic infections in immunocompromised humans.

It can be seen therefore that prior to European settlement, the Tasmanian Aborigines were probably exposed to a relatively small number of diseases, many of which were zoonotic in nature.

With the advent of European settlement at Sydney Cove in 1788, and Van Dieman's Land (later renamed Tasmania) in 1803, the whole disease picture changed. Increasing numbers of European settlers brought with them such childhood diseases as measles, influenza, mumps, and whooping cough, all of which caused epidemics from time to time. Sexually transmitted diseases were introduced soon after settlement, and spread quickly to the Aborigines. (Goldsmid 1988).

In Australia today, most of these childhood diseases are now relatively uncommon (Goldsmid 1988). This has been largely due to the fact that during the course of the Twentieth Century, immunising agents have been developed to prevent many of them. Many have been controlled or eradicated altogether (Goldsmid 1988), although recent fears have been expressed regarding a resurgence due to poor immunisation compliance.

The Europeans brought with them domestic animals (e.g. dogs) and livestock. These animals carried their own diseases, many of which were capable of infecting humans, including anthrax, brucellosis, leptospirosis, and hydatid disease to name but a few.

Anthrax was first recorded in Australia in 1847 in New South Wales, from where it spread to Victoria. It was the first disease of animals to be diagnosed bacteriologically in Australia (Fenner 1990). The disease was successfully controlled by immunisation in 1888, using Pasteur's vaccine (Goldsmid 1988, Fenner 1990). Stevenson and Hughes (1988) note that in the ten years 1977 to 1987, only five isolated cases were reported, and the source of *Bacillus anthracis* infection appears to be confined to Victoria and New South Wales.

Bovine tuberculosis caused by *Mycobacterium bovis* was brought to Australia with British cattle, and was widespread by 1880 (Fenner 1990). Eradication of tuberculosis in dairy cattle was achieved in the late 1960's, brought about by a programme of tuberculin testing and slaughter of positive reactors. Due to logistical problems and non-specific tuberculin test results, there have been difficulties eradicating tuberculosis from beef cattle. The incidence in these animals has however declined (Fenner 1990).

Prior to national eradication programmes, human brucellosis occurred in all states, higher prevalences being found in New South Wales and Victoria. About 94 cases per annum being reported (Stevenson and Hughes 1988).

The brucellosis eradication programme in Australia was implemented in two phases, the first of which was free compulsory strain 19 vaccination. This was designed to reduce the incidence and prevalence of the disease prior to introducing the second phase which involved wide scale testing and slaughter. This programme was effective for *Brucella abortus*, but for *B. ovis*, a combination of *B. abortus* strain 19 vaccine along with formalin killed *B. ovis* saline in oil emulsion was required. This was later changed to *B. ovis* saline in oil emulsion only. Vaccination of sheep for brucellosis is no longer carried out, rather accreditation schemes involving test and slaughter regimes are now used (Fenner 1990).

These programmes have resulted in a decrease in the number of reported cases, and since 1982, only about 20 cases per year have been reported (Stevenson and Hughes 1988). In July 1989, *B. abortus* was provisionally declared to have been eradicated from Australian herds (Fenner 1990).

The first case of human hydatidosis in Tasmania was recorded in 1833 (Goldsmid and Pickmere 1987). It appeared that this disease was most likely imported with sheep or dogs of the European settlers who first settled the island in 1804, and the parasite soon became established as a dog-sheep cycle. Goldsmid and Pickmere (1987) note that by 1961 Tasmania had the highest prevalence of hydatidosis in Australia and New Zealand, with an average of one operation a week being performed to remove hydatid cysts and with one new case of hydatidosis being diagnosed every two weeks.

It has been conservatively estimated that there were 35 to 60 new cases a year up until 1960 (McConnell 1987).

In 1962 -1963, an eradication programme began, which led to the formation of the Tasmanian Hydatid Eradication Council. This programme involved monitoring of dogs, tagging infected animals, controlling dog movement on farms, making it illegal to feed offal to dogs, monitoring sheep slaughtered at abattoirs and quarantining infected properties. It has resulted in a dramatic decrease in the number of cases of hydatidosis being reported. No cases were reported in people under the age of 20 years between 1981 and 1985, with total reported cases over the same period down to approximately 20, compared with 60 cases over the period 1966 to 1970 (Goldsmid and Pickmere 1987). In the early 1990's, government funding for the Tasmanian Hydatid Eradication Council was stopped (Goldsmid 1992). This fact has caused concern due to the fact that there are still a large number of old sheep infected with hydatid, which may act as reservoirs. Thus the potential exists for a resurgence of the disease.

In a small study of dingoes and foxes in north east Victoria conducted by Reichel *et al* (1994), all 17 animals examined at post mortem harboured *Echinococcus granulosus* worms in their small intestines , demonstrating that on the mainland, hydatid diseases is still a very real threat. While no dingoes or foxes are present in Tasmania, the potential surely exists for a resurgence of the disease in Tasmania unless careful surveillance of sheep and dogs is maintained.

Leptospirosis is still a disease of some concern, especially to those employed in the dairy and meat industries. Approximately 200 cases were reported in Australia in 1986. Vaccination of stock has proved to be an effective way of controlling the disease (Faine 1982).



It can thus be seen that, to a large extent, most of these "imported" zoonotic diseases of domestic animals have been successfully controlled or eradicated.

Stevenson and Hughes (1988) state that the Rabies virus does not occur in Australia, but stress that the disease should be considered in humans reporting animal bites acquired overseas. The disease does appear to have entered Australia at least once in the past with a report in 1867 in which a dog, believed to be rabid, bit a young girl who subsequently died with symptoms suggestive of rabies (Goldsmid 1988). Other cases of the disease have been reported in Australia (Faoagali *et al* 1988, Bek *et al* 1992, and Grattan-Smith *et al* 1992), but in all of these three cases, the patients had acquired animal bites overseas, and after with a prolonged incubation period.

Other implicated viral zoonoses have been imported into Australia from time to time, including the arbovirus dengue, which entered the country a number of times between 1873 and 1898, eventually extending its range to Western Australia. It was estimated that at one stage, almost 75% of Queensland school children suffered from dengue (Goldsmid 1988).

With the ease of international travel today, there are very real threats of other viral diseases being imported into Australia. These include the arbovirus O'nyong-nyong (break-bone fever) which causes joint pain and haemorrhagic fever, Marburg virus (green monkey disease), causing haemorrhagic fever which apparently originated in monkeys and Lassa fever a highly infectious disease characterised by fever, vomiting, cough, weakness, mouth ulcers and lymphadenopathy (Timbury 1983). The latter has a reservoir in Africa of rodents such as the multimammate mouse.

One of the major factors responsible for the emergence of new zoonoses, has been man's entering of new geographical or ecological zones in large numbers for the first time to open up lands for cultivation, mining, dams and logging (Schwabe 1984).

In 1885, Tasmania became self governing, and from that time, the boundaries of settlement were pushed back further into areas which were previously considered irrecoverably wild. By the 1880's, the west coast had been opened up to mining, along with other areas such as Beaconsfield and the Fingal Valley, resulting in Tasmania's population increasing by over 80%. In the early 1970's, the Hydro-Electric Commission began to plan dams to provide hydro-electric power for the state (Collentte 1990).

The factors mentioned previously have thus been firmly put into place for the emergence of zoonotic diseases.

With control of the domestic animal-human infections, and with human expansion into more remote areas, we must therefore look to the common natural, newly recognised and potential zoonoses as an increasing potential cause of human disease. Many of these diseases are associated with occupational and recreational activities, where much time is spent out doors, or water is obtained from rivers and creeks.

Stevenson and Hughes (1988) noted that more than 60 zoonoses have been reported in Australia, (APPENDIX 8.1) and that many of these occur at low prevalence, and are of minor public health significance. They also noted however, that some of these infections are of major importance to both human and veterinary medicine.

## **2.3 NEWLY RECOGNISED AND POTENTIAL ZONOSSES**

With the expansion of human activity into previously unexplored regions, more contact has been made with our native animals. This, as already mentioned, has increased the potential for the emergence of newly recognised diseases.

Another factor which may have influenced the emergence of newly recognised zoonotic diseases in Australia is the country's multicultural nature, with different cultures migrating to the country and with different styles of food and food preparation appearing. Some of these involve either minimal or no cooking of meat and fish etc., which has the potential to allow previously unrecognised zoonoses to emerge.

A number of new or potential zoonoses have been described in recent years, some of which may occur in Tasmania's wildlife.

## 2.4 PARASITIC ZOONOSES

Goldsmid (1988) noted that worldwide estimates of parasitic diseases have significantly increased over the last forty to fifty years, and that many of the parasitic diseases reported in Australia are imported either by Australians travelling overseas or to a lesser extent by migrants or visitors to Australia. Of course not all parasitic diseases are imported and many such infections occur naturally in Australia. Goldsmid (1988) reports that hookworm and ascariasis have in the past been reported to be universal amongst Aboriginal children in some areas. It would appear that there are also a number of potential parasitic zoonoses which have been reported on the Australian mainland, including dirofilariasis, angiostrongyliasis, and infections caused by the trichurid nematodes *Capillaria hepatica* and *C. philippinensis* (Stevenson and Hughes 1988). The dog heart worm *Dirofilaria immitis* causes rare infections where humans are accidental hosts, larval stages of the worms lodging in an artery of the pulmonary circulation (Stevenson and Hughes 1988). The infections are usually asymptomatic. *C. hepatica* is a common parasite of rodents. Humans, most commonly children, may become infected where hygiene is poor. There have been no reports of infections with this parasite in Australia, Stevenson and Hughes (1988) describe one study that showed that 79% of rats in a Brisbane factory were infected. *C. philippinensis* lives in the intestines of birds, with fish or crustaceans being the intermediate host. Humans may become infected by eating raw or undercooked seafood. The rat lungworm *Angiostrongylus cantonensis* may infect humans after ingestion of contaminated water, fresh water prawns or land crabs, or vegetables contaminated by molluscs. The most common presentation in humans is that of an eosinophilic meningoencephalitis which usually lasts

about two weeks. Reports from Queensland show that the infection is more prevalent than first thought (Gutteridge *et al* 1971).

Prociv and Croese (1990) have reported an outbreak of eosinophilic enteritis in Townsville, Queensland, and identified the agent responsible as *Ancylostoma caninum*. The basis for this conclusion was the finding of a single hookworm attached to the inflamed ileal segment of one patient out of a reported 93 cases. The worm was not able to be fully identified, however factors such as the worm's obvious hookworm morphology, the fact that most patients had possible contact with dog faeces and the point that *A.caninum* is a common parasite of dogs in the area of Townsville, led them to this conclusion. It would appear that their conclusion may be well founded, however to claim that this parasite is the agent responsible for the outbreak, based on the evidence of a single unidentified worm would seem at this stage to be presumptuous.

Whilst most of these parasites have been described on the mainland only, there may be the potential for them to occur in Tasmania's native animals.

### **Protozoan parasites:**

#### ***Blastocystis hominis***

*Blastocystis hominis* is a protozoan parasite which has emerged in recent years as a potential pathogen in humans in many parts of the world, including Tasmania (Goldsamid 1981). Zierdt (1983) noted that in patients with diarrhoea, without any other cause, the finding of this parasite was significant, and recommended treatment. In one study *B.hominis* was the second most common pathogen isolated after *Salmonella* spp.(Waghorn and Hancock 1991)

The organism has been associated with outbreaks of diarrhoea in humans (Libanore *et al* 1991), and numerous studies have demonstrated the presence of *Blastocystis* spp. in animals including monkeys, birds and domestic pigs (Burden *et al* 1979, McClure *et al* 1980, Yamada *et al* 1987). It is not known if these organisms are *B.hominis* or another species.

It has been suggested that *Blastocystis hominis* may be a zoonosis, although this remains a questionable assertion (Anonymous 1991).

Lee *et al* (1990) described a case of diarrhoea and infective arthritis in an Immunosuppressed patient caused by *B.hominis*. In this case, the patient had been treated for arthritis with prednisone. She had also suffered from diarrhoea, *B.hominis* being identified in her faeces, and also in synovial fluid from her left knee. The organism has been recognised as the cause of chronic diarrhoea in a patient with acquired immunodeficiency syndrome (AIDS) (Llibre *et al* 1989).

### **Cryptosporidium spp.**

*Cryptosporidium* spp. have become recognised as an important enteric protozoan pathogen. It has been reported in a wide range of animals, birds and reptiles, including cattle, sheep, dogs, cats and birds. (O'Donoghue 1984). In the early 1980's most reported infections were in patients with acquired immunodeficiency syndrome (AIDS), the patients having protracted, often severe, watery diarrhoea. At that stage, it was thought that *Cryptosporidium* spp. was an opportunistic pathogen, but the organism has now been identified in cases of acute self limiting diarrhoeal disease in immunocompetent hosts world wide. The parasite has been

identified in 3 to 13% of patients with diarrhoea in developing countries. (Janoff and Barth Reller 1987).

In a study carried out in northern Tasmania, Hawkesford (1989) reported a prevalence of 2.0% in patients with diarrhoea. This was the second most common finding after *Campylobacter jejuni*. It would appear that this is a lower incidence than compared with other parts of the world, Nimri and Batchoun (1994) for instance report an incidence of 4% in Jordanian school children.

The source of infection may be animal or human. Water, unpasteurised milk, offal, even uncooked sausage have been identified as vehicles of transmission. Simmons (1991) notes that waterborne outbreaks of *Cryptosporidium* spp. have become increasingly common, and suggests that these may be due to run off of large amounts of water from surrounding land after heavy rain. Hawkesford (1989) reported that in northern Tasmania, human cryptosporidiosis was more common during the calving and lambing season (spring), and that human cases were common in children and were often associated with animal contact or with the ingestion of unpasteurised milk.

Zoonotic transmission of *Cryptosporidium* spp. is quite probable. The organism has been associated with diarrhoea in animals, and the first human cases had a history of contact with animals (Janoff and Barth Reller 1987). O'Donoghue (1984) reports that the parasite can cross host barriers, and that domestic animals and pets can act as reservoirs of infection for susceptible humans. This is supported by the findings of Milstein (1993), who reported that 11.9% of domestic cats were infected with *Cryptosporidium* spp.

## **Giardiasis**

*Giardia* is the most common intestinal parasite worldwide and is firmly established in human and possibly mammalian reservoirs (Farthing 1992).

In recent years there has been much controversy as to whether *Giardia* is in fact a zoonosis. Thompson *et al* (1988) demonstrated with the use of DNA hybridization, that several feline isolates of *Giardia* were identical or very similar to some isolates from humans. Using three molecular approaches, Boreham *et al* (1988) could demonstrate no evidence for host-specific animal species of *Giardia* and claimed that there is strong evidence for a species complex.

Giardiasis is an important cause of diarrhoea, especially chronic diarrhoea, and particularly in children. Goldsmid (1981) reported a prevalence of 3.3% in patients suffering from diarrhoea in Tasmania.

For many years *Giardia* was considered host specific. It has been shown however that cross-transmission occurs, beavers and musk rats being infected with viable cysts from human faeces (Bemrick and Erlandsen 1988). Faubert (1988), showed that *Giardia* isolated from beavers and calves was shown to belong to the same group of *Giardia* that infects human beings, namely *Giardia intestinalis*, and concluded that *Giardia* is a zoonosis.

In Australia, Thompson *et al* (1988) reported a prevalence of 21% in dogs and 14% in cats in Perth Western Australia. Collyer *et al* (1992) noted a prevalence of 21% to 50% in dogs in Sydney, and suggest that dogs may prove to be a significant source of human infection.

It would appear that no published figures are available to show the prevalence of *Giardia* in domestic or wild animals in Tasmania, but



Davies *et al* (1993) described a case of giardiasis in a dog in Tasmania. The dog was affected by diarrhoea which responded to treatment with metronidazole. In this case, it is interesting to note that the animal's owner had suffered from a bout of diarrhoea, but this was dismissed by his general practitioner as being unrelated, and no faecal specimens were submitted. This demonstrates that some clinicians are unaware of the zoonotic potential of this parasite. Subsequently, a study by Milstein (1993) has shown that 14% of dogs and 19% of cats in Tasmania were infected with *Giardia* spp.

In New Zealand, Marino *et al* (1992) reported that *Giardia intestinalis* cysts were isolated from 12.9% of brushtail possums, 61% of ship rats and 25.3% of house mice. It was noted that no cysts were found in any of the farm turkeys tested! All of the animals were all trapped well away from any human tracks, and it is unlikely that they contracted their infections from a human source.

### **Toxoplasmosis**

*Toxoplasma gondii* is an intracellular protozoan belonging to the subclass Coccidia. Cysts are found in the musculature and organs of a wide range of mammals and birds, with oocysts being shed in the faeces of cats. Humans become infected when they ingest undercooked meat, or come into contact with infected faeces of cats.

Whilst this is a common infection in humans, serious transplacental infections can occur in women who seroconvert during pregnancy. Infection with the parasite causes a glandular fever-like illness, in children and young adults. Munday (1970) noted that approximately 60% of the Tasmanian population had serological evidence of infection with

*Toxoplasma gondii*, with raw mutton being a common source of infection. Milstein (1993) noted that 50% of a group of blood donors in Tasmania had serological evidence of toxoplasmosis.

The parasite remains with the host for life, and clinical disease develops when the immune system is compromised. Toxoplasmosis has become a significant cause of death and disease in patients suffering from Acquired Immune Deficiency Syndrome (AIDS) (Garcia and Bruckner 1993).

In a study of Eastern Barred Bandicoots, Obendorf and Munday (1990) reported that a number of these animals had been found to be infected with *T. gondii*. The source of infection was not established, however it is postulated that the animals had probably become infected through ingesting earthworms or other mechanical transport hosts. Earthworms may be ingested by birds or omnivorous animals, thus facilitating widespread dissemination of the parasite. The role of the earthworm has potentially far reaching implications, from infection of native and rural animals to indirectly increasing the chance of infection to humans. It would appear therefore, that native animals may also assist in the spread of this disease to humans.

## Nematode parasites

### Anisakiasis

The potential disease risk of *Anisakis* spp. in Tasmania was discussed by Clark (1990) at a meeting of The Royal College of Pathologists of Australasia.

Anisakiasis is an infection caused by the larvae of *Anisakis* spp. and related species of nematodes. The disease is characterised by a visceral larva migrans, often mimicking conditions such as duodenal ulcer, carcinoma or appendicitis, with associated allergic symptoms (Garcia and Bruckner 1993)

For human infection to occur with this parasite, there must be infected fish, and humans must ingest live larvae from these fish. The larvae do not develop to maturity in the human host, but burrow into the small intestine wall where they appear to cause eosinophilic phlegmonous enteritis or gastritis, sometimes accompanied by inflammation of the mesentery and pancreas. Subsequent penetration with larvae can cause severe allergic reactions (Stevenson and Hughes 1988).

