Understanding transmission and control of Cystic Echinococcosis and other taeniid infections in the Falkland Islands.

Submitted in partial fulfilment of the requirements of the University of Salford for the degree of Doctor of Philosophy, 2021

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October 2021

Abstract

Cystic echinococcosis, caused by the larval form of the cestode parasite *Echinococcus granulosus*, has been identified as an important public health risk in the Falkland Islands since the early 1940s. This prompted the instigation of an intensive control scheme in the mid-1960s, comprised of regular dosing of domestic dogs with the anthelmintic praziquantel and education of local people about safe disposal of potentially infected offal. This scheme has remained in place to the current day and is generally considered to be a successful programme– resulting in a reduction in the prevalence of infection in sheep has reduced from >50% in the 1950s to less than 1 % now and there has not been a case of human hydatid disease for more than 20 years. However, concerns remain that hydatid cysts are still identified in a small number of sheep at slaughter (0.004% in 2017) and occurring every year subsequently suggesting transmission is still occurring. This is also supported by the observation that sheep continue to be infected at higher levels with the (non-zoonotic) cestode *Taenia hydatigena*, also transmitted by dogs. In 2010, all dogs on the Falkland Islands were tested by Copro-PCR, resulting in eight dogs (1.4%) testing positive. The dog population was tested again in 2012, where there were no cases but when tested in 2014 by Coproantigen testing, six (1.04%) were positive for *E. granulosus* coproantigens.

This project used questionnaires, coproantigen and coproPCR analysis, abattoir data surveillance, DNA sequencing, environmental sample analysis and mathematical modelling to study *Echinococcus granulosus* and other taeniids endemic in the Falklands and investigate how their continued transmission can occur in the face of the prolonged intensive control programme. A questionnaire survey identified possible methods of disposal of offal that in a previous study, were associated with canine coproantigen positivity. The entire dog population was analysed via coproantigen techniques in 2018, and four (0.68%) dogs were coproantigen positive, though none of these were confirmed by PCR. From 2018 to 2020, five cases of CE were identified in sheep at the Sand Bay abattoir in the Falklands (0.01%), with one of the cases coming from a positive farm in 2018. There were two cases from farms with positive dogs in 2010 and one from a farm with a positive dog in 2014. To investigate environmental contamination on farms and potentially identify historical dog infections, soil samples taken from kennel sites were analysed for the presence of coproantigens, with five farms having positive results, one farm matching with a positive dog in 2018. To identify key processes fuelling the transmission of *E. granulosus* in the Falklands, a mechanically informed compartmental model was created, estimating the basic reproduction number (R_0) for the parasite, and identifying scenarios where this estimate increased above one suggesting continued transmission could occur. Seven scenarios where lapses in control measures could result in the R_0 estimate increasing above one and continued transmission of *E. granulosus* could occur.

The results of this project show clear evidence of dogs still being involved in the transmission of taeniid parasites in the Falklands, with key areas of the eradication programme such as the inadequate disposal of offal and dogs gaining access to offal allowing the transmission cycle to be completed and transmission of *E. granulosus* and other taeniids to occur. Rectifying these lapses in control measures and focussing control and surveillance to a more localised control approach will help strengthen the control programme and move the Falklands closer towards the complete eradication of Cystic Echinococcosis.

Table of Contents

Table of Figures

Table of Tables

Acknowledgements

The opportunity to be part of a research project such as this has been an unbelievable experience both academically and personally. Developing my knowledge of parasite diagnostics (and the complications that accompany it), along with entering new grounds with mathematical modelling and adapting to the challenges that have come with it has shown me that coupled with frustration and confusion is the sense of achievement in the success of a simple result eclipses the hardships into a simple memory. Not only that, but this project has also sent me 8000 miles away to a place that feels like home. The Falkland Islands and its community is a special place and has offered me opportunities to meet people and trace family footsteps, 38 years on, that one could only dream of.

Thanks, must start with Professor Mike Rogan and my supervisory team Dr Alex Mastin, Dr Haseeb Randhawa, and Dr Steve Pointing, who have offered support it every possible way. Throughout this project, each and every one of them has been an example of how supervisors should be. Whether that be a push in the right direction, a pull back away from a pointless or impossible idea, or just a beer at the end of a long day, all of my supervisors have been pillars of support and encouragement throughout. While in the Falklands, Dr Randhawa and Dr Pointing have been brilliant in helping me settle into the community, introduce me to farmers and help me understand the environment and farming practices which is the backbone of this project. They have both added greatly to the direction of the project with ideas and expertise unique to their skills sets which have complemented the project as a whole. Once back in Salford both Prof. Rogan and Dr Mastin have been vital support to me throughout this project. Prof. Rogan, from the joke he played on me while informing me I had been chosen to be his PhD student to looking at dead sheep in a field, you have imparted your wealth of knowledge and experience with a great enthusiasm and sense of humour that has encouraged me through this project, helping play to my strengths but encouraging my improvements on my weaknesses. I am privileged to be your last PhD student and I wish you all the best in your retirement. Dr Mastin, thanks doesn't cover the effort you have gone to ensuring the data analysis and modelling have run smoothly, but more importantly, you introduced me to 'The Friday Pub Group'. Many a PhD student has been saved by this group through sharing of difficulties and offering support. Dr Mastin has gone above and beyond his role offering advice during the harder times and I am very grateful for that.

Throughout my time in the Falklands, the members of the Department of Agriculture have been brilliant in welcoming me and helping out wherever possible in this project. I especially want to thank Gordon Lennie for his help in the lab and the transport of samples from the Falkland Islands to the University of Salford. Further support from Katrina Stephenson helping me with the logistics of travel in the Falklands has facilitated all aspects of field work and of course the farming community, who have shared their experience and knowledge of Hydatid disease and farming in the Falklands to make this project as informed as possible.

I have also had the amazing opportunity to work with other researchers who are experts in their field. Early support from Dr Marisol Collins, who shared her knowledge and experience in diagnostics and epidemiology of *Echinococcus* spp. Helped me build the foundations I needed to carry out these techniques on a large scale and her constant support throughout has been incredibly valuable. In the same manor, Dr Tony Bodell has been a constant help throughout this project, offering advice and encouragement. His wealth of knowledge and experience has been invaluable, and I extend my thanks to all the lab technicians at the University of Salford. I would also like to thank Paul Torgerson, who has offered his expertise and provided expert opinion on some of the modelling we have been doing. Having such advice from an expert in the field has only improved this project.

This project has been funded by the Falkland Island Government, who have supported the entire project. I am grateful to be given the opportunity to work on this project to try and help the Falkland Islands in their quest to irradicate *Echinococcus granulosus* and other parasites from the islands and I hope that this project has shown the possibility of doing so going forwards. I would also like to thank the South Atlantic Environmental Research Institute for their support in sharing information and data throughout the project.

I want to personally thank James Bryan, who I lived with in the Falkland Islands and who has become a close friend. He not only helped show me round the farms of the Falklands but also shared his homelife with me as well. Some wonderful days spent fishing the tranquil rivers or enjoying a drink watching the rugby, James you well and truly made my experience all the more enjoyable! Thanks, James.

Finally, to my friends and family, thanks unwavering support and encouragement. Mum, Jo, dad, David, Gilly and Angus, the ruthless sense of humour throughout has kept me going and the gentle comment of ‘shut up we don’t need to hear about more dog poo!’ has reminded

me there are other things outside the world of dog poo and offal! My girlfriend Nicole, you have stood by me in the tough times and put up with me when throughout! I couldn't have finished this without you and thanks doesn't cover it. A truly amazing experience that I am grateful to have shared with you all.

Chapter 1. General Introduction

Parasitic diseases of livestock have a substantial impact on animal health and can lead to significant economic loss through reduced productivity and condemnation of carcasses. Parasitic diseases which are also zoonotic infections have major public health implications. The understanding of such diseases and controlling their spread is vital in reducing their impact on the societies in which they thrive (Torgerson, 2013). Control of any zoonotic infection is challenging and involves coordinated interaction between medical, veterinary, agricultural, and governmental bodies. In addition, control programmes will involve sustained effort over long periods of time to ensure that infection levels in humans and animals remain low. Echinococcosis is one such disease affecting humans and animals. From a domestic, agricultural perspective this is primarily caused by the cestode *Echinococcus granulosus* which causes Cystic Echinococcosis (CE) or Hydatid Disease. In several parts of the world, there have been successful control programmes relating to CE and one of those has been in the Falkland Islands. However, even after more than 50 years of effort, a small number of animals remain infected and maintain the life cycle. The Falkland Islands Government (FIG) is keen to get to the situation where this parasite has been completely eradicated and the risk of re-emergence is low. This study has, therefore, been carried out to try and establish why low levels of infection still exist in livestock and what steps may be required to eliminate the parasite completely.

1.1 Biology and taxonomy of *Echinococcus granulosus*

Echinococcus spp. are cyclophyllidean cestodes (tapeworms) which belong to the family Taeniidae. In their natural life cycles taeniid parasites infect several mammalian species as both definitive and intermediate hosts. Infection is described in a definitive host as the presence of an adult tapeworm in the intestine and in the intermediate host as the presence of the larval metacestode stage of the parasite in the formation of a cyst (Craig, Rogan, & Campos-Ponce, 2003; Moro & Schantz, 2009). The Taeniidae comprises of several species affecting humans such as *Taenia solium* and *T. saginata* and some which are primarily of agricultural importance such as *T. hydatigena* and *T. ovis*. *Echinococcus* is a genus within the

Taeniidae where definitive host species are from the Canidae family including dogs, foxes, wolves, and similarly related species. Eggs from the adult parasite are shed in their faeces and contaminate the environment, infecting the intermediate host orally during feeding or grazing (Eckert & Deplazes, 2004).

A number of different *Echinococcus* species have been historically recognised, including *E. granulosus, E. multilocularis, E. vogeli, E. oligarthra, E. shiquicus* and *E. felidis* (Agudelo Higuita, Brunetti, & McCloskey, 2016). Of these, *Echinococcus granulosus* is the most important species where intermediate host species vary but ungulates are the most common group infected with the larval metacestode parasite forming cysts in the liver and lungs. Humans can act as an accidental intermediate host by consuming viable eggs through unwashed contaminated vegetables or encountering infected dog faeces (Chaâbane-Banaoues, Oudni-M'rad, Cabaret, M'Rad, Mezhoud, & Babba, 2015). *E. multilocularis* is a related parasite causing Alveolar Echinococcosis (AE) in humans, which primarily infects rodents as intermediate hosts with wild or domestic canids acting as definitive hosts. However, the taxonomy of *E. granulosus* is currently being refined due to the identification of genetic and phenotypic variation between isolates from different intermediate host species and geographical locations (Vuitton, McManus, Rogan, Romig, Gottstein, Naidich, Tuxun, Wen, & Menezes da Silva, 2020). At present there are ten recognised genotypes (G1-10) described within the *Echinococcus granulosus sensu lato (s.l.)* complex (Dybicz, Borkowski, Dąbrowska, & Chomicz, 2015; Zait, Kouidri, Grenouillet, Umhang, Millon, Hamrioui, & Grenouillet, 2016). Genotypes *E. granulosus* G1 (Common sheep strain), *E. granulosus* G2 (Tasmanian sheep strain) and *E. granulosus* G3 (Buffalo strain) are known as *Echinococcus granulosus sensu stricto (s.s)* and are the most common cause of human CE (Kinkar, Laurimäe, Acosta-Jamett, Andresiuk, Balkaya, Casulli, Gasser, González, Haag, Zait, Irshadullah, Jabbar, Jenkins, Manfredi, Mirhendi, M'Rad, Rostami-Nejad, Oudni-M'rad, Pierangeli, Ponce-Gordo, Rehbein, Sharbatkhori, Kia, Simsek, Soriano, Sprong, Šnábel, Umhang, Varcasia, & Saarma, 2018). The remaining genotypes are *E. equinus* G4 (Horse strain), *E. ortleppi* G5 (Cattle strain), and *E. canadensis* G6-10 (previously described as *E. granulosus* camel strain-G6, pig strain-G7, cervid strain-G8, and genotypes G9–G10, also referred to as *E. intermedius*) (Nakao, Lavikainen, Yanagida, & Ito, 2013; Thompson & McManus, 2002). *Echinococcus granulosus (s.s), E. equinus, E. ortleppi, E. oligarthra, E. shiquicus, E. canadensis* and *E. felidis* are now,

therefore, often considered as separate species (Nakao, McManus, Schantz, Craig, & Ito, 2007; Romig, Ebi, & Wassermann, 2015; Thompson, 2017; Vuitton et al., 2020).

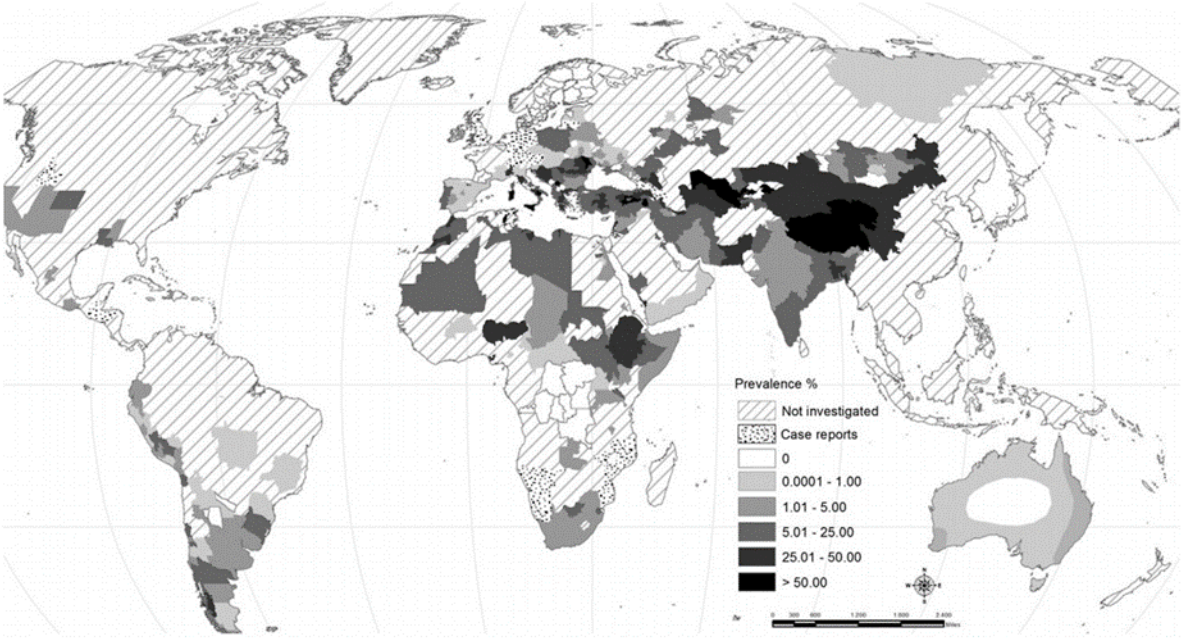


Figure 1: Global distribution of Cystic Echinococcosis in domestic intermediate hosts (Deplazes, Rinaldi, Alvarez Rojas, Torgerson, Harandi, Romig, Antolova, Schurer, Lahmar, Cringoli, Magambo, Thompson, & Jenkins, 2017).

CE has a global distribution (Figure 1) but has been described as a Neglected Tropical Disease (NTD), meaning that the disease has received relatively little attention, and is most common in areas and countries with high levels of poverty and poor healthcare. The parasite is known to exist in both domestic and sylvatic lifecycles, with the disease being most common where agriculture is a main contributor to the country's economy (Piseddu, Brundu, Stegel, Loi, Rolesu, Masu, Ledda, & Masala, 2017) and the highest risk to humans coming from areas with high levels of pastoral agriculture and where humans work and live closely with dogs (Campos-Bueno, López-Abente, & Andrés-Cercadillo, 2000). In North America, one of the main risks to humans is *E. canadensis,* that is normally transmitted between wolves and cervids like moose, elk, caribou, and deer (Lichtenwalner, Adhikari, Kantar, Jenkins, & Schurer, 2014; Rausch, 2003). Indigenous populations in North America are most at risk to this genotype though their rural lifestyle and due to their use of sled dogs (Deplazes, Rinaldi, Alvarez Rojas, Torgerson, Harandi, Romig, Antolova, Schurer, Lahmar, Cringoli, Magambo, Thompson, & Jenkins, 2017). However, CE is generally only a major public health problem in situations where domestic dogs act as the definitive host. In most pastoral agricultural settings in which human CE is an issue, *E. granulosus* s.s. is the most common cause. North

Africa is a highly endemic area for CE with 1.9% of people positive for abdominal CE based on ultrasound testing (Chebli, Laamrani El Idrissi, Benazzouz, Lmimouni, Nhammi, Elabandouni, Youbi, Afifi, Tahiri, Essayd El Feydi, Settaf, Tinelli, De Silvestri, Bouhout, Abela-Ridder, Magnino, Brunetti, Filice, & Tamarozzi, 2017). Lack of education, poor hygiene and sanitation are all important factors in the distribution of CE because understanding of risk and the ability to prevent infection is reduced, leading to a higher prevalence of infection (Buishi, Njoroge, Zeyhle, Rogan, & Craig, 2006; Deplazes et al., 2017). Cultural and religious beliefs also influence the distribution of CE. Tibetan communities have been described as hyper-endemic groups for CE. As with the described influences of rural communities, poor sanitation and education contributing to higher prevalence of CE, it has also been observed that the Buddhist beliefs of leaving older animals to die naturally and offal not being disposed of contributes to the continued transmission of CE in livestock and humans in areas such as Tibet. These are some factors that contribute to the global distribution of CE alongside the factor of global pastoralism and animal husbandry (Yang, McManus, Huang, & Heath, 2009).

The pathology of human CE is caused by the metacestode stage of the parasite and characterised by the development of fluid filled cysts predominantly in the liver and lungs. Cysts develop slowly over a number of years and the patient remains asymptomatic until the cyst reaches a size large enough to cause major pathology (Craig, Budke, Schantz, Li, Qiu, Yang, Zeyhle, Rogan, & Ito, 2007; Eckert & Deplazes, 2004). Symptoms appear as a result of the unilocular cyst development impacting on the function of the organ where they have developed. If located in the liver, symptoms can include enlargement of the liver, abdominal pain, jaundice, and secondary cirrhosis. If located in the lungs, symptoms may present as chest pains and chronic coughing. In other parts of the body, cysts act as space-occupying lesions (Eckert, Gemmell, Meslin, Pawlowski, & World Health, 2001). Treatment of CE is complex as the patient can remain asymptomatic for several years post infection. Diagnosis and estimation of cyst numbers and size in humans is conducted via imaging techniques such as ultrasound, CT scanning and MRI, with ultrasound most commonly used. Molecular diagnosis using Polymerase Chain Reaction (PCR) can be used to definitively confirm the diagnosis from tissues taken at surgery (Zhang & McManus, 2006). Additionally, serology is a tool used in detecting *E. granulosus* in humans, using antibody detection methods such as ELISA to detect parasite-specific antibodies against cyst fluid antigen (Barnes, Deplazes,

Gottstein, Jenkins, Mathis, Siles-Lucas, Torgerson, Ziadinov, & Heath, 2012; Brunetti, Kern, & Vuitton, 2010; Ito & Craig, 2003).

Once a patient is confirmed with CE, there are four treatments used generally to monitor, remove, or reduce the cysts and prevent rupture and further infection; these are surgery, chemotherapy, puncture, aspiration, injection, and re-aspiration (PAIR), and watch and wait (Velasco-Tirado, Alonso-Sardón, Lopez-Bernus, Romero-Alegría, Burguillo, Muro, Carpio-Pérez, Muñoz Bellido, Pardo-Lledias, Cordero, & Belhassen-García, 2018). Surgery is used to reduce the size or remove the cyst and attempt to repair the infected organ. Historically, surgery has been the main treatment, often combined with chemotherapy. Chemotherapy treatment involves the use of albendazole or mebendazole (anthelmintic therapeutics) administered continuously for a prolonged period. The drugs disrupt the glucose absorption of the metacestode parasite, having degenerative effects on the mitochondria (Smego & Sebanego, 2005). Another form of treatment is known as puncture, aspiration, injection, re-aspiration (PAIR). This percutaneous treatment is seen as a less invasive treatment and is restricted to treating hepatic cysts and involves puncturing the cysts and aspirating the cyst fluid then re-aspirating the cyst with scodicidal solution to destroy any protoscoleces and the germinal layer of the cyst (Junghanss, da Silva, Horton, Chiodini, & Brunetti, 2008; Rajesh, Dalip, Anupam, & Jaisiram, 2013). This method of treatment is also combined with chemotherapy to increase chances of patient recovery (Smego & Sebanego, 2005). Watch and wait is the final treatment method, that involves leaving the cyst untreated and monitoring them over time. This is often used in cases where cysts have no major impact on the patient and are not impacting the functions of any major organs. Results from this treatment often result in the cysts remaining stable and calcifying over time and becoming completely inactive. This treatment also removes any risks that are associated with the other treatments (Junghanss et al., 2008).

1.1.1 Economic losses in livestock due to CE.

Infection of intermediate hosts with CE impacts farmers economically by reducing the productivity of the livestock. Cystic Echinococcosis can reduce productivity, fertility and growth rate while animals are being reared as well as reducing product yield and quality of produce, such as milk production, wool growth or carcass weight and quality (Budke,

Deplazes, & Torgerson, 2006). Further losses occur when internal organs such as liver and lungs are condemned, which reduces sales income as well as accruing additional costs associated with the destruction of infected material (Benner, Carabin, Sánchez-Serrano, Budke, & Carmena, 2010). CE is often endemic to poor rural communities that rely on the income of all production from livestock. The economic impact in Peru, where prevalence of CE in sheep is as high as 77% in sheep, identified direct losses from liver condemnation to be USD $196,681 annually. This figure increased to USD $3,846,754 when indirect losses from reduced yields of carcass weight, milk production and wool weight were included in the calculation (Moro, Budke, Schantz, Vasquez, Santivañez, & Villavicencio, 2011). Losses of this scale can have significant impacts on rural farmers income. Kenya recorded similar losses to livestock traders due to condemnation of offal of USD $152,003 from a single county in a year (Kere, Joseph, Jessika, & Maina, 2019). These losses are small in comparison to some areas such as Argentina and Iran where annual costs of USD $4.7 million and USD $457,582 have been estimated respectively (Bingham, Larrieu, Uchiumi, Mercapide, Mujica, Del Carpio, Hererro, Salvitti, Norby, & Budke, 2016; Vafaei, Faridnia, Mostafaei, Azadi, Damod, Fakhar, & Kalani, 2017). The variation in losses between locations can be influenced by the prevalence of CE in a population, the number of animals slaughtered and the respective value of livestock (Kere et al., 2019; Vafaei et al., 2017). Further economic losses can occur in highly endemic regions as a result of trading restrictions and bans on movement of livestock as a result of CE in the populations (Battelli, 2009). The economic impact of CE is amplified by the fact that areas with greater incidences of *E. granulosus* are often some of the most socioeconomically vulnerable countries and communities globally. These communities are more dependent on sale of the entire carcass and small decreases in productivity can reduce the already low income gained from livestock production (Budke et al., 2006).

1.1 Life cycle and transmission of *Echinococcus granulosus*

Echinococcus species causing CE share similar life cycles which involves two hosts, one acting as the definitive host where the adult tapeworm develops and the other as the intermediate host where the larval metacestode develops. The life cycle of *E. granulosus s.s* (Figure 2) in agricultural communities is largely maintained by the ingestion of the offal of infected sheep by domestic dogs and the subsequent ingestion of eggs by sheep, and therefore requires the

presence of both dogs and sheep, as is common in many pastoral communities (Alishani, Sherifi, Rexhepi, Hamidi, Armua-Fernandez, Grimm, Hegglin, & Deplazes, 2017; Craig, Mastin, van Kesteren, & Boufana, 2015). Dogs can become infected by feeding on the viscera of livestock via a number of routes: (i) by actively killing animals in a predator-prey relationship; (ii) by scavenging on animals that have died naturally in open areas; (iii) by being fed raw offal by farmers. This latter route is particularly important in areas where unregulated home slaughter occurs (Craig et al 2007). When dogs consume hydatid cysts, they may ingest several thousand protoscoleces, depending on the size and number of cysts which are eaten. Protoscoleces attach to the wall of the small intestine and develop into adult tapeworms. These grow only to around 5-7 mm in size and become fully mature by around 35-42 days producing a single gravid proglottid containing eggs (Eckert et al., 2001).

