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Economic impacts and epidemiological risks associated with sheep measles

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Abstract

Data generated by the National Sheep Health Monitoring Survey identified a high prevalence of sheep measles (*Taenia ovis*) infection in slaughtered sheep from all sheep producing areas of Australia. Sheep measles has also been identified by processors as causing major financial losses to the Australian sheep meat industry. This study investigated on-farm transmission risk factors for sheep measles, the role of wild and domestic canids in transmission and the financial impact of sheep measles to processors. Throughout the study great emphasis was placed on use of the media to pass information back to producers. The study showed domestic and wild dogs appear not to have a major role in transmission but identified the, hitherto unrealised, role of foxes in transmission. The study also generated data on processor losses due to sheep measles. The results of this study highlighted the need to modify current control strategies and incorporate direct protection of sheep through vaccination. A highly effective experimental vaccine exists but is currently unregistered for commercial use. Controlling sheep measles would conservatively save the Australian sheep meat industry several million dollars per year.

Executive summary

For some time Australian sheep meat processors have been complaining that sheep measles (*Taenia ovis*) in slaughtered sheep is causing major financial loss due to condemnation of affected meat and offal (hearts) and extra carcass inspection and trimming time. Sheep measles is caused by the intermediate stage of a tapeworm that infects domestic dogs. Eggs of the sheep measles tapeworm pass into the environment with the faeces of infected dogs. Sheep become infected through accidentally ingesting these eggs whilst grazing. Once in the intestine of the sheep, the eggs hatch, releasing microscopic larvae that exit the intestine, enter blood vessels and pass to the musculature of the sheep. Larvae are mainly attracted to the diaphragm and heart, but any muscle of the body can be infected. The parasites develop into cysts in the muscles, each cyst containing a tapeworm head. If cysts are eaten by dogs they become infected with a tapeworm. However, the cysts in sheep meat are only infective to dogs for a short time (2-3 months), eventually being killed by the immune response of the sheep. Dead cysts develop into pus-filled lesions, which over time become mineralised, transforming into gritty masses and then into calcified nodules. From a consumer's perspective none of these cystic manifestations is acceptable in meat for human consumption.

The study investigated on-farm risk factors associated with sheep measles transmission through a farmer questionnaire, revisited the lifecycle of the sheep measles parasite through surveys of the tapeworms in farm dogs and wild canids (foxes and wild dogs), using traditional parasitological methods and DNA identification to confirm tapeworm species. In addition, data on the financial losses incurred by abattoirs were collected from 5 abattoirs, one each in Western Australia (WA) and Tasmania and three in New South Wales (NSW).

The questionnaires were completed by farmers that had a sheep measles problem in their sheep and by those that did not. Farmers were identified through the National Sheep Health Monitoring Project (Animal Health Australia) data base. Two hundred and thirty nine farmers were invited to undertake the survey, 56 living in Tasmania, 90 in NSW and 63 in WA. The initial questionnaire mail-out was followed by a follow-up letter to 120 producers who did not respond to the first request. Ninety five farmers returned completed questionnaires 42 NSW, 31 Tasmania and 22 WA (To view the questionnaire, see Appendix 1). The questionnaire asked questions on topics such as working and pet dog care and maintenance, vertebrate pest presence/control, home slaughter/hunting and offal disposal, proximity of national /state parks or forests and supplementary feeding of stock. No identifiable risk factors could be determined. In NSW, there was a weak correlation between farmers who bought in hay compared to those who did not. However, this was not evident in either WA or Tasmania.

The survey of tapeworms in farm dogs was undertaken through identification of eggs in faeces. The eggs of all species of intestinal worm found in the faecal samples were identified and a report sent back to the owners. Of the 245 faecal samples examined only one sample contained eggs of the sheep measles tapeworm, and this dog lived in Tasmania.

In Tasmania farm dogs, particularly in respect of diet, were being maintained similarly to those on the mainland. The absence of sylvatic definitive hosts (wild dogs and foxes*) in Tasmania means transmission has to be via *T. ovis* infection in rural domestic dogs. The only *T. ovis*-infected domestic dog identified during the study was a member of a Tasmanian wallaby hunting pack. We suspected that much of the

transmission in Tasmania centres around the packs of wallaby hunting dogs found commonly in rural areas. These dogs are fed dry food and fresh meat and offal from a range of species, including sheep, and de-wormed rarely. These packs of dogs often have permission to hunt over a number of local sheep properties as an aid to controlling wallaby numbers.

The absence of *T.ovis* in mainland Australian rural dogs and the low prevalence in Tasmanian dogs is likely to be the result of the ready availability of highly efficient "all-wormers" and convenient-to-use, nutritious, palatable, dry dog food. Although, some domestic dogs on mainland Australia may still be transmitting eggs intermittently, their contribution in transmission appears to be small and does not account for the levels of sheep measles currently seen in slaughtered sheep. Clearly some other definitive host has to be involved.

Our survey of tapeworms in 471 foxes recovered in NSW (216) and WA (255) revealed a *T.ovis*-infected fox in one area of NSW (Jugiong) and one area in WA (Katanning). The locations where infected foxes were found were where the larger samples of foxes had been collected, Jugiong 1/102 (1.0%) and Katanning 1/80 (1.2%). These are the first reliable reports of *T. ovis* infection in foxes, to be confirmed unequivocally using molecular methods. In two previous reports of *T. ovis* infection in foxes, the method of identification was not given in one and in the second identification was based on rostellar hook measurements – an unreliable method because of size overlap of rostellar hooks between *Taenia* tapeworm species. Also recovered from one fox in NSW and two from WA were *T. hydatigena* tapeworms. This tapeworm has a similar lifecycle to the sheep measles tapeworm (dogs/sheep). However, with this species the larval stage found in sheep develops in or around the liver as fluid-filled bags commonly known as bladder worms. These tapeworms are contracted by canids through consumption of sheep offal (livers and associated tissues) therefore these foxes had to have been scavenging sheep carcasses.

Gross examination of intestinal contents post mortem revealed sheep wool was commonly present in fox intestines in WA (22.3%), less commonly in NSW (3.7%). However, a microscopic study of stomach and intestinal contents from 36 foxes from Jugiong NSW revealed sheep wool in 8 (22.2%). Therefore our gross observations are likely to be an under estimation as to the level of predation/scavenging of sheep by foxes in NSW and WA.

Fifty two wild dogs collected from around sheep rearing areas in NSW and adjacent areas in the Australian Capital Territory (ACT) were also examined for sheep measles tapeworms. Although no sheep measles tapeworms were recovered, 7 (13.5%) wild dogs were found infected with *T. hydatigena*. The absence of sheep measles tapeworms and the presence of *T. hydatigena* strongly suggests the sheep being predated or scavenged were the older age groups of sheep those less likely to contain viable sheep measles cysts.

The financial losses borne by abattoirs vary between states and the type of enterprise. Processors losing the most are those killing and processing older age groups of sheep, whilst for those processing predominately lambs the losses are lower. Mutton processors in WA appear to be the worst affected, losing an average of $2,138/day, however, daily spikes may be in excess of $4,000/day whilst in NSW losses are lower, averaging around $1,100/day. For lamb processors the impact of sheep measles is lowest at less than $100/day. Our study indicates the impact of sheep measles on the Australian sheep meat industry conservatively amounts to several million dollars per year.