In Tasmania, Clark (1990) noted a prevalence of 91% in Striped Trumpeter, and that 1.6% of the salmonids were infected with anisakid-like larvae.

With the emerging popularity of sushi bars, along with the consumption of raw fish, and the ability of the *Anisakis* spp. larvae to survive many pickling and smoking procedures, Clark (1990), concluded that conditions for infection exist in Tasmania.

### **Visceral Larva Migrans caused by *Baylisascaris tasmaniensis*.**

Visceral larva migrans is a condition occurring where ascarids of carnivores use intermediate or paratenic hosts in their life cycles. It is believed that the number of ascarids causing larva migrans in humans and animals is small, perhaps the best recognised cause being *Toxocara canis*, the dog ascarid *Toxocara cati* and possibly *Toxascaris* spp. (Kazacos 1986). It would appear that in Australia, clinical manifestations are rare, considering that in one survey of blood donors in Canberra, 7.5% of samples had ELISA antibody to *T. canis* (Stevenson and Hughes 1988), however in Tasmania, Goldsmid (1987), recorded a prevalence of 29.3% based on serological studies of random subjects, whilst Milstein (1993), in a similar study of the Tasmanian human population, reported prevalences of 18% in routine blood donors, 33.3% in high risk donors, and 45.5% in patients with eosinophilia.

Kazacos (1986) described a number of human cases of visceral larva migrans, including two fatal cases of cerebrospinal nematodiasis in children caused by the raccoon ascarid *Balisascaris procyonis* in the United States of America. Humans may become infected by ingesting the infective eggs of *B. procyonis*, which are extremely resistant, and may remain viable on the soil for years. In all of the cases described, there was a history of contact with infected animals, or their excreta. One of the children had chewed on pieces of bark from which infective *B. procyonis* eggs were recovered, and infection in the other was believed to be from a fireplace which was contaminated with raccoon faeces.

In Tasmania, *B. tasmaniensis* is a potential aetiological agent of visceral larva migrans

### **Capillaria spp.**

The potential for human infection with the lungworm *Capillaria aerophila* may exist, although no human cases have been reported from Australia (Stevenson and Hughes 1988). The parasite has been found in dogs, and cats. In Sydney, a prevalence of 5.2% was reported in cats. The organism has also been reported from Brisbane and Tasmania (Stevenson and Hughes 1988). In a survey of feral cats in Tasmania, Milstein (1993) recovered unidentified *Capillaria* spp. eggs from 4.76% of these animals, thus the possibility of native animals becoming infected through the feral cat population cannot be dismissed.

### **Fascioliasis**

Whilst fascioliasis has been recognised as a zoonosis for many years, the number of cases reported in Australia have been low.

Humans are infected by ingesting metacercariae of *Fasciola hepatica* (the sheep liver fluke), which have encysted on vegetation or contaminated drinking water (Anon 1982). The parasite is not uncommon in sheep and cattle, and has been found in horses, pigs man as well as wild herbivores (Schwabe 1984). The parasite has been reported in rabbits and deer in America (Faust, Russell and Jung 1974). In one Tasmanian study, Obendorf and Black (1983) reported an 80% prevalence of liver fluke in young home-bred cattle in an area in the north west of the State. The flukes are prolific egg producers, producing up to 50,000 eggs per day, a moderate chronic infection in sheep able to produce up to two million eggs a day (Anon 1982).

Obendorf and Black (1983) noted that liver fluke infections had been recorded in a wide range of Tasmanian animals including the Forester kangaroo, Bennett's wallaby, red-bellied pademelon, common wombat

and brush-tailed possum, and these infections in the macropods may represent a significant problem in the control of liver fluke in domestic livestock.

Recently, two cases of ectopic fascioliasis were reported from Australia by Prociv *et al* (1992). The mode of infection in both of these cases appears obscure and they appear to be the first of their kind in Australia. These cases parallel similar cases elsewhere in the world such as Africa (Perry *et al*, 1972 and Kyronseppa and Goldsmid 1978).

### **Trichinosis caused by *Trichinella pseudospiralis*.**

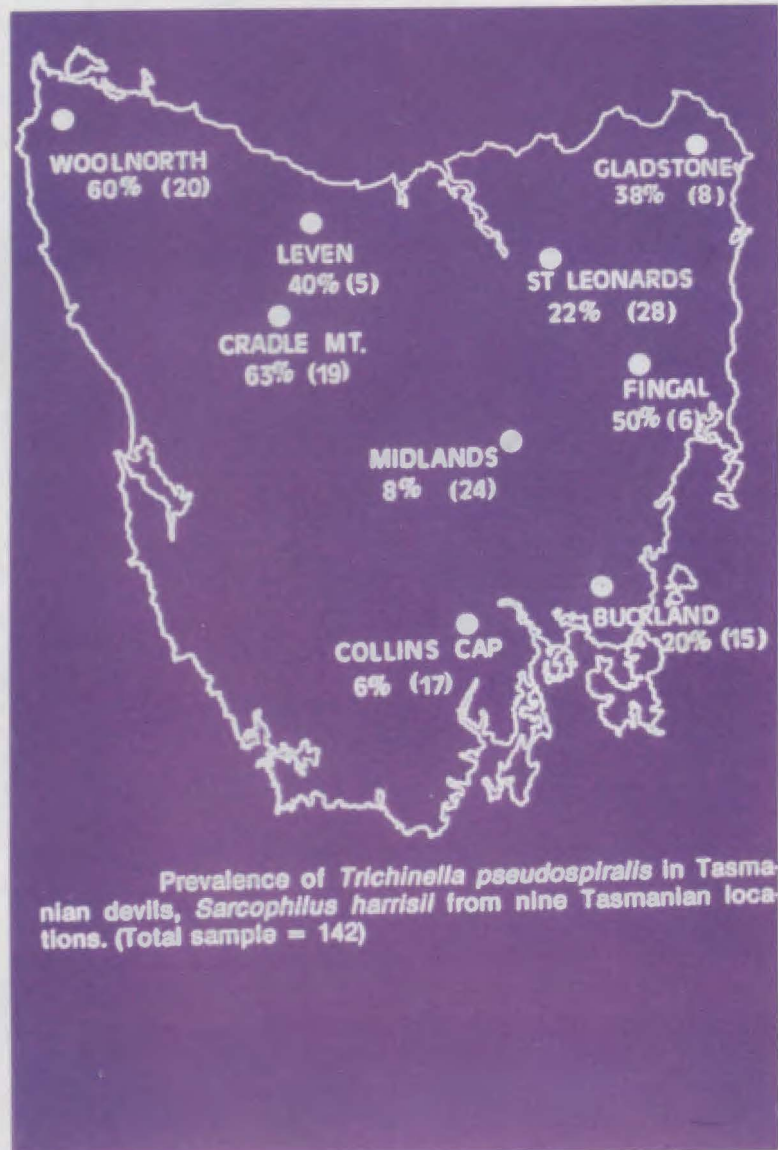
Trichinosis is a disease caused by the nematodes *Trichinella spiralis*, *T.nelsoni* and *T.nativa*. Encysted larvae are ingested in raw or undercooked meat, usually pork. They pass into the small intestine where after excysting the larvae invade the intestinal mucosa, mature into adults and mate. The female deposits larvae into the mucosa and sometimes the lymphatics from which they enter the blood stream, which carries them to all parts of the body. They burrow into the muscle fibres and encyst (Brown 1975).

Most Australian studies have noted that although encysted larvae have been found in humans in Australia, there was no evidence that these people became infected in this country (Seddon 1967).

So too until 1987, *Trichinella* had never been recorded in free living or domesticated animals in Australia, although one case had been reported in a polar bear at the Melbourne zoo (Obendorf *et al* 1990).

However in 1987 worms resembling *Trichinella* were observed in skeletal muscle of a quoll from the Cradle Mountain area. A program of trapping

was carried out in the same area, and the animals examined for evidence of the parasite. Larvae similar to those found in the original animal were detected in 70% of Tasmanian devils, 30% of spotted tailed quolls and 36% of eastern quolls (Obendorf *et al* 1990).



**Fig 1** Distribution of *T.pseudospiralis* in Tasmanian devils  
(Obendorf *et al* 1990)

It became obvious on further testing that the parasite was widespread throughout Tasmania with an overall prevalence of 30% of Tasmanian devils being infected. Retrospective studies were also carried out on museum specimens, and larvae were found in 2 devils collected from different areas in 1976 and 1977. With the use of DNA hybridisation, the parasite was identified as *Trichinella pseudospiralis* (Obendorf *et al* 1990). Milstein (1993) however, using serology, found that there was no evidence of infection in feral cats in Tasmania.

*T.pseudospiralis* has been isolated from wild birds and mammals in North America and Asia. It has been shown experimentally that it is less pathogenic than *T.spiralis*, the lethal dose of *T.pseudospiralis* larvae in mice being over 20,000 compared with only 4,500 for *T.spiralis* (Stewart 1989).

It is interesting that the *Trichinella* story in Tasmania closely parallels that of *T.nelsoni* in Africa, where for many years trichinosis was also believed to be absent (Nelson *et al* 1961).

An isolated outbreak of probable trichinosis occurred in South Australia in the late 1980's, where five members of an "ecotourism" group presented with symptoms of *Trichinella* infection. The diagnoses were made on serological evidence, all five patients refusing muscle biopsies. Included in this group were two Tasmanians. The question of where the infection originated has not been solved, one possibility is that these Tasmanians may have brought infected meat with them (Obendorf 1994).

What is believed to be the first human case of *T.pseudospiralis* infection was recently diagnosed in an adult female living in New Zealand. The



patient had suffered from chronic liver disease with myalgia for a number of years. Muscle biopsies revealed live larvae which were identified as *T.pseudospiralis*. It is interesting to note that the patient had been a student in Tasmania, where some time was spent examining Tasmanian devil faeces. She had also visited the Northern Territory. Her larval concentration was 15 larvae per gram of tissue (Obendorf 1992 pers. com. and Andrews *et al* 1993 and 1994).

It would thus appear that there is the potential for further undiagnosed human infection with this parasite, especially in people with a high risk of infection such as hunters, national parks rangers etc.

## **2.5 VIRAL, RICKETTSIAL and CHLAMYDIAL ZOOSES**

The emergence of a number of previously unreported viral, rickettsial and chlamydial diseases has occurred in Tasmania over the last decade.

Diseases which were thought to occur only on the mainland have been reported with increasing frequency, and the potential exists for others to be recognised.

### **Flinders Island Spotted Fever**

A newly recognised form of tick typhus has been described on Flinders Island in the Bass Strait . The disease has become known as Flinders Island Spotted fever. It is characterised by fever, headache, myalgia, slight cough, transient arthralgia and a macropapular rash (Stewart 1991). Two cases have also been diagnosed from the west coast of Tasmania (Templer 1991). There is serological evidence that the disease is caused by a rickettsia of the spotted fever group, but the aetiological agent remains to be characterised (Graves *et al* 1991). Stewart (1994) reports that rickettsiae have now been isolated from the blood of infected patients. Analysis to date indicates that the Flinders Island rickettsia is similar, but different from *Rickettsia australis*, the causative agent of Queensland tick typhus, and that it represents a unique species.

## **Ross River Virus**

Goldsmid (1988) suggested that Ross River virus, and the disease caused by it, "endemic polyarthritis" may have been present in Australian fauna long before the coming of Europeans to the continent.

Prior to 1965, endemic polyarthritis was an uncommon disease south of the Great Dividing Range, however there are increasing reports of the disease from the south coast of Victoria and Tasmania (Stevenson and Hughes 1988). The first human case of endemic polyarthritis in Tasmania was reported in 1981, with more reports in subsequent years (McManus and Marshall 1986).

McManus and Marshall (1986) have demonstrated high titres of antibody to Ross River virus in a wide range of native fauna including Bennets wallabies, brush tailed possums, wombats and pademelons. In fact in one study, 36 out of 56 marsupials were haemagglutination inhibition (HI) antibody positive, and of those, Bennets wallabies had the highest titres.

The two major proven mosquito vectors of Ross River virus, *Culex annulirostris* and *Aedes vigilax*, are thought to be absent from Tasmania. Experiments using local species have shown that *Ae. camptorhynchus* appears to be as competent a vector as *Ae. vigilax*. Ross River virus has been isolated from *Aedes flavifrons* collected in the Bicheno area. In 1985 the first *C. annulirostris* were recorded from Tasmania (McManus and Marshall 1986).

McManus and Marshall (1986) have concluded that Ross River virus is active in Tasmania, and is probably enzootic in marsupials.

### **Chlamydial Infections.**

Psittacosis, also known as Ornithosis or Parrot Fever is a chlamydial disease caused by *Chlamydia psittaci*. Humans become infected after contact with infected birds, especially the psittacine species (of which Australia has numerous species).

The disease is characterised by an irritating cough, pyrexia, anorexia, headache and photophobia. The disease may present as atypical pneumonia and runs a variable course, lasting seven to ten days, with low mortality in treated patients. Secondary bacterial infections and myocarditis may be complications of the infection (Stevenson and Hughes 1988).

The disease is encountered sporadically in humans in Tasmania and in most of these cases, there has been a clear history of bird contact. (Goldsmid 1994 personal communication)

## **2.6 BACTERIAL ZOONOSES**

A number of bacterial zoonoses have already been discussed along with their control. Other diseases have been reported as causing sporadic outbreaks in humans, but their zoonotic potential has possibly been overlooked in favour of more conventional modes of transmission.

Schwabe (1984) for example, describes a human outbreak of Legionnaire's disease in Pontiac, Michigan, where guinea pigs acquired natural airborne infections with *Legionella pneumophila*.

There are a number of newly recognised bacterial diseases some of which have been reported in Tasmania.

### **Lyme Disease**

In 1982 a case of Lyme disease was reported in the Lower Hunter Valley of New South Wales, following the bite of an unidentified arthropod.

Within a 12 month period, a further six cases were described. Munro and Dickeson (1989), detected antibodies to Lyme disease in 2.2% of 189 blood donors from rural and urban areas of New South Wales, and out of 40 patients from Flinders Island who were being tested for "Flinders Island Spotted Fever", 8 were found to have antibodies to *B. burgdorferi*. There is some evidence that a number of cases have also occurred in Queensland (Stevenson and Hughes 1988).

Lyme disease is a multisystem disorder caused by the spirochaete *Borrelia burgdorferi*. The early stages of the disease are most commonly characterised by skin lesions. Erythema chronicum migrans begins as a small papule at the site of bite by an infected tick. This lesion spreads

to a diameter of several centimetres. Multiple lesions at sites distant from the original rash can occur (Barbour 1985).

In this early stage, the patient may suffer from fever, fatigue, arthralgia and myalgia as well headache and stiff neck. Many untreated patients may suffer from recurrences of erythema chronicum migrans, or other systems may be involved. The most notable being the heart, nervous system and joints, with carditis, chronic meningitis and arthritis being reported (Barbour 1985).

In America, the vectors of the disease are the *Ixodid* ticks. The organism has also been identified in a number of animals including deer and rodents, as well as deer flies, horse flies and mosquitoes. In Australia, the source of the spirochaete and specific vectors have not been identified, although *I.holocyclus* is a common tick in New South Wales where Lyme disease has been reported. If arthropod vectors other than ticks can be incriminated, it may explain why some patients have no history of tick bite (Stevenson and Hughes 1988). Ban (1993) noted a number of reports which discussed the possibility of some species of seabird ticks acting as vectors for this disease.

Hudson *et al* (1994) put forward the hypothesis that there exists a separate Australian strain of the disease. They base their hypothesis on the fact that have been at least three DNA groups, or genospecies, identified overseas, and note that the ecology of Lyme borreliosis in Australia is different from anywhere else in that the borreliae in Australian ticks structurally resemble the classical Lyme borreliosis organism, but differ in their growth requirements. The aetiological spirochaetes may differ significantly in their biological, antigenic and genetic make-up from classical *B.burgdorferi*.

At present research is being carried out by Hudson and colleagues to attempt to define the criteria for adequate case identification in the Australian context, isolate the causative organisms, and analyse them genetically, develop diagnostic procedures that aid case definition, and set in motion an epidemiological surveillance program.

### **Listeriosis**

*Listeria monocytogenes* is a non spore forming gram positive bacillus which is motile at room temperature, but not at 37°C. It has been isolated from both wild and domestic mammals, birds, fish, plants and soil (Clarridge and Weissfeld 1985). It is an uncommon disease with sporadic cases being reported (Stevenson and Hughes 1988). In recent years, there have been case clusters in a number of states, including two cases reported in Tasmania following the ingestion of smoked mussels (Mitchell 1991). These cases were probably due to environmental contamination rather than zoonotic spread.

Infection is acquired from either ingestion of infected food, especially soft cheeses or milk, or as intrauterine or infected birth canal transmission in a mother who may be an asymptomatic vaginal carrier or have had the illness (Stevenson and Hughes 1988).

The incidence of clinical listeriosis is highest in neonates, people over 40 years and the immunocompromised. Paul *et al* (1994) noted that in their study, 86% of patients with this infection had predisposing conditions. The commonest manifestation in adults is meningoencephalitis. In pregnancy, an influenza like illness occurs with fever, chills pyrexia, headache and back pain. A premature stillborn or acutely ill baby may be

born a few days after the mother's illness, and cross infections in neonatal units have been known to occur. The prognosis is poor in neonates and the elderly, in one review of eighty four cases in Sydney, a mortality of 21% was recorded (Paul *et al* 1994).

Whilst being an uncommon infection, *Listeria* has been isolated from many animals, as already mentioned, and has been found in many fresh and processed foods (Public Health Committee Tas.1992). With more people consuming game meat, sometimes obtained from dubious sources, the potential for infection from our native animals should not be ignored.

### **Salmonella infections**

Although not a newly recognised zoonosis, human infections with *Salmonella* spp. continue to be revealed. In the third quarter of 1991, there was a 187% increase in the *Salmonella* case rate per 100,000 head of population in Tasmania (Powling 1992). Speare and Thomas (1988) report that in one study in north Queensland, 26.8% of orphaned kangaroo and wallaby young were infected with *Salmonella* spp.