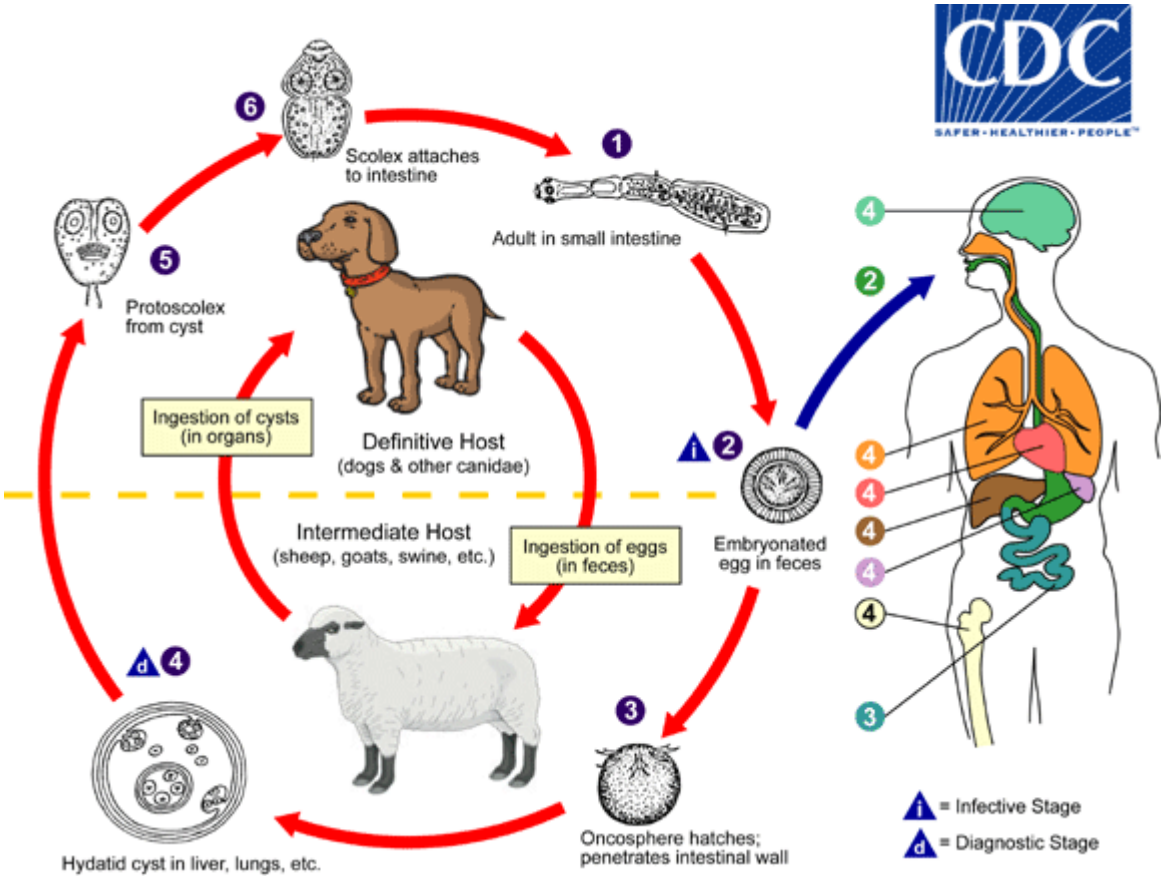


Figure 2: Life Cycle of Cystic Echinococcosis. (Reproduced from the Centres for Disease Control and Prevention at https://www.cdc.gov/parasites/echinococcosis/biology.html. 1. Adult parasite lives in the small intestine of the canid definitive host, releasing eggs via gravid proglottids in the canid's faeces. 2. Eggs are then ingested by an intermediate host and hatch in the small intestine developing into oncospheres that penetrate intestinal wall and enter the blood stream travelling to various organs, most commonly the liver and lungs, developing into hydatid cysts. As the cysts develop and grow, protoscoleces are produced on the interior of the cyst. 5. The definitive host becomes infected when it consumes organs containing the viable cysts. The protoscoleces from the cyst evaginate and attach to the intestinal mucosa, 6. Developing into adult tapeworms in 35 to 80 days.

Eggs excreted in dog faeces contaminate both the dog fur and the environment. The eggs are very resistant to environmental factors such as temperature gradients, sunlight and humidity gradients (Eckert & Deplazes, 2004) and can survive for many months in the environment. Ideal conditions for prolonged survival of taeniid eggs are cool temperatures in areas with plenty of shade and humidity (Thevenet, Jensen, Drut, Cerrone, Grenóvero, Alvarez, Targovnik, & Basualdo, 2005). Livestock become infected by eating *Echinococcus* eggs on contaminated pasture. The eggs hatch and the oncosphere larvae penetrate the wall of the intestine to enter the blood stream. Larvae are then transported to organs such as the liver and lungs where they are filtered out and begin to develop into hydatid cysts. This is a slow process and cysts remain less than a few mm in size for several months. Hydatid cysts are said to be fertile when they produce protoscoleces within the cyst fluid which often takes more than 6 months to occur. The cysts continue to grow slowly and in sheep may reach sizes of 3 cm or more after several years. The mean number of protoscoleces developing within cysts varies with age with animals less than one year producing an average of 215 protoscoleces in cysts 0.5-2cm in diameter, whilst those in animals over six years of age producing an average of 12,603 protoscoleces in cysts 0.5-8cm in diameter (Torgerson *et al* 2009).

Human infection is also a result of oral consumption of *Echinococcus* eggs from contaminated environments. The exact route of transmission can vary depending on a variety of factors. Eggs released in a dog's faeces can adhere to their coats as well as contaminating the ground. Dogs experimentally infected with *T. hydatigena* were found to have up to 210 eggs per cm^2 in the region around the anus and up to 20 eggs per cm^2 across the rest of their body (Deplazes & Eckert, 1988). Humans who come in contact with dogs and do not wash their hands before touching their mouths are at risk of becoming infected accidentally (Alvarez Rojas, Mathis, & Deplazes, 2018). Children in endemic areas are also most likely to be exposed due to their regular hand to mouth contacts. Children's hand to mouth contact exposure showed they can touch their mouths up to 28 times per hour when indoors and up to 14 times per hour outdoors. If they are touching a dog that has rolled in infected faeces or the area in which they are playing is contaminated with infective eggs, children are at a high risk of becoming infected (Xue, Zartarian, Moya, Freeman, Beamer, Black, Tulve, & Shalat, 2007). Human infection could also occur via unwashed fruit and vegetables. Market produce sampled across Europe found that 30 of 141 products were contaminated with cestode eggs, including the

zoonotic eggs of *E. granulosus,* suggesting that the growing environment of the fruit and vegetables has been contaminated with dog faeces. If humans eat the produce without washing it, they expose themselves to infection with *E. granulosus* resulting in CE (Federer, Armua-Fernandez, Gori, Hoby, Wenker, & Deplazes, 2016) .

1.2.1 Other relevant taeniid species.

In studying the transmission of *E. granulosus* it is important to consider other related parasites which share similar life cycles. Species such as *T. hydatigena* and *T. ovis* have the same ovine intermediate and canid definitive hosts (Miran, Kasuku, & Swai, 2017), although the rate of egg production from a single worm (which is related to the "biotic potential" of the parasite) is greater than that of *Echinococcus* due to the larger size of the adult worm (Phythian, Stafford, Coles, & Morgan, 2018b; Torgerson & Heath, 2003). As with *E. granulosus,* adult *T. hydatigena* and *T. ovis* reside in the small intestine of dogs, releasing eggs in the faeces. Once ingested by livestock such as sheep, eggs hatch in the small intestine and the oncosphere migrate in the bloodstream to the liver and other organs such as the kidneys, lungs, heart, and other organs within 3 months (Corda, Dessì, Varcasia, Carta, Tamponi, Sedda, Scala, Marchi, Salis, Scala, & Pinna Parpaglia, 2020; Hajipour, Allah Rashidzadeh, Ketzis, Esmaeili Seraji, Azizi, Karimi, Bagherniaee, & Montazeri, 2020). Once the *T. ovis* oncosphere reach these organs, they develop into small (6-100mm) fluid filled cysts containing a single scolex (DeWolf, Peregrine, Jones-Bitton, Jansen, MacTavish, & Menzies, 2012). *Taenia hydatigena* oncospheres mature into larger (1-10cm) fluid filled cysts, again with a single scolex. These are found in the omentum, mesentery, and peritoneum (Scala, Urrai, Varcasia, Nicolussi, Mulas, Goddi, Pipia, Sanna, Genchi, & Bandino, 2016). Infection with these parasites is generally asymptomatic, and diagnosis occurs at slaughter, however, in the case of *T. hydatigena*, severe infection can cause hepatitis and peritonitis, predominantly in younger animals. Though not causes of major pathology, the presence of these parasites in sheep results in the condemnation of carcasses and economic losses. Transmission of *T. ovis* and *T. hydatigena* is continued when these cysts are ingested by a dog, where the single scolex attaches to the wall of the small intestine and matures into an adult once more (Jenkins, Urwin, Williams, Mitchell, Lievaart, & Armua-Fernandez, 2014). These parasites often infect the same definitive and intermediate hosts as *E. granulosus* and the metacestode stage of these species are often found in livestock at slaughter (Miran et al., 2017). They are endemic

to similar areas due to their association with pastoralism and animal husbandry practices and their similar life cycles (Gessese, Mulate, Nazir, & Asmare, 2015). The presence of *T. hydatigena and T. ovis* in livestock is an indicator that infected dogs have been present. In areas where control programmes exist, this is also an indicator that anthelminthic treatment of dogs has not been fully effective.

1.2.2 Transmission dynamics of *Echinococcus granulosus*

Taeniid parasites have great biotic potential to contaminate the environments in which they are found due to their ability to produce and expel high numbers of eggs from the definitive host (Cabrera, Haran, Benavidez, Valledor, Perera, Lloyd, Gemmell, Baraibar, Morana, Maissonave, & et al., 1995). In the natural environment, transmission of *E. granulosus* and other taeniid cestodes is dependent on several factors. Parasite populations exist in each host and within the environment, therefore, host population size is an important factor influencing the transmission dynamics. The presence of the parasites within their hosts is over-dispersed such that relatively few animals have high levels of infection with most having low or no infection. The biotic potential of worms within dogs will determine the level of environmental contamination and subsequent likelihood of infection to sheep. Within the intermediate host, infection levels are influenced by a variety of factors such as spatial distribution of eggs, the age of the eggs at the time of ingestion, density-independent constraints on egg viability and infectivity (e.g., temperature and humidity), age of the hosts when first exposed to eggs, heterogeneity of individuals within the flock, and density dependent constraints, such as immunity. In a farming setting the factors which contribute to the transmission of taeniid parasites involve both extrinsic and intrinsic factors as well as socioecological factors (see Figure 3).

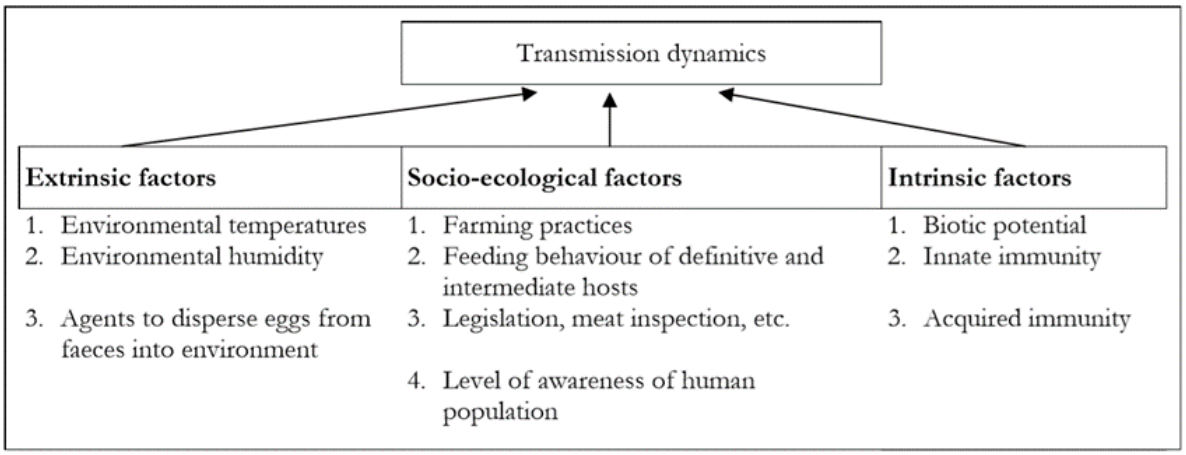


Figure 3: Extrinsic, Socio-ecological, and Intrinsic factors that contribute to the transmission dynamics of *E. granulosus* (Gemmell, 1990).

The persistence and stability of a parasite population within an environment is, therefore, dependent on a number of intrinsic and extrinsic factors and is driven by the Basic Reproductive Ratio (R_0) which in helminths, can be defined as the ratio of the number of adult parasites in the following generation to the number of adult parasites in the present generation. By definition, R_0 is the reproduction ratio in the absence of density-dependent constraints (Gemmell, Lawson, & Roberts, 1986a) but the Effective R_0 (R) accounts for these constraints. If a parasite population is neither increasing nor decreasing with time, then it is in a steady state and its effective R_0 is equal to 1. For cestode parasites epidemiological steady states can be distinguished into three different states: Endemic steady state, Hyperendemic steady state, and Extinction steady state. Endemic steady state occurs when the parasite population size is constant, and the effects of density-dependent constraints are insignificant ($R_0 \cong 1$). Hyperendemic steady state occurs when the parasite population size is constant, and the population is strongly regulated by density-dependent constraints ($R_0 >> 1$). Extinction steady state occurs when the parasite is in decline and moving towards elimination ($R_0 < 1$).

The objective of any control programme aiming to eradicate a parasite from a host population would be to drive R_0 into the Extinction steady state.

1.3 Control of Echinococcosis

1.3.1 Managemental control options

To control cestode parasites, the transmission cycle must be broken to prevent the completion of the life cycle. Simple managemental control measures have been used to do this by preventing the definitive host coming into contact with infective material. Disposal of

offal rather than feeding it to dogs and controlling where dogs can go as well as housing them suitable when not being used for work can stop the transmission between sheep and dogs, therefore preventing adult parasites developing (Heath, Yang, Li, Xiao, Chen, Huang, Yang, Wang, & Qiu, 2006). Knowledge of the life cycle and the routes of transmission are important in controlling cestode parasites, therefore educating the communities at risk of CE is a vital aspect of any control programme, promoting good hygiene and reducing risk factors and behaviours such as feeding offal to dogs when slaughtering animals at home can support the other managemental control measures being successful (Craig, Hegglin, Lightowlers, Torgerson, & Wang, 2017).

1.3.2 Pharmaceutical control options for definitive host.

Early pharmaceutical control methods involved the removal of parasites from the intestine of the dog by purgation, the evacuation of the bowl brought about by the administration of a purgative drug. Arecoline salts purgation was the most common purgation method, with its 100% specificity allowing for visualisation of *Echinococcus* for visual inspection (Craig, Gasser, Parada, Cabrera, Parietti, Borgues, Acuttis, Agulla, Snowden, & Paolillo, 1995b). Arecoline salts do have some major disadvantages, including variable sensitivity and failure rate of between 10-20% (Craig, Gasser, Parada, Cabrera, Parietti, Borgues, Acuttis, Agulla, Snowden, & Paolillo, 1995a; Wachira, Macpherson, & Gathuma, 1990). Moreover, the process is time consuming, taking sometimes 30 minutes for the purge to take place. On some occasions not taking place at all, resulting in a second dose being administered (Lahmar, Lahmar, Boufana, Bradshaw, & Craig, 2007). Another major disadvantage is the biohazardous impact on the dogs, causing serious distress in some cases (Eckert et al., 2001). These issues resulted in the move towards less harmful anthelmintic drugs such as praziquantel and albendazole for the removal of intestinal parasites.

Since the introduction of drugs like albendazole, and praziquantel, most CE control programmes focus in interrupting the parasite life cycle at the definitive host level. The aim of which is to eliminate adult tapeworm by regular anthelmintic treatment and reduce the risk to humans and ungulates over time. The main anthelmintic drug used is praziquantel, due to the broad range of parasites it is effective against. Once administered, praziquantel is rapidly absorbed and can be detected in the blood within one hour (Steiner, Garbe, Diekmann, & Nowak, 1976). Anthelmintic drugs have been used globally in control

programmes. However evidence has shown that control programmes based on treatment of dogs required over ten years of compulsory regular treatment to reduce CE transmission to very low levels (Craig & Larrieu, 2006). The extended course of treatment over many years can be expensive to maintain, especially in poor rural areas where CE is most prevalent (Larrieu & Zanini, 2012).

The continuous repeated use of such drugs can allow for possible resistance to be built up over time. Evidence of such drug resistance has been seen in the canid tapeworm parasite *Dipylidium canium*, where veterinarians in the USA reported failed treatments of the parasite at the prescribed dose. In one case of four infected dogs, none of the infections were resolved by praziquantel at the prescribed dosage and required a combination of therapies as the infections were also resistant to the use of Epsiprantel, another anti-parasitic drug (Jesudoss Chelladurai, Kifleyohannes, Scott, & Brewer, 2018). There is further evidence of praziquantel resistance being seen in *Taenia* saginata, the beef tapeworm, with resistant cases in humans being observed in India, resulting in the use of an alternative, Nitazoxanide, to clear infections after multiple failed praziquantel treatments (Lateef, Zargar, Khan, Nazir, & Shoukat, 2008).

The efficacy of praziquantel is dependent on the correct dosage being administered. Praziquantel has an efficacy of more than 99% against cestode parasites, when administered at the correct dose rate (Altreuther, Radeloff, LeSueur, Schimmel, & Krieger, 2009; Gemmell, Johnstone, & Oudemans, 1977; Schmid, Rohdich, Zschiesche, Kok, & Allan, 2010; Thomas & Gönnert, 1978). This efficacy does reduce when the drug is administered insufficiently, such as too small a dose for the size of the dog, which can result in infections not being removed and risks further transmission. In rural control programmes, the individuals dosing the dogs is often a lay person and so, if not trained or experienced, inaccuracies in dosing could result in the survival of cestode parasites and continued transmission (Šarkūnas, Vienažindienė, Rojas, Radziulis, & Deplazes, 2019).

With the successful use of anthelmintic drugs to kill parasites within the definitive host, there has been limited research on the development of a vaccine against *E. granulosus* in dogs. However, a number of studies have shown that good protective responses can be obtained with recombinant antigens and novel delivery systems (Pourseif, Moghaddam, Saeedi, Barzegari, Dehghani, & Omidi, 2018; Zhang, Zhang, Shi, Li, You, Tulson, Dang, Song, Yimiti, Wang, Jones, & McManus, 2006).

1.3. Control options for intermediate hosts

As well as reducing infection in definitive hosts a number of approaches have been used to determine whether intermediate hosts could be protected against infection. Therefore, immunisation studies began to add further directed controls at specific hosts and stages of the parasite's lifecycle. Early studies used antigens from activated *E. granulosus* onconspheres to attempt to induce resistance to infection in sheep leading to a high degree of immunity to oral infection with eggs. However, it was noted that immunity was only impactful against early-stage infection and prior to cyst development. This was the first evidence of immunisation in sheep using secreted antigens to immunise sheep against *E. granulosus* infection (Osborn & Heath, 1982). It was later discovered that a host-protective recombinant antigen of taeniid cestodes, known as 45W, that was based off *T. ovis* onconspheres. Further studies found no evidence of any cross-protection with *E. granulosus* or a homologous gene (Johnson, Harrison, Lightowlers, O'Hoy, Cougle, Dempster, Lawrence, Vinton, Heath, & Rickard, 1989). Finally, a vaccine for *E. granulosus* derived from a cloned recombinant protein from mRNA using onconspheres, called EG95 was developed in 1996 (Lightowlers, Lawrence, Gauci, Young, Ralston, Maas, & Heath, 1996). Sheep were protected from infection with *E. granulosus* eggs with an efficacy of 96-98%. Further developments were required to establish dosage, length of immunity and other mechanisms of the vaccine prior to its use in control programmes. The vaccine EG95 was trialled in Australia and Argentina to establish the effectiveness of the vaccine in sheep. The results of the trials found protection to be 96-100% when challenged against eggs from New Zealand, Australian and Argentinean isolates (Lightowlers, Jensen, Fernandez, Iriarte, Woollard, Gauci, Jenkins, & Heath, 1999). Further research on the longevity of effective immunity showed that two injections induce 85% protection for 12 months, but if a third injection is administered six to 12 months after the second one, then a much higher level of protection occurs and can last for three to four years (Heath, Jensen, & Lightowlers, 2003). After three years of vaccinating lambs, the prevalence of CE in sheep up to three years of age reduced from 26.2% to 7.8%. As the study only lasted three years, it could not be concluded whether sheep remained protected for their entire lifetime. However, the evidence from the study showed that administering vaccinations to sheep is a viable approach to help support control programmes (Larrieu, Herrero, Mujica, Labanchi, Araya, Grizmado, Calabro, Talmon, Ruesta, Perez, Gatti, Santillán, Cabrera, Arezzo,

Seleiman, Cavagión, Cachau, Alvarez Rojas, Gino, Gauci, Heath, Lamberti, & Lightowlers, 2013).

1.3.4 Examples of historical control schemes

The control and elimination of CE has been attempted for over 100 years in various countries. The first documented control strategy was undertaken by the Icelandic Government in 1864. At the time, Iceland had the highest recorded prevalence of *E. granulosus* in humans, with 22% of autopsies showing the presence of hydatid cysts between 1861 and 1870. The prevalence of dogs infected with *E. granulosus* was 28% in 1863 (Beard, 1973). The Icelandic control programme began in 1863 by educating the public using a detailed document informing the population about hygiene and prevention of infection, as well as introducing Hydatid disease prevention to the school curriculum to educate children. In rural communities, committees were set up, known as Sanitary boards, responsible for control of Hydatid Disease in the area (Beard, 1973).

During the 1870s, 80s and 90s, there were several outbreaks of Canine distemper which reduced the dog population, especially feral dogs (Sigurdarson, 2010), and thereby restricted the availability of hosts to become infected and reduced the rate of transmission. Further control measures were implemented in 1900 that included the building of controlled slaughterhouses to reduce the transmission of *E. granulosus* to dogs. Following this, the sale of home slaughtered meat was prohibited in 1920 and incentives, such as subsidies were offered to farmers who sent all animals to recognised slaughterhouses, thus ending the largescale unsupervised slaughter on farms. A total ban on slaughtering animals on farms was then introduced in 1947, stating that slaughter of any healthy sellable sheep would result in loss of subsidy for the farmer (Beard, 1973).

Restrictions on dog movements were imposed in 1953 when legislation was introduced to update and replace previous legislation. This resulted in dogs being controlled or banned by local law, with taxes brought in for the ownership of farm dogs and increased taxes on ownership of pet dogs. Police were also instructed to shoot any stray dogs found without owners. The legislation also brought in restrictions on farmers, banning all dogs from outbuildings and areas where sheep were enclosed. Offal disposal was also regulated, stating that any farmer that slaughters sheep that have cystic lesions, must bury, or burn offal immediately, with large fines for any individual caught not following the law (Beard, 1973;

Sigurdarson, 2010). Iceland also introduced the dosing of dogs annually with an anthelmintic drug at the end of the abattoir season to ensure if any transmission had occurred to the dogs, the parasite was then killed. This control resulted in the eradication of CE in Iceland from 1979 (Sigurdarson, 2010).