This study has unequivocally identified foxes as an agent in the transmission of sheep measles in Australia. Although prevalence in foxes is low, these data have important implications, not only for Australian sheep meat processors, but also for Australian producers. Although domestic dogs were shown to not to be a major transmission host there is no doubt they still have the potential to be an important conduit for the distribution of sheep measles tapeworm eggs onto pastures. Traditional control strategies of regular de-worming with praziquantel and feeding of "safe" food (commercially produced dry rations or sheep meat and hearts that have been thoroughly cooked or frozen for 10 days) must not be neglected.

Confirmation of foxes as hosts for *T. ovis* highlights the need for a major shift in the focus of currently advocated sheep measles control strategies, namely, direct protection of sheep. A highly efficient recombinant vaccine for sheep was developed in Australia and New Zealand in the late 1980s and the results published in the prestigious scientific journal Nature (Johnson et al 1989). Our results indicate it is high time this vaccine was developed as a commercial product for use in Australia. The commercial benefits to Australian sheep meat producers and processors resulting from the use of this vaccine would be considerable.

(*About 10 years ago foxes were illegally introduced into Tasmania. There followed an intense 1080 baiting program and if foxes still exist in Tasmania their current population is so low as to be irrelevant to current sheep measles transmission in Tasmania)

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1 Background

1.1 Sheep measles

1.1.1 Importance

Infection of sheep with the dog parasite *Taenia ovis* is commonly referred to as "sheep measles" due to the "spotty" appearance of meat containing pale cystic parasitic lesions. Cysts in sheep muscles are small (approximately 4-6mm), blister-like structures, each containing a single tapeworm head. Cysts occur most commonly, in heart and diaphragm muscle, but in heavy infections cysts can be found throughout the skeletal musculature of the animal. However, as time passes (2-3 months) the immune system of the sheep overwhelms the cysts which are killed, developing into a pus-filled abscess. These lesions eventually become mineralised transforming from gritty masses to hard calcified nodules. From a consumer's perspective, pus-filled or calcified lesions in meat for human consumption are unacceptable.

Intermediate stages of *T. ovis* infecting sheep cause financial losses for the Australian sheep meat industry through downgrading and condemnation of meat and hearts. Recent data collected through the National Sheep Health Monitoring Project (NSHMP; AHA 2011) showed *T. ovis* to be widespread and common in slaughtered sheep from all sheep rearing areas of Australia.

Taenia ovis infections in sheep are of no apparent veterinary importance and in contrast to the hydatid tapeworm (*Echinococcus granulosus*) dogs infected with adult *T. ovis* pose no risk to human health. The importance of sheep measles to the Australian meat industry is purely aesthetic leading to a negative impact on carcass value and a potential impediment to international trade.

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

1.1.2 Lifecycle

Taenia ovis tapeworms reside in the small intestine of canids, commonly domestic dogs. Dogs become infected through eating the intermediate cystic stage of the parasite residing in the muscles of sheep, particularly in the diaphragm and heart, but in heavy infection cysts can occur in any muscle of the body. Each cyst contains a single tapeworm head and once eaten by a dog, tapeworms take approximately 42 days to become mature and begin producing eggs. Transmission of the parasite to sheep occurs via tapeworm eggs passing into the environment with the faeces from infected dogs. Eggs are further spread in the environment by agents such as flies (Lawson and Gemmell 1985), rain and wind. They remain viable on pasture for at least 300 days (Arundel 1979) from where they are accidentally ingested by sheep whilst grazing. From the time of infection it takes about two months of development before cysts in sheep muscle are infective to dogs. Cysts remain infective to dogs for only 2-3 months. As described above, senescent cysts calcify and remain in sheep musculature for the life of the animal.

1.1.3 Control

Current recommended control strategies for *T. ovis* focus on ensuring domestic dogs remain uninfected with sheep measles tapeworms. Control strategies rely on regular (ideally monthly) de-worming with a product containing praziquantel and only feeding commercially prepared dog food. However, if sheep meat and/or hearts are to be fed to dogs they should be either thoroughly cooked or frozen for 10 days prior to feeding.

Currently there is no commercial treatment to kill infection in sheep or to protect sheep from becoming infected. However, an experimental vaccine for sheep against sheep measles has been developed (Johnson et al 1989 – see Appendix 3)

1.1.4 Role of wild carnivores in transmission

Until now, it has generally accepted by parasitologists that foxes, wild dogs and feral cats do not contribute to transmission of sheep measles to sheep in Australia.

Foxes: In three major surveys (New South Wales (NSW), Victoria and Western Australia (WA)) the intestines of over 2,000 foxes were examined for helminths. Although several species of *Taenia* tapeworms were recovered *T. ovis* was never recorded (Coman 1973; Ryan 1976; Dybing 2013). Despite an old report of *T. ovis* in foxes (Pullar 1946), Coman and Ryan (1974) concluded foxes did not act as hosts for *T. ovis.* More recently, Howkins (1986) reported *T ovis* in foxes from the Australian Capital Territory (ACT) and adjacent areas in New South Wales. The tapeworm identification method was not revealed in Pullar (1946) and Howkins (1986) relied on rostellar hook measurement, a diagnostic method considered unreliable due to considerable of overlap in hook length between species (Coman 1973; Beveridge and Gregory 1976; Edwards and Herbert 1981).
Experimental infection studies of foxes with *T.hydatigena*, a parasite closely related to *T. ovis*, also using sheep as its intermediate host, were undertaken by Coman (1973). Although parasites established in the foxes, none reached maturity and produced eggs, compared with concurrent infections in dogs. Based on these infection data and others using *Taenia pisiformis* (Beveridge and Coman 1978) it was concluded foxes were poor hosts for *Taenia* species. Coman and Ryan (1974) suggested an additional reason for the absence of *T. ovis* and *T.hydatigena* in foxes was they rarely ate fresh sheep meat/offal, mainly eating carrion and small vertebrates.

Wild dogs: The situation regarding the role of wild dogs as definitive hosts for *T ovis* is also unclear. As with rural domestic dogs, past studies concerning intestinal helminth infections of Australian wild dogs commonly focused only on *E. granulosus* (reviewed by Jenkins et al 2006). However, none of the authors who reported on the presence of other helminths in the wild dogs, ever reported *T. ovis* (Durie and Riek 1952; Coman 1972; Reichel and Gasser 1994; Brown and Copeman 2003; Jenkins et al 2008), however, as with the identification of *Taenia* species recovered from foxes, identification of tapeworms was based on tapeworm morphology and/or rostellar hook measurements, therefore the results remain equivocal.