Tasmania has a wide range of *Salmonella* spp. causing infections in humans, including *S.typhimurium*, *S.paratyphi* B, *S.bovismorbificans* and *S.mississippi*. In a paper presented at an conference on parasitology of wildlife, Goldsmid *et al* (1992), reported that 14 different species of *Salmonella* had been isolated from wallabies, snakes, devils, quolls, wombats, pademelons, tiger cats and some birds. Davies and Goldsmid (1994) noted that there were a wide range of Tasmanian animals infected with *S.mississippi*. This species appears to be almost unique to Tasmania. In the National *Salmonella* Surveillance scheme annual report for 1993,



Powling *et al* (1994) noted that Tasmania accounts for approximately 96% of *S.mississippi* cases reported in Australia.

### **Infections due to *Campylobacter* spp.**

*Campylobacter jejuni*, and less commonly *C.coli* are associated with diarrhoea and enteritis in humans. The source of infection is usually poultry as well as a wide range of wild and domestic birds and animals, with infection occurring after ingestion of contaminated food or water. Infections with *Campylobacter* spp. are a prominent cause of infectious diarrhoea worldwide. In 1986, there were more notifications of diarrhoea caused by *Campylobacter* spp. than either *Giardia* spp. or *Salmonella* spp. It is also a well recognised cause of "Traveller's Diarrhoea" (Stevenson and Hughes 1988).

Since 1989, infections with *Campylobacter* spp. have been notifiable. A steady increase in the number of *Campylobacter* isolations has been noted. In 1991 in Tasmania, there were 729 campylobacteriosis notifications, the second highest notification rate in Australia following the Northern Territory . After April 1992 however, there was a rapid, sustained decline in notifications, between April and December 1992, a 50% decline was reported. This was largely due to an increased intensity of infection control programmes in most commercial chicken farms in Tasmania (Henderson 1994).

### **Infections caused by atypical *Mycobacteria*.**

As already discussed, bovine tuberculosis has largely been controlled. There remain however a number of so called "atypical mycobacteria" which are able to cause disease in humans. This group of organisms are present in the environment (eg. water and soil), and also infect animals. Strains of *Mycobacteria intracellulare*, *M.avium* and *M.scrofulaceum* as

well as strains of *M. fortuitum* and *M. kansasii* *M. xenopi* are the commonest atypical mycobacteria causing pulmonary disease and lymphadenitis. Immunosuppressed patients are often at risk of developing systemic infections with these organisms should they become infected. It is now common to find mycobacterial infections without granuloma formation in immunocompromised patients, particularly those with acquired immunodeficiency syndrome (AIDS). With the exception of *M. marinum*, which is usually associated with cuts or scratches whilst handling fish or fish tanks, the role of these infections as zoonotic diseases are unclear (Stevenson and Hughes 1988). It is interesting that Cartledge (1994 personal communication) reports that in Tasmania, lymphadenopathy due to *M. avium* is particularly common.

In their Synopsis of Zoonoses in Australia, Stevenson and Hughes (1988) list a number of other bacterial diseases which have been reported after contact with animals, most of which are rare and have only occurred in isolated cases. These include self limiting enterocolitis caused by *Yersinia enterocolitica*, a range of clinical conditions caused by *Y. pseudotuberculosis*, skin infections caused by *Dermatophilus congolensis* and *Erysipelothrix rhusiopathiae*, Melioidosis caused by *Pseudomonas pseudomallei*, and wound infections caused by *Pasteurella* spp.

### **Diseases affecting Aborigines prior to European settlement**

There were probably insufficient numbers of Tasmanian Aborigines to maintain human epidemic diseases such as measles, however, the Aboriginal population were probably affected by diseases having animal sources (zoonoses) as well as being exposed to other infections transmitted by ectoparasites such as lice and ticks (Goldsmid 1988).

As the dingo did not reach Tasmania, it is unlikely that Aborigines had been exposed to parasites transmitted by these animals such as hydatid, *Dirofilaria immitis* and *Toxocara canis*.

Goldsmid (1988) noted that it is probable that a number of diseases such as Ross River virus, Murray Valley encephalitis and some fungal infections, were endemic to Australia during that period, and Tasmanian Aborigines may have been exposed to a few of these diseases.

### **Diseases affecting Aborigines after European settlement.**

With the advent of European settlement, the picture changed. Common childhood diseases such as measles, influenza, whooping cough and mumps were introduced. Sexually transmitted diseases were also spread to the Aborigines.

A large range of immunising agents have been developed during the twentieth century. These have succeeded in controlling or in some cases as with smallpox, eradicating most of these diseases.

The European settlers brought with them domestic animals, which in turn brought their own diseases, many capable of infecting humans, such as anthrax, brucellosis, leptospirosis and hydatid. Again most of these diseases have been eradicated or controlled during this century.

To a large extent, it is evident that most imported diseases of humans and their animals have been controlled.

With human expansion into previously uninhabited areas for recreation, mining cultivation of crops and logging, contact has been made with native animals.

It can be seen therefore that potential exists for human infection to occur through our contact with native fauna.

New zoonotic diseases are being recognised in humans for the first time (e.g. Flinders Island spotted fever, and Ross River virus), and previously unrecorded diseases are being reported in native animals which may have the potential to be transmitted to humans (e.g. *Trichinella pseudospiralis*).

Further, diseases previously thought to be restricted to one species of mammal are now being shown to be potentially zoonotic (e.g. giardiasis).

There is thus a need therefore to study and evaluate the overall zoonotic potential of Tasmania's wildlife, and it is this need that has prompted this present investigation in an attempt to discover what part, if any, our native fauna play in human disease.

**CHAPTER 3**  
**TASMANIAN MAMMAL SURVEY -**  
**INTESTINAL PATHOGENS**

### **3.1 Bacterial Pathogens**

#### **3.1.1 Introduction.**

Large numbers of visitors to Tasmania visit the state to experience the beauty of the numerous national parks and bush walks. An unpleasant side effect of their pastime has been diarrhoea. Even in recent months, bushwalkers have reported cases of diarrhoea to National Parks officials (Driessen 1994 Personal communication). The aetiology of these cases is unknown, although *Giardia* spp. has been implicated. Newspaper reports have emphasised the need for hygiene standards to be maintained by bushwalkers (APPENDIX 8.12).

Unfortunately, very few faecal specimens from bushwalkers are submitted for examination, with many general practitioners preferring to treat empirically, so the causative agents of these diarrhoeas are unknown. While it is not known for certain whether the agents are bacterial, viral or parasitic, it is suspected that Tasmania's native animals may play a part in the transmission of bacterial and parasitic diseases to humans.

In order to determine the zoonotic potential of Tasmanian wildlife, a survey of native mammals was carried out to establish what potentially zoonotic bacteria might be present.

### 3.1.2 Materials and Methods

#### 3.1.2a Specimens

In all, 180 specimens of animal faeces (scats) were collected from around the State by National Parks rangers, bush walkers, fishermen. A small number of autopsy specimens were obtained from road deaths. Each specimen was split, part being placed into 10% formalin for microscopy, and the remainder being placed into a sterile container for culture. Faecal specimens were identified on the basis of their macroscopic appearance and with the aid of the figures provided by Triggs (1992) and Morrison (1981).

The number and origins of these specimens are detailed in Table 1:

<b>Animal</b>	<b>Number Tested</b>
Bandicoot	4
Dasiurid (Unspecified)	5
Pademelon	2
Platypus	2
Possum (Unspecified)	72
Potaroo	2
Quoll	11
Devil	12
Wallaby	45
Wombat	24
Water Rat	1
<b>NUMBER TESTED</b>	<b>180</b>

**Table 1**      Origin and number of specimens analysed.



### 3.1.2b Methods

All samples were routinely cultured for *Salmonella*, *Shigella* and *Campylobacter*. The samples were plated directly onto Hektoen Enteric Agar (Oxoid CM419), Xylose Lysine Desoxycholate (XLD) medium (Oxoid CM469) and *Campylobacter* selective agar (Preston's) (Oxoid SR117). A portion was also placed into Selenite Broth (Oxoid CM395 and L121). (APPENDIX 8.2-8.5)

All plates and the broth, with the exception of the *Campylobacter* agar, were incubated for 24 hours in air at 37°C. The *Campylobacter* agar was incubated for 48 hours at 42°C in an Oxoid anaerobic jar with a *Campylobacter* gas generating kit (Oxoid BR60).

The Selenite Broth was sub-cultured after 24 hours onto a further XLD plate, which was incubated for 24 hours at 37°C.

Plates were examined for the presence of clear pink colonies on the XLD agar and clear green colonies on the Hektoen agar. These colonies are unable to ferment the carbohydrates in the medium. Colonies demonstrating the production of hydrogen sulphide gas by the presence of black colouration, were also sought.

Any colonies fitting the above criteria were sub-cultured to obtain a pure growth, and then presumptively identified biochemically using the Microbact 12E system (Disposable Products, Adelaide, South Australia), and serologically using the Wellcolex *Salmonella* latex agglutination kit (Murex International, ZC 50). Any isolate that was identified as a probable *Salmonella* spp. was sent for full identification to the Institute of Medical and Veterinary Science in Adelaide South Australia.

In addition, unpublished data on *Salmonella* isolates from Mt. Pleasant Laboratories in Launceston was kindly obtained from Mr Bevan Peal, for analysis.

Due to an extremely poor result for the isolation of *Campylobacter* spp., a further experiment was designed to establish the period of time an isolate of *Campylobacter* spp. remained viable. Twenty colonies of an isolate of *Campylobacter* spp. obtained from a human, were inoculated into 1 gram of faeces. The specimen had previously been cultured routinely, and found to be negative for *Campylobacter* spp. The faecal specimen was left on the bench at room temperature, which varied between 8°C and 18°C, and plated out daily onto *Campylobacter* selective medium, and cultured for 48 hours at 42°C in an Oxoid anaerobic jar, with a *Campylobacter* gas generating kit.

A technique described by Kaplan *et al* (1983) for improving the recovery rate of *Campylobacter* spp. involved the inoculation of a "Campy thio" broth which was refrigerated overnight prior to sub culture onto selective media, and incubation at 42°C. Using this method, Kaplan *et al* (1983) estimated that 10% more *Campylobacter* spp. were isolated.

With this information in mind, a small number of specimens (20 ) were collected from a variety of animals, predominantly wallabies, possums and bandicoots, directly into a selective enrichment broth, made from Nutrient Broth (Oxoid CM67), and containing Preston's Selective Supplement (Oxoid SR117), *Campylobacter* Growth Supplement (Oxoid SR84), and Lysed Horse Blood. The broths were incubated for 24 hours at 42°C and then sub-cultured onto Preston's *Campylobacter* Agar, and incubated for 48 hours at 42°C, in an Oxoid anaerobic jar with

*Campylobacter* gas generating kit. The results of this experiment are summarised in Table 4.

### 3.1.3. Results

The results of this survey are summarised in Tables 2 and 3

<b><i>SALMONELLA SPECIES ISOLATED</i></b>		
<b>SPECIES</b>	<b>ANIMAL</b>	<b>NUMBER</b>
<i>Salmonella mississippi</i>	Bandicoot	1
	Spotted Tail Quoll	1
<i>Salmonella orion</i>	Wallaby	1
<i>Salmonella</i> subsp 1 6,8 :	Wallaby	1
	<b>TOTAL</b>	<b>4</b>

<p><b>Table 2</b>     <i>Salmonella</i> spp isolated in this study (180 animals tested)</p>
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An evaluation of data supplied by Mount Pleasant Laboratories, Launceston, demonstrated that a wide variety of *Salmonella* isolates are found in the raven population and in many other animals including snakes. This data is summarised in Table 3.

SALMONELLA SPECIES ISOLATED FROM TASMANIAN WILDLIFE	
SPECIES	ISOLATED FROM:
<i>S.give</i>	Raven
<i>S.give</i> v15+ (Newbrunswick)	Raven
<i>S.victoria</i>	Tiger Cat
<i>S.typhimurium</i>	Raven, Quoll, Possum, Devil, Wallaby
<i>S.mississippi</i>	Quoll, Devil, Wallaby, Wombat Pademellon, Copperhead snake, Raven
<i>S.bovismorbificans</i>	Devil, Raven, Wallaby.
<i>S.infantis</i>	Devil
<i>S.muenchen</i>	Snake, Devil, Wombat
<i>S.oraniburg</i>	Raven
<i>S.arizoniae</i>	Wombat, Quoll, Devil Tiger Snake
<i>S.sofia</i>	Raven

Table 3: Analysis of data supplied by Mount Pleasant Laboratories, Launceston.

The results of the experiment to attempt to isolate *Campylobacter* spp after collection into selective enrichment broth is summarised in Table 4.

---

<b>Total Number of Specimens Tested :</b>	<b>20</b>
<b>Number of <i>Campylobacter</i> spp Isolated:</b>	<b>0</b>

<p><u>Table 4</u> Results of culture for <i>Campylobacter</i> spp. after incubation in a <i>Campylobacter</i> enrichment broth</p>
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The experiment to determine the period of viability of *Campylobacter* spp. showed that the isolate remained viable for 6 days. Even though temperatures in the outdoors may vary more than they did in this experiment, the results demonstrate that the organism appears to be more hardy than originally believed.

#### 3.1.4. Discussion

The results of the bacterial survey appear disappointing, with an overall prevalence of *Salmonella* spp. of just 2.3%.

Other studies have shown a much higher prevalence of *Salmonella* in some animal groups, for example Ball (personal communication 1993) noted that approximately 50% of Tasmanian devils were infected with *Salmonella mississippi*. The majority of these specimens were collected from road kills, as Ball's study was to determine the *Salmonella* infection rates of animals. The present study however, has been carried out using faecal material from animals to establish what role faecal contamination of the environment from animals plays in the overall picture of bushwalker's diarrhoea.

The most likely explanation for the relatively low numbers of positive cultures would probably be due to a time delay from collection to processing of the specimens. The faecal samples were not collected into any transport medium, and could therefore have dried out enough to render the organisms non viable. Balows *et al* (1991) states that faecal specimens should be cultured within two hours of collection in order to recover fastidious organisms. The nature of the study made this impossible, some specimens, collected in isolated bush areas taking two to three days to reach the laboratory. Although they were collected as fresh as possible, it is not known how fresh the specimens were when collected.

A surprising result in this study is the fact that no *Campylobacter* spp. were isolated. Due to the fastidious nature of the organism, again a possible explanation is that the organisms were non-viable at the time of culture.

Yet another explanation should be considered. Jones *et al* (1991) describe the recovery of "viable but non-culturable" *Campylobacter jejuni*. These strains were identified using electron microscopy after four suspensions of *C.jejuni* were stored in sterilised pond water at 4°C. After storage for six weeks, the suspensions were found to be non-culturable. They were then fed to suckling mice, and it was found that colonisation of the animals occurred in two of the four strains.

It would appear unlikely however that this could be an explanation for the lack of *Campylobacter* spp. in this study of the Tasmanian native animals. The strains referred to by Jones *et al*, (1991) had been stored for up to six weeks, in a sterile environment, at 4°C. All specimens cultured in this study were collected as freshly as possible, the untreated faecal specimen was cultured. No other treatment of the specimen was carried out.

It may well be that the organisms had in fact died before being cultured, however this again would appear to be unlikely as an experiment in the present study has shown that *Campylobacter* spp. remains viable for up to six days at ambient temperature, and the longest delay in receipt of faecal specimens was in the region of three days.

Efforts were made to culture *Campylobacter* spp. after the collection of faeces into a selective enrichment broth, again without success. It is apparent therefore, that the most likely explanation for the lack of *Campylobacter* spp. found in the native animal population, is that native

animals do not play a great part in the transmission of this disease, rather it is the domestic stock that plays the major role.

This explanation appears to be supported by a survey of *Salmonella* spp. and *Campylobacter* spp. in river and dam waters in Tasmania carried out by DeBoer (1994 personal communication ). *Campylobacter* spp. was isolated from 35% of river and dam water from rural areas, but there were no isolates of *Campylobacter* spp. from any of the sites tested within the national parks.

Henderson (1994) noted that the majority of human infections with *Campylobacter* spp. in Tasmania were associated with cattle, sheep, dogs, cats and poultry. So too, the majority of chicken carcasses from retail outlets in the United States have been found to be contaminated with *Campylobacter* spp. Henderson (1994) obtained twenty non-human isolates from poultry carcasses.

There appears to be little data relating to the incidence of *Campylobacter* spp. in Australian native animals. One report of a notification of native animals being infected was found in the Annual Report of the National Salmonella Surveillance Scheme for 1988, where a kangaroo and a wombat were found to be infected with *Campylobacter* spp. It was noted however, that the wombat was in fact a pet (Powling 1989), and therefore had contact with humans, the animal may therefore have been infected by those humans. No details were available on the origin and location of the wallaby.



In summary, this study has shown that a wide range of Tasmanian native animals are capable of harbouring some intestinal bacterial pathogens. Data obtained from this study and from Mt Pleasant laboratories in Launceston demonstrate that many native animals, including birds and reptiles are infected with a variety of *Salmonella* species. Data obtained in this study appears to confirm previous reports that many native animals and birds are infected with *Salmonella mississippi*.

Despite all efforts to culture *Campylobacter* spp. no animals appeared to be infected with this organism. It appears therefore, that native animals do not play a part in the transmission of *Campylobacter* spp. in Tasmania, rather, it is domestic companion animals that are responsible.

## **3.2 Parasitic infections**

### **3.2.1 Introduction.**

A survey was carried out using the same faecal specimens which were cultured for bacterial zoonoses, to attempt to establish what zoonotic intestinal parasites were infecting Tasmanian native animals.

There is much anecdotal evidence of bush walkers in Tasmania suffering from diarrhoea after returning from walks within the State's national parks. Many general practitioners are treating this condition empirically with metronidazole assuming that the causative organism is *Giardia* spp. (Leeb 1992). No studies of the state's wildlife have been carried out to date in order to ascertain their part in this scenario, or whether other parasites such as *Cryptosporidium* spp. are implicated. Hawkesford (1989) noted that *Cryptosporidium* spp. was the most common parasite recovered from humans with diarrhoea in Tasmania.

Davies *et al* (1993) described what appears to be the first Tasmanian report of a case of giardiasis in a host other than a human. This case was one where a dog was passing large numbers of trophozoites. This report prompted the question of the ability for Tasmanian wildlife to become infected with, and transmit human strains of *Giardia*. Faubert (1988) believes that most publications on the subject present rudimentary evidence that domestic and wild animals have been infected with *Giardia duodenalis*.

It was decided therefore, to conduct a survey of the specimens already collected, to establish whether evidence of any potentially zoonotic parasitic infections including *Giardia* spp. and *Cryptosporidium* spp., existed in native animals.