More recent control programmes have been implemented with varying success. In 1971 Cyprus implemented similar measures as Iceland to try and reduce the widespread cases of CE in sheep. Prevalence in adult sheep ranged from 40-100% and from 4-50% in lambs. There were also 12.9 human cases per 100,000 people between 1933 and 1968. The main causes of this high prevalence were a high population of stray dogs, uncontrolled slaughter and offal disposal, and limited understanding of the lifecycle and transmission of *E. granulosus*. Education of the public began in 1971 with home visits and public lectures by veterinarians. With the improved understanding of *E. granulosus,* dead animals and stray dogs were avoided and reported to authorities. Alongside this, a total of 41,670 stray dogs were euthanised over a five-year period. In the years from 1972 to 1974, 98% of the dog population was tested annually through purgation with Arecoline hydrobromide, discovering prevalences as high as 6.8%. All infected dogs were destroyed with the permission of their owners.

During a five year time span, there was a 84% decrease in infections from 6.8% to 1.1% (Polydorou, 1977). As in Iceland, fees for ownership of dogs were implemented. However, in Cyprus, the registration fee for owning un-spayed bitches was increased, with the cost for a spayed bitch remaining the same as a male dog, resulting in the reduction of the un-spayed bitches to just one-third of the dog population. The construction and regulation of abattoir facilities and the introduction of legislation requiring all animals to be slaughtered in such facilities also contributed to the success of the campaign by increasing inspection and appropriate disposal of offal (Polydorou, 1977). Though the control programme in Cyprus showed success in the attack phase of implementing control measures and educating the population, the eradication campaign was only continued in the Republic of Cyprus after the Island was divided in 1974. In the government-controlled areas where disease control was active, cases continued to decline until 1985. The control programme was therefore ceased as the parasite was thought to have been eradicated in livestock and dogs (Economides & Christofi, 2000; Economides, Christofi, & Gemmell, 1998). Conversely, active transmission continued at high rates in the non-government controlled Northern Cyprus. Following the

termination of the control programme, sporadic cases were discovered in the Republic of Cyprus. The government was unable to establish whether the cases stemmed from the introduction of animals smuggled illegally from Northern Cyprus, or in cases far from the border, residual infections that were not discovered during the attack phase of the programme.

In 1989, *E. granulosus* was discovered in 79 villages in both dogs and livestock, resulting in the control programme being reintroduced in 1993. The focus was now on surveillance of livestock, restricting movement of animals and the treatment of dogs in areas where infection was recorded. Rates of infection began to decrease once again to less than 1%. However, the government maintained the control measures and entered a permanent consolidation phase (Economides & Christofi, 2000).

New Zealand has many similarities in agricultural practices to the Falkland Islands, with large numbers of sheep bred for the wool industry. In the 1950s, New Zealand's dog population was approximately 200,000, with a local *E. granulosus* prevalence of 10-37% when tested by Arecoline purgation (Gemmell, 1990). In 1959, the New Zealand government set up the National Hydatid Council, whose role was to create and implement policies to control Hydatid disease. The campaign began by educating communities by administering individually adapted programmes for the needs of each community. This, along with arecoline testing, was relied upon for the first ten years of the campaign (Gemmell et al., 1986a; Lawson, 1994). At the same time as the education programme was rolled out, New Zealand began registering all urban and rural dogs including a compulsory fee for ownership. This helped finance the National Hydatid Council and its control programme supporting arecoline/anthelminthic six-weekly treatments. From 1959 to 1972 Arecoline purging was used, before moving to the use of anthelminthic drugs such as niclosamide and ultimately praziquantel.

Annual testing of dogs by specially trained Hydatid control officers observed a decrease in infections in dogs from as high as 8% in some regions to less than 1% between 1960 and 1975 (Burridge & Schwabe, 1977). The Ministry for Agriculture (MoA) reviewed annually the control programme, monitoring the success, and in 1990, the MoA introduced the control of livestock movements for farms that were diagnosed with Hydatid disease through slaughter surveillance.

In 1991, the National Hydatid Council working at a local level was disbanded after a review of the control programme, with focus switching to slaughter surveillance and tracing infections to individual farms. This marked the move into the consolidation phase; however, Hydatid control officers continued to be employed on a local level to carry out farm inspections and praziquantel dosing of dogs, which was maintained until 1996. The last outbreak of Hydatid disease involving large numbers of animals was discovered in 1990 with infection in multiple neighbouring farms discovered via abattoir surveillance, however these farms were subsequently cleared of infection in 1993. In the years following, calcified cysts were discovered from a single farm, but no new infections were discovered. New Zealand then entered a maintenance of eradication phase, continuing control measures such as offal disposal and abattoir surveillance permanently (Burridge & Schwabe, 1977; Economides et al., 1998; Lawson, 1994; Pharo, 2002).

As a result of the discovery of high prevalences of CE in Australia, investigation into controlling the worst affected areas began in 1963. Between 1946 and 1958 there were 92.5 cases per 100,000 during the decade (Gemmell, 1961). In a later study by Meldrum and McConnell (1968) discovered a figure of 151 cases per 100,000 over a similar time period (discrepancy comes from the possible under reporting from surgeons). The prevalence of CE in adult sheep was greater than 50% which prompted the initiation of Tasmania's Hydatid Control programme in 1965 (Meldrum & McConnell, 1968). Tasmania followed similar practices to New Zealand, focusing on prevention of the life cycle being completed in the definitive host. Tasmania is unique in the fact that there is only one species suitable to act as a definitive host. Unlike the mainland of Australia, the island state is free of dingoes, therefore only domestic dogs can transmit the adult parasite.

The main difference in the control programme in Tasmania, compared to other nations, was that the focus on control, rather than eradication, based on the favourable conditions that support transmission and the government described it as improbable based on the existing information at the time. The initial focus was to reduce the prevalence in humans – the cause of a serious public health problem – as well as to reduce the financial losses due to the condemning of offal. The Tasmanian Government established The Tasmanian Hydatids Eradication Council that represented farmers and organisations associated with the agricultural industry and was made up of influential local community members that helped

increase the community involvement in the programme. The Council began by introducing Arecoline testing annually from 1965 along with anthelmintic treatment targeting the tapeworm itself. The Department of Agriculture also introduced a series of recommendations to prevent the reinfection of dogs following treatment. These began with the prohibition of offal being fed to dogs, even when cooked. There was clear emphasis on improving the hygiene standards around home slaughter of animals, especially when disposing of offal, thus ensuring it could not be accessed by dogs.

Following the control programme, there were no human cases in children under the age of ten from 1974, and no cases under the age of 20 by 1976. This success was also seen in the reduction in prevalence within the sheep population to below 1% by 1982. The Council then made the decision to move into the consolidation phase after ten years of the control programme, continuing to test dogs, only on farms where infected sheep were discovered at slaughter surveillance. The discovery of infected sheep resulted in quarantine of the farm, and sale restrictions on sheep, only allowing sale to official abattoirs (Craig et al., 2015; Economides et al., 1998; Meldrum & McConnell, 1968).

The Tibetan Communities of Western Sichuan Province, China, offer an example of how control measures for CE can be implemented in more challenging settings. These Tibetan communities hold strong Buddhist beliefs and allow old animals to die naturally, as well as having no restrictions on offal disposal during slaughter. As a result, CE prevalence is high in both the livestock and human populations. Coupled with this, hygiene and suitable sanitation are limited in the remote and rural communities, further increasing the public health risk of human CE (Yang et al., 2009). Control in areas of China such as the Datangma District, Ganzi County, was based on six-weekly dosing of praziquantel, alongside euthanising stray or unwanted dogs with efforts focused on highly endemic areas, though it was decided that to avoid religious or cultural conflict, the focus of anthelmintic treatment of dogs rather than euthanasia was more appropriate moving forward.

A small study conducted in two counties (Hutubi and Wensu) in the Xinjiang region of China also included selecting individuals as Hydatid control officers to dose all dogs in their village every six weeks as well as educating their community to the risks of CE (Zhang, Zhang, Wu, Shi, Li, Zhou, Wen, & McManus, 2015). Following the four-year dosing scheme in the two counties, the prevalence of *E. granulosus* in dog populations was reduced from 14.7% and

18.6% to zero dogs detected respectively. The prevalence of CE in sheep born after the introduction of the control scheme was also reduced by 90-100% in these counties (Zhang, Zhang, Yimit, Shi, Aili, Tulson, You, Li, Gray, McManus, & Wang, 2009). In 2005 the control measures were expanded to 217 counties in the Western region of China. The controls were made up of five factors. As with other control programmes, health education around CE was given to these communities. The government also introduced ultrasound screening of communities as well as treatment for those people infected with CE. The final two steps were praziquantel dosing of all registered dogs and offal inspection and controlled disposal at slaughterhouses. In some regions, the control measures resulted in a 50% decrease in prevalence in sheep and a reduction of human cases from 7.1 per 100,000 in 2006 to 2.4 per 100,000 in 2011 (Feng, Wu, Ma, Duan, & Yang, 2012).

More recent studies looking at *E. granulosus* prevalence in dogs have shown that the prevalence has increased to as high as 18.1% in some counties, with several dogs showing co-infection with *E. multilocularis*. This increase was attributed to the rise in trade of meat and neglect of offal disposal as well as dogs being allowed to roam freely (Liu, Xu, Cadavid-Restrepo, Lou, Yan, Li, Fu, Gray, Clements, Barnes, Williams, Jia, McManus, & Yang, 2018).

For control programmes to be successful, the measures implemented to prevent transmission must be continued for an extended period to ensure the life cycle of *E. granulosus* is broken and reinfection of intermediate or definitive host does not occur. In areas where *E. granulosus* control programmes are combatting high prevalence of infection, reinfection of hosts can occur rapidly if control measures are not adhered to or are missed. Control programmes in South America, for example, face such issues, with socioeconomic, epidemiologic, and environmental conditions favouring reinfection of dogs and sheep (Cabrera, Parietti, Haran, Benavidez, Lloyd, Perera, Valledor, Gemmell, & Botto, 1996). Early control programmes in South America were based on successful ones conducted in New Zealand and Tasmania, *i.e.* by using anthelmintic treatment to deworm the dog populations. This widespread dosing plan had a number of major drawbacks. However, the greatest challenge was how to logistically administer praziquantel to the large and widespread dog populations found in South America. Furthermore, another challenge was in accessing remote locations where roads and infrastructure are limited. The programmes must also be funded for at least ten years, which considering the logistical difficulties, is a large financial undertaking. Various approaches to

implement control measures were taken from different countries. Argentina's control programme was carried out by individual provinces. In 1970, the first control programme began in the small Department of Huiliches, Neuquén Province, dosing dogs every 45 days with Arecoline bromhidrate. Later, with the introduction of praziquantel this was extended to the entire province, with relative success.

From 1995 to 2004, the incidence of CE in children ages 0-14 dropped from 22.1 per 100,000 to 6.2 per 100,000. Though there is significant decrease in incidence, cases remain high considering a prolonged control programme had been in place. Also, in other municipalities, incidence rates were as high as 29.2 per 100,000 for the same age group, suggesting high levels of recent transmission. This is thought to be caused by the varying geographic and socio-cultural characteristics of the municipalities of the province, with higher incidence found in the areas of more agricultural practices (Pierangeli, Soriano, Roccia, Giménez, Lazzarini, Grenóvero, Menestrina, & Basualdo, 2007).

In the Province of Río Negro, a control programme was launched in 1980, focusing on community involvement by healthcare assistants conducting home visits and providing praziquantel tablets to homes with dogs, relying on dog owners to administer them. This control programme saw a significant decrease in human cases (5.6% to 0.3% in 12 years). However, though there was a decrease in prevalence in dogs and sheep, it remained as high as 20% in sheep (Larrieu, Costa, Cantoni, Labanchi, Bigatti, Pérez, Araya, Mancini, Herrero, Talmon, Romeo, & Thakur, 2000; Larrieu & Zanini, 2012).

A different approach was taken in Chile, where a national control programme was established in 1982. Though described as a national control programme, it was only implemented in two regions of the country, and once again was focused on anthelmintic treatment. Veterinarians were tasked with deworming dogs using praziquantel eight times annually, over a 15-year period. The approach focused on distributing praziquantel based on the ecological and geographical characteristics of the area. The control programme was initially successful, reducing human incidence to just six per 100,000. However, in 1998, the control programme was dismantled and deworming of dogs was made voluntary for farmers (Larrieu & Zanini, 2012). As a result, prevalence in dogs has been observed at 48.2% (Alavarez, Tamayo, & Ernst, 2005).

A subsequent control programme began in Uruguay in 1992; once again taking inspiration from the New Zealand control programme. The establishment of the Commission for Combating Hydatidosis coordinated dosing of dogs every 30 days, conducted by individuals employed specifically for the programme, much like Hydatid control officers employed in New Zealand, and was funded by dog registration fees. By 1995, over 90% of the dog population was being dewormed and consequently the *E. granulosus* prevalence in dogs dropped to less than 1% (Larrieu & Zanini, 2012). However, analysis of the purgation dosing regimen of dogs in the Durazno region, an agricultural heartland of Uruguay, that had a baseline prevalence of 13.2% infection with *E. granulosus* prior to dosing of dogs had reinfection rates of 5.4% after just four months. This increased to 18.6% after six months and by the following year reached 27.9%, over double the baseline prevalence. The same increase was seen in *T. hydatigena* and *T. ovis*, both returning to over baseline or baseline levels. Common practices by farmers such as feeding of offal to dogs from home slaughter as well as sheep and hunting dogs are rarely confined when not working and scavenging, and ranging behaviours are common. These behaviours contribute to the reinfection of definitive hosts (Cabrera et al., 1996).

1.4 Cystic Echinococcosis in the Falkland Islands – an historic perspective

The Falkland Islands are an archipelago situated approximately 500km off the east coast of Argentina, South America (Upson, Williams, Wilkinson, Clubbe, Maclean, McAdam, & Moat, 2016). The climate is cool with mean summer and winter temperatures of 9.4°C and 2.2°C respectively (McAdam, 1985). The archipelago consists of two main islands, West Falkland, and East Falkland, and 776 smaller islands with a total area of 12,000 square kilometres. The population is currently 2563 (2020) of whom 2115 live in the main town of Stanley. Around 200 people live on the remainder of East Falkland with around 130 people on West Falkland (Government, 2020). Farmland in the Falklands is low in nutrients and fertility, making production of crops on a commercial scale non-viable. Agriculture is therefore based upon pastoralism, which is dependent on the maximisation of native grassland and plant species with grazing systems based on increasing productivity of sheep for the wool industry (Huw Ll, 1983). The sheep population is approximately 500,000 and farming is organised in 81

individual farms, with an average size of 13,850 hectares (ranging from 75 to 152,320 hectares).

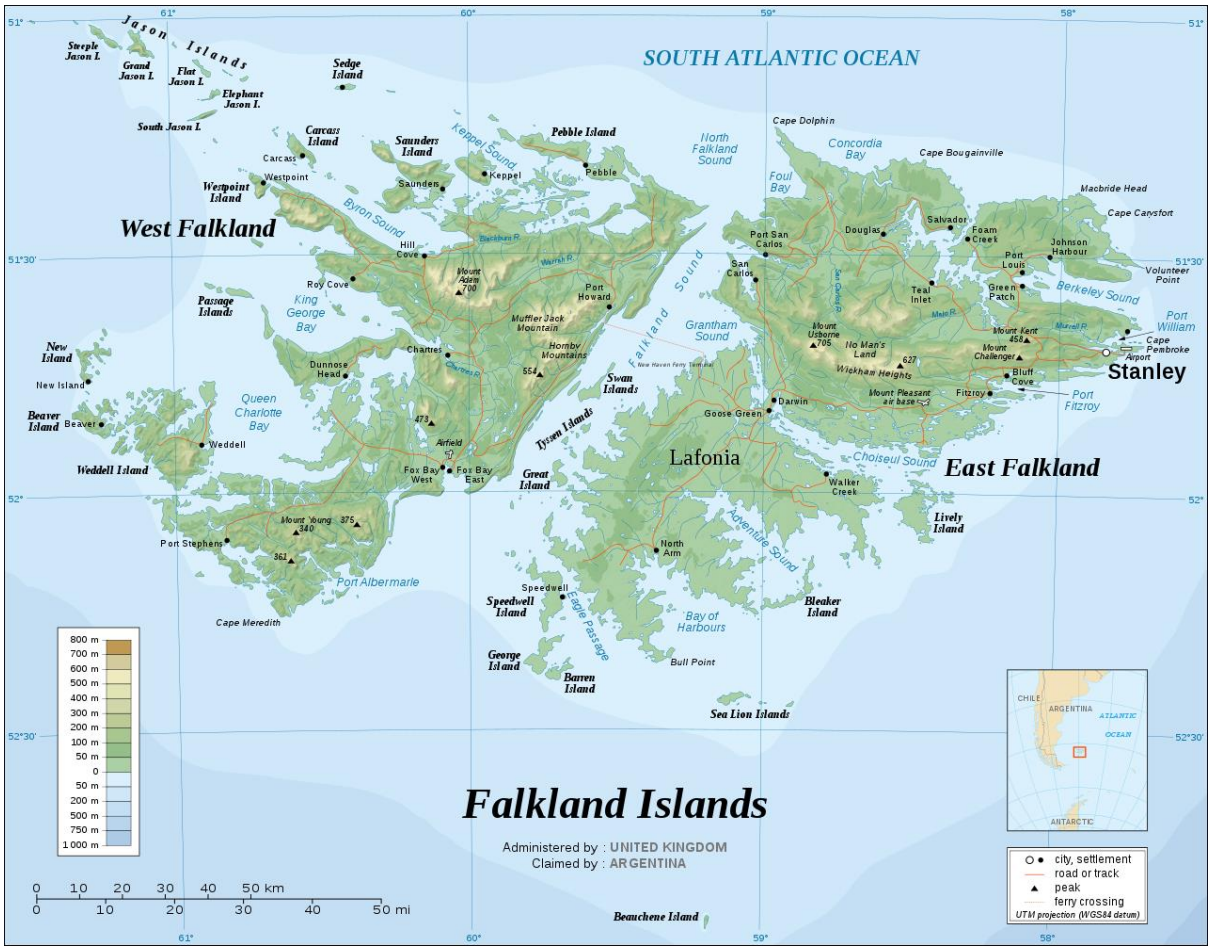


Figure 4: Map of the Falkland Islands (Gaba, 2007).

It is thought that *E. granulosus* was first introduced via live sheep imports from South America (Whitley, 1983). The first recorded case of CE discovered in the Falkland Islands was in 1941, when a single sheep out of a group of 2,000 that were inspected was found to have a hydatid cyst (Gibbs, 1946). *Echinococcus granulosus* subsequently spread rapidly throughout the sheep and dog population, aided by the regular feeding of sheep offal to dogs and the lack of dog controls. The prevalence of infection in sheep peaked in 1969 when 59.3% of sheep slaughtered at the abattoir were found to be infected with CE (Reichel, Baber, Craig, & Gasser, 1996). As the parasite prevalence in sheep increased, human cases were discovered. Between 1965 and 1975, 11 human cases of CE were diagnosed in the Falkland Islands. This represents a high prevalence amongst a population of less than 2,000 people, especially considering that early asymptomatic infections would be likely to be missed. In 1977 the population was subjected to serological testing and a further nine cases were diagnosed (Whitley, 1983). The

Falkland Island population was tested again in 1988 revealing another 18 humans testing positive for *E. granulosus* (Reichel et al., 1996). In 1965, the first control measures were put in place.

1.4.1 Control of cestode parasites in the Falkland Islands

The high and increasing prevalence described above led to the farming community starting to implement control measures. These measures were focused on reducing the prevalence of *E. granulosus* infection in dogs. The first area to focus upon was the definitive host, with dog's regular contact with both sheep and humans, leading to infection of both these intermediate hosts (Macpherson, 2005; Otero-Abad & Torgerson, 2013). This was initiated by the farming community taking responsibility. However, in 1965 after the first human case was identified and CE was identified as a public health risk, the government implemented the Tapeworm Eradication (Dogs) Order No.1. This was the first legal action taken in the Falklands and involved purging of dogs using Arecoline acetarsol (Tenoban).

In 1970 the Tapeworm Eradication (Dogs) Order No. 2. Was introduced. This order was the first order where restrictions on what dogs could be fed was put in place. As well as updating the purging drug to Bunamidine hydrochloride (Scolaban), the order banned the feeding of offal including hearts to dogs unless they had been stored in a dog proof container for a minimum of 28 days, though not recommended after this length of time. Restriction of feeding offal to dogs removed the transmission from the intermediate host, (sheep) to the definitive host, (dogs) (Li, Wu, Wang, Liu, Vuitton, Wen, & Zhang, 2014). There were also restrictions on dog movements, banning dogs from any area where home slaughtering practices take place or slaughterhouses and enforced a £25 fine to anyone found to be breaking this order. This fine was increased to £200 in 1973.

The next major change to the control programme came in 1975, with the Hydatids Eradication (Dogs) Order. This order enforced the disposal of all offal in dog proof containers and banned feeding of any offal to dogs. It also restricted the movement of dogs by confining them to kennels or dog runs when not being used for farm work. Fines were increased to £500 for breaking of these laws. In 1977, the government started a programme of supervised dog dosing every six weeks with praziquantel (Droncit and Drontal) as an anthelmintic drug to prevent the parasite from surviving in the dogs and producing eggs. Praziquantel is a drug widely used as treatment for dogs, with six-weekly dosing implemented to prevent the

completion of the life cycle (Eckert et al., 2001; Gemmell et al., 1986a). In the same year, public education was introduced in the form of presentations and slide shows, pamphlets, and the release of a news sheet called 'Hydatid News'. These were released sporadically over a 6-year period from 1977 to 1983 (Whitley, 1983). This was made law in 1981 with the Hydatid Eradication (Dogs) Order 1981 (Falkland Island Government, 1981). This order combined and finalised previous orders along with the dog dosing scheme and is still in place today, with the only change coming in 2010 when the dosing schedule was reduced from six to five weeks to ensure the gap between dosing of dogs was significantly shorter than the maturation time of *E. granulosus*. The focus of CE control in the Falklands is on control of the definitive host, thereby stopping the life cycle from continuing through controls on what dogs can be fed as well as the now five-weekly dosing with praziquantel (Eckert et al., 2001; Gemmell et al., 1986a; Li et al., 2014).

1.4.2 Outcomes of prolonged control programme in the Falkland Islands

As a result of the implementation of the control programme, there was a decline in the prevalence of CE in the Falkland Islands. From 1970 to 1982 data was collected at the butchery in Stanley by staff from the Department of Agriculture when inspecting the offal of sheep killed for mutton (Whitley, 1983).