Feral cats: *Taenia ovis* has never been reported in feral cats in Australia (Arundel 1970) or in New Zealand (Sweatman and Williams 1962), however Arundel (1970) reported natural infection in a cat which was passing segments that contained no fully formed eggs. In an experimental infection study with *T. ovis* in 24 cats (Sweatman and Williams 1962) tapeworms in only 7 animals produced eggs. Although this study demonstrated *T. ovis* can establish and develop to patency in

some cats, in most cats the parasite does not establish or fails to reach patency. These experimental data and the lack of natural infection data from feral cats suggest cats are not sylvatic definitive hosts for *T. ovis.*

2 Project objectives

2.1 Parasite transmission

There were four main study objectives:

2.1.1 Determination of on-farm risk factors contributing to the transmission of sheep measles

2.1.2 Determination of the role of domestic and wild canids in the transmission of sheep measles

2.1.3 Determination of processor financial losses due to sheep measles

2.1.4 Development of an education package to improve producer understanding of the importance of sheep measles

3 Methodology

3.1 Methodology

3.1.1 Farmer questionnaires

Our questionnaire had four sections (A-D) and was designed to obtain information on the care and maintenance of farm dogs (pets, hunting and working dogs), home slaughter/hunting practices, carcass/offal disposal, livestock water sources and buying–in feed, proximity of properties to parks and forests, presence and control of vertebrate pests (especially foxes and wild dogs) and other dogs (with hunters, drovers or visitors or roaming neighbour's dogs) entering properties (copy of questionnaire attached Appendix 1).

The questionnaire was approved for use by the Charles Sturt University Human Experimentation Ethics Committee, reference 416/2012/01. A copy of the approval letter is attached see Appendix 2). A condition for the CSUHEEC approval was that we could not know the names and addresses of the producers to whom the questionnaires were to be sent until the farmers had completed and returned the questionnaires. Therefore, all the questionnaires had to be delivered through a third party who matched PIC numbers with producer name and address and mailed out the questionnaires.

The questionnaire was "road-tested" by 10 NSW sheep producers, who commented on question clarity and relevance. Their comments and suggested changes were incorporated into the final version of the questionnaire before it was used in the study.

To determine risk factors, the goal was to survey 90 properties, with a minimum of 30 from each state. Within each state, where possible, the farms consisted of (at least) 20 positive properties with a prevalence greater than 5% determined in at least 3

lines of sheep examined at slaughter during 2011 (identified through the NSHMP data base) and 10 farms with no reported infection as negative controls. These farms were also selected from the data base of the NSHMP. Since we expected a return rate of around one third we sent out three times more questionnaires than required.

The results of the questionnaire were analysed in two steps. Firstly, a descriptive overview of all the three States (NSW, TAS, WA) was undertaken followed by a comparison of variables between the positive and negative farms using an student T-test for continuous variables, a Chi-square test on contingency tables for categorical variables and a Mann Whitney U rank test for ordinal data. All data were included in an Excel sheet before being analysed with PAWS 18.0 (SPSS, Chicago, IL, USA).

3.1.2 Collection of foxes and wild dogs

Wild dogs were collected in NSW and the ACT and foxes were collected in the ACT, NSW and WA. All animals were obtained from professional vertebrate pest control officers who trapped or shot the animals during the normal course of their duties or from recreational shooters or farmers who were removing potential predators from the vicinity of sheep flocks. Foxes from Western Australia (WA) were shot by farmers and recreational hunters during an official state-wide fox control program, in sheep-rearing areas of Western Australia (WA) (Fox, Cat and Rabbit Red Card Days - info@redcard.net.au). As soon after death as possible, intestines were removed from the animals and kept on ice until examined, sometimes up to 48 hours later. Some intestines were frozen prior to tapeworm collection.

Animal ethics approval for this study was not required since all animals used in the study were being culled as part of approved vertebrate pest control activities by licensed personnel.

3.1.3 Identification of tapeworm infection in domestic dog faeces

3.1.3.1 Faecal flotation

Helminth eggs in faeces were visualized using a standard flotation methodology incorporating saturated sodium nitrate as the flotation solution. Eggs of all species, except *Taenia* eggs, were identified to species microscopically using morphological criteria. Taeniid eggs were identified using molecular methods.

3.1.3.2 Molecular identification of *Taenia* tapeworm eggs

Taeniid egg isolation was performed using a flotation and sieving method (Mathis *et al* 1996). Briefly, 8 mL of zinc chloride solution (1.45g/mL) were added to 2 g of each faecal sample. The samples were homogenized by vortex and centrifuged at 1000xg for 30 min. The supernatant was passed through 41µm and 21µm mesh sieves. The taeniid eggs were collected from the 21µm mesh and resuspended in water in a 10mL flat tube. Egg identification was carried out under an inverted microscope. DNA extraction was performed using a Qiagen multiplex PCR kit (Qiagen, Hilden, Germany) (Štefanić 2004). A multipex-PCR (Trachsel 2007) targeting two mitochondrial genes, NADH dehydrogenase subunit 1 (*nad1*) for *E. multilocuaris* and small subunit of ribosomal RNA (*rrnS*) for both *E. granulosus* and *Taenia* spp. was used for species identification of the taeniid egg-positive samples. For *Taenia* spp positive samples, species level was achieved by direct sequencing of the amplicons. Sequencing was performed by Synergene Biotech GmbH, Biotech Center Zurich,

Switzerland (http://www.synergene-biotech.com) with the primer Cest5seq (Trachsel 2007). The sequences obtained were compared with those available in the GenBank nucleotide database by BLAST search (http://www.blast.ncbi.nlm.nih.gov).

3.1.4 Collection of tapeworms

The section of intestine from about 10-20mm below the stomach to the caecum was separated from the omentum and retained separately in a labelled clip-lock plastic bag on ice from each fox and wild dog. The intestine was slit longitudinally using gut scissors and the contents washed out into a black plastic tray with water. The intestine was discarded and the intestinal contents washed through a sieve (350µ mesh) with running tap water. The contents of the sieve were back-washed into the black tray with tap water. All tapeworms present were identified to genus morphologically. All *Taenia* species were collected, blotted dry on absorbent paper and placed in 80% ethanol for later identification using molecular methods.

3.1.4.1 Molecular identification of tapeworms

Identification of *T. ovis* was through sequencing a mitochondrial gene for the small subunit of ribosomal RNA (rrnS). DNA was extracted from tissue samples using a Qiagen DNeasy kit, and PCR techniques of Trachsel et al (2007). Using “nBLAST”, sequenced samples were compared to defined species sequences from the ‘genebank’.

3.1.5 Processor financial impact study

Abattoir management were contacted at abattoirs in NSW, Tasmania and WA. Five abattoirs agreed to provide data as required for the financial impact study. Southern Meats, Goulburn, NSW; Fletcher International, Dubbo, NSW; Gundagai Meat Processors, Gundagai, NSW; Tasmania Quality Meats, Cressy, Tasmania; Fletcher International, WA. A data collection form was developed in collaboration with meat inspection staff at Southern Meats at Goulburn and circulated to the other establishments for comment/modification. It was agreed to have a 5 day data collection trial run, refine the data collecting form and procedure as necessary, then collect data from each abattoir for a five day period four times per year coinciding as far as possible with each season.

4 Results and discussion

4.1 Results and discussion

4.1.1 Farmer questionnaires

The overall questionnaire return rate of all three states was, 36.9% (89 out of 241), consisting of 40 negative farms and 49 positive farms. Per state, the response rate was NSW 42.2% (38 out of 90, equating to 20 negative, 18 positive farms), WA 31.3% (20 out of 64, equating to 3 negative, 17 positive farms) and TAS 35.6% (31 out of 87 equating to 17 negative, 14 positive farms). Not all farmers answered all questions.