### 3.2.2 Materials and Methods

The samples were examined for parasites using a direct wet preparation, and then processed using a standard Formalin-ethyl acetate concentration technique utilising the FPC parasite concentrator (Evergreen Scientific , Los Angeles, Calif.) according to the manufacturer's instructions. (APPENDIX 8.6)

Direct smears were prepared from fresh faeces from 73 of the specimens, which were then stained using an acid fast stain for *Cryptosporidium* spp. as described by Balows *et.al.* (1991). (See APPENDIX 8.7).

A direct immunofluorescent stain for *Cryptosporidium* spp. was also used to test for this parasite (APPENDIX 8.8).

The Crypto-Cel IF Test (Celllabs Diagnostics Pty Ltd Brookdale N.S.W.) utilizes direct staining of faecal smears with a monoclonal antibody labelled with fluorescein. The labelled mouse monoclonal antibody binds specifically to the *Cryptosporidium* oocysts in the specimen. A washing step removes unbound antibody, and when observed under a fluorescence microscope, the oocysts appear bright green against a background of yellow or red contaminated material.

It was found however, that this method was not satisfactory due to the nature of the specimens tested in this survey, as they were generally a few days old when received, and were formalin preserved, which is not ideal for preserving *Cryptosporidium* spp.

Hawkesford (1992), had noted that di-chromate was the preservative of choice for *Cryptosporidium* spp. and therefore it was decided to perform only the acid fast stain on those specimens which had direct smears made when collected.

In the case of *Giardia*, the fact that the success rate for the microscopic detection of cysts of from a single faecal specimen is only 50 - 70% (Burke 1977), all samples were tested for specific *Giardia* antigen using the *Giardia* Celisa enzyme linked immunosorbent assay (ELISA), (Cellabs Diagnostics Pty. Ltd. Brookvale Australia) in addition to being examined microscopically.

This method detects *Giardia* antigens in a capture enzyme immunoassay. Antigens from faecal specimens are bound to microplates which have been coated with purified mouse monoclonal antibodies to *Giardia*. These antibodies react with the *Giardia* antigens of molecular weights 30,000 and 66,000, which are the major antigens shared by both cysts and trophozoites (Janoff *et al.* 1989). After a washing step, a second antibody, purified rabbit antibody to *Giardia* is added, followed by an anti rabbit IgG conjugated to horseradish peroxidase. Enzyme activity is directly proportional to the concentration of *Giardia* antigen present in the sample and control. The enzyme activity is measured by adding the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB) which produces a colour which can be detected either visually or by measuring the absorbance in a spectrophotometer.

In humans, the ELISA test for *Giardia* has been shown to have a sensitivity of 97% with a specificity of 96% (Rosenblatt *et al.* 1993). In a study in Perth and Melbourne to detect antigens in the faeces of both humans and dogs, this method demonstrated a specificity of between 91 and 95%, with a sensitivity of 64% (Hopkins *et.al.* 1993).

The manufacturer claims that the test will not cross react with , *Blastocystis hominis*, *Chilomastix mesnili*, *Cryptosporidium* spp. *Dientamoeba fragilis*, *Endolimax nana*, *Entamoeba hartmanni*, *Entamoeba coli*, *Entamoeba histolytica*, *Iodamoeba butchlii*, *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Enterobius vermicularis*, *Hymenolepis nana*, *Strongyloides stercoralis* and *Trichuris trichiura*. This claim was confirmed by Hopkins *et.al.* (1993), who state that other parasites observed in *Giardia* free samples failed to produce a false positive reaction with this method.

All specimens were processed in accordance with the manufacturer's instructions (APPENDIX 8.9). Once the reaction had been completed, the plates were read using a Flow Laboratories Titertek MCC EIA reader, with a 450nm wavelength filter.

To determine the results of the test, a cut-off was calculated in accordance with the test instructions, by adding 100% to the mean of the absorbance of negative controls. Any specimens with absorbance values greater than the cut-off value were considered positive. Specimens with absorbance values less than, or equal to the cut-off value were considered negative.

A small number of specimens (38 in all) had smears made from fresh faeces, which were fixed in Schaudinn's fixative immediately, and stained with trichrome stain, as described by Garcia and Bruckner (1993) (APPENDIX 8.10). These smears were examined microscopically for parasites.

### 3.2.3 Results

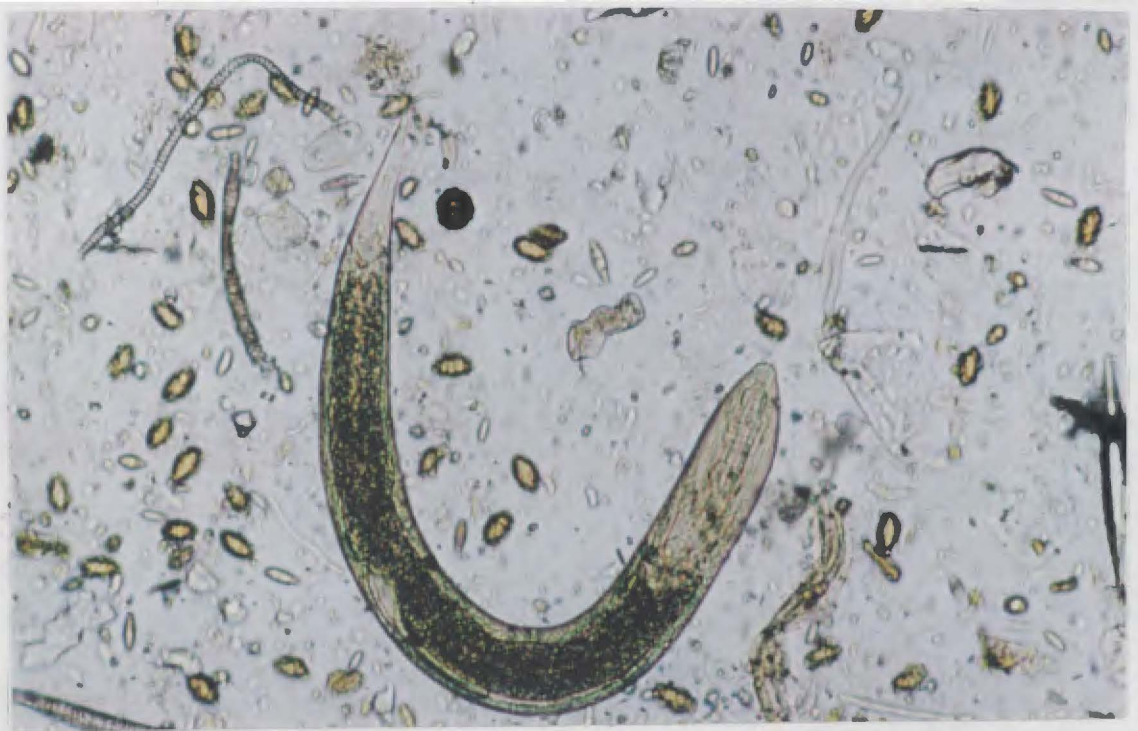
Of the 180 samples examined for parasites, 110 contained at least one parasite, with many containing multiple larvae, eggs or cysts.

Many contained larvae of possibly free living nematodes, some strongyle (hookworm - like) eggs, some tapeworm eggs and a few sporocysts of *Eimeria* spp.



**Fig 2** *Eimeria* oocyst - sporulated with 4 sporocysts. X1000

*Eimeria* spp. (Fig 2) are coccidian parasites that have occasionally been reported in humans, but they are spurious parasites, ingested in a non viable condition in sardines or other fish (Faust *et al* 1974). *Eimeria* spp. are differentiated from *Isospora* spp. by the four sporocysts produced by *Eimeria* spp. *Isospora* spp. only produces two sporocysts.



**Fig 3** Possible free living nematode larvae (top) X400 (bottom) X1000

A number of different strongyle-type nematode eggs and larvae were seen on microscopy (Fig 3). The identification of these larvae has been difficult, some may be parasitic or free living. No definite conclusion could be reached on their significance.



A large number of the animals tested had multiple parasite infections, as can be seen in Figure 4, which contains a nematode egg (left), as well as an unidentified tapeworm egg (right).



**Fig 4** Unidentified Nematode (L) X1000 Tapeworm (R) X 1000



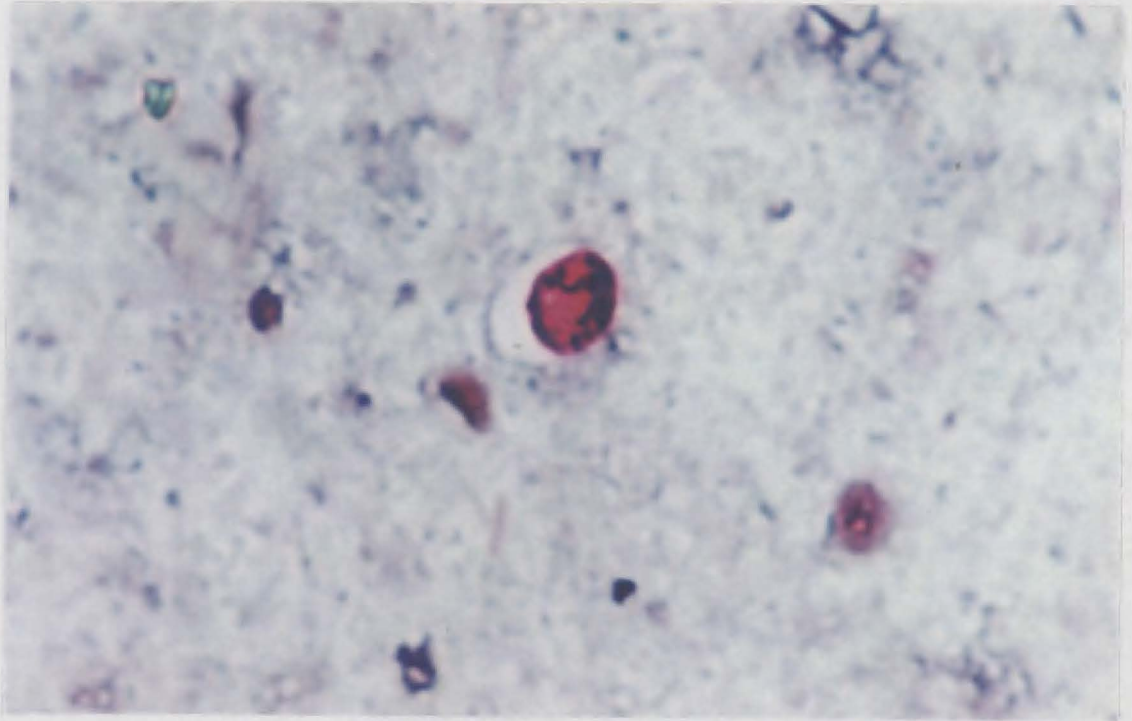
The majority of these were not species that are recorded as infecting humans, however, there were a number of parasites found which were recognised as human pathogens, or potential human pathogens.

These are summarised in the Table 5.

Animal	Number Tested	<i>Giardia</i>	<i>E.coli</i>	<i>Trichuris/ Capillaria</i>	<i>Baylis- ascaris</i>	<i>Fasciola</i>	<i>Crypto- sporidium</i>	<i>Blasto- cystis</i>
Bandicoot	4	1		1				
Dasiurid	5			1	1			
Pademelon	2							
Platypus	2							
Possum	72	4					6	
Potaroo	2							
Quoll	11			3				
Devil	12	1			1			
Wallaby	45	2	1			1	4	3
Wombat	24	2					1	
Water Rat	1							
<b>No. EXAMINED</b>	<b>180</b>	<b>10</b>	<b>1</b>	<b>5</b>	<b>2</b>	<b>1</b>	<b>11</b>	<b>3</b>

Table 5: Parasites Isolated from Native Tasmanian Animals  
Figures for *Giardia* obtained from microscopy and antigen detection.

*Cryptosporidium* spp. (Fig 5) appeared to be the most common parasite, with 11 animals of the 73 having samples tested by the Acid Fast stain, positive , giving a prevalence of 15%.



**Fig 5** *Cryptosporidium* spp. Acid fast stain X1000

*Giardia* spp. (Fig 6) was the next most commonly found parasite, with a prevalence of 5.55%. This figure was a combined result of microscopy and the *Giardia* antigen test,

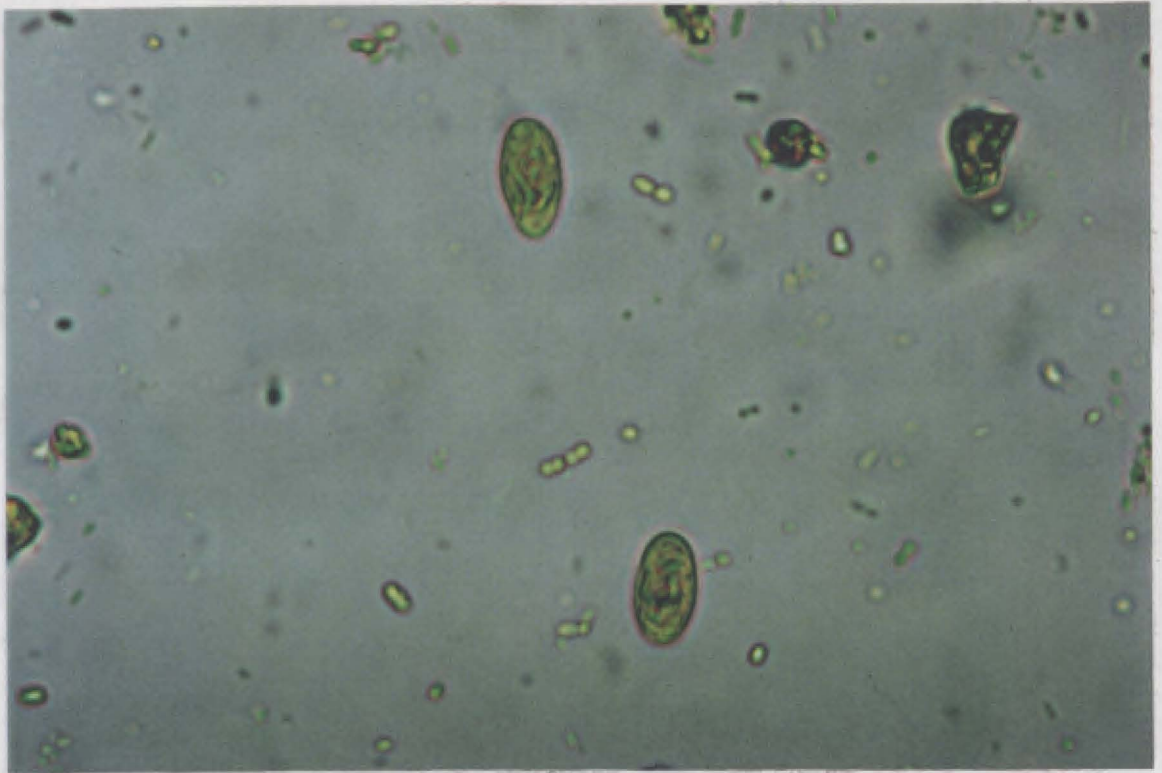
The results for the combined *Giardia* antigen test and routine microscopy are extracted from Table 5, and are summarised as follows:

TOTAL NUMBER TESTED	NUMBER POSITIVE
180	10
%POSITIVE	<u>5.55</u>

These results for *Giardia* are analysed further in Table 6:

ANIMAL	TOTAL NUMBER	NUMBER POSITIVE	%POS.
Possum	72	4	5.55
Wombat	24	2	8.33
Wallaby	45	2	4.44
Bandicoot	4	1	25.00
Devil	12	1	8.33

**Table 6** Analysis of sources of positive microscopy and positive *Giardia* antigen tests



**Fig 6** Cysts of *Giardia* spp. X1000

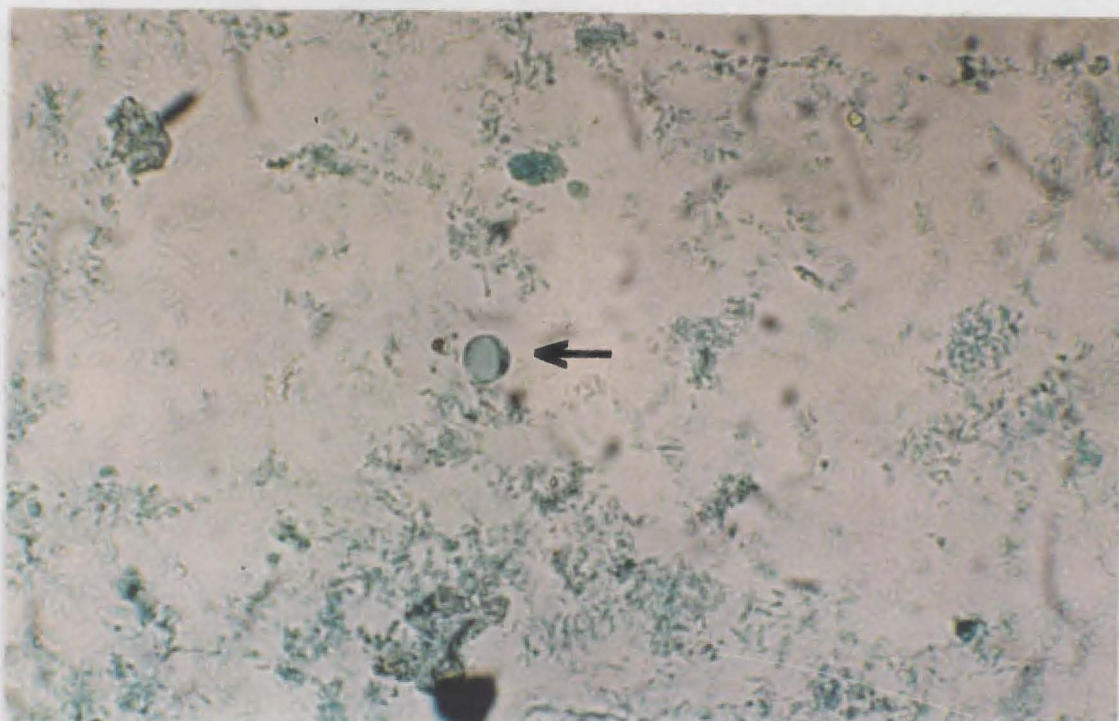
Although accurate identification was not possible, five animals (2.7%) were found to be passing eggs of a probable *Trichuris* spp.(Fig 7)



**Fig 7** Probable *Trichuris/Capillaria* spp. egg X1000

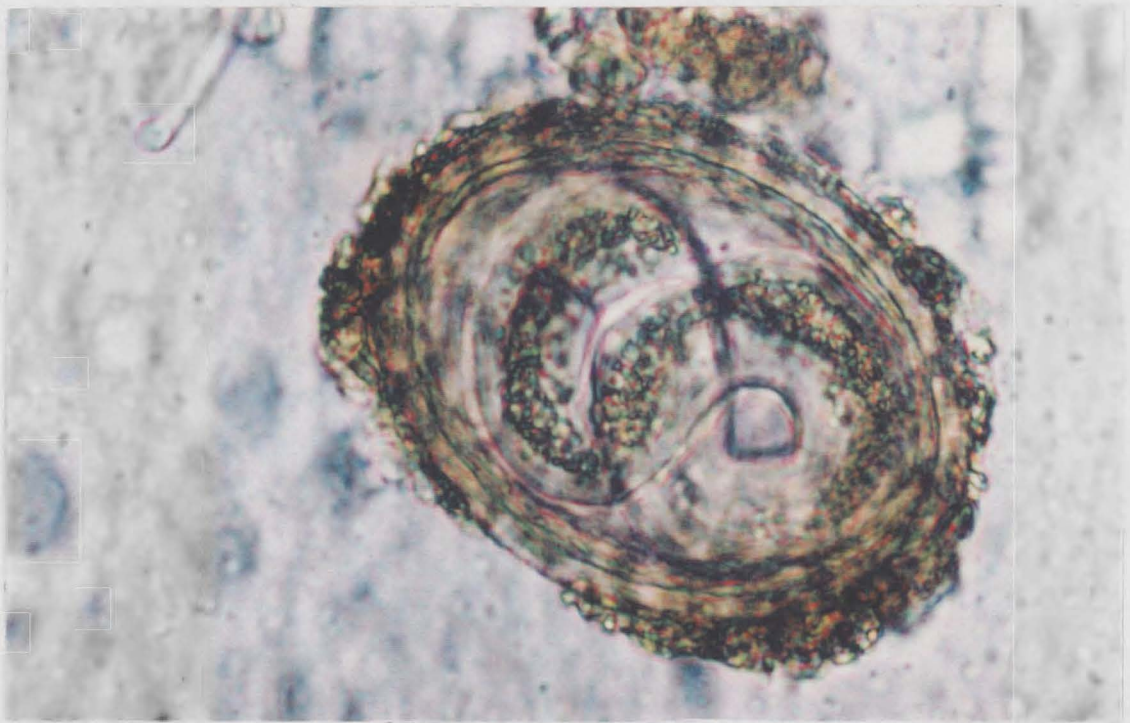


A *Blastocystis* like organism (Fig 8) was found in 3 animals (1.6%).