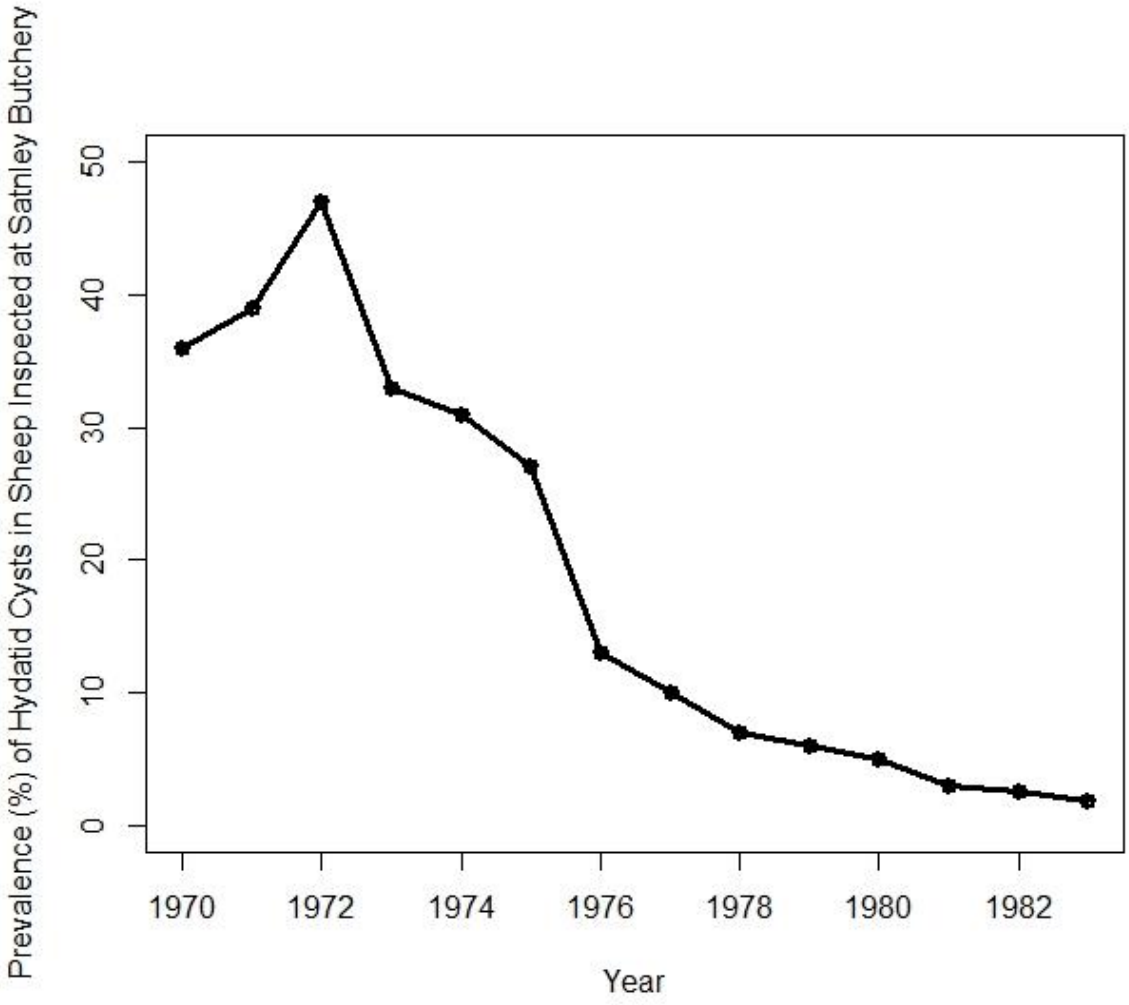


Figure 5: Prevalence of hydatid cysts in Sheep killed for mutton at Stanley butchery between 1970 and 1983 by members of the Department of Agriculture. (Data collected over this period published by Whitley (1983), graph was developed from this data).

The results in figure 5 show that the prevalence was increasing and remained high until 1972, two years after restrictions were put in place on what could be fed to dogs. This restriction coupled with the purging of dogs began to reduce the prevalence of CE in sheep through the breaking of the life cycle of the parasite. Further education and dog dosing accelerated the decline, reducing the prevalence below 10% by 1978.

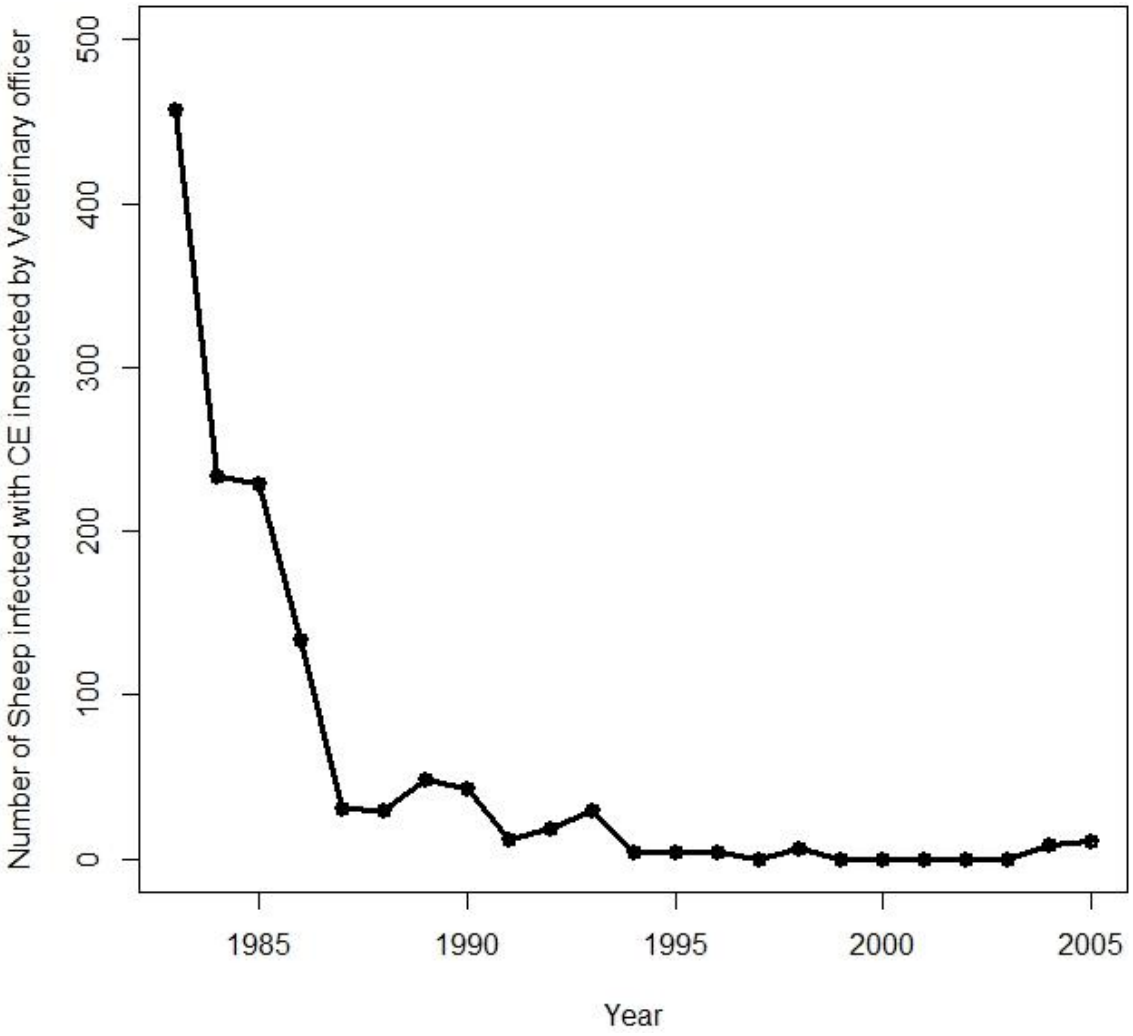


Figure 6: The number of sheep infected with CE from 1983-2005. All inspections were carried out by a veterinary officer or EU registered meat inspector.

There was a further decline in the number of sheep infected with CE slaughtered in Stanley. Prevalence dropped to less than 2% of all sheep slaughtered in 1983 (Whitley, 1983) and it continued to drop, and records of individual sheep infected with CE was less than 100 per year (Figure 6). By 1993, the prevalence of CE in sheep in the Falkland Islands falling to below 1% of all sheep slaughtered (Reichel et al., 1996). Though the control measures showed clear success, a documented a case of CE in a two-year-old sheep demonstrated continued transmission. During the same study, all dogs were serologically tested for anti-*E. granulosus* antibodies with 2.1% testing positive, suggesting that exposure to *E. granulosus* was continuing despite the control programme (Reichel et al., 1996).

1.5 Aims and objectives

Awareness of the public health importance of CE and attempts to reduce transmission have been ongoing in the Falkland Islands for over 40 years. To date the prevalence in sheep at slaughter is very low but the parasite still persists. Similarly, other taeniid cestodes with similar life cycles (eg *T. hydatigena*) occur in sheep at slightly higher levels. The FIG is keen to ultimately eradicate Echinococcosis. In order to achieve this, it is important to understand its low-level persistence during intensive control. Possible contributing factors include: (1) mismanagement of control processes to prevent dogs being infected with adult worms; (2) resistance to praziquantel; (3) long term egg survival and environmental contamination; alternative hosts for adult worms.

The overall aim of this project was to investigate and understand the current pattern of taeniid cestode infections in dogs and sheep in the Falkland Islands. The main objectives of the project are:

i. To establish current farming practices which may influence transmission of taeniid cestodes.

ii. To establish the level of *E. granulosus* in dogs and to identify other possible definitive hosts for Echinococcus.

iii. To evaluate differences in prevalence of *E.granulosus* and other taeniid cestodes in sheep at slaughter over time.

iv. To evaluate approaches for detecting parasite eggs and biomolecules in soil samples.

v. To apply mathematical models to explain the geographical distribution of Taeniid infections in the Falklands and to simulate control and transmission of these infections.

Because of the low availability of adult *Echinococcus* worms from the Falkland Islands it was decided not to investigate the possibility of resistance to praziquantel in the current study. The outcome of the project is to develop a series of recommendations to reduce the risk of

transmission of *E. granulosus* and help the Falkland Islands Government move towards eradicating *E. granulosus* entirely.

Chapter 2. Current Management of Farming Practice

2.1 Sheep Farming in the Falkland Islands.

Agriculture in the Falklands makes up a large proportion of the economy, with the main income coming from the wool industry. There are 466,364 sheep on the Falkland Islands (according to 2020 farming statistics) farmed across 81 farms with a combined total of 1,129,723 hectares (Department of Agriculture, 2020; Epstein, Pointing, & Halfacre, 2007). Farms are run by the owners, or in some cases by farm managers working for the landowner (government owned farms or privately owned land farmed by a tenant).

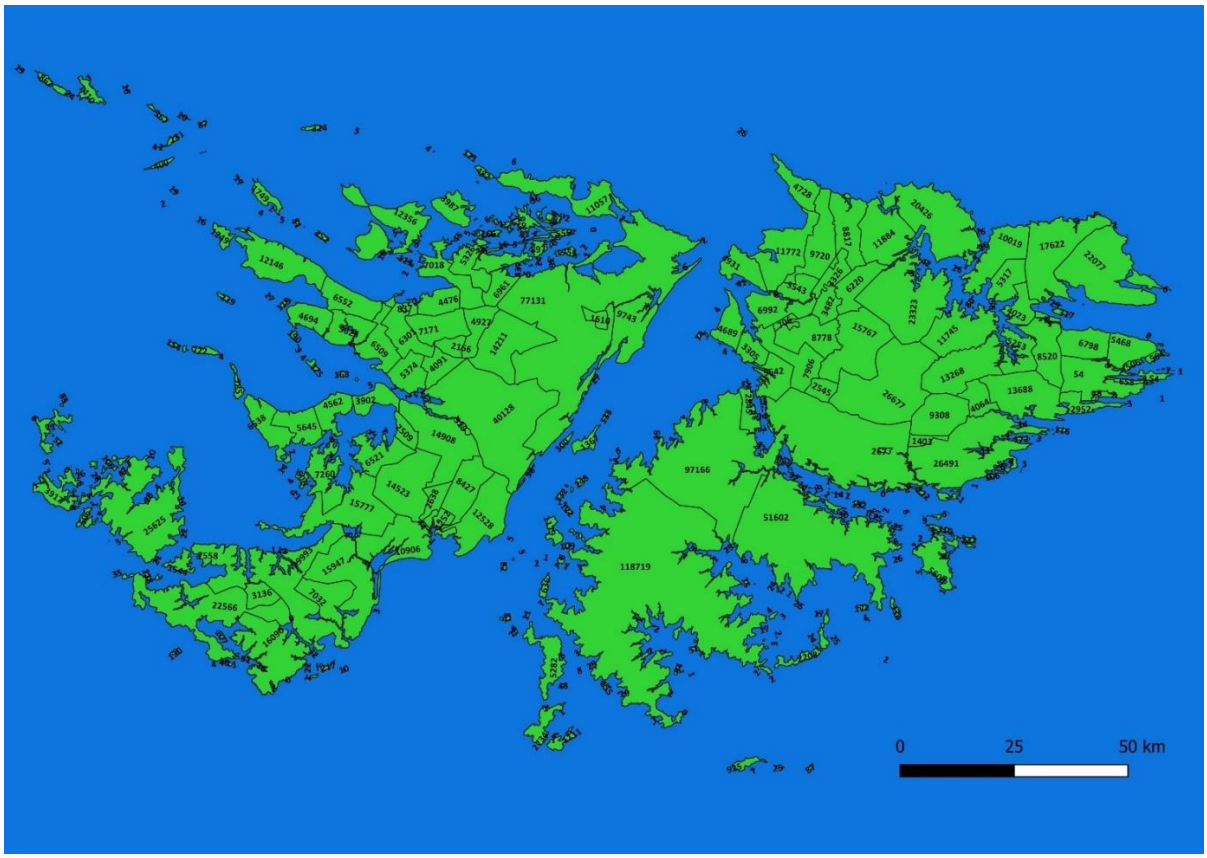


Figure 7: Farm boundaries of the Falkland Islands with the size of each farm in Hectares (Department of Agriculture, 2020).

Farming techniques in the Falkland Islands are like ranching, i.e sheep spend most of their lives in 'camp' (open pasture). Farmers gather sheep several times a year, using sheep dogs to herd large flocks of sheep and drive them towards the farm settlement. The main breeds are Merino and Polworth sheep, selected for characteristics that allow for their survival and productivity in the tough Falkland Island environment, as well as their fine wool micron.

Typical flock structures would contain approximately 40% Breeding Ewes, 40% yearling/young stock, 18% mature wethers (castrated male sheep) and 2% Rams (Department of Agriculture, 2020). This is to maintain breeding potential, maximise wool production and to ensure that stock numbers are maintained within the carrying capacity of the land available (Department of Agriculture, 1998).

2.1.1 Import and export of animals and animal products into the Falklands

Up until 2004, trade of sheep between South America and the Falkland Islands was common. Animal products as well as live animals were regularly traded with South America, most commonly with Chile. It is thought that the import of sheep from South America, where Cystic Echinococcosis is endemic in many countries (Cucher, Macchiaroli, Baldi, Camicia, Prada, Maldonado, Avila, Fox, Gutiérrez, Negro, López, Jensen, Rosenzvit, & Kamenetzky, 2016), was the cause of the outbreak and prolonged endemic of CE in the Falklands (Whitley, 1983). This trade stopped in 2004 when the last of 70 cattle were imported from Chile (Epstein et al., 2007). Live animals have also been imported from New Zealand and Australia in the 1990s when different breeds of sheep were imported to maximise wool production by selective breeding of flocks. More recently, all imports have been of genetic material for artificial insemination to reduce risk of diseases being introduced while improving genetic traits of flocks such as no black markings, fine wool micron, and hardiness. In contrast, there is no export of live animals from the Falkland Islands (Epstein et al., 2007).

Sheep spend most of their life on the farm they are born on. In some rare cases, smaller farms may purchase older sheep from larger farms that would have been culled in order to get another year's wool production from them. When animals move between farms, animal movement documents must be filled out and submitted to the DoA. In practice, this is not always completed, and sheep are moved or sold without informing the DoA. When live sheep are taken to the abattoir, these same movement sheets are completed to show that the abattoir has received these animals, as they are not always slaughtered on the day they arrive at the abattoir.

Falkland Island wool is highly regarded due to its natural white colour as well as being very soft. It is also popular because there is very vegetation contamination, making it more versatile for use in manufacturing. As a result of the eradication of ked and lice in the 1980s,

dipping no longer occurs, meaning there is no chemical traces in the wool. In 2020, a total of 1,641,819kg of wool was produced for export.

In 2003, the Sand Bay abattoir was opened. This is an EU registered abattoir, operating from early January to the end of May for the export of lamb, mutton and beef to the EU. The abattoir runs all year for domestic services. In 2020, a total of 44,202 sheep were slaughtered at the abattoir for export and 5,091 sheep were slaughtered for domestic consumption (Department of Agriculture, 2020). As a result of abattoir opening, there was a shift in stock structure to diversify and supplement income from wool exports. An increase in breeding ewes, as well as a reduction in the age of older stock. There was also an increase in the number of farmers producing beef.

2.1.2 Home and Abattoir Slaughter of animals in the Falkland Islands

Meat production is limited due to the productivity of the pasture, so the main source of income is from wool. As a result, the average age of the sheep is higher than other parts of the world at seven years old (Whitley, 1983). From the early 1950s, animals were slaughtered at the Stanley Butchers, situated on the outskirts of Stanley. Animals were slaughtered twice a week, on a Tuesday and a Friday for local consumption, and should demand require, an extra day per week. A member of the Veterinary Department was present during slaughter to inspect the offal (liver, lungs, and heart), for hydatid cysts and other ailments, documenting the number of cysts discovered. Once inspected, the offal, along with the rest of the internal organs was disposed of into concrete pits on a nearby beach for a minimum of 21 days before being tipped onto the beach and allowing the tide to rise above the pits and remove the rotten remains. The Stanley butcher slaughtered and supplied meat for the local population until 2003, when the Sand Bay Abattoir built. The EU accredited abattoir operates every weekday between January and the end of May (known as the export season) when animals are slaughtered for exportation to multiple countries within the EU and the UK, as well as sale domestically. Throughout the non-export season, slaughter is only carried out once per week, which only supplies the local market. During the busy export season, the abattoir can slaughter up to 1000 sheep per day, with all carcases and offal inspected by an EU accredited Meat Hygiene Inspector (MHI) and/or veterinarian. All conditions discovered, including

hydatid cysts and other cestode cysticerci are marked with a coloured tag that remains with the carcass until it is digitally weighed where the condition is recorded. Any suspected hydatid material is removed and sent to the laboratory at the Department of Agriculture where microscopic diagnosis is carried out looking for the presence of protoscoleces to confirm if the cyst is a hydatid cyst or not. Once offal has been inspected, healthy livers, heart and lungs remain with the carcass and the guts are loaded into a lorry and driven to a disposal site 4km from the abattoir and deposited into the sea. In terms of infection status of each animal, the coloured tag recorded at the weighing scale is then added to the disease report under the Lot in which the animal was slaughtered in.

As described, the age of the sheep population in the Falklands is higher than other parts of the world with an average age of approximately seven years old (Whitley, 1983). Once older sheep have come to the end of their wool producing life, they are often slaughtered on the farm for dog meat, or if there are large numbers of older sheep, they are culled and left on the pasture. When the carcasses are left, they are deposited whole, and the offal is not inspected. These older animals are more likely to be infected with CE and so there could be more cases of the disease going unrecorded (Torgerson, Williams, & Abo-Shehada, 1998). Culling of older sheep normally occurs in April, after shearing is completed to ensure maximum yield from stock. The culling of older stock reduces the stock numbers on a farm to the winter carrying capacity. The reason these animals are not taken to the abattoir is that the cost of transportation is often greater that the price of the animal (Department of Agriculture, 1998). However, in 2018, the Sand Bay abattoir began to promote the sending of cull ewes to the abattoir for slaughter and export rather than them being slaughtered on farm, which reduced the amount of home slaughter culling. This has the added benefit of all animals being slaughtered at the abattoir being inspected by a vet and/or meat hygiene inspector, potentially identifying cases of CE that would be missed if older animals are culled and not inspected (Torgerson et al., 1998).

2.1.3 Dog Rearing, shepherding and control.

The number of dogs on a farm varies greatly (0-40) depending on the size and requirements of the farm (Reichel et al., 1996). Historically this will have been a major route of transmission of *E. granulosus* between dogs and sheep due to the interaction between the two species

(McManus, Zhang, Li, & Bartley, 2003). Depending on the size of the farm, dogs may be owned and worked by one farmer, who runs the farm alone, or, on larger farms, individual shepherds who are employed and live on the farm, may use their own dogs. There are various breeds , though all have shepherding pedigree (Reichel et al., 1996) with breeding occurring on farms to produce offspring from well trained and skilled working dogs.

Figure 8: Images of Dog kennels or runs used to house working dogs when not being used for farm work.

Dogs are required by law to be kept in kennels while not working since 1970 (see chapter 1). Farmers work with dogs most days and will exercise them on days when not being used for sheep work. Farmers may also have them with them while carrying out non-sheep related tasks such as mechanical work or fencing. Figure 8 shows examples of different kennel

structures in which dogs are housed. Kennels vary in size and structure, with some simply being a small kennel with a chain to large, gated structures, and in some cases, fenced areas that allow for dogs to have space with shelters in as well. In 1975, dogs were banned from any area where home slaughter of livestock takes place to ensure that when carcasses are dressed and offal is removed, it can be disposed of properly into containers without the risk of dogs accessing it. When animals are slaughtered, the offal must be disposed of in dog proof containers for a minimum of 28 days. There is no specific container specified, with farmers using old oil drums, water tanks, and concrete pits as methods of disposal. After the 28 days, the offal can be disposed of. This disposal varies from farm to farm, with some burying the containers, others burning them, and some being deposited into the sea. Only the offal is to be stored for 28 days, and often the guts and stomach are deposited into the sea immediately after slaughter. Figure 9 shows different offal storage methods, as well as the basic slaughter facilities and methods of disposing of offal into the sea.

Figure 9: Examples of the basic on farm slaughter facilities and containers used to store and dispose of offal after slaughter, as well as disposal methods of depositing offal into the sea.

In 1977 the Falkland Island government developed the control programme further by introducing the anthelmintic treatment of all dogs every 5-weeks, using praziquantel. This responsibility is on the dog's owner to administer the drug and it is recommended by the

veterinarians that the owner watches the dog for a period after dosing to ensure that the pill is not regurgitated. On farms with more dogs, the responsibility is often on the farm manager to ensure all dogs are dosed by the shepherds. These practices are still in place legally currently, brought together by the Hydatid Eradication (Dogs) Order 1981 which collated all the legislation and control measures under a single act (Falkland Island Government, 1981).

2.1.4 Eradication of ectoparasites from the Falkland Islands.

The Falkland Islands farming community has successfully eradicated ectoparasites such as Sheep lice and Keds. Dipping on farms was conducted annually from the early 1900s, and in 1908 it was reported that the Falkland Islands were free from sheep Lice *Trichodectes sphaerophalus*. However, it wasn't until 1939 that this became official after the last of five farms were released from government-imposed quarantine (Department of Agriculture, 1946). Keds, *Melophagus ovinus,* is a blood sucking ectoparasite (Small, 2005), survived in the sheep population until 1980. With the eradication of these ectoparasites, annual dipping was no longer necessary and was stopped (Epstein et al., 2007). The eradication of these ectoparasites demonstrate the capability for the livestock populations to be controlled and protocols to be adhered to for control of parasites to occur.

2.2 Information gathering on current farming practices and dog ownership.

In order to provide further understanding of the current management of Echinococcosis control measures, visits, conversations and questionnaires were established with individual farms during the current study (Ethics approval STR1920-09).

2.2.1 Farm visits

Throughout the current study it was necessary to visit farms for the collection of dog faecal samples and other material. During these visits there was opportunity to talk to farm owners or farm managers in an informal way to establish their views on Echinococcosis transmission and control. Brief notes were kept of any relevant details.

2.2.2 Questionnaires

A detailed questionnaire survey was carried out in the first year of the project in 2018 (See appendix I-a). The questions were designed around recommendations on the ownership and management of dogs and livestock set by the Falkland Island Government. Questions were not solely based on farming practice and animal slaughter. They also asked about the role of the dog and how they are kept, as well as the diet to gain an idea of possible routes of transmission of parasites or gaps in the control measures that were not being adhered to properly.

Questionnaires were distributed by email prior to the arrival of the author to only farms (51) which had registered dogs based on the documentation from the Department of Agriculture about dog ownership and dosing information. The questionnaires were filled in by the farm manager, as farms often had several workers each with their own dogs. However, the managers oversee all aspects of the farm and so are the most knowledgeable about all aspects covered by the questionnaire. Farm Managers were asked to return the questionnaires within two weeks.

2.3 Questionnaire survey results

2.3.1 Observations and comments

In total 50 out of 81 farms were visited by the author. The reason for not visiting all of the farms were because of availability of farmers, logistical difficulties in accessing the farms such as poor weather conditions, and some farms are not lived on throughout the year and the farmer only goes to tend sheep at certain times of the year, e.g shearing. This was carried out during two study periods (07/03/2018 to 27/04/2018 and 23/01/2019 to 28/05/2019). Farm settlements in the Falkland Islands comprise of the living accommodation, that includes the main farmhouse, and on larger farms, farm worker's houses as well, making them into small villages. The settlement also includes a shearing shed and other farm buildings such as a workshop and equipment sheds.