Section A - producer details

Table 1. Farm type, other livestock and feral species

Farm type (n,%)	All farms	positive	negative	P value
Sheep (wool)	79 (88.8%)	45 (57.0%)	34 (43.0%)	0.248
Sheep (meat)	79 (88.8%)	42 (47.2%)	37 (41.6%)	0.254
Beef cattle (pasture crop)	61 (68.5%)	34 (38.2%)	27 (30.3%)	0.514
Beef cattle (feed lot)	4 (4.5%)	4 (4.5%)	0 (0.0%)	0.087
Wheat and other crops	51 (57.3%)	34 (38.2%)	17 (19.1%)	0.010
Other Livestock				
Horses	31 (34.8%)	18 (20.2%)	13 (14.6%)	0.424
Donkeys	1 (1.1%)	1 (1.1%)	0 (0.0%)	0.551
Domestic Goats	2 (2.2%)	2 (2.2%)	0 (0.0%)	0.300
Alpacas	6 (6.7%)	6 (6.7%)	0 (0.0%)	0.024
Llamas	1 (1.1%)	1 (1.1%)	0 (0.0%)	0.551
Deer	6 (6.7%)	2 (2.2%)	4 (4.4%)	0.247
Pigs	6 (6.7%)	2 (2.2%)	4 (4.4%)	0.247
Feral Species				
Horses	-	-	-	-
Donkeys	-	-	-	-
Goats	12 (13.6%)	3 (3.4%)	9 (10.2%)	0.028
Feral Cats	72 (81.8%)	39 (44.3%)	33 (37.5%)	0.552
Deer	24 (27.3%)	10 (11.4%)	14 (15.9%)	0.107
Pigs	21 (23.9%)	7 (8.0%)	14 (15.9%)	0.023
Predation				
Wild dogs onto property	11 (12.5%)	6 (6.8%)	5 (5.7%)	0.592
Wild dogs predating on sheep	5 (5.7%)	4 (4.6%)	1 (1.1%)	0.260
Foxes onto property	58 (66.7%)	36 (41.4%)	22(25.3%)	0.055
Foxes predating on lambs	53 (61.6%)	32 (37.2%)	21 (24.4%)	0.129
Drinking water obtained from				
Dams	80 (90.9%)	44 (50%)	36 (40.9%)	0.536
Troughs	68 (77.3%)	37 (42%)	31 (35.2%)	0.584
Bore	23 (26.1%)	10 (11.4%)	13 (14.8%)	0.159
Creek	36 (40.9%)	20 (22.7%)	16 (18.2%)	0.524
River	33 (37.5%)	17 (19.3%)	16 (18.2%)	0.412
Spring	20 (22.7%)	9 (10.2%)	11 (12.5%)	0.235
Bought sheep the last 5 years	79 (89.8%)	43 (48.9%)	36 (40.9%)	0.616
Purpose of sheep bought in				
Grazing for slaughter	18 (21.7%)	11 (13.3%)	7 (8.4%)	0.306
Feedlot	5 (6.0%)	4 (4.9%)	1 (1.1%)	0.220
Replacement	35 (42.2%)	20 (24.1%)	15 (18.1%)	0.337
Rams	73 (88.0%)	41 (49.4%)	32 (38.6%)	0.112
During an average year buy in hay	12 (13.8%)	9 (10.3%)	3 (3.4%)	0.103
Do you own dogs	87 (98.8%)	47 (53.4%)	40 (45.5%)	0.545

Table 2. Property size, land usage and number, type of dogs

Variable (mean, Standard Deviation)	All farms	positive	negative	P value
Mean property size in acres	8,525 (+/- 28,904)	3,951 (+/- 5,155)	13,989 (+/- 41,906)	0.110
Mean grazing area in acres	7,245 (+/- 29,185)	2,922 (+/- 4,210)	12,234 (+/- 42,335)	0.146
Mean cropping area in acres	1,180 (+/- 3,233)	801 (+/- 1,399)	1,617 (+/- 4,495)	0.251
Dogs				
Number of working dogs	4.7 (+/-5.0)	4.6 (+/-4.8)	4.8 (+/-5.3)	0.856
Number of hunting dogs	0.3 (+/-1.7)	0.2 (+/-0.65)	0.4 (+/-2.4)	0.439
Number of pet dogs	0.8 (+/-1.3)	0.7 (+/-1.1)	1.0 (+/-1.5)	0.220

Section B – husbandry related factors

Table 3. Husbandry related factors

Farm type (n,%)	All farms	positive	negative	P value
Home slaughter sheep	57 (66.3%)	31 (36.0%)	26 (30.2%)	0.564
Regular deworming of dogs	82 (93.2%)	46 (52.3%)	36 (40.9%)	0.256
Do you weigh your dogs for deworming	43 (51.8%)	24 (55.8%)	19 (22.9%)	0.558
What is the normal diet for your dog(s)				
Dry food	85 (96.6%)	47 (53.4%)	38 (43.2%)	0.431
Canned food	18 (20.5%)	11 (12.5%)	7 (8.0%)	0.361
Fresh meat from butcher	8 (9.1%)	7 (8.0%)	1 (1.1%)	0.052
Fresh meat home slaughter	41 (46.6%)	20 (22.7%)	21 (23.9%)	0.212
Fresh meat from death lamb sheep	11 (12.5%)	7 (8.0%)	4 (4.5%)	0.376
Feeding offal of any species	10 (11.6%)	5 (5.8%)	5 (5.8%)	0.538
Is the offal before feeding				
Cooked	1 (9.1%)	1 (9.1%)	0	-
Raw	9 (81.8%)	4 (36.4%)	5 (45.5%)	0.273
Frozen	4 (36.4%)	4 (36.4%)	0	0.045
Utilitise your working dogs for moving sheep				
Only Yards	6 (7.0%)	2 (2.3%)	4 (4.7%)	0.274
Only Mustering	5 (5.8%)	2 (2.3%)	3 (3.5%)	0.433
Both Yards and Mustering	78 (90.7%)	45 (52.3%)	33 (38.4%)	0.018
Livestock guarding dogs with any of your stock	3 (3.5%)	1 (1.2%)	2 (2.3%)	0.447
Are pet dogs confined at the property	18 (20.9%)	10 (11.8%)	8 (9.3%)	0.371
Are pet dogs allowed to roam freely	36 (41.9%)	16 (18.6%)	20 (23.3%)	0.332
Frequency of deworming (Mean, SD) in months	5.6 (3.4)	5.5 (3.4)	5.7 (3.5)	0.779
If working dogs not working how many hours confined	7.5 (0.8)	7.3 (1.1)	7.7 (1.2)	0.978

Section C - Climate and Environmental related factors

Table 4. Climatic, environmental and "other dog" data

Variable (mean, Standard Deviation)	All farms	positive	negative	P value
Distance to the closest National Park or State Forest (kilometers)	31.3 (+/-32)	22.2 (+/-25.9)	41.6 (+/-35.4)	0.005
Average rainfall (millimetres)	594 (+/-216)	594 (+/-225)	594 (+/-208)	0.999
Farm type (n %)	All farms	positive	negative	P value
Access to carcasses of sheep	48 (55.5%)	29 (33.7%)	19 (22.1%)	0.143
Property protected by a wild dog proof fence	2 (2.3%)	1 (1.1%)	1 (1.1%)	1.000
Wild dog/fox numbers controlled on property	47 (55.3%)	28 (32,9%)	19 (22.4%)	0.126
Attacks on your livestock from neighbour's dogs or town dogs	27 (31.8%)	17 (20.0%)	10 (11/8%)	0.189
Hunter', drover's/shearer's or neighbour's dogs ever come onto property	49 (57.6%)	27 (31.8%)	22 (25.9%)	0.403

Section D - Communication and awareness

Overall 38 farmers (44.2%) indicated they knew how *T. ovis* was transmitted (21 farmers were from positive farms and 17 from a negative farms). From all options given only one question regarding "Feeding cooked/frozen sheep meat and hearts" was identified as a positive risk factor between the negative and positive group of farmers. The negative group of farmers ranked this as a more important factor compared to the positive farmers (P value 0.04).