**Fig 8** *Blastocystis* like organism X1000

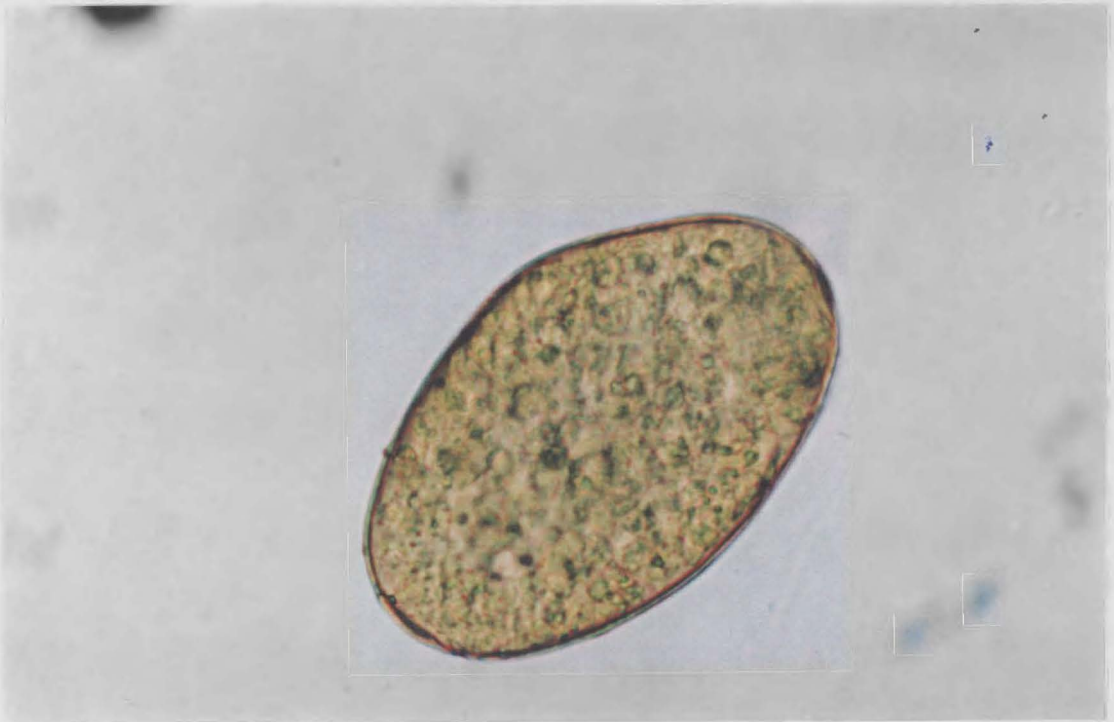
Eggs of *Baylisascaris* spp. (Fig 9) were found in 2 dasyurids. This represents an prevalence of 1.1% in the native animals.



**Fig 9** Egg of *Baylisascaris* spp. X1000

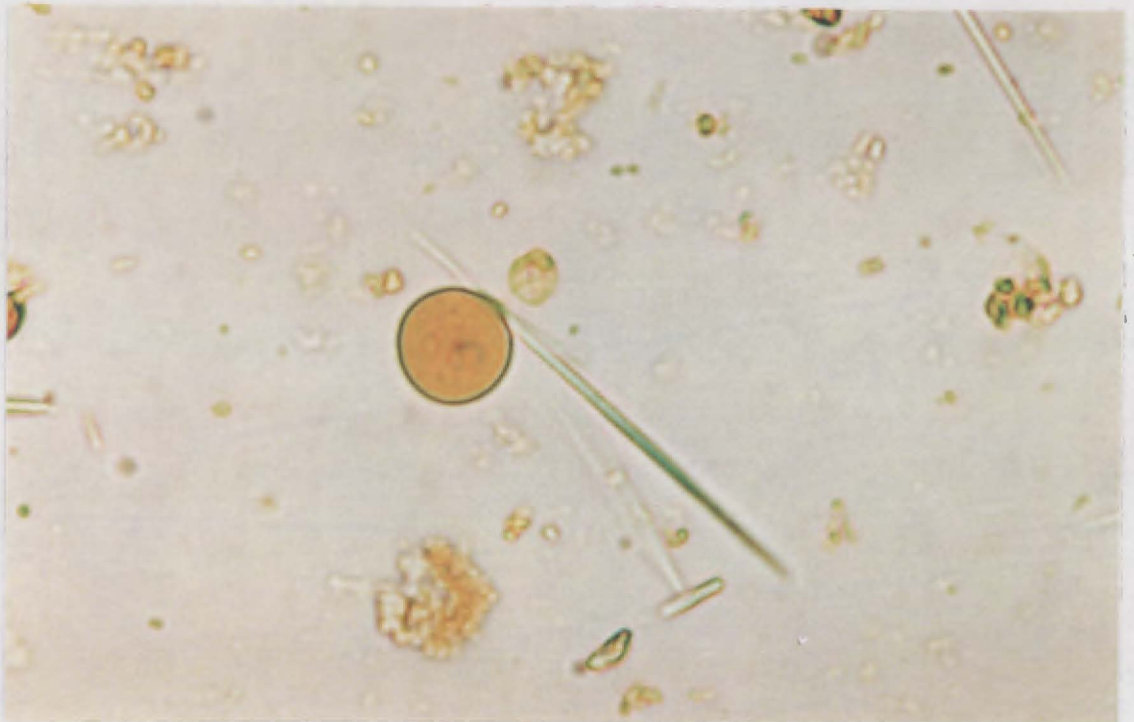


Eggs of *Fasciola hepatica* (Fig 10) were found in faeces from a wallaby. The egg is a typical trematode egg, being large (ranging from 130-150um x 60-90um), and operculate.



**Fig 10** *Fasciola hepatica* egg X400

Cysts morphological identical to *Entamoeba coli* (Fig 11) were found in a wallaby. This parasite is not considered pathogenic in humans, but does indicate ingestion of faecally contaminated matter.



**Fig 11** Cyst morphologically identical to *Entamoeba coli* (X400)  
(Iodine Stained)

### 3.2.4 Discussion

An experiment was designed, similar to that described by Faubert (1988), involving the infection of a range of young native animals with cysts of *Giardia* spp obtained from humans. Young animals were chosen because there was less likelihood that they would be naturally infected. The animals were to be fed a standardised load of cysts harvested from human faeces, and monitored for a period of time to establish whether they began to pass cysts themselves. After completion of the experiment, the animals were to be treated with metronidazole before being released. Animal Ethics Committee approval was sought, and obtained, however the experiment did not proceed as no suitable place to house the animals could be found.

The general pattern of this survey parallels that of Hawkesford (1989) in a study of the human population of Tasmania, where *Cryptosporidium* spp. was the most commonly isolated parasite. The next most common parasite isolated in this survey, like that of Hawkesford (1989), was also *Giardia* spp., with an overall prevalence of 5.5%. This figure was obtained after not only microscopic examination of faecal specimens, but also by the use of the commercial enzyme linked immunosorbent assay (ELISA) for *Giardia* antigen.

The data obtained from this study shows that *Giardia* was found in 5.5% of Tasmanian possums. This is a somewhat lower incidence than possums from the North Island of New Zealand. Marino *et al* (1992) report an incidence of 12.9% in possums trapped in specific areas of New Zealand, namely Auckland's Hawke's Bay, the Wairarapa and Wellington.

The faeces examined in the Tasmanian study were obtained from a variety of environmental areas, some were collected in the national parks around the State, while some were collected in suburban and urban areas.

It is interesting to note that other native animals had a significant infection rate with *Giardia* spp. Of course the figure of 25% of bandicoots may not be very accurate due to the very small number of specimens tested (4), a larger survey may well serve to produce a more realistic result. There were two wallabies which were infected with *Giardia*, giving a positive rate of 4.4%.

Both wombats and devils had an infection rate of 8.33% with *Giardia*, but again the numbers of animals tested were small, and a larger study may provide more accurate data.

Despite the fact that in some cases only small numbers of animals were tested, the data from this study demonstrates that 5.5% of Tasmanian native animals are infected with *Giardia* spp., and may well serve as reservoirs for this parasite, contributing to the understanding of the causes of bushwalker's diarrhoea.

The identification of some of the eggs recovered was difficult.

It was initially thought that eggs of *Capillaria* spp. were found in a number of quolls, unspecified dasyurids and a bandicoot, a prevalence of 2.7%. The fact that the polar plugs protrude, however, led to the presumptive identification of *Trichuris* spp. Further investigations need to be carried out to ascertain the identity of these parasites, and their zoonotic potential.

The *Blastocystis* like organism identified in 3 wallabies (1.6%) follows the trend of Hawkesford's survey of humans in 1989, but again, it appears that the prevalence in native animals is much higher than the 0.3% described for *B.hominis* in humans in that survey.

Two animals, a Tasmanian devil and an unspecified dasyurid were infected with *Baylisascaris* spp. (1.1%). In the United States *Baylisascaris procyonis* is a common parasite of the raccoon. There have been numerous reports of cases of visceral larva migrans caused by this nematode (Kazacoz 1986), including a fatal case of eosinophilic meningoencephalitis in an eighteen months-old boy who became infected when he ingested the eggs of *Baylisascaris procyonis* after chewing on a piece of bark which had been contaminated with raccoon faeces (Fox *et al* 1985)

*Fasciola hepatica* has been reported in a wide range of marsupials, in Tasmania. In this survey, one wallaby was found to be infected (0.55%). There have been recent reports of ectopic fascioliasis from the mainland.

The finding of cysts morphologically identical to *Entamoeba coli* in one wallaby (0.55%) from a national park, indicates that the animal has ingested matter (food or water) contaminated with human faeces. This contamination is occurring despite that fact that improved toilet facilities have been provided in the national parks, and bush walkers are instructed in waste disposal prior to embarking on walks. Thus there is a potential for other human pathogens to be present.

It is obvious therefore that native animals are capable of being infected with parasites such as *Trichuris* spp., *Cryptosporidium* spp., *Fasciola hepatica*, *Baylisascaris* spp. and *Blastocystis* like organisms.

The finding of many of these parasites shows that the potential exists for the animals to contribute to some of the cases of diarrhoea suffered by humans after venturing into the national parks.

The information gained from this survey may be used to advise people venturing into the national parks on bush walking expeditions. It is now possible to advise bush walkers that all water should be boiled before consumption.

Nematode larvae were found in numerous animals. Whilst it is believed that these are probably free living species, with some parasitic species, accurate identification was not possible. The larvae lacked the prominent pseudobulb found in *Rhabditis* spp., a free living nematode which has been reported in humans (Goldsamid 1967), and it is therefore possible that they may be parasitic. The morphological features of the nematodes however, lead us to believe that it is unlikely that any of the nematodes found were parasites of humans, such as *Strongyloides* spp. or hookworms.

**CHAPTER 4**  
**EVALUATION OF HUMAN TRICHINOSIS**

## 4.1 Introduction

The first report of trichinosis in a range of carnivorous animals in Tasmania (Obendorf *et al* 1990), (Fig 12) led to a number of questions being asked.

What was the species of *Trichinella*?

How did the parasite get to the island?

How long had it been there?

Was there any evidence of human infection with this parasite?

It was subsequently established that the species was *Trichinella pseudospiralis*, but many of these questions remain unanswered.

Evidence exists from a retrospective study of animals in museums that the parasite was present in some animals as early as 1976 (Obendorf *et al* 1990).

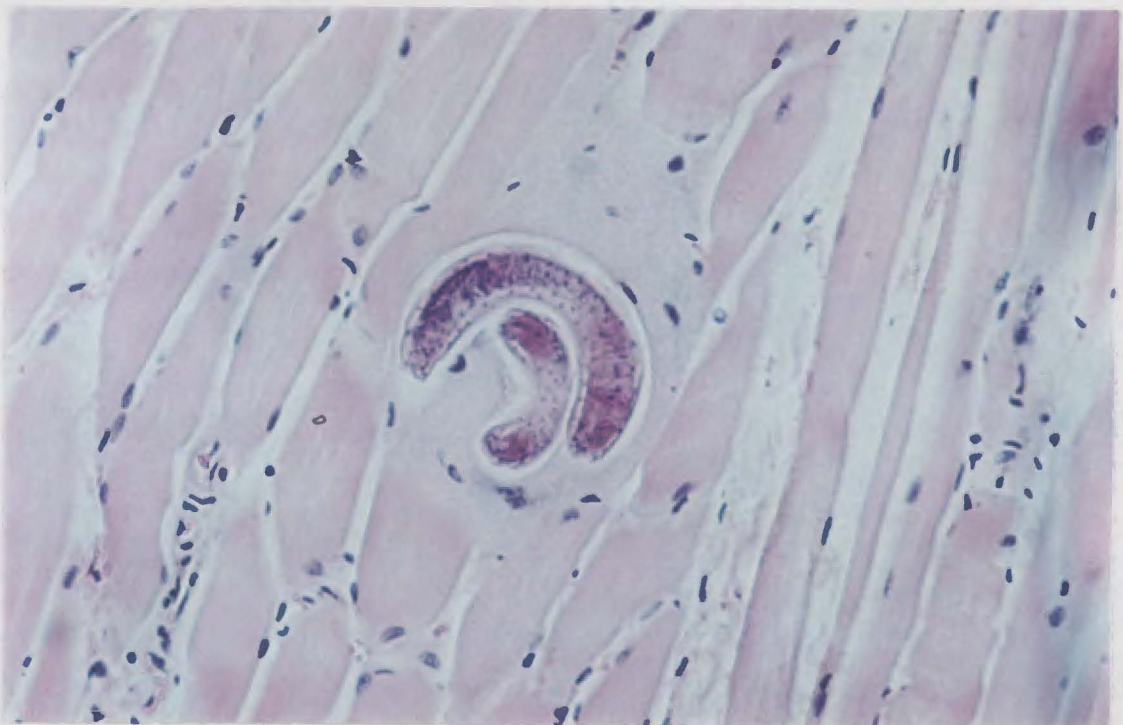
Obendorf (1993), showed by experimental infection, that many species of native animals and even feral cats are susceptible to infection with this parasite. Rats, mice, cats, brushtail possums (*Trichosurus vulpecula*) and two species of raptors (marsh harrier *Circus aeruginosus* and brown falcon *Falco berigora*) were infected by either being fed infected muscle or larvae of *T.pseudospiralis*.

In a survey of feral cats in Tasmania, Milstein (1993) showed that none of 34 feral cats were positive for antibodies to *T. pseudospiralis* using the indirect fluorescent antibody test. These findings are significant in that although these animals would not play a direct role in the transmission of the parasite to humans as they are not eaten by humans, they could, by their scavenger habits, assist in the spread amongst native animals, both



regular scavenging species (eg. Tasmanian devils, Quolls etc.) and also occasional scavengers (eg. possums and even wallabies) (Obendorf 1994 personal communication)

Whilst data is available for the prevalence in humans in Tasmania of *Salmonella* spp., *Campylobacter* spp. and *Giardia* spp., no data is available for the prevalence of human trichinosis in Tasmania. Because of this fact, and with the first report of a human case of infection with *T.pseudospiralis* probably originating in Tasmania (Andrews *et al* 1993 and 1994), it was decided that a survey should be carried out to attempt to establish whether there was any serological evidence of human infection with this parasite.