Figure 10: An example of a larger farm settlement, with multiple houses, shearing shed and other farm buildings.

The landscape is primarily open grassland, and sheep graze freely in these grasslands (see Figure 10), with large, fenced subdivisions ensuring sheep remain in the areas designated by the farmer. On average, there is one sheep per 2.4 hectares. Sheep remain outside all year round, only being gathered, and brought into the settlement for specific reasons such as shearing, lambing, and being brought in to be taken to slaughter. Sheep share the pasture with cattle bred for beef, as well as geese, that also feed on the grass and are considered pests by farmers.

Figure 11: Photos of sheep spread out over typical grassland pasture, including sheep being gathered with dogs and vehicles for shearing.

2.3.1.1 Dog housing

As described, farmers bring in sheep for various reasons throughout the year. In September and October, ewes are moved to their lambing pasture and begin lambing. From November, the first of the non-breeding sheep are brought in to be shorn. Between December and March, all sheep are brought for shearing and finally in March/April, animals due to be culled are brought in. Over the winter months from April to September there is less gathering of sheep, and they are left roaming looking for grazing. Throughout the year, sheep may be brought in and taken to the abattoir. Historically, working dogs were used on all farms to gather sheep. Over time, the number of farmers using dogs has reduced, due to the more regular use of machinery such as motorbikes and quadbikes, and by using more advanced fencing structures that make it easier to bring sheep in for jobs such as shearing and lambing.

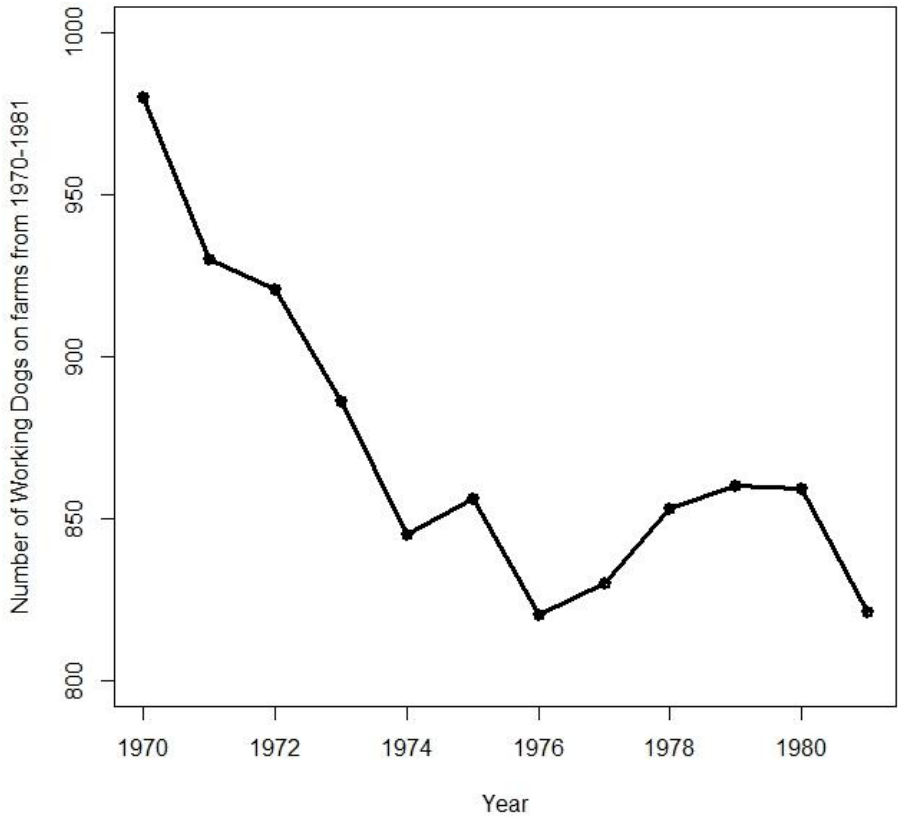


Figure 12: The number of working dogs on farms in the Falkland Islands between 1970 and 1981.

Figure 12 shows the decrease in the number of working dogs over an 11-year period from 1970 to 1981. Over 11 years, the working dog population decreased by 16.2% (980 in 1970 to 821 in 1981). Further decrease occurred between 1993 and the beginning of this study.

In 1993, there were 908 working dogs in the Falkland Islands (Reichel et al., 1996). At the time of this study, in 2018 there were only 365 working dogs on farms, a decrease of 39.2%. Of the 81 farms on the Falkland Islands, 62.9% (51 out of 81) still use dogs, compared to 100% of farms in 1993 (Reichel et al., 1996).

Dog kennels are situated within the settlement, often close to the shearing shed or homestead. On larger farms, there are often multiple kennels located across the settlement. Kennels can comprise of individual runs containing a single dog, or larger fenced areas with shelters where all dogs are contained together and were observed to be in varying degrees of condition. On some farms, brand new kennels had been built to replace old ones whereas on other farms, kennels needed repair. The functionality of the kennels on whether dogs could escape was not observed. When on farms, dogs were often observed with farmers when not doing sheep work, often in the workshop or out fencing. Dogs were observed away from the farmers on occasion, though not for extended periods of time.

Figure 13: Examples of dog kennels. Kennels A and B are in good condition whilst C and D are rundown and in need of maintenance.

2.3.1.2 Home slaughter and disposal of offal.

Home slaughter in the Falkland Islands is common, with farmers slaughtering sheep for home consumption, dog meat as well as culling. Slaughter is conducted by holding the sheep on the

slaughter bench (see figure 14) and cutting its throat. The animal is then hung upside down and allowed to bleed out before it is skinned.

Figure 14: An example of the simple home slaughter facilities and slaughter blocks used in the Falkland Islands.

The carcass is the eviscerated and the liver heart and lungs are removed and placed in dog proof containers, in line with legislation. The intestines and stomach are not required to be disposed of in this way, and therefore farmers often throw it down chutes that lead to the sea (see figure 15).

Figure 15: An example of the way stomachs and intestines are disposed of out to sea close to the settlement.

There is no specification on what container is needed to dispose of offal, and methods range from using old oil drums to 1000L water containers. Once stored for the prescribed length of time, the containers can then be disposed of. In some farms these are placed in landfill with the rest of the settlements rubbish, whereas other farms burn the contents of the drums

when full (see figure 16). The only requirement for these containers is that dogs cannot gain access to the offal at any stage.

Figure 16: Examples of 1000L water container used as an offal container and an example of offal being burned in an old 150L oil barrel.

2.3.1.3 Farming community perception of alternative hosts involved in the life cycle of *E. granulosus.*

During informal conversations with farmers, it was mentioned multiple times that their belief was that dogs are no longer involved in the transmission of *E. granulosus* and that there must be another definitive host causing infections in sheep. Farmers mentioned that they had seen tapeworm in geese shot on the farm, as well as suggesting that species such as Turkey Vultures (*Cathartes aura*) and Giant Petrels (*Macronectes giganteus*) as they often feed on carrion and offal disposed of after slaughter. These species have been seen to kill lambs and ewes during the lambing season, however, are protected, so are not popular species within the farming community.

Due to the practice of depositing intestines and stomachs into the sea, suggestions of marine species have also been brought up as acting as definitive hosts, such as South American Sea Lions (*Otaria flavescens*) and Leopard Seals (*Hydrurga leptonyx*). Though there is no evidence of terrestrial cestodes infecting marine species such as these, there are cestode tapeworm infecting seals and sea lions such as from the *Diphyllobothrium* genus (Hernández-Orts, Kuzmina, Gomez-Puerta, & Kuchta, 2021). Though definitive hosts for *Diphyllobothrium* can include dogs and humans as well as pinnipeds, the intermediate hosts are crustaceans and

small fish, so do not infect sheep (Scholz, Garcia, Kuchta, & Wicht, 2009; Scholz, Kuchta, & Brabec, 2019). There is also very little overlap in habitat of these pinnipeds and sheep, making regular transmission of any parasites between the species unlikely.

After multiple discussions and time spent in the Falkland Islands, farmers regularly mentioned the large numbers of feral cats in the Falklands. Some farmers even suggesting they numbered in the thousands on their farm. Though it is known that *E. granulosus* does not mature in cats, they have been documented to act as definitive hosts for similar species such as *T. hydatigena* and *T. ovis* (Borji, Razmi, Ahmadi, Karami, Yaghfoori, & Abedi, 2011; Hajipour et al., 2020). These thoughts and opinions of farmers helped open other areas of interest within the project and ensure as many areas of investigation as possible were looked at.

2.3.2 Questionnaire results and feedback.

A total of 23 out of 51 (45%) questionnaires were returned from the surveyed farms, that included 49% of the working dog population on the Falklands (179 of 365).

Question one to four were informational questions that provided information about the farm

1. *Serial Number:* (Created by the Author that linked to farm dog identification)
2. *Name of Farm:*
3. *Name of Farmer:*
4. *Location of Farm (Circle one): East Falklands- [North] [South] [East] [West] [Central]*

   West Falklands- [North] [South] [East] [West] [Central]

<u>Section 1: Dog ownership and management.</u>

Question 5: Do you own a dog? a. [Y] b. [N]

All farms who filled in the questionnaire had working or pet dogs on the farm as the questionnaire was only distributed to farms with dogs registered with the Department of Agriculture.

Question 6: How many dogs do you own?

a. [1] b. [2] c. [3] d. [4] e. [More than 4 (Please specify)]

The number of dogs on farms that completed the questionnaire ranged from one to 31 (mean number of dogs was 8 (7.78)). This figure is not reflective of the number of dogs on all farms, only those who completed the survey.

Question 7: What is your dog's role?

a. [Pet] b. [Working animal] c. [Both]

All 23 farms described their dogs as working animals, with six (26%) also describing them as pets.

Question 8: Where do you keep your dog when not working? (Figure 17)

a. [In the house] b. [Locked in Kennel] c. [Tied up outside] d. [Free roaming on property]

Dogs are required to be locked or tied up when not working, though there is not a specific requirement as to how this is done. Most farms kept dogs locked in kennels (17 of 23, 73.9%), with others keeping dogs inside the home (2) or tied up outside (1) or a combination of housing (4). One farm kept dogs in the house or free roaming on the property.

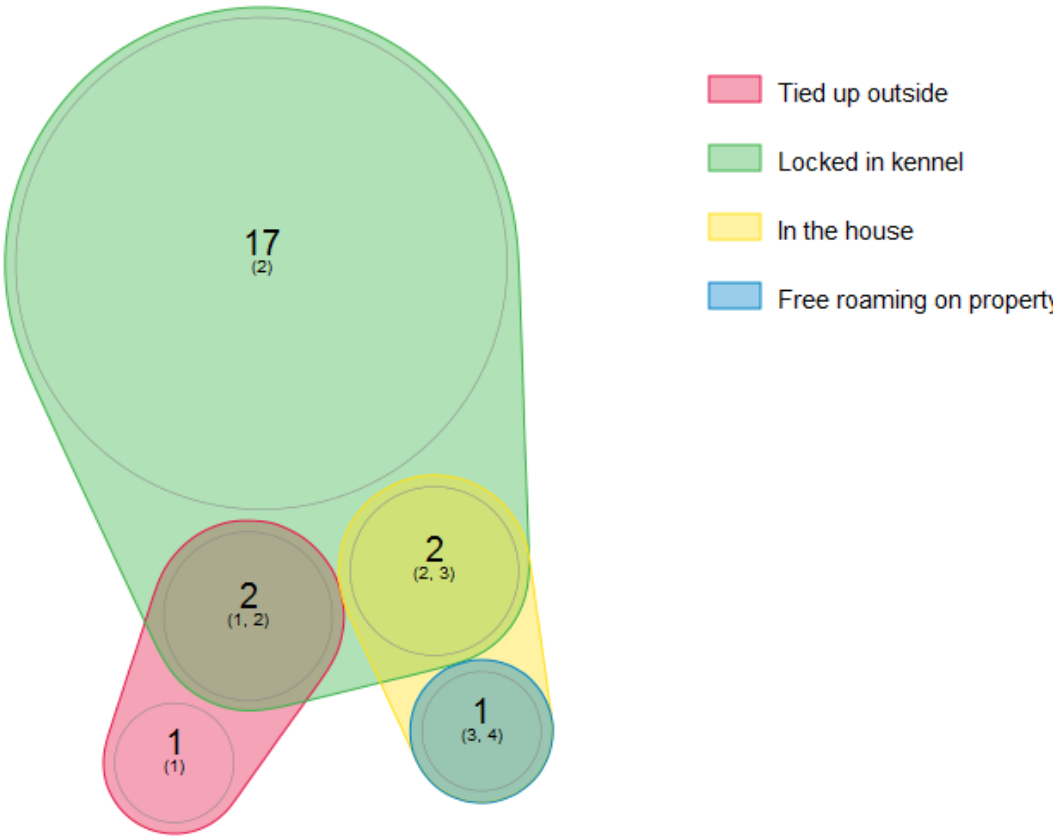


Figure 17: A Venn diagram of the various ways dogs are controlled on farms when not working with the farmer. (n=23).

Question 9: What do you feed your dog? (Figure 18)

a. [Processed dog food] b. [Raw meat] c. [Raw offal] d. [Other (Please specify)]

Working dogs' diet in the Falkland Islands comprised of process dog food and raw meat. A total 96% of farms fed their dogs raw meat as part of their diet (22 of 23), with 26% (6 of 23) of farms feeding only raw meat (mutton) to dogs and only one farm fed a diet of only processed dog food. No farms fed their dogs raw offal and no other food sources were disclosed.

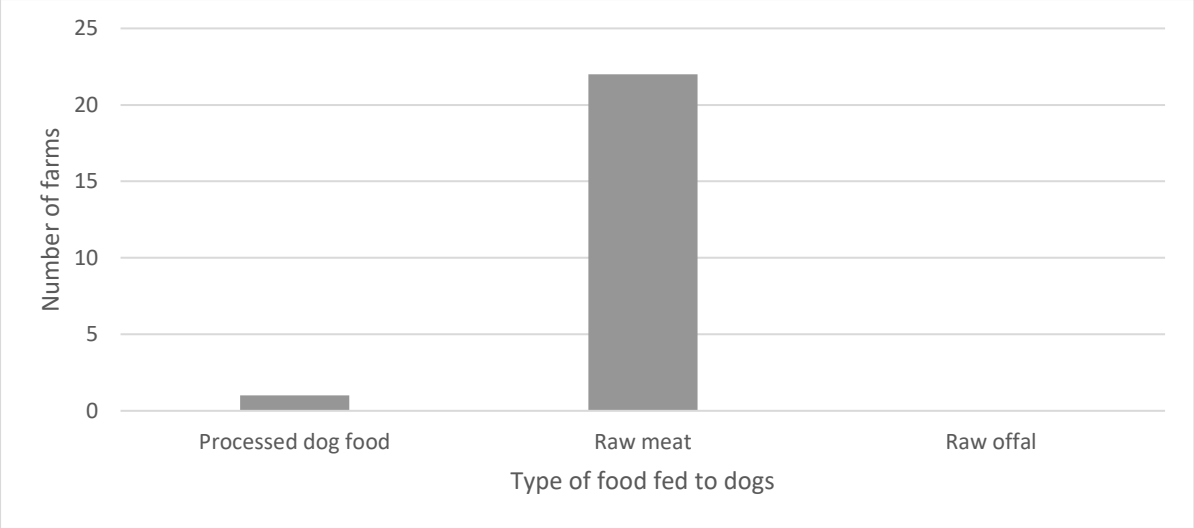


Figure 18: Different food types used by farmers to feed their dogs (n=23).

Section 2: Livestock management.

Question 10: Do you have Seasonal pastures? a. [Y] b. [N]

Open farmland known as 'camp' is the open grazing land of a farm and is often subdivided into separate pastures. Farmers were asked whether they used seasonal pasture for grazing. In total, 69.6% (16 out of 23) of farms used seasonal pastures.

Question 11: How do you rotate these pastures? (Please explain)

In relation to question ten, farmers were asked how they rotated their pasture. This is a subjective question, and the responses were varied but were dependant on food availability, holistic grazing and one farm had preferred pasture for specific ages of sheep. No further details were given.

Question 12. What water sources do your sheep use to drink?

a. [Natural streams/rivers] b. [Drinking Troughs]

c. [Available standing water] d. [All of the above]

Farmers were asked where sheep would drink from on the farm. All farmers stated that sheep used natural water sources for water, with just two farms having water troughs for sheep to drink from.

Section 3: Hydatid control

Question 13. Where is the slaughter of your animals conducted? (Figure 19)

a. [Abattoir] b. [Home Slaughter] c. [Both]

Results indicated that all farms (100%) do some slaughter on the farm to provide meat for animal and human consumption or for disposal of old sheep, with the majority (78.3%, 18 of 23) also sending sheep to the abattoir for slaughter. Five farms (21.2%) only slaughtered at home and did not send any animals to the abattoir.

Figure 19: The location where animals are slaughtered per farm (n=23).

Question 14. Do you home slaughter animals for use on your farm?

a. [Y] b. [N]

As described in question 13, 100% of farms home slaughter animals on their farm.

15. How many animals do you home slaughter per year? (Figure 20)

a. [0-20] b. [21-40] c. [41-60] d. [61-80] e. [More than 80 (please specify)]

In total approximately 3200 sheep are slaughtered at home on the farms sampled. The average number of sheep home slaughtered annually was 140 sheep, with the lowest number being 20 animals and the highest being 800. Three of the largest farms, that responded to the survey, killed between 200 and 800 animals per year.

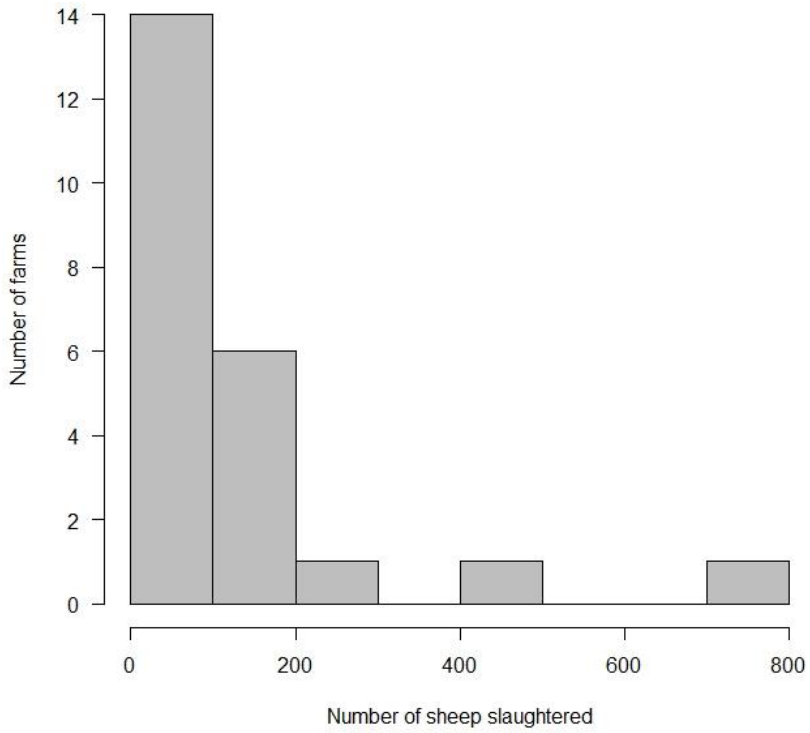


Figure 20: Number of sheep home slaughtered annually per farm (n=23).

16. What time of year do you home slaughter animals? (Figure 21)

a. [Spring] b. [Summer] c. [Autumn] d. [Winter]

The majority of farmers (14 of 23, 60.9%) slaughter all year round, with some famers conducting home slaughter at more specific times of the year such as only in Autumn and Spring in preparation for busier periods or harsher weather.

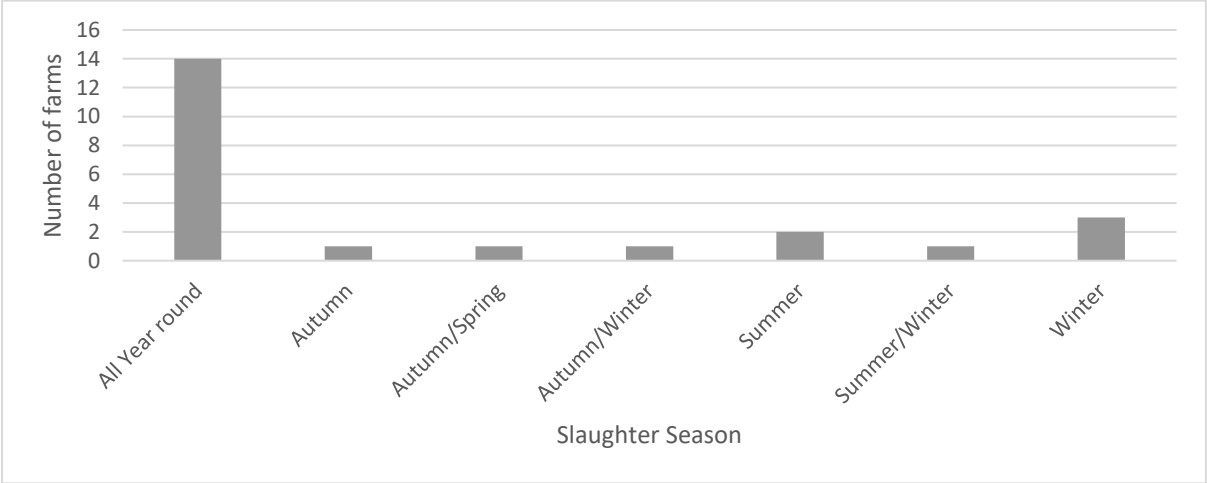


Figure 21: Season when home slaughter takes place (n=23).

Question 17. What do you do with offal? (Figure 22)

a. [Incinerate and bury] b. [Throw in the oil drums] c. [Boil] d. [Feed to dogs] e. [Freeze]

f. [Combination of above (Please explain)]

After slaughtering sheep for use on the farm, farmers were asked how they dispose of offal. Most farmers place the offal in oil drums or similar containers (9 of 23, 39%) or incinerate and bury it (5 of 23, 21.7%), or a combination of the two methods (4 of 23, 17.3%). One farmer froze the offal before disposal and four farms used other methods of disposal. These were to deposit it onto the beach where birds would feed on it immediately (2 of 23, 8.7%) and one farmer fed all offal to their pigs. A single farm simply described offal disposal as 'Disposed of away from dogs so they cannot access it'.

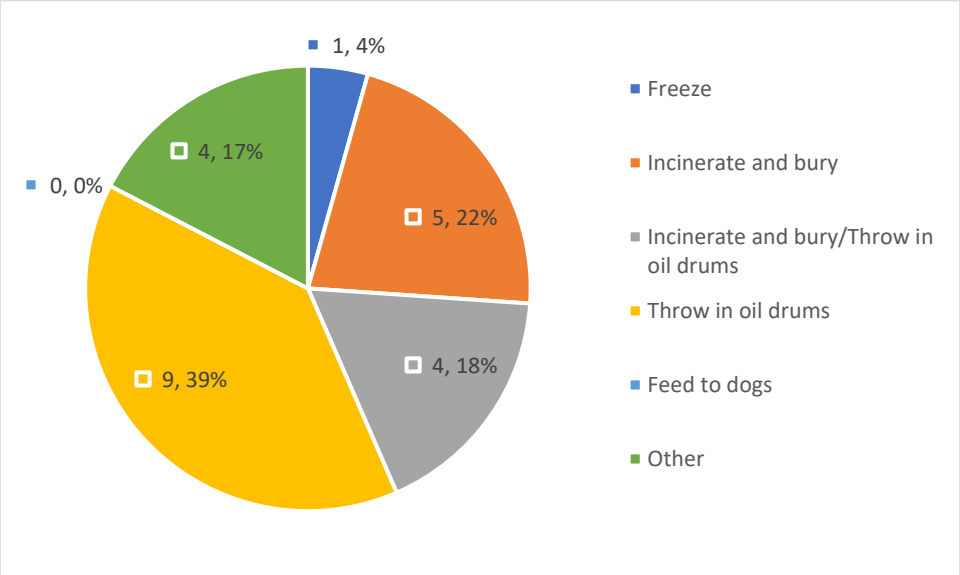


Figure 22: Method of disposing of offal after home slaughter (n=23).