General comment: No other identifiable on-farm risk factors for *T.ovis* transmission could be identified, except in NSW, where there was a weak correlation between farmers who bought in hay compared to those who did not. However, this correlation was not evident in either WA or Tasmania.

4.1.2 Identification of tapeworm infection in domestic dogs and wild canids

4.1.2.1 Faecal flotation

Table 4. Helminth eggs in the faeces of rural domestic dogs visualised by faecal flotation using saturated sodium nitrate.

	NSW	Tasmania	WA	Totals
n dogs (% infected)	125 (38.4)	101 (29.0)	19 (16.0)	245 (33.0)
Helminths n(%)				
Taenia spp	0	1* (1.0)	0	1 (0.4)
S.erinacei	2 (1.6)	0	0	2 (0.8)
D. caninum	1 (0.8)	0	0	1 (0.4)
T. vulpis	10 (8.0)	11 (11.0)	0	22 (9.0)
Hookworm spp	33 (26.4)	14 (14.0)	3 (16.0)	50 (20.4)
T. canis	2 (1.6)	3 (3.0)	0	5 (2.0)

**Taenia ovis*

Overall about one third of the dogs were infected with intestinal worms, with the highest incidence in NSW (38.4%) and the lowest in WA (16.0). Only one dog infected with a taeniid cestode was identified. This dog lived in Tasmania and the

cestode was identified as *T. ovis*. The most commonly found intestinal worm infection in dogs from all states was hookworm. Hookworm species were not identified. Tapeworms *Spirometra erinacei* and *Dipylidium caninum* were only identified in dogs from NSW. Although most farmers reported de-worming their dogs regularly (most commonly two or four monthly) few de-wormed their dogs frequently enough to ensure their dogs were worm–free.

The role of domestic dogs in the transmission of sheep measles in Australia has not been investigated for over 40 years, during which time palatable and nutritionally balanced dry dog foods have been developed and the highly efficient cestocidal drug, praziquantel, has become widely available, being included in many brands of commercial dog de-worming products. These developments alone have had a profound effect on the prevalence of intestinal worms in Australian dogs. During the current study 245 faeces samples were examined for eggs of intestinal helminths and only about one third of the faecal samples contained eggs of intestinal worms. Eggs of *Taenia* tapeworm species were found only once. The almost complete absence of *Taenia* of any species in all jurisdictions is of interest, suggesting that the role of domestic dogs in the transmission of *T. ovis,* and also *T. hydatigena,* is currently less important than in previous decades. These data are corroborated by other recently obtained data (Jenkins 2013, unpublished data, submitted for publication) from a study of 1,425 rural domestic dogs from all states of eastern Australia (1,119 from mainland Australia and 306 from Tasmania). Only 11 dogs in this cohort were found infected with *Taenia* species, and of these, four were infected with *T. hydatigena* and none was infected with *T. ovis*. This low incidence of *Taenia* species infection in rural domestic dogs in our study and that of Jenkins (2013, unpublished) is a likely reflection of wide use of commercial dried dog food and modern “all-wormers” being available through supermarkets, large pet stores, stock and station agents and on-line, together with improved farmer awareness of the importance of de-worming dogs because of the risk of hydatid tapeworm infection.

4.1.2.2 DNA identification

Tapeworm DNA identification data for all infected foxes and wild dogs are presented in Table 2 and 3 below.

Table 2. Taenia species recovery data from foxes collected at various sites in the Australian Capital Territory, New South Wales and Western Australia

Foxes	ACT	New South Wales								NSW Totals
Location	Baroomba/Stromilo	Brindabella / Wee Jasper	Bathurst	Lithgow	NSW various	Jugiong/ Tarcutta	Taralga	Tumbarumba	St Marys	
n examined	11	27	72	8	12	80	5	3	9	216
n infected with *Taenia* spp (%)*	0	7(25.9)	3(4.2)	0	0	4(5.0)	1(20.0)	0	0	15(46.9)
n infected with *T. ovis* (%)	0	0	0	0	0	1(1.2)	0	0	0	1(0.5)
n infected with *T.hydatigena* (%)	0	1(3.7)	0	0	0	0	0	0	0	1(0.5)
n infected with *T. pisiformis* (%)	0	1(3.7)	1(1.4)	0	0	0	0	0	0	2(0.9)
n infected with *T. serialis* (%)	0	6(22.2)	2(2.7)	0	0	4(5.0)	1(20)	0	0	13(6.0)
Sheep wool in intestine (n%)	0	0	7(8.6)	0	0	1(1.2)	0	0	0	8(3.7)

Foxes	Western Australia					WA Totals
Location	Quairading	Boddington	Katanning	Niabing	Williams	
n examined	87	15	102	7	44	255
n infected with *Taenia* spp (%)*	3(3.4)	0	7(6.9)	0	3(6.8)	13(5.1)
n infected with *T. ovis* (%)	0	0	1(1.0)	0	0	1(0.4)
n infected with *T. hydatigena* (%)	1(1.1)	0	1(1.0)	0	0	2(0.8)
n *T. pisiformis* (%)	0	0	0	0	0	0
n *T. serialis* (%)	2(4.5)	0	5(4.9)	0	3(6.8)	10(3.9)
Sheep wool in intestine (n%)	16(18.4)	3(20)	28(27.4)	1(14.3)	9(20.4)	57(22.3)

Figure 3. Taenia species recovery data from wild dogs collected at various sites in the Australian Capital Territory and New South Wales.

Wild Dogs	ACT	New South Wales								NSW Totals
Location	Baroomba	Brindabella	Dingo Dell	The Follies	The Mullion	St Marys	Nottingham	Taralga	Adelong	
n examined	4	20	3	2	4	3	13	2	1	50
n infected with *Taenia* spp (%)	2(50.0)	11(55)	1(33.3)	0	2(50)	2	3(23.0)	0	0	21(40.4)
n infected with *T.ovis* (%)	0	0	0	0	0	0	0	0	0	0
n infected with *T. hydatigena* (%)	1(25.0)	5(25.0)	1	0	0	0	0	0	0	7(13.5)
n infected with *T. pisiformis* (%)	1(25.0)	8(40.0)	0	0	2(50.0)	2(66.6)	2(15.4)	0	0	15(28.8)
n infected with *T. serialis* (%)	0	1(10.0)	0	0	1(25.0)	0	1(7.7)	0	0	4(7.7)
Sheep wool in intestine (n%)	0	0	0	0	0	0	0	0	0	0

Taenia ovis tapeworms were found in two foxes, one from NSW (Jugiong) and one from WA(Katanning). *T. hydatigena* was present in three foxes, one from NSW and two from WA. In addition, sheep wool was identified grossly in over 20% of WA foxes examined and in almost 4% of NSW foxes. In addition, a microscope study of stomach and intestinal contents of 36 foxes from Jugiong, NSW revealed 8 (22.2%) contained sheep wool fibres indicating that scavenging carcasses and/or predating on lambs by foxes is probably more common than our gross findings indicate.