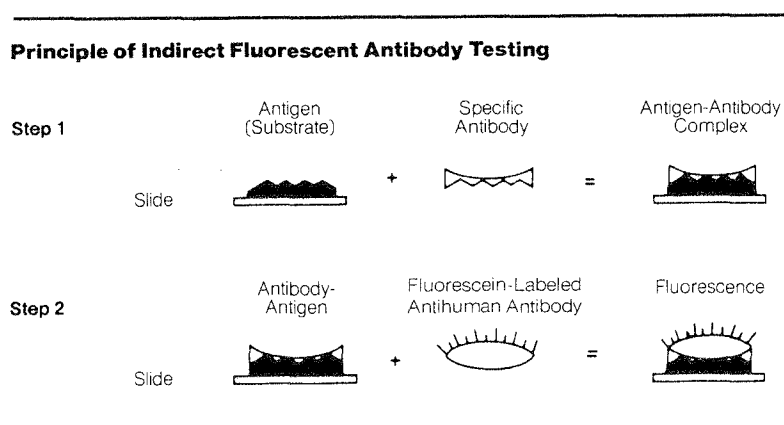
**Fig 12** Larva of *Trichinella pseudospiralis* - Haematoxylin and Eosin Stain (X1000)

## 4.2 Methods

### 4.2a Experimental Techniques - development of a diagnostic test.

Faust *et al* (1974), and Feingold and Baron (1986) note that the indirect fluorescent antibody test has been described as the method of choice for the diagnosis of trichinosis by a number of workers. Due to the fact that no commercial kits for *Trichinella pseudospiralis* exist, together with the problem of local unavailability and expense involved in importing kits for the serological diagnosis of *Trichinella spiralis*, it was decided to attempt to use larvae obtained from native animals as a crude antigen for an indirect fluorescent antibody test. (IFAT) (for details of method see APPENDIX 8.11) The principle of indirect fluorescent antibody testing are summarised in

Figure 13



**Fig 13** - Principle of Indirect Fluorescent Antibody Testing.

The *T. pseudospiralis* larvae for use as antigen were obtained in two forms from Mount Pleasant Laboratories, Launceston.

1) Larvae extracted from muscle by rapid digestion of infected tissue in 1% pepsin/ 0.5% concentrated hydrochloric acid solution were washed in buffered saline pH 7.2, and processed using routine histological embedding techniques. Sections were cut, and placed on glass slides, and the wax removed, again using standard histological techniques. These slides were used as antigen for the IFAT.

2) Wax embedded sections of infected tissue were cut, placed on glass slides, and the wax removed by routine histological techniques. These slides were used as antigen slides for the IFAT.

A commercially available *Trichinella spiralis* antiserum (Difco) was used as a positive control, and serum from cord blood was used as a negative control. The *T. spiralis* antiserum appeared to react with the antigen, giving apple green fluorescence around, and within the larvae. The serum from cord blood failed to react.

Initially, a series of positive and negative controls were run to establish that:

- a) The *T. spiralis* antiserum cross-reacted with *T. pseudospiralis* antigen,
- b) the test actually worked,
- c) which of the two antigens was preferable, namely, giving a good result with the positive serum, without any fluorescence in the negative control.

Having run this series of experiments it was established that the extracted larvae appeared to have been changed antigenically, both positive and

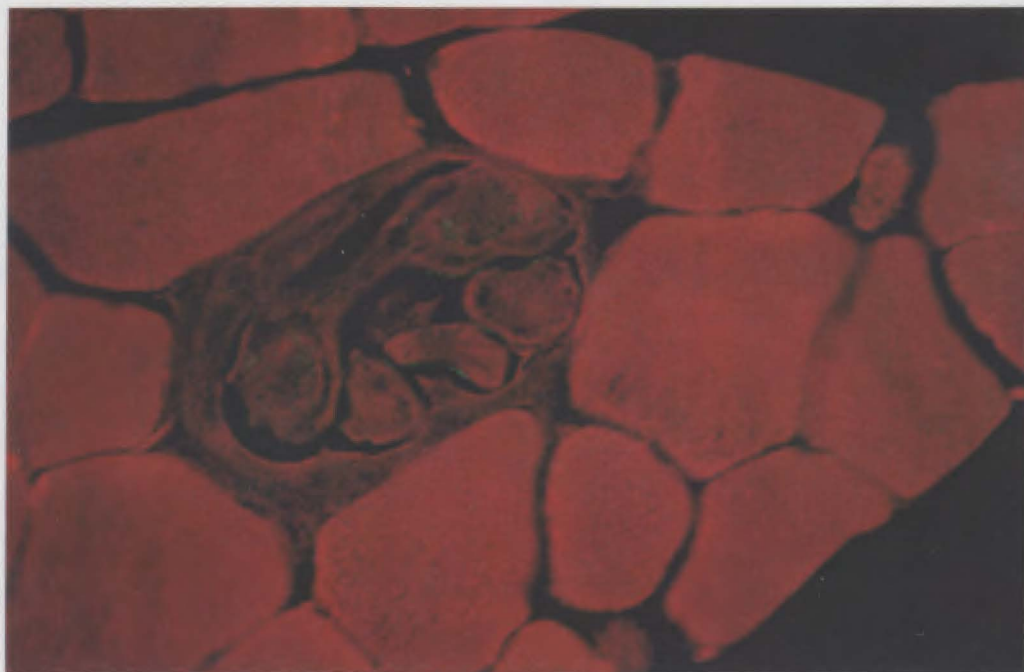
negative sera showing bright green fluorescence. This rendered them unsuitable for further testing.

The tissue sections, however, gave very promising results. There was good apple green fluorescence of the larvae in the positive serum (Fig 14), whilst the cord blood showed no green fluorescence at all (Fig 15).

The disadvantages of using tissue sections were that relatively large amounts of serum were required to be placed onto the sections, and only one section could be placed on each slide. This made the test rather cumbersome.

It did however demonstrate that the *T.spiralis* antiserum cross-reacted with the *T.pseudospiralis* antigen.

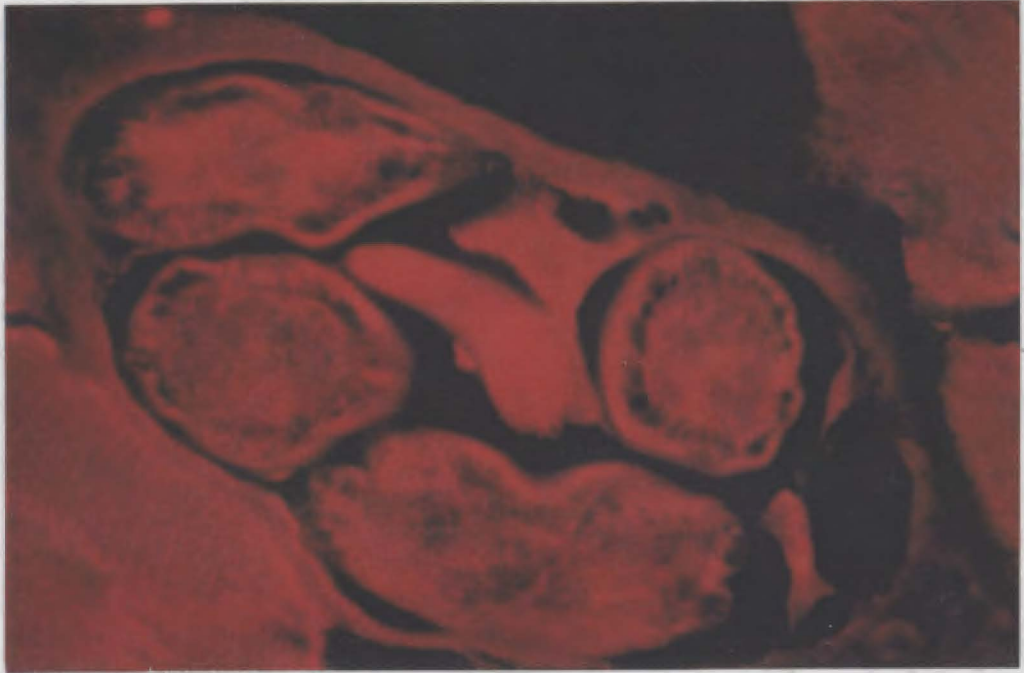
The positive serum was then diluted to find an optimum titre. A final dilution of 1 in 5 was chosen. There was good fluorescence within the larva, with no erroneous fluorescence in the tissue.



**Fig14** *T.pseudospiralis* I.F.A.T.- Tissue Section POSITIVE CONTROL  
(X400)

The pattern of fluorescence was interesting. There appeared to be speckled fluorescence within the worm, which appeared to correspond with the parasite's gut.





**Fig 15** *T.pseudospiralis* I.F.A.T Tissue Section NEGATIVE CONTROL  
(X1000)

This is interesting, as Berggren and Weller 1967, Gold *et al* 1969, Bawden and Weller 1974 had observed circulating antigens in schistosomes, with Lichtenberg *et al* (1974) eventually arriving at the conclusion that these antigens originated in the worm's gut. This finding eventually led to the development of an indirect fluorescent antibody test

for schistosomiasis, using sections of adult worms as antigen (Nash *et al* 1974).

A similar antigen / antibody reaction may be occurring with the *T. pseudospiralis* antigen, and this could explain the pattern of fluorescence obtained with this test.

#### 4.2b Survey of Human Population.

In order to establish whether serological evidence of *T. pseudospiralis* infection existed in the human population of Tasmania, a survey was carried out using the I.F.A.T. as described above.

Subjects were divided into three main categories, these being

- a) blood donors,
- b) people who were regarded as being in a "high risk" group either by occupation (eg. National Parks Rangers) or habits (eg. eating meat from native animals),
- c) those with clinical symptoms resembling trichinosis (eg. unexplained eosinophilia, myalgia etc).

The largest group of people tested were the blood donors. In all 224 donors were randomly selected from donations over the period of a week throughout the state by the Red Cross Blood Transfusion Service.

The "high risk" group of 11, comprised mainly National Parks rangers, researchers working with native animals and a small number of other individuals who, on questioning, admitted to eating meat from native animals.

A noteworthy inclusion in this latter group were three members of one family who had shared wallaby stew with the patient referred to by Andrews *et al* (1994), in their report of the first human case of infection with *T.pseudospiralis*. This meal was believed to be the only one of meat from native animals that this person had consumed.

In addition, a small group of fifteen people with symptoms suggestive of trichinosis, comprised mainly patients with unexplained eosinophilia was investigated. There were two patients with eosinophilia and other symptoms such as myositis, where the consultant physician (who had a special interest in "exotic" diseases) had specifically requested that serology for trichinosis be performed.

Two specimens of serum were obtained from the patient in New Zealand, and were also tested.

As described by Matossian *et al* (1975), all sera that were positive with the trichinosis IFAT, were also tested using antigen to *Schistosoma mansoni* and a commercial enzyme immunoassay for *Toxocara* spp. to ensure that there were no cross-reactions with these parasites.



### 4.3 Results

Sera which demonstrated fluorescence, were retested on two further occasions. Any serum reacting on three occasions was interpreted as positive.

As can be seen from the Table 7, 5 sera were found to be repeatedly positive to *T.pseudospiralis* antigen. All of these sera were from blood donors.

<b>TRICHINELLA PSEUDOSPIRALIS FLUORESCENT ANTIBODY TEST</b>			
<b>Category of Subject</b>	<b>No. Tested</b>	<b>Pos</b>	<b>% Pos</b>
Blood Donors	224	5	2.23
High Risk Occupations (e.g National Parks Rangers Hunters etc.)	11	0	0
Patient with: a) Unexplained Eosinophilia or b) Other symptoms suggestive of Trichinosis	15	0	0
<b>TOTALS</b>	<b>250</b>	<b>5</b>	<b>2</b>

**Table 7:** Results of survey for *T.pseudospiralis*

The positive rate for this study was 2.0%.

None of sera from the three subjects who had shared the meal of wallaby stew, or any of the other "high risk" volunteers, reacted with the antigen. This was also the case with the patients with unexplained eosinophilia or other symptoms suggestive of trichinosis.

In an attempt to validate these results, the serum obtained from the New Zealand patient was tested using the IFAT. The two specimens were collected at the same time, however, they were tested as separate samples. Both specimens were weakly, but definitely positive at a dilution of 1 in 5. When tested both neat and diluted 1 in 2 in PBS., the reaction was much stronger.

All positive sera were also tested using a commercial latex agglutination method for *Trichinella spiralis* (Difco), and all failed to react. This suggests that the IFAT is a more sensitive test, detecting lower levels of antibodies and that it detects antibodies persisting after acute infection. All sera failed to react with both the *Schistosoma mansoni* and *Toxocara* spp. antigens.

#### 4.4 Discussion

The indirect fluorescent antibody test has been described as the method of choice for the diagnosis of acute trichinosis (Faust *et al* 1974), although some workers regard the bentonite flocculation test to be the method of choice (Stites and Terr 1991).

Matossian *et al* (1975) described an outbreak of trichinosis in Lebanon in which the IFAT was used to make an early serological diagnosis of the disease. They concluded that the method is easy to perform, and that it is sensitive and specific. Of course these reports refer to the test being used to diagnose acute infections, not for screening for evidence of past infection.

Rose *et al* (1992) state that :

***"The ideal serological test for trichinosis should detect antibody response within 2 weeks of infection at which time chemotherapy is beneficial, however no test meets this requirement",***

and that

***" .....almost all individuals are negative 2 to 5 years after infection".***

These comments indicate that there appears to be no ideal serological test for either the routine diagnosis, or more especially for the screening of humans for evidence of past infection.

Although the IFAT is perhaps not the most ideal method for diagnosing chronic trichinosis, the cost, and lack of ready availability of commercial test kits forced the assessment of alternative methods using materials locally available.

The New Zealand patient was thought to have become infected sometime between early 1984 and mid 1985. The sera tested from her were collected in August 1993, some eight to nine years after she became infected. In view of the above comments, it seems probable that this time delay may account for the weak reactions when testing with the IFAT.

None of the positive results obtained appeared to be false reactions due to the fact that they were repeatedly positive with the IFAT, and they all failed to react when tested with other parasitic antigens.

A technique for enhancing antigenicity from a range of tissue antigens by the use of microwaves has been described by Leong and Milios (1993). This method involves the microwaving of tissue sections, and the authors claim that fluorescence is markedly improved. This technique may be useful for the *T.pseudospiralis* fluorescent antibody test, especially for enhancing fluorescence in weakly reacting positives, such as in the case of the New Zealand patient.

**CHAPTER 5**  
**DISCUSSION**

Overall, this study has established that the native animals do harbour a range of diseases potentially infective to the human population of Tasmania.

Worldwide, salmonellosis remains an important enteric disease. One recent outbreak of *Salmonella typhimurium* in Chicago had an estimated 150,000 to 300,000 cases (Balows *et al* 1991). In 1970, the Centre for Disease Control estimated that 14% of *Salmonella* infections in the United States were turtle-associated (280,000), a situation which decreased when restrictions were placed on the sale of turtles as pets.

Elsewhere in the world, thirteen serotypes of *Salmonella* were isolated from 31 frogs which had been imported into France as human food from Asia. In parts of Africa, *Salmonellae* were isolated from almost half of 301 lizards tested, and of the 33 serotypes isolated from the lizards, 21 were isolated from humans in the same area, which serves to emphasise the zoonotic nature of the disease (Schwabe 1984).

The National *Salmonella* Surveillance Scheme reports that in Australia, there were 4587 Australian acquired cases of *Salmonella* reported in 1993, which represented an increase of 3% over 1992. *Salmonella typhimurium* is the most common serotype, accounting for 29% of all cases reported (Powling 1994). Speare and Thomas (1988) report that in Queensland, sixteen different serotypes of *Salmonella* were isolated from 26.8% of orphaned kangaroo and wallaby young, and that these animals are a potential source of zoonotic infection for *Salmonella* spp.

It is interesting to note that *S. typhimurium* was also the most common serotype in Tasmania, accounting for 42.9% of all Tasmanian cases, (4.3% of Australian cases), followed by *S. mississippi*, which was responsible for 34.1% of Tasmanian cases, however the Tasmanian isolates of this serotype accounted for 93.9% of Australian cases. for that year. This indicates that *S. mississippi* infection is almost unique to Tasmania.

Ball has observed that 50% of Tasmanian devils examined were infected with *S. mississippi* (pers com 1993), and he felt that these animals play an important part in the maintainance of this *Salmonella* sp. infection in Tasmania, probably by indirectly contaminating water supplies when their faecal material is washed into rivers and creeks with rainwater.

While the number of *Salmonella* spp. isolates in this study is relatively small (4), an analysis of records from Mount Pleasant Laboratories in Launceston, show that many of Tasmania's native animals are infected with a wide range of *Salmonella* serotypes and the potential to infect water supplies is present. A total of eleven different serotypes were identified in that series.

A possible reason why Ball's isolation rate for *Salmonella* spp. was greater than this present study was that Ball obtained many of his specimens in the form of tissue from road kills, in an effort to determine the overall infection rate. This study however, has been concerned with the potential for faecal contamination of water supplies, and therefore the majority of specimens tested were obtained from the national parks, and comprised of faeces only.

This study confirms therefore, that in Tasmania, as is true elsewhere in the world, and on mainland Australia, native animals certainly have the potential to act as reservoirs for human *Salmonella* infections.

*Campylobacter* spp. are a major cause of bacterial diarrhoea in the developed world, and in developing countries. They are a serious cause of diarrhoea in infants and young children. In the United States, the majority of cases occur in people between the ages of 10 - 29 years, and in developing countries, almost half of the infections are asymptomatic, in children under the age of six months (Henderson 1994).

Most human infections with *Campylobacter* spp. are not usually due to direct exposure to infected animals (Elliott *et al* 1985). Balows *et al* (1991) report that infections have occurred after handling of animals, including pets, and that infections generally occur through the consumption of contaminated water, or food products, including poorly cooked meat, or unpasteurised milk. Waterborne outbreaks have occurred where purification and chlorination of water supplies has broken down.

*Campylobacter* was the most common notifiable cause of diarrhoea in Australia between 1991 and 1992, with over 8000 cases being notified. This was almost double that of salmonellosis for the same period.

In 1991, Tasmania had the second highest notification rate in Australia for *Campylobacter* spp. after the Northern Territory, however, there was a fifty percent decrease in notifications that year after there was an increase in infection control programmes in commercial chicken farms in the State (Henderson 1994).



The principal reservoirs of infection with *Campylobacter* spp. are young animals. including cattle, sheep, dogs, cats, poultry, wild birds, horses, goats, rodents and monkeys. There is little data relating to infection rates in native animals, however, an interesting fact that has emerged from this study is that the native animals do not appear to contribute towards the transmission of *Campylobacter* spp. in Tasmania. Despite all efforts to culture this organism by routine methods and enrichment methods , there were no isolates of *Campylobacter* spp. obtained from any of the two hundred samples tested. This conclusion was confirmed by DeBoer (personal communication 1994) in his study of river water, which showed that whilst 35% of rural supplies were contaminated with *Campylobacter* spp., the organism was not isolated from any of the supplies tested in the National Parks.

There is also evidence that Tasmanian native animals are capable of transmitting a range of parasitic infections.

*Giardia* spp. is the most common intestinal protozoan parasite worldwide (Farthing 1992). Debate continues as to the parasite's zoonotic potential (Boreham *et al* 1988, Faubert 1988, Thompson *et al* 1988, Eckert *et al* 1989), however, it now is being increasingly accepted as a zoonotic infection (Bemrick and Erlandsen 1988, Faubert 1988, Eckert *et al* 1989, Mahbubani *et al* 1992).and it now appears to accepted that *Giardia* is common in companion animals worldwide (Thompson *et al* 1988).