18. Do you know the difference between hydatid cysts and other taeniid cestode cysts? (Figure 23 & 24)

a.[Y] b. [N]

19. Please identify the Hydatid cysts.

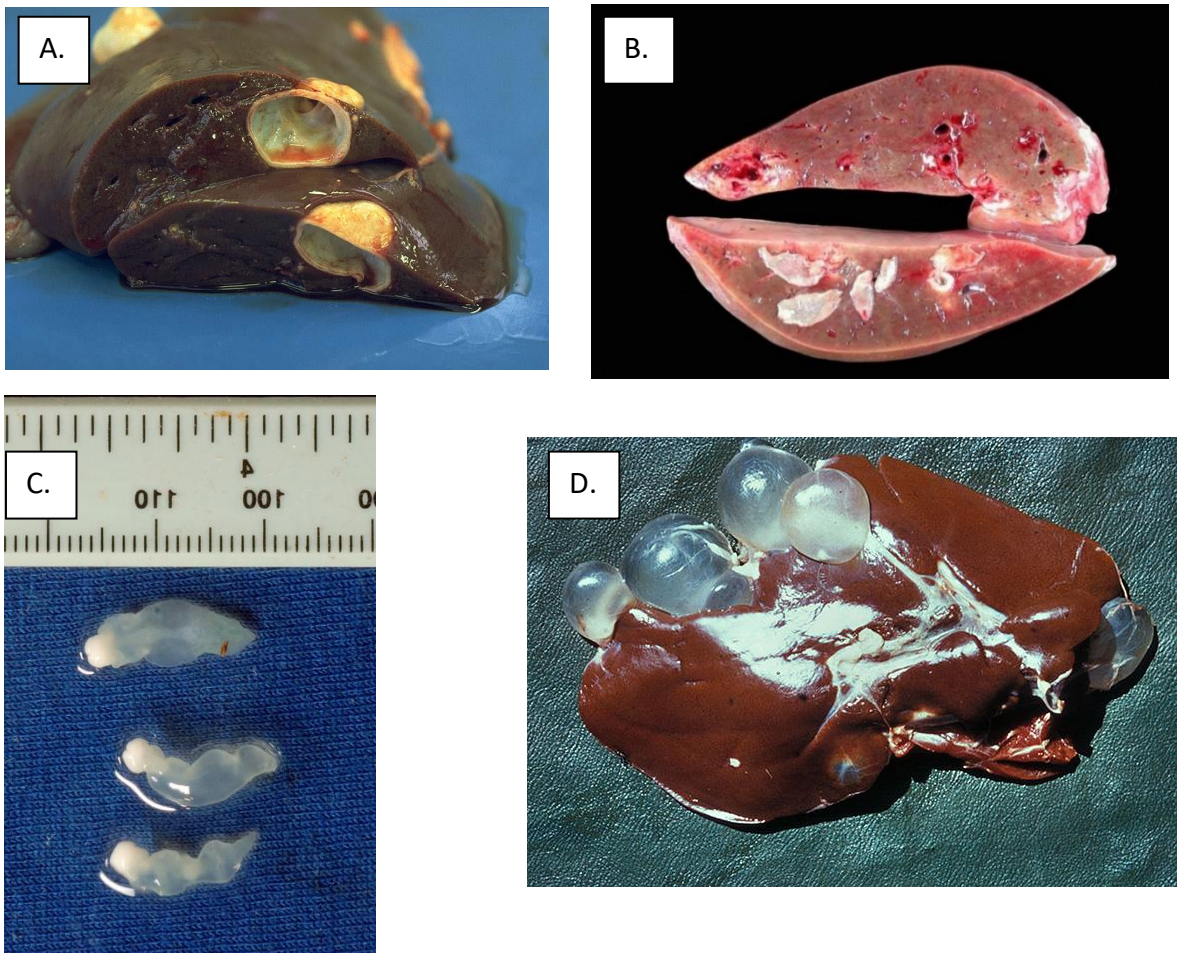


Figure 23: Images shown to farmers to identify hydatid cysts (A) from other cestodes (C. *Taenia pisiformis*, D. T. *hydatigena*), as well as a non-related parasite (B. *Fasciola hepatica*).

To gain understanding of farmers knowledge of hydatid cysts and the ability of farmers to identify hydatid cysts, they were asked to identify a known hydatid cyst from a series of images of cysts and parasites from a variety of species (figure 23). Only 56.5% (13 of 23) of the farmers felt they could differentiate between hydatid cysts and other cestodes, however, when asked to identify hydatid cysts from four images, only 47.8% (11 of 23) were able to. Farmers were not given any other information about the images or were told whether there was a hydatid cyst as one of the images.

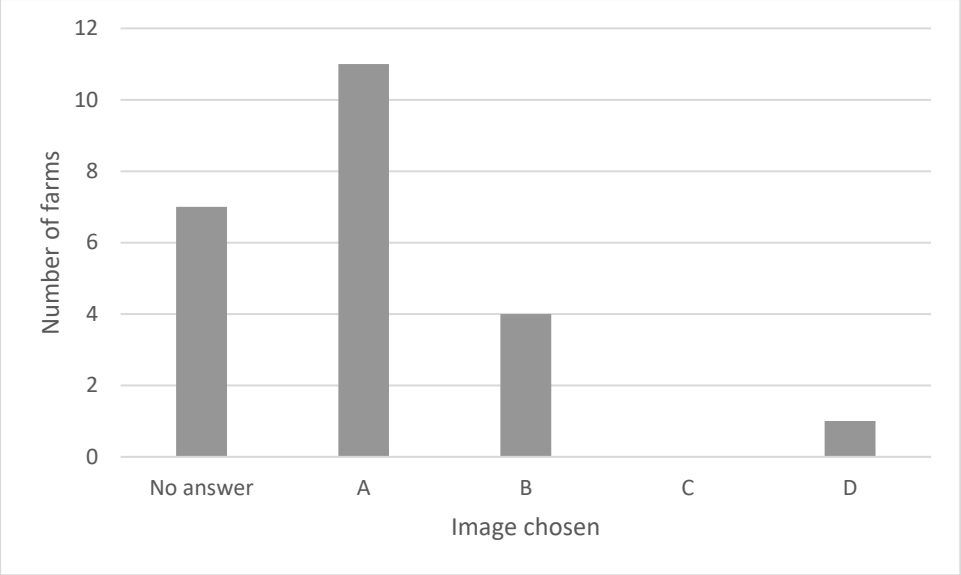


Figure 24: The number each image chosen as hydatid cysts by farmers (n=23).

20. Have you seen cysts in any of the following? (Figure 25)

A. [Home slaughtered animals] b. [Geese] c. [Other (Please specify)]

Farmers were asked about where they had seen cysts in various species. Most (16 of 23, 69.6%) stated that they didn't see cysts in any of their home slaughtered animals or geese (that had been reported prior to the project beginning). Six farms reported seeing cysts in the animals that they had slaughtered at home, with one of those reporting to have found a suspected hydatid cyst in a bullock 35 years ago. Others reported having not seen cysts in 8-10 years in home slaughtered animals. One farmer reported seeing lots of 'worms' in geese shot on the farm, though no further information was given.

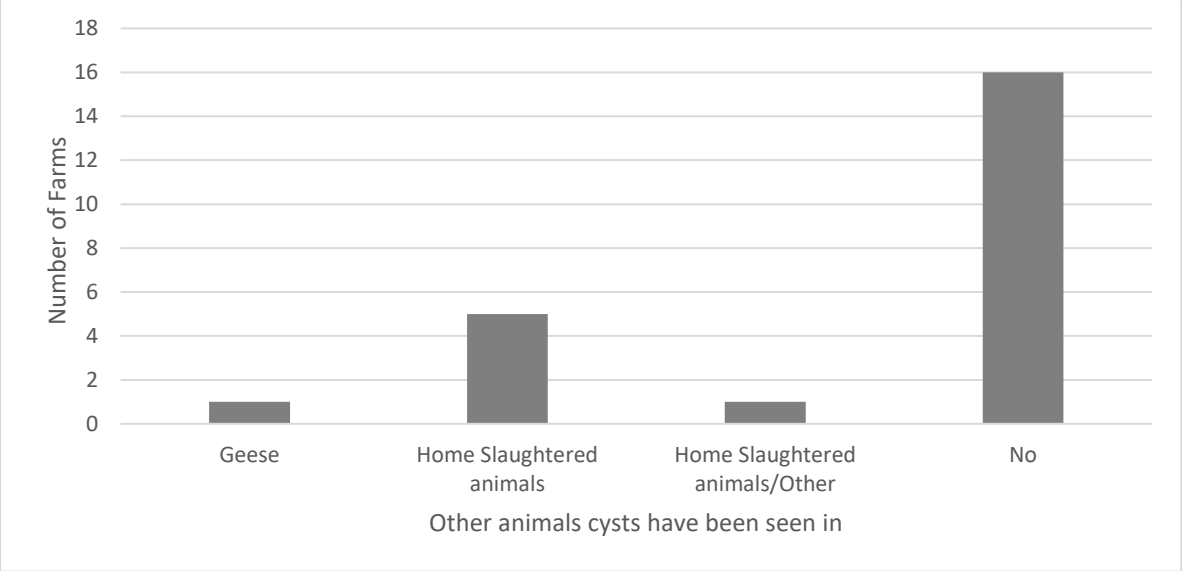


Figure 25: Other possible areas where farmers see cysts present in animals (n=23).

In relation to the information obtained from questionnaires two practices stood out as being of potential risk in relation to transmission of *Echinococcus* and other cestodes to dogs. These were (i) disposal of offal on beaches or in pig pens without any other treatment and (ii) allowing dogs to roam in farm settlements. Figure 26 shows the position of farms where this activity took place, as well as the locations of farms that have dogs and those that do not and the farms that responded to the questionnaire.

A quantitative analysis of attitudes and behaviours concerning sustainable parasite control practices from Scottish sheep farmers

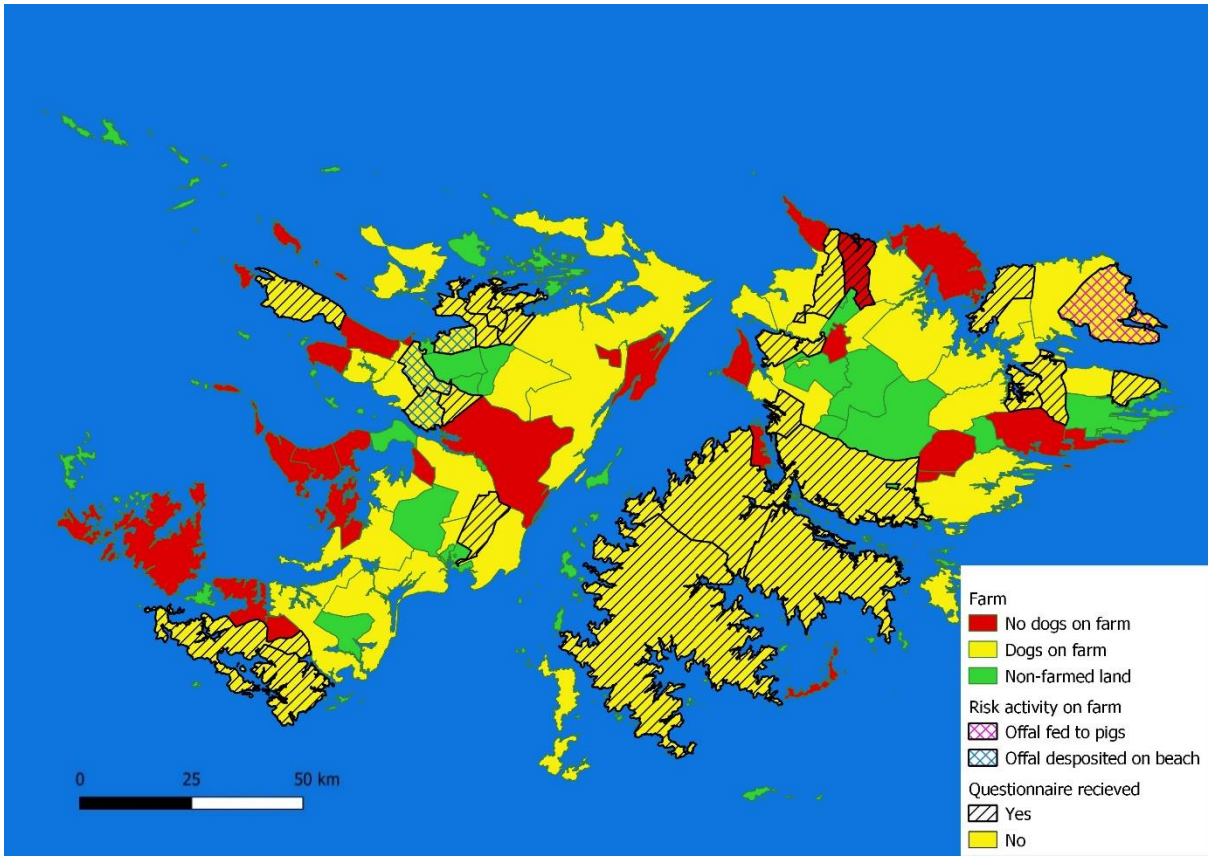


Figure 26: Farm boundary map of the Falkland Islands displaying which farms have dogs or not, which farms responded to the questionnaire and risk activity answered in the questionnaire.

2.4 Discussion

In the current study there was a 45% response rate to the questionnaires and although a target of around 60% would be more acceptable (Fincham, 2008), this could be considered a reasonable response. However, with a small population size of 51 farms a more meaningful response number could be calculated as $n = N/1+N(e)^2$ where N is the population size and e is the level of precision (Jack, Hotchkiss, Sargison, Toma, Milne, & Bartley, 2017) which in this case would be 45 responses (88%). Despite reminding farmers on several occasions to return their questionnaires there was still some disinterest. The responses would have been better if they had been carried out on a face-to-face basis rather than email. However, even allowing for a limited response, some useful information was obtained. It was also important to raise the profile of the project and encourage their involvement. Also, their opinions and historical knowledge is important in building a picture of the environment as a whole.

Obtaining meaningful information on farming practice can be difficult and the use of questionnaires can be useful but is dependent on appropriate and well-structured questions

and on high response rate and honest answers. In the current survey the questions were designed before the author made the first visit to the Falkland Islands and were based on previous studies carried out in Kyrgyzstan (Van Kesteren, Mastin, Mytynova, Ziadinov, Boufana, Torgerson, Rogan, & Craig, 2013). On reflection a number of questions such as 'What is the role of your dog?' and 'Do you own a dog?', provided limited information as the responses from each farm were the same, and the questionnaire was only distributed to farms with dogs. In addition, it would have been better to ask other questions such as "When were dogs last kept on that farm?" or "Do you use dogs from other farms?" Questions on seasonal pasture and drinking sources were useful in understanding the landscape and how farmers use it, however, were less focused on *E. granulosus* and control of parasites. In addition, the responses to some questions such as those on dog roaming and offal disposal could have been explored more extensively with additional questions.

The aim of the questionnaire was to identify practices that could lead to failures in the current control campaign that could allow the transmission of *E. granulosus.*

In relation to dog housing, it was evident that most farmers keep their dogs in locked kennels or at least tied up when not working. On farm visits, this was the case as dogs are seen as working animals for the most part, and so are managed in that way. The condition of the kennels varied between farms with many being well constructed and in good order. However, some showed that they required some repair and could potentially allow dogs to escape for a short time. In addition, one farm indicated that their dogs were allowed to roam around the farm property when not working and kept in the house at night. This is a violation of the control programme as free dogs could get access to material from sheep around the slaughter area and on local beaches. In addition, a dog was observed feeding on sheep carcases on cull sites associated with this farm (see Chapter 3).

Information on the feeding of dogs indicated that, in all but one farm, dogs were fed on raw meat at least some of the time. However, the majority of farms (73.9%) have now introduced commercial processed dog food at least some of the time. This represents a change in farming practice since 1993 when a survey indicated that all dogs were fed only on raw meat (Reichel et al., 1996). This approach can help lower the risk of transmission by reducing the amount of raw meat (and potentially infective material) fed to dogs.

Information obtained on slaughter practice indicated that in all farms home slaughter was carried out to some extent but in only five farms (21.7%) was this the only location. Most farms (78.3%) now send sheep to the abattoir. This is in contrast to 1993 when over 50% of farms only slaughtered at home (Reichel et al., 1996). This is linked to the development of the Sand Bay abattoir in Stanley which can process large numbers of animals. In terms of monitoring levels of infection this is beneficial as more animals are being inspected by professional staff where CE or other cestode infections can be detected. Only slaughtering animals on farm results in these farms not being included in abattoir surveillance long term and allow for potential infections to be missed.

The numbers of animals slaughtered on farms varies with most killing less than 100 animals per year but in three of the bigger farms between 200 and 800 animals are killed on site. In total around 3200 sheep each year are killed on farms which have dogs, for either food (human and animal) or for disposal of old animals. The infection status of these animals is unknown as they are not assessed by recognised meat inspectors. In addition, those older animals which are culled as a mechanism of disposal are not inspected at all. Farmers were asked where they regularly see cysts. Several farmers described seeing cysts in animals slaughtered on the farm, with one farmer saying they saw a hydatid cyst in a bullock 25 years previously, but no recent hydatid cysts. The majority, however, described not seeing any cysts in sheep slaughtered at home, which, based on previous abattoir data is unusual as *T. hydatigena* has been seen in sheep at prevalence's as high as 10% annually. Farmers are asked to inspect offal to pick up any CE infections in sheep not sent to the abattoir, however, farmers are not trained in what to look for. To understand farmers ability to identify hydatid cysts, they were asked to choose from a series of images, which one was a hydatid cyst. Only 56.5% were able to correctly identify hydatid cysts, with some farmers stating that they do not know what they are to look for, and so didn't answer the question. If farmers are not able to diagnose CE in sheep they slaughter, CE infections could be missed and as a result underestimating the prevalence of CE in the Falkland Islands. This was seen in a case of CE in a sheep on a farm that was found by an experienced farmer when slaughtering sheep on his farm, that was then brought to the department of agriculture for microscopic analysis and was confirmed as a hydatid cyst.

In relation to disposal of offal, 19 farms (82.6%) reported carrying out a procedure that would kill hydatid cysts and cysticerci i.e. long term storage, incineration or freezing before material was dumped on the shore or buried. However, four farms reported either dumping untreated offal directly on the shore or feeding it to pigs. Pigs can act as an intermediate host for taeniid but have not been reported to be susceptible to infection by eating the metacestode larvae of *E. granulosus* (Li, Chen, Budke, Zhou, Duan, Wang, Zhong, Liu, Luo, He, Shang, & Ito, 2021). This activity is therefore not a risk in relation to these animals producing adult worms but could be a problem if dogs get access to the offal as well. The dumping of untreated offal on beaches is a significant risk especially if these are close to where dogs are kept. The scavenging of this material by birds is also a risk as they could potentially carry parasitic tissue over a wider area where dogs may pick it up. This disposal method was used by 36% of the farmers in 1993 that had seropositive dogs, and on a single farm where five positive dogs were found, this was the only method of disposal (Reichel et al., 1996).

It must also be remembered that it is only the heart, lungs, and liver which is "treated" prior to disposal. Other organs such as the intestines may be deposited directly on the beaches without any processing. Whilst this is not an issue for *E. granulosus* it is significant for *T. hydatigena* as cysticerci are often found in the mesentery around the intestine (Singh, Sharma, Gill, & Sharma, 2015). Dogs which are temporarily roaming around slaughter areas or beaches could therefore eat such material. The concern that some farmers had over sea lions consuming disposed offal was not investigated. Although sea lions can harbour adult tapeworms such as *Diphyllobothrium* spp (Hernández-Orts et al., 2021) there are no records of these being hosts for taeniid cestodes.

In conclusion the questionnaire data highlighted a number of high-risk activities in a small number of farms which could allow dogs to become infected with *E. granulosus* and other taeniid worms. These are:

(i) The potential for some dog to wander unaccompanied in the immediate farm surrounding as allowed by the owner or through possible escape from kennels which are in need of repair.

(ii) The depositing of untreated livers, lungs and intestines on shores close to the settlements where dogs could potentially gain access.

(iii) The culling of old animals at cull sites distant from the settlements with intact carcases being left for scavengers. This could potentiate infection of other wild definitive hosts (See chapter 3).

In addition, education of farmers on the identification of cestode cysts and recording processes could be improved.

The results from the questionnaire survey show that there is still potential risk of infection in dogs through the access of offal not being disposed of properly, the possibility of dogs roaming freely on the farm and the culling of older animals without offal inspection or proper disposal. This supports the investigation into the prevalence of infection in dogs and the prospect of other hosts being involved in taeniid transmission in the Falklands.

Chapter 3. Current Prevalence of Echinococcus in definitive hosts.

3.1. Introduction

The definitive hosts of *Echinococcus* genus come primarily from the Canidae family, including dogs, foxes, wolves and other related species (Eckert & Deplazes, 2004). Domestic dogs, as part of a domestic life cycle, are the main host for *E. granulosus (sensu lato)* globally and the most important in relation to human infection. Important intermediate hosts in such cycles include sheep, goats, cattle, pigs, horses, alpacas and camels. Parasite genotype can vary in these domestic cycles although the G1 genotype may be found in most intermediate host species (Deplazes et al., 2017). A number of wildlife cycles also exist in different parts of the world, with wolves (*Canis lupus*), foxes (*Vulpes* spp. and *Lycalopex* spp.) coyotes (*Canis latrans*), jackals (*Lupulella adusta, Lupulella mesomelas* and *Canis aureus),* hyaenas (*Hyaena hyaena, Hyaena brunnea, Crocuta Crocuta,* and *Proteles cristata*) and dingoes (*Canis lupus dingo*) being involved as definitive hosts and wild cervids, wild boar, wallabies acting as intermediate hosts (Deplazes et al., 2017). Domestic cats are known to be poor hosts for *E.granulosus* (Smyth & Smyth, 1964) though they have been shown to be able to act as intermediate hosts in rare cases, sometimes related to Feline Immunosuppressive Virus (FIV) (Armua-Fernandez, Castro, Crampet, Bartzabal, Hofmann-Lehmann, Grimm, & Deplazes, 2014; Avila, Maglioco, Gertiser, Ferreyra, Ferrari, Klinger, Barbery Venturi, Agüero, Fuchs, & Jensen, 2021).

Apart from domestic dogs, the only major carnivores to occur in the Falkland Islands are feral cats, distributed across the entire Falkland archipelago, and the Patagonian fox (*Lycalopex griseus*), that is located on six satellite islands (Weddell, Beaver, Staats, Teal, Split and River Islands). There was also the now extinct Falkland Island Wolf, also known as the Warrah (*Dusicyon australis*), which was endemic to the Falklands until 1876 when it was hunted to extinction (Austin, Soubrier, Prevosti, Prates, Trejo, Mena, & Cooper, 2013; Slater, Thalmann, Leonard, Schweizer, Koepfli, Pollinger, Rawlence, Austin, Cooper, & Wayne, 2009).

Of the species still found today, both cats and Patagonian Foxes are known definitive hosts of multiple taeniid species, though only the Patagonian Fox is a known definitive host for *E. granulosus* (Zanini, Laferrara, Bitsch, Pérez, & Elissondo, 2006). As mentioned above, these

foxes are isolated to six islands where their numbers are estimated to be 3000 at the start of the breeding season (unpublished data, South Atlantic Environmental Research Institute (SAERI)), and den sites are mainly located in coastal areas of the islands. The Patagonian fox is described as an opportunistic carnivore, who's diet is mainly comprised of small mammals, birds, and their eggs, arthropods, and carrion (Muñoz-Pedreros, Yáñez, Norambuena, & Zúñiga, 2018; Zapata, Travaini, Delibes, Rolando, Nez-Peck, Austral, Deseado, Cruz, & Doñ, 2005). Of the islands where foxes inhabit, only on island, Weddell Island, is run as a working farm, with a sheep population of 5,600. This is the only island where foxes could become infected through consuming infected offal through scavenging on dead sheep, though reports of foxes hunting or even scavenging on dead sheep has not been reported by the farmers during this project.