Eggs of *T. ovis* were recovered from the faeces of one of the *T.ovis*-infected foxes. The tapeworm in the other fox was fully developed with egg-laden segments but its faeces were not retained for examination. Nevertheless, it is likely both these animals would have been spreading eggs into the environment.

Curiously infection with *T. ovis* in wild dogs was absent despite 7 being infected with *T. hydatigena*. This absence of *T. ovis* and presence of *T. hydatigena* in wild dogs may be largely influenced by the biology of the parasites, namely the short time cysts in sheep remain infective for definitive hosts and wild dogs more commonly predating on older age groups of sheep, those less likely to contain viable sheep measles cysts. *T.ovis* cysts are only infective to definitive hosts for a few weeks whilst those of *T. hydatigena* remain infective for several years.

Our data indicate conclusively that wild carnivores are acting as definitive hosts for *T. ovis* and also *T. hydatigena* providing a regular source of pasture contamination with tapeworm eggs.

4.1.3 Processor financial impact study

Gundagai Meat Processors, Gundagai, NSW					
Data collection month	Sept 2012	Feb 2013	July 2013	Days monitored	Total mean daily loss ($)
Duration of data collection (days)	3	5	5	13	
Mean loss/day ($)	24.56	39.82	87.20		50.52
Approx mean kill/day	2500 (lambs)	2500 (lambs)	2500 (lambs)		

Fletcher International, Dubbo, NSW			
Data collection month	Sept 2012	Days monitored	Total mean daily loss ($)
Duration of data collection (days)	2	2	
Mean loss/day ($)	1,106.99 (mutton)		1,106.99
Mean kill/day			

Southern Meats, Goulburn, NSW				
Data collection month	July 2012	Jan 2013	Days monitored	Total mean daily loss ($)
Duration of data collection (days)	1	5	6	
Mean loss/day ($)	1,617.24	762.94		1,190.09
Mean kill/day	2,800 (43.55% lambs)	4,374 (35% lambs)		

Fletchers International, Narrikup, WA						
Data collection month	Sept 2012	Feb 2013	May 2013	August 2013	Days Monitored	Total mean daily loss ($)
Duration of data collection (days)	5	5	5	5	20	
Mean loss/day ($)	2,836.70	1,322.55	2,106.60	2,009.73		2,137.79
Approx mean kill/day	(7,500 mutton)	(7,500 mutton)	(7,500 mutton)	(7,500 mutton)		

Tasmania Quality Meats, Cressy					
Data collection month (duration days)	June 2012	Feb 2013	June 2013	Days monitored	Total mean daily loss ($)
Duration of data collection (days)	3	5	8	16	
Mean loss/day ($)	32.82	37.90	52.90		41.20
Mean kill/day	1,182 (lambs)	1,699 (72.9% lambs)	1,122 (88.6% lambs)		

Despite initial interest and enthusiasm to contribute to the study, data on the financial impact of sheep measles experienced by processors were not supplied equally by all processors. The only abattoir supplying data around the times requested was Fletcher international in WA. Despite the shortcomings of the data set, some trends are evident. The establishments concentrating mainly on lambs (TQM and GMP) suffer the least financial impact, those processing mainly mutton the most. Within the mutton processing establishments, it was evident that the impact in WA was about double that experienced in NSW.

5 Success in achieving objectives

5.1 Success in achieving objectives

5.1.1 Determination of on-farm risk factors for sheep measles

5.1.1.1 Identifying questionnaire recipients

The number of returned questionnaires, following the initial mail-out and the reminder letter, was good giving us about the number of returns we expected. The breakdown of farms with sheep measles infection and those without was 49 to 40, close to 50:50. We had hoped to have a ratio of closer to 3:1 of infected versus uninfected, therefore from this perspective the questionnaire was less successful. Nevertheless, we achieved our objective in obtaining sufficient data from which to obtain a statistically robust data set for reliable identification of on-farm risk factors for transmission of sheep measles.

Without the exceptional support from AHA in allowing us access to the sheep measles database of the NSHMP selecting sufficient appropriate farms would

have been almost impossible. We gratefully acknowledge the crucial contribution of AHA in this component of the study.

5.1.2 Determination of the role of domestic and wild canids in transmission

5.1.2.1 Obtaining foxes and wild dogs to examine

From links established during past collaborations with LHPA vertebrate pest control officers and trappers with ACT Parks and Conservation and Forests NSW, and tapping into farmer networks we were able to obtain sufficient study animals from a number of areas in NSW and the ACT. The opportunity to collaborate with the Western Australian "Fox, Cat and Rabbit Red Card Days" was crucial in being able to obtain sufficient animals to examine from WA. I would like to acknowledge the exceptional help and enthusiastic support from the state organiser of this event Mr Graeme Murray and from all local organisers in the areas where we collected foxes. In addition having access to the post mortem room at Murdoch University to process the WA fox intestines and harvest the tapeworms was also important in enabling us to more easily achieve our objectives.

5.1.2.2 Additional funding for students assisting with the study

An important aspect of this study was the contribution of my students which really helped to ensure the success of the molecular study. This aspect of the study was not identified as being of major importance until the discovery of the first *T.ovis*-infected fox, thereafter becoming a major focus. Additional financial support was sought through the competitive small grant arrangements at CSU for student to assistance with project activities.

Kate Mitchell undertook a 10 week work period during the first year of the project for which she received a CSU Graham Centre Summer Scholarship worth $4,000. During this time she organised the first batch of foxes, collected the tapeworms, extracted their DNA and found our first *T. ovis*-infected animal.

Thomas Williams did his Honours year with the project during year two following up on the work undertaken by Kate Mitchell. Thomas received two grants, a CSU Graham Centre New Initiative Grant worth $9,000 and a CSU Faculty of Science Honours Project Operating grant worth $2,000. During his Honours year Thomas provided crucial input into all aspects of the fox study in WA and south eastern Australia and identified the second *T. ovis*-infected fox from WA.

5.1.3 Determination of processor financial losses

Despite initial processor enthusiasm to contribute to this aspect of the study, maintaining interest and obtaining the data required became problematic in several establishments. The best data set was obtained from Fletcher International in WA. This was entirely due to the presence of a highly motivated and well organised Operations Manager (Mr Justin Cuthbert). Despite the difficulties, sufficient data were obtained confirming sheep measles to be most prevalent in WA, causing approximately double the daily financial loss compared to processors in NSW and that establishments processing mainly lambs were least affected. However, further better organised data collection needs to be undertaken before a truly clear picture of the degree of financial loss to processors can be obtained.