In some developing countries, it has been shown that almost all children have been infected with *G. duodenalis* by the age of three years. In the United States, *Giardia* is the leading cause of outbreaks of diarrhoea associated with drinking water (Savoli *et al* 1992). In the United States and elsewhere, *Giardia* has been identified in beavers, rats, voles and muskrats (Bemrick and Erlandsen 1988), whilst in New Zealand, 12.9% of brushtail possums, 61% on ship rats, and 25.3% of house mice were found to be infected with *Giardia* (Marino *et al* 1992). In London, 12% of stray dogs were found to be infected with *Giardia* (Sykes and Fox 1989).

In Australia, Swan and Thompson (1986) demonstrated that 21% of dogs and 14% of cats were infected with *Giardia* in Perth, Western Australia, and Collins *et al* (1987) recorded prevalences of *Giardia* of between 21% and 50% in dogs, and 16% in cats. Foreshaw *et al* (1992) recorded the first Australian cases of giardiasis in wild birds, identifying the parasites in the small intestine and faeces of straw-necked ibis in Western Australia.

In Tasmania, Goldsmid (1981), noted that *Giardia* was the most common protozoan parasite recorded in humans in the State. Davies *et al* (1993), describe what appears to be the first case of giardiasis in an animal in Tasmania. The case occurred in a dog, an interesting point of this case was that the dog's owner suffered from a bout of diarrhoea which was dismissed by his general practitioner as being unrelated to the finding in the dog. Milstein (1993), reported that in Tasmania, 14% of dogs and 19% of cats were infected with *Giardia*.

General practitioners have generally believed that cases of diarrhoea experienced by bushwalkers were caused by *Giardia* spp., and treated accordingly with metronidazole. To date, there has been no local data available to confirm this assumption.

This study has shown that 5.5% of Tasmania's native animals are infected with the parasite. This confirms that giardiasis may certainly be the cause of some of the cases of diarrhoea suffered by bushwalkers.

*Cryptosporidium* spp. is now considered to be an important enteric pathogen occurring in a wide range of vertebrate hosts. The organism is acknowledged to be a cause of diarrhoeal disease worldwide, at rates often rivalling those of *Campylobacter* spp. *Salmonella* spp. and *Shigella* spp. In India, where exposure begins at birth through contact with infected cattle, *Cryptosporidium* spp. was isolated from 13.1% of patients with diarrhoea, and 9.8% of controls (Janof and Barth Reller 1987). O'Donoghue (1984), describes the organism as a newly-emergent zoonosis, being transmissible between a wide range of hosts.

In Tasmania, Hawkesford (1989) recorded a prevalence of *Cryptosporidium* spp. of 2.0% in patients with diarrhoea, this was the second most common intestinal pathogen after *Campylobacter* spp., and the most common parasite found.

The present study has demonstrated that a wide range of animals are infected with *Cryptosporidium* spp. Whilst only a small number of specimens (73) were stained using the acid fast stain, the number of positives (11) reveals a prevalence of 15% in the native animals.

It appears therefore, that native animals in Tasmania have the potential to be significant reservoirs of this parasite, with the potential to infect water supplies in rural areas.

There were five animals which were infected with *Trichuris* spp. Eggs were presumptively identified by the fact that the polar plugs protruded, which would differentiate them from *Capillaria* spp. where the polar plugs do not protrude. The common whipworm on humans, *Trichuris trichiura* has a worldwide distribution, and is common in the tropics and areas where sanitation is poor. In areas of Asia where human faeces is used as fertiliser, prevalence rates may reach 50% -80%. However, in North America, whipworm infection is seen primarily in immigrants from tropical areas (Markel *et al* 1986). The dog whipworm, *Trichuris vulpis* is widespread world-wide, including Australia. Hall and Sonneberg (1956), and Kenney and Eveland (1978), describe cases of human infection with *Trichuris vulpis*, although no such human infections have been described from Australia. As the dog whipworm has been shown to cause human infection, this may indicate that other non human species of *Trichuris* spp. are capable of infecting humans, again posing the possibility of human infections with *Trichuris* spp. from native fauna.

Two animals were found to be infected with *Baylisascaris* spp. Kazacos (1986) noted that human infections with *B.procyonis* occurred in the United States. It would appear that there are no reports of human infection with *Baylisascaris* spp from Australia. The animals that were found to be infected in the present study, an unspecified dasyurid and a Tasmanian devil, are scavengers. In their nocturnal search for food members of these species might well frequent bushwalker's camps . They could contaminate the areas with their faeces which, in turn, could be the potential source of human infection with this parasite.

A single wallaby was found to be infected with *Fasciola hepatica*. Ectopic infections with this parasite have been reported both on the Australian mainland by Prociv *et al* (1992) and overseas by Kyronseppa and Goldsmid (1978) as previously described. Infections with *F.hepatica* would seem to be rare in Australia (Stevenson and Hughes 1988) but again may be underreported as many such infections are clinically silent.

It would be reasonable to assume that potential exists for humans to become infected in Tasmania, especially in areas where sheep and wallabies come into contact with one another when grazing, and where humans ingest watercress or other aquatic vegetation upon which the metacercariae have become encysted. Most human cases can be traced to infected watercress, which has been grown in water to which herbivores have had access (Markell *et al* 1986).

Obendorf and Black (1983) report that marsupials may act as reservoirs of this parasite. *F.hepatica* has been reported in Forrester kangaroos (*Macropus giganteus tasmaniensis*), Bennett's wallaby (*M. rufogriseus*), the red-bellied pademelon (*Thylogale billardierii*), the common wombat

(*Vombatus ursinus*) and the brush-tailed possum (*Trichosurus vulpecula*) (McManus 1979).

Again wallabies appear to be the only native animals which were found to be infected with parasites morphologically resembling *Blastocystis hominis*. Epidemics of diarrhoea caused by *B.hominis* have been described, usually in subtropic countries (Zierdt 1983). There still remains much debate as to whether this parasite is pathogenic in humans. It appears however, that where no other cause of diarrhoea can be found, *Blastocystis hominis* may well be a causative agent of disease. The potential therefore exists for these animals to contaminate water supplies. Of course, this parasite may also be responsible for some of the cases of diarrhoea found in bushwalkers. As most medical practitioners assume that diarrhoea in bushwalkers is caused by *Giardia* spp., they have successfully treated these people with metronidazole. This drug is also the drug of choice for diarrhoea caused by *Blastocystis hominis*, therefore it is possible that many cases may have been mis-diagnosed.

A single animal was infected with an *Entamoeba coli* like species. While the species is not considered pathogenic in humans, it is conceivable that if an animal can become infected with *E.coli*, probably from a human source, it also could become infected with other pathogenic amoebae, should water supplies or pastures become contaminated with human faeces. With large numbers of overseas travellers visiting Tasmania's national parks, this would not be beyond the realms of possibility.

With the first case of human infection with *Trichinella pseudospiralis* being reported, and probably originating in Tasmania, it was important to establish to what extent human infection occurred in the State. Andrews *et al* (1994), believe that the patient infected with *T.pseudospiralis* had become infected in Tasmania, possibly from eating infected pork. The patient had worked in Tasmania, conducting research, some of which involved the examination of faecal material from Tasmanian devils. Although unusual, this may have been a potential source of her infection, Obendorf (1994) failed to find evidence of infection with this parasite in pigs in Tasmania. It is interesting that feral cats, while harbouring a range of zoonotic parasitic infections, were found in a joint study with Milstein (1993), not to harbour *T.pseudospiralis*, thus supporting the parasitological findings of Obendorf *et al* (1990) in their study of *Trichinella pseudospiralis* in Tasmanian wildlife.

The results of the present study demonstrate that there is evidence that human infection with *T.pseudospiralis* does occur, although, at a low level, with only 2% of blood donors having serological evidence of infection with *T.pseudospiralis*. It is interesting to note that none of the subjects who had a history of eating meat from native animals, were positive using the IFAT, despite a number of workers describing it as the method of choice for diagnosing trichinosis (Faust *et al* 1974 and Stites and Terr 1991). An explanation for these negative findings may be due to the fact that most subjects revert to a seronegative state after a number of years (Rose *et al* 1992). If these people had been consuming meat from native animals for any length of time, it is conceivable to assume that they may indeed have been infected in the past, and have reverted to a seronegative state.

One would however expect serological tests to be positive in the acute stage of infection where patients are symptomatic. None of the patients with eosinophilia or other symptoms suggestive of trichinosis were found to be positive. These negative findings in symptomatic patients would indicate that the low positive rate found in this study are in fact an accurate reflection of the state of human infection, and that the negative findings in subjects who eat meat from native animals just reinforce the finding that there is only a low level of human infection with *T.pseudospiralis*.

Tasmania has recently been officially recorded as being a source of trichinosis (WHO 1994). An infected farm was mentioned as probable source of infection of the only human case of *T.pseudospiralis*. It is interesting to note that this report does not distinguish between *T.spiralis* and *T.pseudospiralis*, rather reporting, with reference to Australia:

***"Only one human case of human trichinellosis has been reported lately".***

The implications of this statement could be far reaching. If it were assumed that the infection was caused by *T.spiralis*, there could be serious implications for the pork industry in the state. If Tasmania was thought to be a source of *T.spiralis* infection, other mainland and overseas markets would be reluctant to support the State's pork industry.

There are trends towards the commercial production of meat from native animals. Again, with this report of Tasmania being a potential source of trichinosis, this industry could be jeopardised.



**CHAPTER 6**  
**CONCLUSIONS**

This study has served to confirm or clarify a number of points which were previously either suspected, unclear, or unknown.

The role played by native animals as reservoirs of bacterial disease has been clarified.

Evidence obtained in this, and other studies, shows that Tasmania's native animals comprise a significant potential reservoir for a wide variety of *Salmonella* species. It is also clear that almost all cases of *S. mississippi* reported in Australia are found in, or can be traced back to Tasmania and this seems to reflect the wild reservoir for infection.

It is apparent that no single species of animal is more commonly implicated in the transmission of *Salmonella* infection, rather, a wide range of mammals, reptiles and birds are responsible.

It is apparent that Tasmanian native animals do not serve as reservoirs for *Campylobacter* spp. Techniques used in this study were unable to isolate *Campylobacter* spp. from these animals. However, DeBoer (1994), using the same culture techniques, carried out a study of water supplies and found that the organism was isolated from water in rural farming areas with comparative ease. The fact that *Campylobacter* spp. was not isolated in any water supplies from national parks by De Boer appears to confirm the conclusions reached in this study.

From this study, it is clear that Tasmanian native animals may well play a part in the transmission of a number of parasites which have been responsible for outbreaks of diarrhoea in bushwalkers.

It was previously unknown whether native animals in the State were capable of becoming infected with *Giardia* spp. The study has shown a prevalence of *Giardia* spp. of 5.5% in these animals.

A significant number of Tasmanian native animals are infected with *Cryptosporidium* spp. In previous studies involving the human population, this organism was found to be the most common parasite in patients with diarrhoea. Of the animals surveyed, 15% were infected with *Cryptosporidium* spp., which again suggest their potential role as reservoirs of this parasite.

The knowledge that *F.hepatica*, and *Baylisascaris* spp. are found in some of the native animals may be worthy of note for clinicians in the diagnosis of cases of visceral larva migrans, or in patients with unexplained subcutaneous swellings and eosinophilia.

It is apparent from this study that trichinosis caused by *Trichinella pseudospiralis* does occur in the human population, although at a low prevalence (2%). It appears that symptoms of *T.pseudospiralis* infection are generally mild, and will pass unnoticed if the load is low. However, as demonstrated in the patient from New Zealand, infection can be severe, and become chronic in nature. The risk of becoming infected with this parasite by consuming meat from native animals appears to be very small, although with the development of expanding markets for native animal meat, including export, the danger must be recognised. The threat of this nematode becoming established in pigs has also to be borne in mind.

The overall conclusions arrived at from this study are that Tasmanian native animals probably do contribute to a number of *Salmonella* infections, as well as contributing to other diarrhoeas caused by *Cryptosporidium* spp., and *Giardia* spp.

It is also apparent that the native animals do not play any part in the transmission of *Campylobacter* spp., rather it is domestic stock which is responsible for the spread of this organism.

Whilst there is a risk of human infection with *Trichinella pseudospiralis*, it is a small risk, with generally mild symptoms. It is further evident that the native animals can harbour other zoonotic helminths species (e.g. *Fasciola hepatica*) and other potentially zoonotic helminth species (e.g. *Baylisascaris* spp. and *Trichuris* spp.)

The knowledge gained from this study is valuable in that it is now possible to develop strategies to prevent zoonotic infections from occurring.

Bushwalkers for instance must now be advised about the dangers of drinking unboiled water, along with the need to avoid eating aquatic vegetation.

People consuming meat from native animals should be advised of the potential for becoming infected with *Trichinella pseudospiralis*, and therefore the need to adequately cook or at least freeze all meat prior to consumption.

People hand feeding native animals should be aware of the potential danger of becoming infected with *Salmonella*. Speare and Thomas (1988)

noted that joeys groom themselves by licking, possibly contaminating their mouths with *Salmonella* spp. Some joeys tended to lick their adopted human minders, and that this could result in the ingestion of *Salmonella* spp. Visitors to the state should be advised to wash their hands thoroughly after any contact with native animals.

**CHAPTER 7**  
**PROSPECTIVE STUDIES**

There are a number of prospective studies which could be carried out as a result of this study:

1) Survey of population for circulating antigens of *Trichinella pseudospiralis*.

It appears that there is no ideal serological test for the diagnosis of past infections with *Trichinella* spp. (Rose *et al* 1992). This may be due to the fact that larvae of *T.spiralis* encyst, protecting the parasite from detection by the host's immune system, thus the host becomes seronegative after a period of time. Nishiyama *et al* (1992) have described a "sandwich" enzyme-linked immunosorbent assay to detect circulating antigens to *T.spiralis* in an outbreak in Japan. Circulating antigens were detected in 29.9% of patients with clinical symptoms, compared with other serological tests in which only 18.9% were positive. In patients without clinical symptoms, circulating antigens were detected in 21.4% compared with antibody detection in only 5.0%.

Again, this method was used by Nishiyama *et al* (1992) to diagnose acute infections, and it would be presumed that once the larvae had encysted, the antigens would no longer be detectable.

One of the major differences between *T.spiralis* and *T.pseudospiralis* is the fact that the larvae *T.pseudospiralis* do not encyst, and are able to migrate through the muscles. This method could well prove to be a more accurate way of evaluating past exposure to *T.pseudospiralis* due to the fact that the larvae do not encyst, but remain mobile, and move through the host muscle. One might therefore assume that antigens from these larvae would continue to circulate, making this method more reliable for

detecting any evidence of past or present infection especially with *T.pseudospiralis*. It is possible that because they do not encyst, they may still be recognised by the host immune system for a longer period of time.

A future project involving the detection of circulating antigens to *T.pseudospiralis* therefore may prove to be more useful.

## 2) Survey to establish the prevalence of fascioliasis in the human population.

As evidence exists of *Fasciola hepatica* infection in wallabies (Obendorf and Black 1983), a survey to establish the prevalence of infection in the human population would be useful. Two techniques could be used in this survey, an indirect fluorescent antibody test using sections of adult fluke as antigen, as with the *Trichinella* I.F.A.T. used in this project, could be attempted. Alternatively, another approach could be the utilisation of a "sandwich" E.L.I.S.A. test for the detection of circulating antigen as described by Espino and Finlay (1994).

## 3) A study of the fungal zoonoses of Tasmania.

This study has concentrated on intestinal pathogens, both bacterial and parasitic, as well as infection with *T.pseudospiralis*. A future project to examine the mycology of native animals, concentrating especially on the zoonoses would be of value.



4) A study of the ectoparasites of Tasmanian wildlife.

Whilst data is now available for the intestinal bacterial and parasitic zoonoses of Tasmanian wildlife, little information exists for the ectoparasitic zoonoses. It is known that animal scabies may be transferred to humans, as can some mite infections (Stevenson and Hughes 1988). A study of the ectoparasites of Tasmanian wildlife may well be of value.

5) A study of the role of water in the transmission of *Giardia* spp., *Cryptosporidium* spp. and other intestinal pathogens. This study has shown that native animals are reservoirs of some of these diseases. We now need to know the mechanisms of transfer of the infection from the potential reservoir to the human. *Giardia* in water supplies could be investigated using flow cytometry.

6) Studies on the human, native marsupial, dog and cat isolates of *Giardia* are needed to establish their relationships. This could involve the use of DNA hybridization as used by Thompson *et al* (1988); molecular methods as used by Boreham *et al* (1988) including such biotechnological techniques as the "Random Amplified Polymerase DNA PCR" (RAPD) methods described by Clark (1994). This would require the development of *Giardia* culture techniques but would be a really worthwhile study.

**CHAPTER 8**

**APPENDIX**

## 8.1 ZOONOSSES AND POTENTIAL ZOONOSSES FOUND IN AUSTRALIA

(FROM STEVENSON & HUGHES 1988)

\*\* denotes zoonoses mentioned in this thesis

### a) Bacterial Diseases:

DISEASE	ORGANISM	SOURCE
Anthrax **	<i>Bacillus anthracis</i>	Cattle
Brucellosis **	<i>Brucella</i> spp.	Cattle, Pigs, Goats
Campylobacteriosis **	<i>Campylobacter</i> spp	Wide host range
Cat-Scratch Disease	<i>Rochalimaea</i> spp.	Cat
<i>Corynebacterium pseudotuberculosis</i>		Sheep, Goats, Cattle
Dermatophilosis	<i>Dermatophilus congolensis</i>	Wide host range
Erysipeloid	<i>Erysipelothrix rhusiopathae</i>	Pigs, Fish, Poultry
Leptospirosis **	<i>Leptospira</i> spp.	Cattle, Pigs, Dogs, Rats
Listeriosis **	<i>Listeria monocytogenes</i>	Common in marsupials, ruminants
Lyme Disease **	<i>Borrelia burgdorferi</i>	Deer and rodents
Melioidosis	<i>Pseudomonas pseudomallei</i>	Wide host range
Pastuerellosis (other than animal bites)	<i>Pasteurella</i> spp.	Wide host range.
Psittacosis **	<i>Chlamydia psittaci</i>	Birds

<i>Rhodococcus equi</i> infections		Wide host range
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Salmonellosis **	<i>Salmonella</i> spp.	Wide host range (see table in text)
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Tuberculosis **	<i>Mycobacterium</i> spp.	Cattle, pigs, deer
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Yersiniosis	<i>Yersinia pseudotuberculosis</i>	Wide host range
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*Yersinia enterocolitica*

**b) Mycotic Diseases:**

Cryptococcosis **	<i>Cryptococcus neoformans</i>	Birds
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Histoplasmosis	<i>Histoplasma capsulatum</i>	Birds, Bats
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Dermatophyte infections	<i>Microsporum</i> spp.	Wide host range
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*Trichophyton* spp.