Feral cats are the only uncontrolled carnivore found across the Falkland Islands, with farmers reporting to see them around the settlements, as well as far away in the open pasture. The numbers of feral cats in the Falkland Islands are unknown, though reports from farmers estimate their numbers to range between 50 and over 1000 per farm. Cats are not known to act as definitive hosts for *E. granulosus*, however, they are known to be suitable definitive hosts for other taeniid species such as *Taenia hydatigena* and *Taenia ovis* that are prevalent in the Falkland Islands (Borji et al., 2011; Smyth & Smyth, 1964). While not acting as definitive hosts, cats have been documented to act as intermediate hosts, with cysts being discovered in the peritoneal cavity and can be environmentally infected with *E. granulosus* G1 (Avila et al., 2021). There is also evidence of an association between the suppression of the immune system of cats caused by FIV and Cystic Echinococcosis infections in cats, which is important as feral cats in the Falklands have been diagnosed with FIV in the past (Armua-Fernandez et al., 2014).

The Falklands is also a marine haven, with species such as the South American Sea Lion (*Otaria flavescens*) South American fur seals (*Arctocephalus australis*) and Leopard Seals (*Hydrurga leptonyx*) found in the waters around the islands. Though not known to be responsible for transmission of terrestrial cestode parasites such as *E. granulosus*, they do harbour tapeworm species from the diphyllobothriid genus, that include common fish tapeworm that have been known to infect humans (Hernández-Orts et al., 2021; Hernández-Orts, Montero, Juan-García, García, Crespo, Raga, & Aznar, 2013; Scholz et al., 2019; Waeschenbach, Brabec,

Scholz, Littlewood, & Kuchta, 2017). Due to their similarities with dogs, suggestions were made that these species may be involved in *E. granulosus* transmission in the Falklands, as locals thought that they may feed on material disposed of into the sea. Sea Lions diet comprises of a variety of marine life including Cephalopod species such as squid and octopus, as well as various species of fish and rays (Koen-Alonso, Crespo, Pedraza, García, & Coscarella, 2000). Fur seals and leopard seals diet also consists of similar species such as squid and fish, but Leopard seals have also been documented hunting penguins and sea lion pups (Casaux, Baroni, Ramón, Carlini, Bertolin, & DiPrinzio, 2009; Naya, Arim, & Vargas, 2002). There are no reports of such species scavenging on offal or carcasses of terrestrial mammals, however, it is possible that these species may come into contact with material deposited at sea. Due to the lack of focused interest in understanding whether these species play a role in transmission of taeniid parasites, there is no information on this possibility, however, the lack of overlap of home ranges further suggests that transmission of *E. granulosus* between pinniped species like sea lions fur seals and leopard seals and sheep in the Falklands is highly unlikely.

3.1.1 Prevalence and Worm Burdens in dogs.

Cystic Echinococcosis has a global distribution and is closely associated with agriculture and pastoralism. Where CE is endemic, the prevalence of *E. granulosus* in definitive hosts varies, and in some cases, prevalence can remain very low yet remain endemic in a region (Buishi et al., 2006; Deplazes et al., 2017; Mastin, Brouwer, Fox, Craig, Guitián, Li, & Stevens, 2011). The variation in prevalence is caused by a variety socio-cultural, economic and environmental factors which influence the transmission of the parasite and the prevalence in dogs.

Table 1: Examples of Prevalence, worm burden and detection method of global studies in *E. granulosus* infection in definitive hosts.

Region	Prevalence (%)	Mean Worm Burden (range)	Detection method	Citation
Turkana, Kenya	27, 26 (n=42, n=161)	540 (2-4080)	Necropsy, Copro-ELISA	(Buishi et al., 2006)
Sichuan Province, China	27 (n=22)	(4-100)	Necropsy	(Yang et al., 2009)
Kazakhstan	23 (n=630)	631 (399-1086)	Arecoline hydrobromide purgation	(Torgerson, Shaikenov, Rysmukhambetova, Ussenbayev,

				Abdybekova, & Burtisurnov, 2003)
United Kingdom	25.6, 2.8 (n=364)	N/A	Copro-ELISA, Copro-PCR	(Lett, Boufana, Lahmar, Bradshaw, Walters, Brouwer, Fraser, Maskell, & Craig, 2018)
Coquimbo region, Chile	28 (n=93)	N/A	Copro-ELISA	(Acosta-Jamett, Weitzel, Boufana, Adones, Bahamonde, Abarca, Craig, & Reiter-Owona, 2014)
La Rioja, Argentina	30.5 (n=269)	N/A	Copro-ELISA	(Amaya, Moreno, Salmaso, Bazan, Ricoy, Córdoba, & Santillan, 2016)
Lima, Puru	18 (n=22)	2 (2)	Copro-ELISA, Copro-PCR, Arecoline bromhydrate purgation	(Reyes, Taramona, Saire-Mendoza, Gavidia, Barron, Boufana, Craig, Tello, Garcia, & Santivañez, 2012)

Kenya is a country where CE is highly endemic, with some of the highest prevalence in dogs (see table 1) (Buishi et al., 2006). The Turkana keep large numbers of dogs using them for various roles such as protecting and herding livestock. Dogs are regularly fed food scraps and when livestock slaughter occurs, dogs are fed the offal which is often infected. This results in the high prevalence of infection as well as a high worm burden (Buishi et al., 2006).

Another area where CE is highly endemic is in the Tibetan communities in China. There are large numbers of stray dogs in the region due to the Buddhist beliefs. The nomadic herdsmen allow dogs to die naturally and when they are too old to work, they are considered free animals, roaming constantly. This combined with feeding them offal in the slaughter season allows for the chance of reinfection of dogs even after the 6-monthly praziquantel dosing (Yang et al., 2009). Cystic Echinococcosis is endemic to many countries across central Asia, with the environment lending itself to livestock farming and the communities often living semi-nomadic lifestyles, herding of sheep and cattle is often conducted with the use of dogs, it provides the ideal situation for CE to be endemic. Regular feeding of offal to dogs, lack of knowledge around the lifecycle of *E. granulosus* as well as regular interaction between sheep and dogs are key contributors to the transmission of cestode parasites in these regions (Zhang et al., 2015). Prevalence in dogs across Asia and the rest of the world is associated with key risk factors such as home slaughter or the use of sheepdogs. Places where similar practices

are shared, result in high levels of canine infection (Mastin, van Kesteren, Torgerson, Ziadinov, Mytynova, Rogan, Tursunov, & Craig, 2015).

South American countries such as Uruguay and Chile, where livestock production is also an important contributor to the economy, are also locations where CE is endemic (Irabedra, Ferreira, Sayes, Elola, Rodríguez, Morel, Segura, Santos, & Guisantes, 2016). Studies in both areas have found differing prevalence of infection in dogs. In Chile, the areas with the highest rates of infection are rural and underdeveloped areas, with prevalence in dogs as high as 28% in some areas. There were also similar key risk factors for infections in dogs such as home slaughter, which resulted in the likelihood of finding infected dogs over three times more likely (Acosta-Jamett et al., 2014). The prevalence in Uruguay however is considerably lower as a result of a control programme in 2005 that focused on health care education, risk factor-based focus and targeting the rural small villages and poor urban communities where data shows are most affected (Irabedra et al., 2016).

The mean worm burden found in dogs is around 300, but infections are often over dispersed, with very few animals having upwards of 1000 individual worms, and a larger number of dogs having relatively low intensity of infection (Craig et al., 2015). Estimating worm burden is very difficult without visual inspection of the small intestine, which requires euthanisation of the dog being examined, and so strict ethics approval. This was not going to be suitable during this study as all the dogs sampled were either pets or working dogs belonging to farmers or from Stanley.

In the early 1990s, there was 908 dogs in the Falkland Islands, the majority of which were working farm dogs used for gathering sheep. At the time, 3.5% of the dog population were seropositive for *E. granulosus* antibodies. A late round of copro-antigen testing revealed 1.8% of 464 dogs tested were also positive (Reichel et al., 1996). Dogs were again tested in 2010, 1.4% of 568 dogs tested at the University of Salford were found to be PCR positive for *E. granulosus* (unpublished data). Despite the control measures in place and no stray or wild dogs in the Falklands, there is evidence that dogs are still becoming infected with *E. granulosus* and transmission to sheep is still able to occur. There are strict controls on how dogs are kept and fed in the Falklands to improve control, so evidence of continued infection of dogs is a key area of investigation.

3.1.2 Diagnostic approaches for detection of *Echinococcus granulosus* in definitive hosts.

Diagnosis of infection is a key concept in epidemiology, and vital in understanding transmission of taeniid cestodes with the view of surveillance and control. The earliest, and to this day the gold standard approach to diagnosing cestode parasites is through post-mortem animal necropsy. Dissection and inspection of the small intestine of canid hosts washed and scraped in saline solution to remove parasites from the intestinal wall has been a long-standing technique to identify infection (Deplazes, Gottstein, Eckert, Jenkins, Ewald, & Jimenez-Palacios, 1992). The technique involves laborious examination of intestinal washings on a black tray and confirmation of specimens under a microscope. Careful examination allows detection of low numbers of worms, although species specific identification may be difficult in areas where *E. granulosus* and E. *multilocularis* are co-endemic (Craig et al., 2015). However, this approach is not applicable to communities which intend to keep dogs alive. A sample is therefore required to be taken from the dogs for diagnostic analysis and in most cases, this is a faecal sample although some approaches involve blood samples for antibody detection. The approaches which have been used are as follows:

i) Purgation

The administration of a purgative to dogs will result in the full evacuation of the intestine in 30-60 minutes and therefore can be used as both a detection and a treatment method. Arecoline hydrobromide has been used for over 100 years both for diagnostics and to eliminate the parasites from the dog's intestine in control programmes such as those used in Iceland, New Zealand and Tasmania (Craig & Larrieu, 2006) . The technique is potentially hazardous to the operator and requires the faecal purge to be collected from the ground and placed in 10% formalin. The purged material would then be examined in black-backed trays under dissection microscopes. The advantage of this approach is in the specificity of diagnosis being close to 100%. However this method lacks sensitivity particularly at low worm numbers with an estimated sensitivity of 40-75% (Lahmar et al., 2007). Additional problems may also occur when some dogs do not purge. In addition, arecoline hydrobromide is potentially toxic to dogs and may lead to cardiovascular collapse (Craig et al., 2015). Its use is therefore discouraged.

ii) Faecal egg detection

Faecal egg detection can be a useful diagnostic technique for many helminths but in the case of *Echinococcus*, the eggs, although of characteristic shape and size to the genus, are identical to all other taeniid species making diagnosis genus specific rather than species specific (Maurelli, Bosco, Pepe, Ianniello, Amadesi, Cringoli, & Rinaldi, 2018). The technique involves the use of flotation media such as zinc sulphate or zinc chloride for egg flotation and microscopical examination. This basic technique alone is known to have a low sensitivity of between 3-33% recovery of cestode eggs, depending on the chosen flotation media. Carnivore faeces also contains high levels of fats which can float on top of the flotation media and significantly reduce the sensitivity of the technique (Széll, Sréter-Lancz, & Sréter, 2014). More efficient techniques that involve the use of centrifugation and sequential sieves to remove other particles and debris such as bone and plant material, as well as allowing only the smallest items such as parasite eggs to pass through and then using flotation medias to separate cestode eggs can increase sensitivity of diagnosis to 94% (Mathis, Deplazes, & Eckert, 1996). Combining this method with other detection methods such as Copro-PCR provides both highly sensitive and specific diagnostic test.

iii) Detection of specific antibodies in serum.

Development of a diagnostic technique to identify antibody production from experimentally infected dogs with cestode antigens from the protoscolex somatic (Px-SM) antigen as well as hatched oncospheres found that immune response could be detected using Enzyme-Linked Immunosorbent Assay (ELISA). This technique found no cross reactivity with other cestode parasites (Gasser, Lightowlers, Obendorf, Jenkins, & Rickard, 1988; Jenkins & Rickard, 1985) and had a specificity of 97-100%, however the sensitivity of the ELISA varied depending on the geographic area from which the infected dogs came from, though was still over 72% (Gasser, Jenkins, Paolillo, Parada, Cabrera, & Craig, 1993). This technique was found to be a useful diagnostic technique, but a major drawback was the difficulty in distinguishing between current infection and residual serum antibodies post infection (Gasser et al., 1993). In addition, blood sampling is a more invasive process than faecal samples and more costly compared with other methods.

iv) Coproantigen detection.

Detection of *E. granulosus* antigen in faeces provides a non-invasive technique to test larger population sizes without the need for specialised staff such as vets to collect serum samples.

Early attempts to detect *E. granulosus* antigen found cross reaction with *Taenia saginata* (Babos & Nemeth, 1962). Further research found raising antisera against non-gravid adult proglottids by immunizing hyperimmune rabbits and using a capture antibody system, binding a capture antibody to a flat bottomed microtitre plate, adding the faecal supernatant, and then adding the peroxidase-conjugate antibody. This method had high sensitivity, detecting infected dogs with worm burdens as low as 15 individuals (87.5%) as well as 96.5% specificity in detection of naturally infected dogs (Allan, Craig, Garcia Noval, Mencos, Liu, Wang, Wen, Zhou, Stringer, Rogan, & et al., 1992). This optimised technique is still used globally in field and lab-based studies (Lahmar et al., 2007; van Kesteren, Qi, Tao, Feng, Mastin, Craig, Vuitton, Duan, Chu, Zhu, & Wen, 2015). This technique has further been used to identify coproantigens in environmental samples such as soil and water from areas containing previously infected dogs, allowing for detection of environmental contamination even when dogs are not present in the area (Sánchez Thevenet, Alvarez, Torrecillas, Jensen, & Basualdo, 2019). Presence of infection is based off the optical density (OD) value produced when a reactive substrate is added. There is evidence that there is a positive correlation between the OD value of a sample and the intensity of worm burden, with heavier infection causing a higher OD value (Mastin, 2015).

v) Copro-PCR Detection

Specificity of diagnostic techniques is vital in detection and classification of cestode parasites, as worms such as *E. granulosus* and *E. multilocularis* are morphologically very similar and other taeniid species may co-infect the same host. Polymerase chain reaction (PCR) had been used for diagnosis of pathogens previously through bacterial and viral gene amplification. The first PCR amplification on cestode DNA from faecal samples amplified the previously isolated *E. multilocularis* UlsnRNA gene (Bretagne, Guillou, Morand, & Houin, 1993; Bretagne, Robert, Vidaud, Goossens, & Houin, 1991). One major difficulty involving DNA amplification from faecal samples is the presence of PCR inhibitors that prevent components of the PCR reaction from working properly, for example, calcium inhibits the function of Taq polymerase, a heat resistant enzyme involved in the incorporation of nucleotides to a replicated DNA strand, resulting in false negatives through no replication of DNA (Opel, Chung, & McCord, 2010). To avoid this, further steps using commercial DNA extraction kits are needed to remove

inhibitors from the sample being tested (Maksimov, Isaksson, Schares, Romig, & Conraths, 2019).

Once extracted from fox faeces, the DNA was successfully amplified by PCR and showed high sensitivity, detecting a single egg from 4g of faeces (Bretagne et al., 1993). Once *E. granulosus* DNA was isolated by Cabrera, Canova, Rosenzvit, and Guarnera (2002), the same technique was used to identify *E. granulosus* DNA from faecal material. This technique showed high sensitivity and specificity (100%), detecting a single egg spiked in a control faecal sample and showed no amplification of any other helminth DNA that was added, including *E. multilocularis* and 3 other *Taenia* species. (Abbasi, Branzburg, Campos-Ponce, Abdel Hafez, Raoul, Craig, & Hamburger, 2003). Over time, the taxonomy of *Echinococcus* species has been modified as genetically different species have been found and sub-species established such as *Echinococcus shiquicus*. It was observed in unpublished data by B. Boufana that there was some cross reaction of primers between *E. multilocularis* and *E. shiquicus* DNA. This resulted in the development of new primers targeting the nucleotide sequences of the NADH dehydrogenase subunit 1 (ND1) mitochondrial gene of *E. shiquicus, E. granulosus* genotype 1 (G1), and *E. multilocularis* (Boufana, Umhang, Qiu, Chen, Lahmar, Boué, Jenkins, & Craig, 2013). The specificity and sensitivity of these results were high and are still commonly used. The PCR protocol is also the same protocol to be used in this study. As described, Copro-PCR provides a highly specific and sensitive diagnostic approach to parasitological studies, however, is labour intensive, with multiple separation and extraction steps prior to the PCR reaction itself. These extra steps make the testing of larger populations of dogs more expensive and time consuming compared to methods such as copro-ELISA. PCR as a diagnostic technique also requires the presence of parasite eggs in the faecal sample,, therefore infections would have to be patent in order to be detect. (Craig et al., 2003).

Another detection method is Loop-mediated isothermal amplification (LAMP). More recent than PCR, LAMP is a sensitive and quicker alternative to PCR that is less affected by biological inhibitors, reducing the need for DNA extraction methods from biological samples (Kaneko, Kawana, Fukushima, & Suzutani, 2007). This technique uses a single incubation temperature of between 60-65°C (assay dependant) and can amplify DNA within two hours. This method has proven suitable for field studies with its cost effective and simple protocol, as well as its high sensitivity and specificity in detecting *E. granulosus* (Ni, McManus, Lou, Yang, Yan, Li, Li,

Liu, Li, Shi, Fan, Liu, Cai, Lei, Fu, Yang, & Jia, 2014). For this project, with the prior availability of PCR equipment and the authors prior knowledge of this technique, this was the preferred diagnostic test.

3.1.3 Other Cestode definitive hosts on the Falkland Islands.

The Falkland Islands has strict controls on dog numbers, with all dogs registered as pets or working dogs, as a result, there are no stray dogs. There are two other species in the Falklands that can act as definitive hosts for cestode parasites, the Patagonian Fox (*Lycalopex griseus*) and feral cats (*Felis catus*). The Patagonian Fox is confined to six of the outer islands of the Falkland archipelago (Weddell, Beaver, Staats, Teal, Split and River islands, see figure 27), where only Weddell Island is a registered commercial farm. There have been cases of CE in sheep from Weddell Island, last documented in a single sheep in 1996. A parasitological study of the foxes on Weddell, Beaver, Split and River Islands was carried out by the Department of Agriculture in 2007, shooting and dissecting 23 foxes, inspecting their intestine visually on the island, but no cestode like parasites were found in any of the individuals dissected.

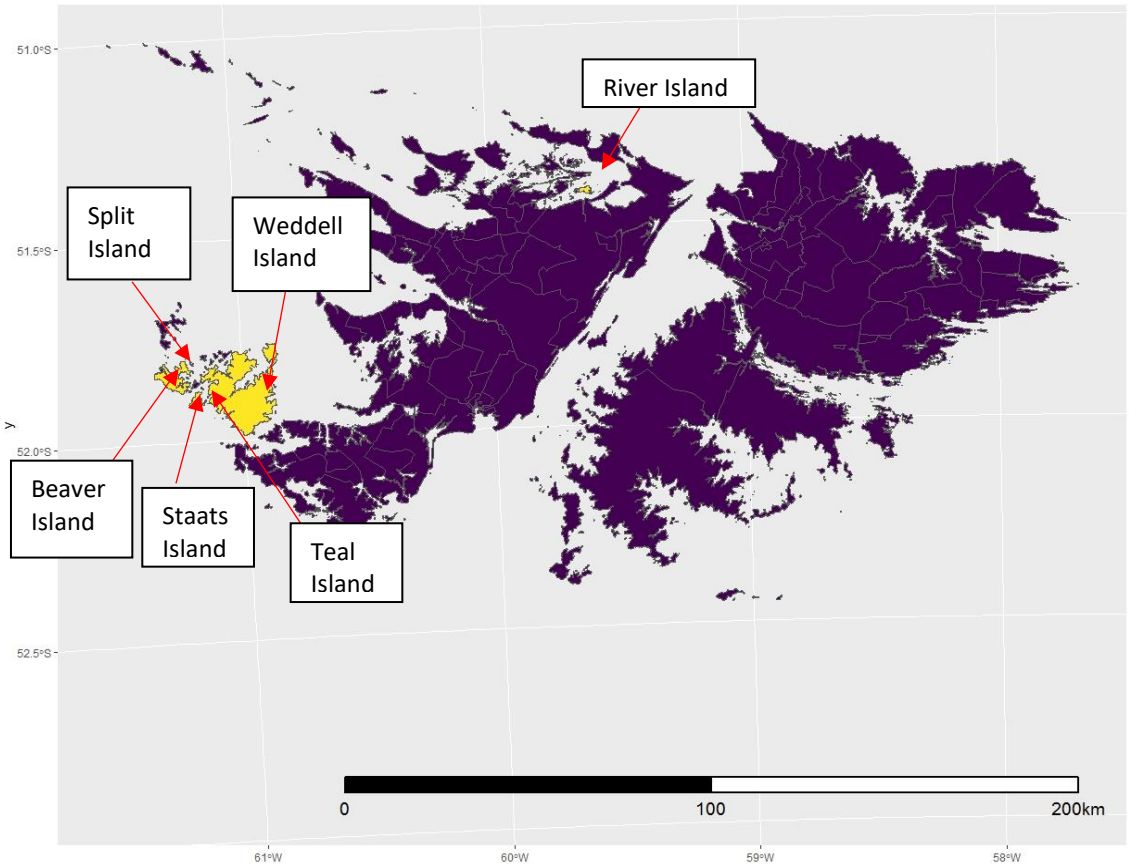


Figure 27 Locations of Islands inhabited by Patagonian Foxes (*Lycalopex griseus*).

The Patagonian Fox is known to act as a definitive host to both *E. granulosus* and *T. hydatigena* across Patagonia, Argentina (Zanini et al., 2006) and so has the potential to contribute to the transmission of such parasites in the Falkland Islands. Due to the remote location of foxes and the limited number of sheep on the islands in which they inhabit, their role is not thought to be significant, however it is an important area of investigation that must be explored. Feral cats are found across the Falkland Islands, with farmers suggesting their numbers to be over 100 per farm, with one farmer suggesting it could be as high as over 1000 on interview. Cats have been described as poorly competent hosts for *E. multilocularis* (Kapel, Torgerson, Thompson, & Deplazes, 2006) and in *E. granulosus* worms do not reach maturity in cats deliberately infected (Konyaev, Yanagida, Ivanov, Ruppel, Sako, Nakao, & Ito, 2012), nevertheless, there is evidence that stray cats can be hosts of various other cestode parasites, including *T. hydatigena* (Borji et al., 2011). Though unlikely to contribute to transmission of *E. granulosus*, feral cats could be a factor contributing to the continued transmission of *T. hydatigena*, that is found at a prevalence of approximately 2% in sheep slaughtered at the abattoir.

3.1.4 Natural death of sheep and deposition of carcasses on pasture

To maintain sheep populations within the carrying capacity of the farmland, farmers either send stock to the abattoir to be sold commercially, or, when animals are older and less productive, they are culled and left on pasture as whole carcasses at localised cull sites. These animals are open to scavenging by other carnivores and therefore provide a potential risk for transmission of cestodes and other parasites. Many species are thought to feed on carcasses left on open pasture both on cull sites, as well as when individual animals die throughout the year. These range from sea birds and raptors, to rats and feral cats. There is also potential for dogs to access sheep carcasses that have not had their offal inspected. As described, the culled animals or animals that have died naturally do not have their offal removed or inspected, making them a potential transmission risk. The highest risk category is culled animals that are older, and more likely to be infected with CE making these areas of increased numbers of dead sheep a major risk of infection (Torgerson et al., 1998).