5.1.4 Development of an education package for producers

This aspect of the project was successfully achieved through the production of a 10 minute power-point slide show with voice over that can be loaded onto Youtube and/or the MLA website. The slide show was based on most frequently asked questions but also covered what constitutes sheep measles, transmission in both the domestic and sylvatic environments, why the parasite is important and how to control it.

6 Impact on Meat and Livestock Industry – now and in five years time

6.1 Impact on Meat and Livestock Industry – now and in five years time

6.1.1 Impact on Meat and Livestock Industry – now and in five years

In the short term, the impact of the data collected in this study on the Australian meat and livestock industry will be minimal. However, the important outcome of this study is that we now know there is sylvatic transmission of *T. ovis* in Australia and that to control transmission we need to do more than de-worming dogs and ensuring they are fed safe foods, we need also to protect sheep. In addition we now have a clearer idea of the financial impact sheep measles is having on the Australian sheep meat industry adding justification for further work to develop more effective sheep measles control.

The major positive impact will arise from a follow-up study to undertake trial work needed to prepare the sheep measles vaccine for registration and commercialisation. The availability of this vaccine as a commercial product in Australia will mean for the first time there will a practical means of generating high-level immunity in sheep against infection with sheep measles. This vaccine provides a realistic prospect for the control of sheep measles in Australian sheep with associated savings of several million dollars per year for the Australian sheep meat industry.

7 Conclusions and recommendations

7.1 Conclusions and recommendations

7.1.1 Conclusions and recommendations

7.1.1.1 Conclusion

Since foxes have been conclusively shown to be definitive hosts for sheep measles tapeworms conventional sheep measles control strategies, namely, regularly de-worming farm dogs and preventing them eating uncooked or unfrozen sheep meat or offal (hearts) are inadequate to prevent the transmission of ovine cysticercosis. These traditional control activities should not stop but will need to be complemented with directly protecting sheep from infection through vaccination.

7.1.1.2 Recommendations

Identify a commercial vaccine maker interested in the concept of producing this vaccine as a commercial product and initiate a 2 year follow-up study to undertake

the experiments necessary for registration of the vaccine for use in sheep in Australia.

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9 Appendices

9.1 Appendix 1 - Sheep Measles Questionnaire

Sheep Measles Transmission Study

Funded by Meat and Livestock Australia

with support from the EH Graham Centre for Agricultural Innovation
(A Charles Sturt University & NSW DPI initiative)

(Participation in this study is completely voluntary. You are free to opt-out at any time)

Questionnaire

Contact address to send you the test results

Name and postal address
……..
……..
Email…….
PIC number……..
Phone number……..

General Information farm and household

1. What commercial activities are currently undertaken on your farm (**Tick one or more options as appropriate**)

   ☐ Sheep (wool)
   ☐ Sheep (meat)
   ☐ Beef cattle (pasture/crop)
   ☐ Beef cattle (feed lot)
   ☐ Wheat and other crops
   ☐ Other ……

2. What **other** livestock species are present on your property **(Tick appropriate box(es)**

   ☐ Horses ☐ Donkeys ☐ Domestic Goats ☐ Alpacas
   ☐ Llamas ☐ Deer ☐ Pigs ☐
   Other……..…

3. Do wild dogs come onto your property? ☐Yes ☐No

4. Do you have wild dogs predating on your sheep? ☐Yes ☐No

5. Do you have foxes on your property? ☐Yes ☐No

6. Do you have foxes predating on your lambs? ☐Yes ☐No

7. What **feral** species are present on your property **(Tick appropriate box(es)**

☐ Horses ☐ Donkeys ☐ Goats ☐ Feral cats
☐ Deer ☐ Pigs ☐ Other……..

8. Where do your stock obtain water? **(Tick appropriate box(es)**

☐ Dams ☐Troughs ☐ Bore ☐ Creek
☐ River ☐Springs ☐ Other……..

9. In the last **5** years, have you ever bought in sheep from elsewhere

☐Yes ☐ No (if **No** continue to question **11**)

10. If you bought sheep in, were they for? (**Tick one or more options as appropriate**)

☐ Grazing for slaughter ☐ Feedlot for slaughter ☐ Replacement ☐ Rams

11. In an average rainfall year do you ever buy-in additional hay for your sheep? ☐ Yes ☐ No

12. How big is your property? .....hectares/acres(circle one)

13. Area for grazing……. hectares/acres(circle one); Area for crops……hectares/acres(circle one)

14. Do you own dogs? ☐ Yes ☐ No (if **Not** continue to question **30**)

15. How many?
Number of working dogs ……Number of hunting dogs… Number of pet/house dogs……

Husbandry related factors

16. Do you home slaughter sheep? ☐ Yes ☐ No

17. Do you de-worm your dogs? ☐ Yes ☐ No (if **No** continue to question **21**)

18. How frequently do you de-worm your dogs? Every …… month(s)

19. De-worming product name……...

20. If you de-worm your dog, how do you calculate the dose to de-worm your dog?

☐ Weigh ☐ Estimate

21. What is the normal diet for your dog(s) **(Tick one or more options as appropriate)**

☐ Dry food ☐ Canned food ☐ fresh meat/shanks/hearts/flaps from the **butcher**

☐ fresh sheep meat/shanks/heart/flaps from **home slaughter** ☐ Other……

22. Do you ever feed fresh meat (eg. shanks, hearts,flaps) from sheep/lambs that die in the paddock?
☐ Yes ☐ No

23. Do you ever feed offal of any species? ☐ Yes ☐ No (if **No** continue to question **25**)

24. Is the offal you are feeding ☐ Cooked ☐ Raw ☐ Frozen

25. When your **working** dogs are not working, approximately, how many hours/day are they confined? ……hrs

26. How do you utilitise your working dogs for moving sheep?

☐ N.A. ☐ Only in the yards ☐ Only mustering in the paddocks ☐ Both

27. Do you use livestock guarding dogs with any of your livestock? ☐ Yes ☐ No

28. Are **pet dogs** on your property confined? ☐ NA ☐ Yes ☐ No

29. Are your **pet dogs** allowed to roam freely through the property? ☐ NA ☐ Yes ☐ No

Climate and Environment related factors

30. Estimate the distance in kilometers from your house to the closest National Park or State Forest boundary? ……. Kilometres

31. What was the rainfall in your area the last 12 months? ……mm

32. Could your dogs ever get access to the carcasses of sheep? ☐ NA ☐ Yes ☐ No

33. Is your property protected by a wild dog proof fence? ☐ Yes ☐ No

34. Are wild dog/fox numbers controlled on your property? ☐ Yes ☐ No (if not continue to question 38)

35. If yes how? **(Tick one or more options)**

☐ Aerial baiting ☐ Bait stations ☐ Buried baits ☐ Hand surface baiting
☐ 1080 ejectors ☐ Traps ☐ Shooting ☐ Other ……..

36. If you used 1080, what type of bait is used? (**Tick one or more options**)
☐ Fresh meat ☐ dried meat ☐ liver ☐ chicken heads
☐ Lamb tongues ☐"Doggon" ☐ "Foxoff" ☐ Other ……..