**c) Viral and Rickettsial infections:**

Arthropod borne viral diseases

Epidemic polyarthritis **	Ross River Virus	Wide host range
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Sindbis virus infection		Wide host range
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Australian encephalitis	Murray Valley Encephalitis Virus	Birds
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Kunjin virus infection		Wide host range
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Kokbera virus-like infection		Horses, Cattle, macropods
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Other arbovirus infections		Wide host ranges
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Cowpox	Cowpox Virus	Rodents, cats, cattle
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Murine typhus	<i>Rickettsia typhi</i> ( <i>R.mooseri</i> )	Rats
Parapox (paravaccinia) virus infections		
Orf		Sheep
Pseudocowpox/bovine papular stomatitis	Paravaccinia virus	Cattle
Q Fever	<i>Coxiella burnetti</i>	Sheep, cattle, goats, bandicoots
Queensland tick typhus	<i>Rickettsia australis</i>	Marsupials
Scrub Typhus	<i>Rickettsia tsutsugumishi</i>	Mite

#### **d) Parasitic Diseases:**

##### Nematodes:

Angiostrongyliasis	<i>Angiostrongylus cantonensis</i>	Rats
Ascaris suum infection	<i>Ascaris suum</i>	Pigs
Dirofilariasis **	<i>Dirofilaria immitis</i>	Dogs
<i>Gnathostoma larva migrans</i>	<i>Gnathostoma spinigerum</i>	Carnivores
<i>Physaloptera</i> infestation		Bandicoots, Lizards, Rodents
Strongyloidiasis	<i>Strongyloides stercoralis</i>	Man, Dogs, Primates
Trichostrongylidiasis	<i>Trichostrongylus</i> spp.	Wide range animals & Birds

##### Trematodes:

Fascioliasis **	<i>Fasciola hepatica</i>	Sheep, Cattle (Wallabies)
Schistosome dermatitis	<i>Cercaria</i> spp.	Various Molluscs

Cestodes:

Hydatid disease **	<i>Echinococcus granulosus</i>	Dogs, Dingo, Foxes
<i>Hymenolepis nana</i> infection		Mice & Fleas
<i>Taenia saginata</i> infection		Cattle(Intermediate Host)
Dipylidiasis	<i>Dipylidium caninum</i>	Dogs
Sparganosis	<i>Spirometra erinacei</i>	Reptiles & Mammals

Protozoa:

Balantidiasis	<i>Balantidium coli</i>	Pigs
<i>Cryptosporidium</i> infection **	<i>Cryptosporidium</i> spp	Domestic Animals
<i>Entamoeba polecki</i>		Pigs, monkeys
Giardiasis **	<i>Giardia duodenalis</i>	Domestic/Wild Animals
Pneumocystosis	<i>Pneumocystis carinii</i>	Wild/Domestic Animals
Toxoplasmosis**	<i>Toxoplasma gondii</i>	Mammals (esp Cats), Birds
Sarcosporidiosis	<i>Sarcocystis hominis</i>	Cattle, Pigs
	<i>S.porcihominis</i>	

Ectoparasites

Acariasis	<i>Sarcoptes</i> spp.	Domestic/Wild Animals
<i>Cheyletiella</i> infestation		Cats, Dogs, & Rabbits
Myiasis	<i>Oestrus ovis</i>	Sheep Bot-fly

Larva migrans

Visceral larva migrans	<i>Toxocara canis, T.catti</i>	Dogs, Cats, Dasyurids
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Cutaneous larva migrans	<i>Ancylostoms caninum</i>	Dogs, Cats
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*A.braziliensis, Uncinaria stenocephala*

<i>Linguatula serrata</i> infestation		Dogs, Sheep, Goats
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**e) Potential Zoonoses:**

Bacteria:

<i>Actinomyces pyogenes</i>		Cattle
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<i>Mycobacterium paratuberculosis</i>		Cattle
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<i>Streptococcus suis</i>		Pigs
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<i>Vibrio parahaemolyticus</i>		Seafood
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Viruses:

Encephalomyocarditis virus		Rodents
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Newcastle disease		Birds
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Equine infectious anaemia		Equines
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Lymphocytic choriomeningitis virus		Rodents
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Rotaviruses		Wide range of Animals
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Fungi:

<i>Sporothrix schenckii</i>		Cats
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Nematodes:

<i>Anisakis</i> **	Marine Fish
<i>Capillaria aerophilia</i> **	Foxes, Cats, Dogs
<i>Capillaria hepatica</i> **	Dogs, Cats, Pigs
<i>Gongylonema pulchrum</i>	Ruminants
<i>Spirocerca lupi</i>	Dogs, Cats, Foxes
<i>Syphacia obvelata</i>	Mice
<i>Trichuris vulpis</i> / <i>Trichuris suis</i>	Dogs/Pigs

Trematodes:

<i>Echinostoma revolutum</i>	Aquatic Birds
Heterophyidiasis <i>Halplorchis</i> spp. and other species	Fish eating birds

Cestodes:

<i>Taenia solium</i>	Pigs
<i>Taenia taeniaeformis</i>	Rodents

**f) Zoonoses not discussed by Stevenson & Hughes (1988):**

Flinders Island Spotted Fever ** <i>Rickettsia</i> spp.-unidentified	Tick
Trichinosis** <i>Trichinella pseudospiralis</i>	Devils, Quolls
Larva migrans caused by <i>Baylisascaris</i> spp. **	Dasyurids
Infections with <i>Balstocystis hominis</i> **	Wide Host Range



## 8.2 HEKTOEN ENTERIC AGAR

(OXOID CODE CM419)

### FORMULA:

Protoese peptone	grams/litre	12.0
Yeast extract		3.0
Lactose		12.0
Sucrose		12.0
Salacin		2.0
Bile salts No.3		9.0
Sodium chloride		5.0
Sodium thiosulphate		5.0
Ammonium ferric citrate		1.5
Acid fushsin		0.1
Bromothymol blue		0.065
Agar		14.0

pH 7.5 +/- 0.2

### 8.3 XYLOSE LYSINE DESOXYCHOLATE MEDIUM

(OXOID CM149)

FORMULA:

Yeast extract	grams/litre	3.0
L-Lysine HCL		5.0
Xylose		3.75
Lactose		7.5
Sucrose		7.5
Sodium desoxcholate		1.0
Sodium chloride		5.0
Sodium thiosulphate		6.8
Ferric ammonium citrate		0.8
Phenol red		0.08
Agar No.1		<b>12.5</b>

pH 7.4 +/- 0.2

## 8.4 *CAMPYLOBACTER* AGAR AND BROTH(PRESTON'S)

(OXOID CM689 SR117 & SR84)

### FORMULA:

#### Base:

"Lab-Lemco" powder	grams/litre	10.0
Peptone		10.0
Sodium chloride		5.0
Agar		12.0

pH 7.5 +/- 0.2

#### Selective Supplement (Preston's):

Vial contents (each vial sufficient for 500ml medium)

Polymixin B	2500 IU
Rifampicin	5mg
Trimethoprim Lactate	5mg
Cycloheximide	50mg

#### Growth Supplement:

Vial Contents (each vial sufficient for 500ml medium)

Sodium pyruvate	0.125g
Sodium metabisulphite	0.125g

Ferrous sulphate	0.125g
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For Selective/Enrichment broth, make 500mls nutrient broth No.2, to which is added 1 vial of Selective Supplement above, Growth Supplement above, and 50ml lysed horse blood.

Formula for Nutrient Broth No.2:

"Lab Lemco" Powder	grams/litre	10.0
Peptone		10.0
Sodium chloride		5.0

pH 7.5 +/- 0.2

## 8.5 SELENITE BROTH

(OXOID CM395 & L121)

### FORMULA FOR BASE:

Bacteriological Peptone	grams/litre	5.0
Lactose		4.0
Sodium phosphate		10.0

pH 7.1 +/- 0.2

19 grams of powder is added to 1 litre of base which contains:

Sodium biselenite	grams/litre	4.0
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## **8.6 PARASITE CONCENTRATION WITH THE FPC SYSTEM.**

- 1) Place a spoonfull of faeces into 9ml of formal saline in the flat-bottomed tube provided.
- 2) Add 3 drops of 20% triton X-100 and mix well.
- 3) Fit the conical tube including the filtration unit to the flat-bottomed tube and invert.
- 4) Shake the contents through the filter until transferred to the conical tube.
- 5) Add 3ml of ethyl acetate and shake well.
- 6) Centrifuge at 2000rpm for 2-4 minutes.
- 7) Decant the supernatant after carefully releasing the ethly acetate / faecal debris plug.
- 8) Examine the sediment for ova and cysts.

### **8.7 ACID FAST STAIN FOR *CRYPTOSPORIDIUM* SPP.**

- 1) Pick a portion of material with an applicator stick, mix the material in a drop of saline, spread it on a glass slide and allow it to dry.
- 2) Fix the dried film in absolute methanol for one minute, and air dry the slide.
- 3) Flood the slide with Kinyoun carbol-fuchsin, and stain for 5 minutes.
- 4) Wash the slide with 50% ethyl alcohol in water, and immediately rinse with water.
- 5) Decolourise the smear with 1% sulphuric acid for 2 minutes or until no colour runs out from the slide.
- 6) wash the slide with water.
- 7) Counterstain the smear with Loeffler methylene blue for 1 minute.
- 8) Rinse the slide with water, dry it and examine the smear with oil immersion.

*Cryptosporidium* oocysts stain bright red and background materials stain blue or pale red.

## **8.8 CRYPTO-CEL IF TEST FOR *CRYPTOSPORIDIUM* SPP.**

- 1) Place 20ul of concentrated faecal specimen onto a microscope slide.
- 2) Allow specimen to air dry.
- 3) Fix the slides for 5 minutes in acetone, and allow to air dry.
- 4) Place 25ul of the fluorescent reagent onto the fixed specimen, and the positive control slide.
- 5) Incubate the slide in a humidified chamber for 30 minutes at 37°C.
- 6) Wash the slide in phosphate buffered saline with gentle agitation.
- 7) Drain the slide, remove excess moisture, and allow to air dry completely.
- 8) Add one drop of mounting fluid, and apply a coverslip to the slide, removing all air bubbles.
- 9) Examine the slide using a fluorescence microscope, initially at X400 magnification, and then at X1000 for confirmation.

Read immediately or store at 2-8°C in the dark for up to 24 hours.

Interpretation:

- 1) *Cryptosporidium* oocysts are 2-6µm in diameter, round or oval in shape, with bright green fluorescence. The test is considered positive if one or more oocysts are present. The positive control slide is used as a comparison with the appearance and size of the oocysts.
- 2) Negative specimens should not show any organisms of green fluorescence and characteristic shape and morphology.



- 3) Nonspecific fluorescence may occur in some specimens but this may be distinguished morphologically from the oocysts.
- 4) When less than 5 oocysts are seen, the test should be repeated with a lower dilution of specimen.
- 5) Algae may be present in environmental specimens, these fluoresce red-orange.

## **8.9 TEST PROCEDURE FOR THE "CELLABS *GIARDIA* CELISA" KIT.**

Formalin preserved specimens were used. These were centrifuged, and the supernatant used in the assay.

- 1) Wash buffer was prepared prior to testing. 15 mls. of concentrate was added to 735 mls of distilled water.
- 2) The required number of microwell strips is removed. The remainder are sealed in the foil bag and stored at 2-8°C.
- 3) Fit the strips into the plate holder and press firmly. Number the strips on the plate and record the samples by reference to the numbers on the plate frame.
- 4) Centrifuge the formalin-treated specimens at 600g for 5 minutes. Do not agitate the tubes after centrifuging.
- 5) Dispense 100ul of supernatant from each specimen, a known negative specimen (as a negative control), and the undiluted positive control into separate wells.
- 6) Cover the plate loosely with foil and incubate in a humidified atmosphere for 10 minutes at 37°C.
- 7) Rinse the plate four times with the wash buffer. Empty the wells completely. Fill them with the wash buffer close to the rim of each well. After the last wash, invert the plate onto a paper towel and tap vigorously to remove excess moisture.
- 8) Dilute the anti-Giardia antibody 1/200 with diluent buffer. Dispense 50ul per well and incubate for one hour at 37°C, as in step 6.

9) Repeat step 7.

10) Dilute the enzyme conjugate 1/100 with diluent buffer. Dispense 50ul per well and incubate for one hour at 37°C as in step 6.

11) Repeat step 7.

12) Combine equal volumes of the TMB substrate (Solution A and B) and mix. (If the solution turns blue after mixing, discard and prepare fresh reagent). Dispense 50 ul per well and incubate 20 minutes at room temperature. add 50ul of the stopping solution to each well.

13) Read the results visually or measure the absorbance in a spectrophotometer at dual wavelengths of 450nm and 620nm, or at 450nm only.

#### Interpretation of Results.

1) Visual: If Giardia antigens are present in the stool specimens, the positive wells will show a yellow colour. Negative wells will have the same or less colour as the negative control.

2) Spectrophotometer:

Calculation of a Cut-off value.

Calculate the mean absorbance value for the negative controls and add 100% to obtain the cut-off value.

i) Specimens with absorbance values greater than the cut-off value are considered positive.

ii) Specimens with absorbance values of less than or equal to the cut-off value are considered negative.

## **8.10 TRICHROME STAIN FOR PARASITES**

- 1) Prepare a smear of faecal material and place it into Shaudinn's fixative immediately. Allow it to fix for a minimum of 30 minutes.
- 2) Remove the slide from the fixative and place it in 70% ethanol for 5 minutes.
- 3) Place the slide into 70% ethanol plus iodine for 1 minute.
- 4) Place the slide into fresh 70% ethanol for 5 minutes.
- 5) Place the slide into a second 70% ethanol for 3 minutes.
- 6) Place in Trichrome stain for 10 minutes.
- 7) Place in 90% ethanol plus acetic acid for 1-3 minutes.
- 8) Immediately drain and dip several times in 100% ethanol. Use this step as a rinse.
- 9) Place in 2 changes of 100% ethanol for 3 minutes each.
- 10) Place in xylene for 5-10 minutes.
- 11) Place in a second xylene for 5-10 minutes.
- 12) Mount with a coverslip using a mounting medium.
- 13) Allow the smear to dry overnight or for 1 hour at 37°C.
- 14) Examine the smear using the 100X objective and oil immersion.

### **8.11 INDIRECT FLUORESCENT ANTIBODY TEST FOR *TRICHINELLA PSEUDOSPIRALIS*.**

- 1) Dilute control and test sera 1 in 5 in phosphate buffered saline pH7.2 (PBS), and flood individual tissue sections with diluted serum.
- 2) Incubate slides at 37°C for 30 minutes.
- 3) Wash slides in PBS for 10 minutes with 2 changes of wash.
- 4) Prepare Anti-Human Globulin/FITC conjugate - 2 drops of AHG in 10mls of PBS. Add 2 drops Evan's Blue.
- 5) Blot slides dry, and flood each slide with the conjugate.
- 6) Incubate as in 2.
- 7) Repeat wash as in 3.
- 8) Blot slides dry, and mount with buffered glycerol.
- 9) Examine microscopically using Ultra Violet light source.
- 10) In a positive test, larvae demonstrate apple green fluorescence in the gut area as well as some surrounding the worm. There is no fluorescence in a negative test.

# 8.12 NEWSPAPER REPORTS WARNING BUSHWALKERS OF HEALTH DANGERS

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Sunday Extra 7

THE SUNDAY TASMANIAN AUGUST 7, 1984

## PARASITE LURKS OUT ON THE TRAIL

WHAT is giardiasis? No, it isn't any kind of pneumonia. Giardiasis has the obvious distinction of being the most talked about and most feared micro-organism among bushwalkers.

It is a parasite that usually lives in the intestines and can cause serious stomach distress. Giardiasis is spread by the cysts being passed in the faeces and entering a new host through the mouth.

These cysts remain dormant for months in cold, running water, even in the presence of chlorine. Giardiasis cysts are more resistant to very cold water. Giardiasis occurs the moment the contaminated water is drunk. It is both a nuisance and a health hazard, which can be taken care of only through the doctor.

In several recent years, large numbers of bushwalkers in the Tasmanian National Park, although there are no direct reports of it there, have been reported to have been passed in parts of the Tasmanian National Park.

Although giardiasis has been a problem for a long time, it is a relatively new and common disease in Tasmania, and elsewhere recently. Why does the parasite live in the water?

### INGESTION CAUSES STOMACH ILLNESS

In the Bush

By John Cannon

Giardiasis is a common, but often overlooked, cause of stomach distress. It is spread by the cysts being passed in the faeces and entering a new host through the mouth.

The parasite is found in the water supply. It is a nuisance and a health hazard, which can be taken care of only through the doctor.

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The trouble of filtering in the most sophisticated models of water purifiers is to remove the way of most people to get rid of the parasite. The most sophisticated models of water purifiers are to remove the parasite. The most sophisticated models of water purifiers are to remove the parasite.

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Water purifiers, a bit over the top in carrying out the Tasmanian bush but they can be a life-saver for bushwalkers in mountainous areas with poor-quality drinking water.

In countries like India and other places where water quality cannot be relied upon.

The signs and symptoms of giardiasis are bad diarrhoea, stomach cramps, bloating, loss of appetite and weight loss. It may take three weeks for symptoms to be noticed after you have been infected. In

## Doctor stresses importance of bush hygiene

**BUSHWALKERS** have been warned to take extra care to protect themselves from the increasing incidence of gastroenteritis.

The chief medical officer with the Department of Health, Dr John Sparrow, said yesterday there had been increasing numbers of bushwalkers being struck down by diarrhoea and vomiting in recent years.

Dr Sparrow said the problem had been worse in the more popular wilderness areas and was probably caused by exposed faecal waste.

To avoid problems, Dr Sparrow said bushwalkers should use

toilets where they were available, and where not they should bury all faecal waste.

"Faecal waste and toilet paper should be buried within the soil's organic layer," Dr Sparrow said.

Waste should be disposed of in a hole about 15 centimetres deep which was at least 100 metres away from campsites and watercourses.

Other precautions included boiling drinking water for three minutes and protecting food from contact with flies.

Food should not be placed directly on to tables in huts or any other places where flies may have landed.

**CHAPTER 9**  
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