37. How many times/year do you bait for wild dogs/foxes?…… times

38. Do you ever have attacks on your livestock from neighbour's dogs or town dogs?
☐ Yes ☐ No.

39. Do pig/deer hunter', drover's/shearer's or neighbour's dogs ever come onto your property?
☐ Yes ☐ No

Communication and awareness

40. Do you know how sheep get infected with sheep measles? ☐ Yes ☐ No

41. How important are the following actions in exposing your sheep to the risk of sheep measles

Answers can be given on scale ranging from **'strongly agree'** to **'strongly disagree'**
Cross only one option per action

Actions	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly Agree
Feeding sheep meat to dogs					
Feeding sheep hearts to dogs					
Feed cooked/frozen sheep meat and hearts					
Feeding feral goat meat and offal to dogs					
Not de-worming of your dogs with an all wormer					

Neighbours dogs/hunters dogs/shears dogs entering your property					
Presence of wild dogs and/or foxes on your property					
Not controlling dogs when not working					
Buying in sheep from elsewhere					
Not drenching your sheep					

Any other comments.
……
……
……
……
……
……

NOTE: The School of Animal and Veterinary Sciences Ethics Committee has approved this project. If you have any complaints or reservations about the ethical conduct of this project, you may contact the Committee through the Executive Officer:

Name: Dr R Freire
Address: School of Animal & Veterinary Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga NSW 2678

Tel: (02) 69334451
Fax: (02) 6933 2991
Email: rfreire@csu.edu.au

Any issues you raise will be treated in confidence and investigated fully and you will be informed of the outcome.

For any further information, please contact

Dr David Jenkins,
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9.2 Appendix 2 – CSU Human Experimentation Ethics Committee approval letter

Charles Sturt University

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5 December 2013

Dr David Jenkins
School of Animal and Veterinary Sciences

Dear David,

Thank you for submitting your revised research proposal on sheep measles to the SAVS Ethics in Human Research Committee.

The School of Animal and Veterinary Sciences Ethics in Human Research Committee has approved this project till the 31/10/13.

The protocol number issued with respect to this project is 416/2012/01.

We would like to wish well with your project.

Yours sincerely

Dr Raf Freire
Chair, School of Animal and Veterinary Sciences Human Ethics Committee
Direct Telephone: 34451, Email: rfreire@csu.edu.au

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9.3 Appendix 3 – First report of a recombinant antigen used to protect sheep from sheep measles

tively labelled cRNA derived from cloned rat γ2 cDNA (manuscript in preparation). Our results show that γ2 mRNA is prominently expressed in neuronal subsets throughout the CNS which include neurons in the olfactory bulb, anterior olfactory nuclei, preoptic area, neocortex, globus pallidus, hippocampus, dentate gyrus, thalamus, inferior colliculus, substantia nigra, pontine nuclei, cerebellar cortex and cerebellar nuclei. Four of these regions have been chosen to illustrate the cellular location of γ2 mRNA (Fig. 4). Significantly, all of these regions contain high-affinity binding sites for benzodiazepines[15,16] and also express α- and β-subunit mRNAs (refs 17, 18; B.D.S., unpublished observations), further supporting the hypothesis that the γ2 subunit is an integral part of GABA/benzodiazepine receptors.

The existence of the γ2 subunit was not anticipated from biochemical studies as subunits of affinity-purified GABA/benzodiazepine receptor are electrophoretically resolved into only two main bands corresponding to relative molecular mass (M_r) 48,000–53,000 (48K–53K) (α) and M_r 55–57K (β) (ref. 1). These bands, however, are heterogeneous, consisting of variants of the α-subunits[3,4] and β-subunits, and of additional $GABA_A$ receptor subunits, including γ2 and a related γ1 subunit, whose cloning preceded that of γ2 and which shows glial localization (manuscript in preparation). The mature γ2-polypeptide (unglycosylated, $M_r \approx 48K$) may co-migrate with α-subunits (M_r, 48–53K)[1,4], which have been postulated to carry the benzodiazepine site on the basis of photoaffinity labelling with flunitrazepam[21–23]. Our results on benzodiazepine responsiveness indicate that the γ- subunit contributes to the formation of the benzodiazepine site and thus may also be photoaffinity-labelled. In fact, it is probable that a flunitrazepam-labelled γ2 subunit would be indistinguishable from certain labelled α-subunits by present methods.

We note that other subunit combinations may also create benzodiazepine responsiveness. Indeed, recent cDNA cloning experiments in our laboratory provide evidence for the existence of additional $GABA_A$ receptor subunits (manuscript in preparation). It seems likely therefore that the true diversity of GABA/benzodiazepine receptor subtypes has been only partly revealed by classical pharmacology[12,19,20]. □

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Vaccination against ovine cysticercosis using a defined recombinant antigen

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CYSTICERCOSIS caused by larval tapeworms is a major public health problem and a cause of substantial economic losses in the farm-animal industries. *Taenia ovis* in sheep is a particularly important example. Immunity to reinfection with the larvae has a central role in regulating natural transmission of the parasites[1], and vaccination with antigens from the early larval oncosphere stage can induce complete protection against infection[2]. As it is impractical to obtain enough oncospheres for a commercial vaccine against these tapeworms, an alternative approach is to use recombinant DNA methods to generate a cheap and plentiful supply of antigens. We report here the expression in *Escherichia coli* of complementary DNA encoding *T. ovis* antigens as fusion proteins with the *Schistosoma japonicum* glutathione S-transferase. Vaccination of sheep with these fusion proteins gave significant, although not complete, immunity against challenge infection with *T. ovis* eggs. Commercial development of a vaccine is being pursued.

Oncospheres of *T. ovis* were chosen as the source of messenger RNA for constructing a cDNA library because they are known to be a rich source of host-protective antigens[3,4]. Furthermore, *in vitro* culture techniques[5] have shown that hatched and activated *T. ovis* oncospheres secrete potent host-protective antigens[6,7], suggesting synthesis is active. Oncospheres were therefore treated with artificial gastric and intestinal fluid and incubated before extraction of mRNA. To reduce the number of antigen-expressing cDNA clones to be tested in vaccination trials in sheep, it was first necessary to identify probable candidates for host-protective antigens. Preliminary experiments with sera from immune sheep showed strong antibody recognition of oncosphere antigens of relative molecular mass (M_r) 47,000–52,000 (47–52K) (unpublished observations). The host-protective nature of the 47–52K antigens was demonstrated directly when lambs immunized with an SDS-PAGE cut-out of this region were protected significantly (98%) against challenge infection (Fig. 1). To isolate the genes encoding these antigens, rabbit antibodies specific for the 47–52K region were eluted from western blots and used to screen the expression library[8]. Figure 1 shows that the antibody probe was specific for the 47–52K region, and enabled us to isolate two clone types, designated 45S and 45W. Antigen-β-galatosidase (β-gal) fusion proteins prepared from the clones (β-gal–45S and β-gal–45W) failed to stimulate host-protective immunity in sheep even though antibody was produced to the 47–52K antigens (Fig. 1). This experiment, however, demonstrated that the 47–52K region contains a series of serologically-related molecules, and that the antigens, or parts of them, had been cloned.

Plasmid vectors have been constructed recently which express antigens as fusion proteins with the enzyme glutathione S-transferase (GST) of *Schistosoma japonicum*, thereby enabling

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