The use of camera traps can be a useful approach in establishing which carnivores are feeding on carcases, and this has been done for other animal systems. To observe Cougar scavenging behaviour in Canada, remote camera trap technology was used to capture the rare scavenging

behaviour of cougars (*Puma concolor*) feeding on an Elk (*Cervus elaphus*) carcass. Camera traps were attached to a tree 1m off the ground approximately 3m from the carcass, with another at the same height on a game trail leading to the carcass. Using distinct markings on the Cougars body, 3 different individuals were identified visiting the carcass over a three-and-a-half-month period (Bacon & Boyce, 2010). To investigate scavenging species feeding on Golden snub-nosed monkey (*Rhinopithecus roxellana*) carcasses in Central and Southern China, similar technology was used over a 25-day period, chronologically documenting four different scavenging species over the given timeframe (Huang, Qi, Garber, Jin, Guo, Li, & Li, 2014).

In the current study camera traps were used to identify the species that scavenge on the dead sheep in the Falkland Islands and establish if any species could potentially contribute to the continued transmission of *E. granulosus*. Scavenging species can be important contributors to disease dynamics by acting as hosts or vectors for various pathogens, facilitating the spread of disease by opening carcasses and exposing infected organs/tissues, moving infected material to where hosts or vectors can access it (Carrasco-Garcia, Barroso, Perez-Olivares, Montoro, & Vicente, 2018).

3.2 Methodology for field sample analysis

3.2.1 Copro-ELISA

Faecal Samples

Faecal samples were collected from each farm between 12th March 2018 and 27th April 2018 after consultation with the farm manager. Information on the age, sex and name of each dog was also recorded. Fresh samples (3-5g) were collected from around the individual kennels of each dog (Figure 28) and placed into a 35ml universal tube and 10ml of 0.3% Phosphate Buffered Saline (PBS) containing 0.3% Tween and 10% formalin (Fisher Scientific, Loughborough, UK). (Allen et al 1992). In the laboratory, samples were homogenised with a wooden spatula, shaken, and then centrifuged at 2500 r. p. m (1125G) for 5 minutes. The supernatant was then removed using a Pasteur pipette and placed into a 1.5ml Eppendorf. Supernatant samples were stored -20^{o}C in the Department of Agriculture Laboratories until

transfer to the University of Salford under Animal & Plant Health Agency import licence (ITIMP18.1114). Here samples were stored at -80^{o}C for 7 days to ensure that no viable eggs were present and then kept at -20^{o}C.

Control positive samples were obtained from purged dogs from a previous study in Kyrgyzstan (Van Kesteren et al., 2013). Control negative samples were obtained from UK pet dogs, from previously screened dogs from the Falkland Islands and from post-mortem autopsied dogs form China (van Kesteren et al., 2015).

Copro-Antigen ELISA antibodies.

Copro-Antigen ELISAs were carried out using purified IgG antibodies produced in a previous study at the University of Salford by Dr Freya Van Kesteren (van Kesteren, 2015) and stored at-80^{o}C. This was based on production of two polyclonal rabbit antibodies. The capture antibody (Rb91) was prepared from hyperimmune rabbit IgG raised against a surface extract from adult *Echinococcus granulosus* worms (Elayoubi and Craig, 2004), and the peroxidase-conjugated antibody (Rb5px) was *anti-E. granulosus* whole worm somatic (Allan et al., 1992). The specificity and sensitivity of this capture antibody system was tested using a panel of faecal samples from necropsied dogs in China (van Kesteren, 2015)and was found to be 93% sensitive as tested with 31 known positive samples and 100% specific using 43 known negative samples including four samples with *T. hydatigena* and two with *T. multiceps*. Subsequently using a different panel based on purge samples from Kyrgyzstan dogs a sensitivity and specificity of 95% and 88% respectively was recorded. The *Echinococcus* detection was genus specific as both *E. granulosus* and *E. multilocularis* could be detected and the false negative samples were associated with low worm burdens (generally <20 worms).

Figure 28: The author collecting faecal samples from under the kennel of a dog in the Falklands.

Standardisation of a Capture ELISA system for detection of *Echinococcus* antigen.

The Copro-ELISA involves the detection of antigen between two layers of antibodies: The 'capture' and the 'conjugate' antibodies. Rabbit 91 (Rb91) was the capture antibody and Rabbit 5 (Rb5px) was the peroxidase-conjugated antibody. In order to check the activity of both antibodies separately, a checkerboard titration was carried out. As adult *Echinococcus* antigen was not available, activity was checked using a PBS extract of *E. granulosus* protoscoleses (EgP) obtained from hydatid cysts in sheep from UK abattoirs (protein concentration 0.75 mg/ml).

Checkerboard ELISA technique

To standardise antibody activity Immunon 4HB, 96 well ELISA plates (Fisher Scientific, Loughborough, UK) were coated with EgP antigen diluted initially at 1 in 100 in carbonate bicarbonate buffer (BCB) (Sigma-Aldrich, Dorset, UK) at 100 μL/well. Doubling dilutions were made on each column across the plate to a final dilution of 1 in 6,400 and one column was left as a no antigen control. Plates were then covered in cling film and incubated overnight at 4°C. After this incubation, the plate was then washed 3 times for 5 minutes each using 0.1% PBS Tween and blocked for 1 hour in PBS containing 0.3% Tween (200 μL/well). After removal of the blocking solution. The rabbit antibodies were added at a starting dilution of 1 in 200 (100 μL/well) in PBS/0.1% Tween and double diluted down the plate to a final dilution of 1 in 6,400. A final row was left as a no antibody control. Plates were incubated for 1 hour at room

temperature before washing (3 x 5 min) with PBS/0.1% Tween. For Rb91 antibody (unconjugated) plates where then incubated with an alkaline phosphatase labelled goat anti-Rabbit IgG second antibody (Sigma-Aldrich, Dorset, UK) diluted at 1 in 10,000 in PBS/0.1% Tween (100 μL/well) for 1 hour at room temperature. After a further 3 washes plates were incubated with the alkaline phosphatase substrate p-nitrophenyl phosphate at 1mg/mL in Diethanolamine buffer, pH 9.8. After 20 min, the O.D. of plates was read on a ThermoScientific Multiscan FC platereader at 405nm. For antibody Rb5px (conjugated) no second antibody was used, and plates were incubated directly with 100μL/well of SureBlue® TMB substrate (Insight Biotechnology, Wembley, UK) for 20 min before reading at 620 nm.

Capture ELISA technique

To test the success of the capture antibody system at the chosen antibody dilution, known serial dilutions of EgP antigen were used. An Immunon 4HB, 96 well ELISA plate (Fisher Scientific, Loughborough, UK) was coated with 100μL/well of the Rb91 capture antibody diluted at 1:500 in BCB. A number of wells were left uncoated to act as blanks. Plates were then covered in cling film and incubated overnight at 4°C. After this incubation, each plate was then washed 3 times for 5 minutes each using 0.1% PBS Tween. One hundred μL of 0.3% PBS Tween was added to each well as a blocking agent and incubated at room temperature for 1 hour. The 0.3% PBS Tween was discarded, and EgP antigen was added at 100 μL/well and doubling dilutions were made down each of five columns used to a final dilution of 1 in 6,400 and the final row contained no antigen as a control. The plate was covered and left for 1 hour at ambient temperature. The contents of the plate were then discarded, washed 3 times for 5 minutes with 0.1% PBS Tween buffer and then 100μL of the Rb5px conjugated antibody was added to each well, except blanks, at a 1:500 dilution in 0.1% PBS tween. This was again covered and left for 1 hour at ambient temperature. The conjugated antibody was discarded and washed 3 times for 5 minutes with 0.1% PBS Tween. The final step was to add 100μL of SureBlue® TMB substrate (Insight Biotechnology, Wembley, UK) to all wells including blanks and was incubated for 20 minutes in the dark and then read on a ThermoScientific Multiscan FC platereader at 620nm.

For faecal antigen detection, the same protocol as above was used, however, once the 0.3% PBS Tween was discarded, the plate patted dry before 50μL of Foetal calf serum (Fisher Scientific, Loughborough, UK) was added to each well. This step is required as faecal samples

can contain protease enzymes which can damage the antibodies. Foetal calf serum (FCS) can act as a source to mop up protease activity (Allan et al., 1992). For test and control samples 50µL of each faecal supernatant was then added in triplicate wells and mixed with the FCS. The plate was then covered for one hour and continued as the above protocol describes.

In order to establish what was positive and negative result, a cut off value was estimated as the mean OD value plus three standard deviations of a panel of 12 negative dogs from the Falkland Islands which had been previously tested by Copro-PCR and Copro-ELISA.

3.2.2 DNA extraction

To conduct CoproPCR on the faecal samples, DNA had to be extracted using a QIAamp® Fast DNA Stool kit (Qiagen, Hilden, Germany) following manufacturer's instructions. These kits are designed specifically for extraction of DNA from faecal samples (Qiagen, 2014).

Samples were weighed out (0.18-0.22g) in 2ml microcentrifuge tubes and placed on ice. One ml of InhibitEX Buffer (Qiagen, 2014) was added to each faecal sample and vortexed continuously until the sample was thoroughly homogenised. The suspension was heated at 70°C for 25 minutes to help lyse cells and eggs present, then vortexed for 15 seconds. Once vortexed, the sample was centrifuged for 1 minute at 14,000rpm to pellet the faecal particles. Alongside this, 15µL Proteinase K was added to a new 1.5ml microcentrifuge tube and 200µL of the supernatant was added. 200µL of buffer AL was added and vortexed for 15 seconds before incubating the sample for 10 minutes at 70°C. Following this incubation, 200µL of 96-100% ethanol was added to the lysate and the sample was mixed by vortexing. The entire 600µL of lysate was then added to QIAamp spin column and centrifuged for 1 minute. This allows for DNA present to be absorbed onto the QIAamp silica membrane (Qiagen, 2014). The DNA bound to QIAamp membrane was washed with 500µL of Buffer AW1 and centrifuged for 1 minute. The membrane was washed again using 500µL of Buffer AW2 and centrifuged for 3 minutes then put in a new collection tube and centrifuged again for three minutes to ensure removal of all washing buffers. The spin column was then transferred to a labelled 1.5 ml microcentrifuge tube and 200µL of Buffer ATE is added directly to the membrane. The spin column was incubated at ambient temperature for one minute and was then centrifuged for one minute. This will elute the DNA ready for PCR. Prior to PCR, an ethanol precipitation was performed to concentrate DNA in the extracted samples. 3M sodium acetate pH5.5 was

added at one tenth the volume of the original extracted sample and mixed by inverting the eppendorf, followed by three volumes of 95-100% ethanol and mixed. The samples were then placed in a -80°C freezer for 30 minutes before being spun in microcentrifuge at 14,000rpm 4˚C for 15 minutes. The supernatant was then aspirated off, avoiding disturbing the pellet and 500 µl of 70% ethanol that had been stored at -20°C and is spun in microcentrifuge at 14,000rpm 4˚C for 15 minutes. The resulting supernatant is aspirated off and the pellet then left to dry at room temperature for 10 minutes at ambient temperature. The pellet is then re-suspended in TE (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) to be used for PCR analysis.

3.2.3 PCR analysis

3.2.3.1 G1 primers

The PCR methodology for *E. granulosus* faecal material collected in the Falkland Islands was that as described by Boufana et al. (2013) with some minor alterations in the PCR reaction mix. The primers follow the specific nucleotide sequence of the *E. granulosus* genotype 1 (G1) NADH Dehydrogenase subunit 1 (ND1) mitochondrial gene. The protocol uses a 50µL reaction volume containing 50µL reaction volume and 5 +manufacturers Flexi reaction buffer (Promega Ltd. Southampton, UK.), 200 mM of each deoxynucleoside triphosphate (dNTPs; Bioline, London, UK), 0.3 mM 0.5 µl of each primer (Eg1F81, 5' GTT TTT GGC TGC CGC CAGAAC 3' and Eg1R83, 5' AAT TAA TGG AAA TAA TAACAA ACT TAA TCA ACA AT 3'), 2 mM MgCl2 and 2.5 U GoTaq polymerase (Promega Ltd. Southampton, UK). However, for this experiment the reaction mix was comprised of 23µl Molecular grade H_2O (Sigma-Aldrich, Dorset, UK.), 25µl MyTaq^{TM} Red Mix (Bioline, London, UK), 0.3 mM 0.5 µl of the forward and reverse primers (Forward- Eg1F81, and Reverse- Eg1R83,) and 1µl of Template DNA. Using a master mix reduced the number of pipetting steps and so reduced the risk of contamination. The reaction mix was then put through a PCR cycle that consists of an initial incubation period of 5 minutes at 94°C for 1 cycle, then 36 cycles consisting of 30s at 94°C, 50s at 62°C, and 30s at 72°C amplifying a *E. granulosus*-specific 226 bp fragment.

3.2.3.2 Universal primers

The Universal Cestode primers were designed by (von Nickisch-Rosenegk, Silva-Gonzalez, & Lucius, 1999) targeting the mitochondrial 12S rDNA region and can be used to detect various *Taenia* and *Echinococcus* species, including *E. granulosus* and *T. hydatigena*. Though not as

specific as the Boufana et al. (2013) primers, their ability to detect multiple species made them ideal for screening all the faecal samples for any other cestodes, not just *E. granulosus*. As with the previous protocol, minor alterations were made to the reaction mixture. For the PCR reaction mixture, a 50 µl reaction mixture comprising of 23µl molecular grade H_2O (Sigma-Aldrich, Dorset, UK.), 25µl MyTaq^{TM} Red Mix (Bioline, London, UK), 0.3 mM 0.5 µl µl of the forward and reverse primers (P60F, 5'-TTAA GATA TAT GTG GTA CAG GAT TAG ATA CCC-3' and 5'-AAC CGA GGG TGA CGG GCG GTG TGT ACC-3') and 1 µl of template DNA was added per sample. The reaction mixture was then put through a PCR cycle of 5 minutes at 94°C for 1 cycle then 40 cycles of 30 seconds at 94°C, 1 minute at 55°C, and 30 s at 72°C to amplify a fragment of DNA 373 bp in length.

Included in the PCR experiment were negative controls using PCR-Grade water (Sigma-Aldrich, Dorset, UK) to identify and contamination as well as positive controls from sequenced genomic DNA to ensure that the PCR reaction had worked. The PCR product was visualised using gel electrophoresis in a 1.5% agarose gel (Bioline Ltd, London) 1x Tris-Borate-EDTA buffer (Severn Biotech, Kidderminster, UK) at 110 V, stained with gel red (Cambridge Biosciences, UK) DNA dye and visualized using Syngene G:Box gel documentation system. To concentrate DNA samples from positive ELISA samples were concentrated using ethanol precipitation. In the presence of salt, ethanol efficiently precipitates nucleic acids, allowing the purified precipitate to be collected by centrifugation to later be re-suspended in a chosen solution. To do this, one tenth the volume of DNA solution of 3M sodium acetate pH 5.5 was added to the eppendorf containing the extracted DNA and gently mixed by inverting the tube followed by adding 3 times the volume of 95-100% ethanol and mixed using the same technique. The solution was then stored at -80°C for ten minutes then spun in a 4˚C microcentrifuge at 14,000rpm for 15 minutes and the supernatant is aspirated off (without disturbing the pellet). 500 µl of 70% ethanol that has been pre-stored at -20°C is added to the pellet and again spun in a 4˚C microcentrifuge at 14,000rpm for 15 minutes. Once again, the solution is aspirated off and the pellet is left to dry for 10 minutes at room temperature before it is re-suspended in TE (10 mM Tris-HCl, pH 7.5, 1 mM EDTA). This ensures the concentrated nucleic acids remain at a neutral pH and the EDTA will bind to any remaining trace metals.

3.2.3 Faecal sample microscopy for egg detection

Copro-ELISA positive faecal samples from 2018 were analysed using microscopy to look for the presence of Taeniid eggs. Initially the sample was put through a flotation protocol using sodium nitrate ($NaNO_3$ Specific Gravity 1.18-1.20). This method was used due to is high specific gravity and availability in the Falkland Islands. Five g of faecal sample was placed into 50ml universal tube and mixed with 10ml of $NaNO_3$ and homogenised, so all the sample was saturated. The solution was then passed through a sequential series of sieves beginning at 100µm to 40µm and the remaining solution was added to a 15ml centrifuge tube. The tube was filled up with $NaNO_3$ to create a positive meniscus and a cover slip was placed on top. The samples were centrifuged at 1,200rpm for 5 minutes, then removed and left to stand for 10 minutes. The cover slip was removed and placed onto a microscope slide and examined under 40x objective. Both the material on the cover slip and the sediment left after centrifugation was then used in DNA extraction as described previously to identify any eggs present on a species level (Dryden, Payne, Ridley, & Smith, 2005; Shaikenov, Rysmukhambetova, Massenov, Deplazes, Mathis, & Torgerson, 2004).

3.2.5 Camera trap surveillance of cull sites

Camera traps were used to identify the main species feeding on the carcasses and look at possible host species that could be leading to further infection of sheep with cestode parasites by the removal and dispersal of potentially infected material, as well as identify potential other species that could be acting as definitive hosts for *E. granulosus*. To document the species feeding on the carcasses, 2 Bushnell Nature View CAMHD Essential cameras were positioned next to the cull site at right angles to each other to ensure the entire site was within the camera's field of view. They were attached to wooden posts 1m above the ground and 3m from the carcasses (Bacon & Boyce, 2010). The cameras were programmed to take 10 photographs, day, and night, after each trigger, with a 10 second delay after each trigger. This was then reduced to 1 photograph after the first cull site due to the large number of images taken reducing the memory capacity. Cameras were left in position for up to 7 days due to the level of decay beyond this point, however some had to be removed earlier due to logistical difficulties in collecting them. The images were observed, and species were documented chronologically using the date and time stamp on the image. Where large numbers of animals were observed in the image, an estimation of numbers were made. The

four cull sites were selected based on the farms that had animals to cull. All farmers were contacted and asked to notify the Department of Agriculture that they planned to cull animals and were acceptable for camera traps to be set up.

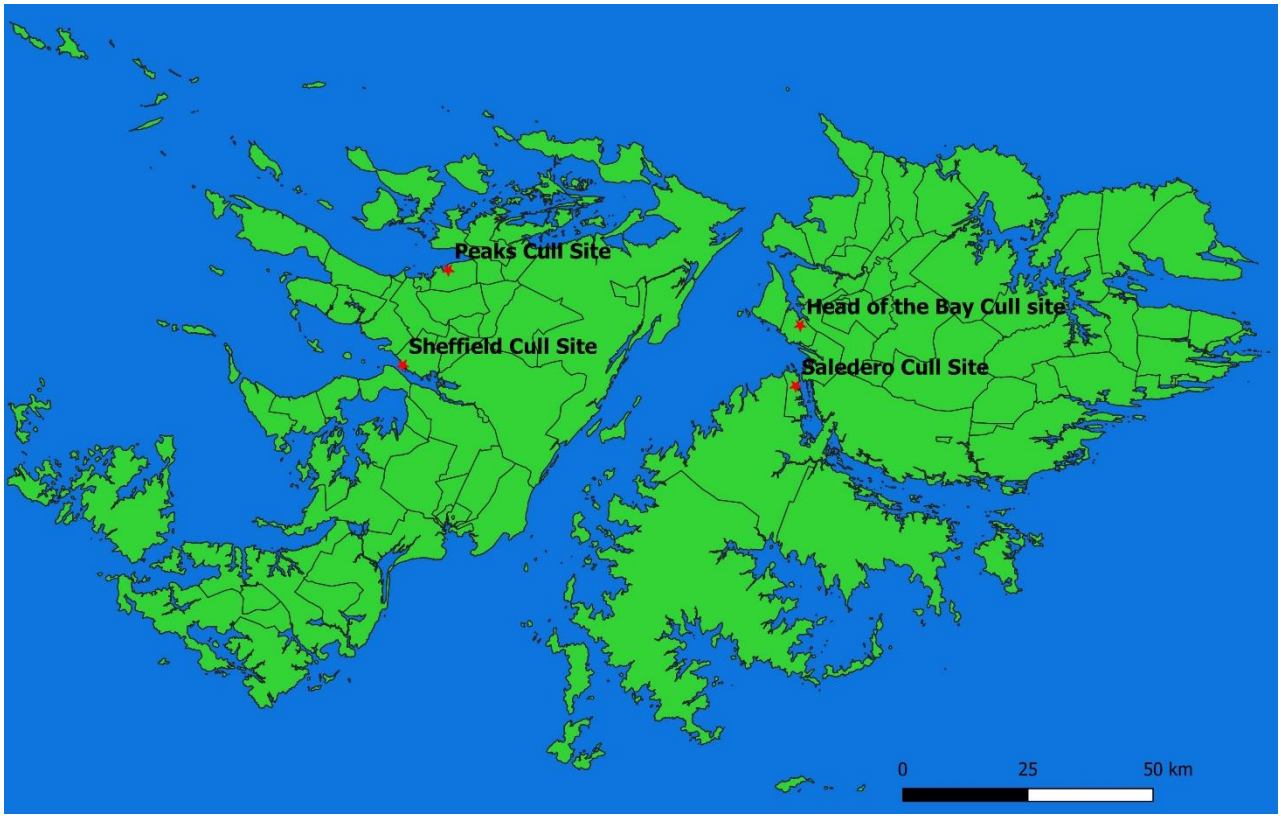


Figure 29: Four cull site locations monitored using Bushnell Nature View CAMHD Essential cameras.

3.2.6 Other cestode definitive hosts on the Falkland Islands

3.2.6.1 Feral cat dissection

Farmers that regularly controlled the numbers of feral cats on their farm were asked to bring any cats they culled into the Department of Agriculture laboratories to be dissected. In total 14 cats were obtained. A cardiac puncture was taken to collect blood for testing with TEST-IT FELV/FIV CAT 10'S (Forte Healthcare Ltd, Meath, Ireland) following manufacturer's instructions then the intestine was removed for dissection. The intestine was cut into 10cm segments and submerged in Phosphate Buffered Saline (PBS) (Fisher Scientific, Loughborough, UK) before being opened and the contents scraped and examined under a dissection microscope. Any parasites found were placed into 15ml bijou tubes and fixed in 99-100% Ethanol. Parasite samples were then imported to the UK and DNA was extracted using a Qiagen DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). From each tapeworm, 25mg

of tissue was cut into small segments and put into a 1.5ml microcentrifuge tube, then 180µl of ATL Buffer and 20µl Proteinase K was added and mixed by vortexing. The sample was then incubated at 56°C until the tissue was completely lysed (1-3 hours). 200µl of AL Buffer was added before incubating again at 56°C for 10 mins. 200µl was then added and mixed using a vortex before the entire solution was added to a DNeasy Mini spin column with a 2ml collection tube. The spin column was centrifuged at 8000 rpm for 1 minute and the flow through discarded and a put into a new 2ml collection tube. Then 500µl of AW1 buffer was added to the spin column and again centrifuged at 8000 rpm for one minute and the flow through discarded. The spin column was again placed in a new 2ml collection tube and 500µl of AW2 Buffer was added and centrifuged at 14,000 rpm for 3 minutes. Finally, the spin column was added to a 1.5ml microcentrifuge tube and 200µl of AE Buffer is added to elute the DNA. The spin column was left at ambient temperature for 1 minute then centrifuged at 8000 rpm for 1 minute, leaving the eluted DNA in the 1.5ml microcentrifuge tube.

Extracted DNA was subjected to PCR analysis to confirm species. Faecal samples were also collected directly from the posterior end of the small intestine for Copro-analysis in the same way as dog faecal samples were collected.

3.2.6.2 Fox faecal analysis

A total of 22 scat samples were collected from around fox dens on Weddell by the farmer and sent to the Department of Agriculture where they were prepared using the same protocols as for the Copro-analysis of the domestic dog samples.

3.3 Results

3.3.1 Faecal sample analysis

3.3.1.1 Copro-ELISA

Antibody standardisation

Analysis of the reactivity of each of the antibody separately indicated that both Rb91 (Figure 30) and Rb5 (Figure 31) showed strong activity against *Echinococcus* protoscolex antigen. For Rb91 dilutions of 1/200 to 1/800 had a good signal to at least a 1 in 3200 dilution (234ng/ml protein concentration). In relation to finding a suitable dilution for use in the capture ELISA system for coproantigen detection it was important to ensure that there was sufficient

antibody available to carry out the entire study. Based on reactivity, a working dilution of 1 in 500 was therefore chosen as acceptable.

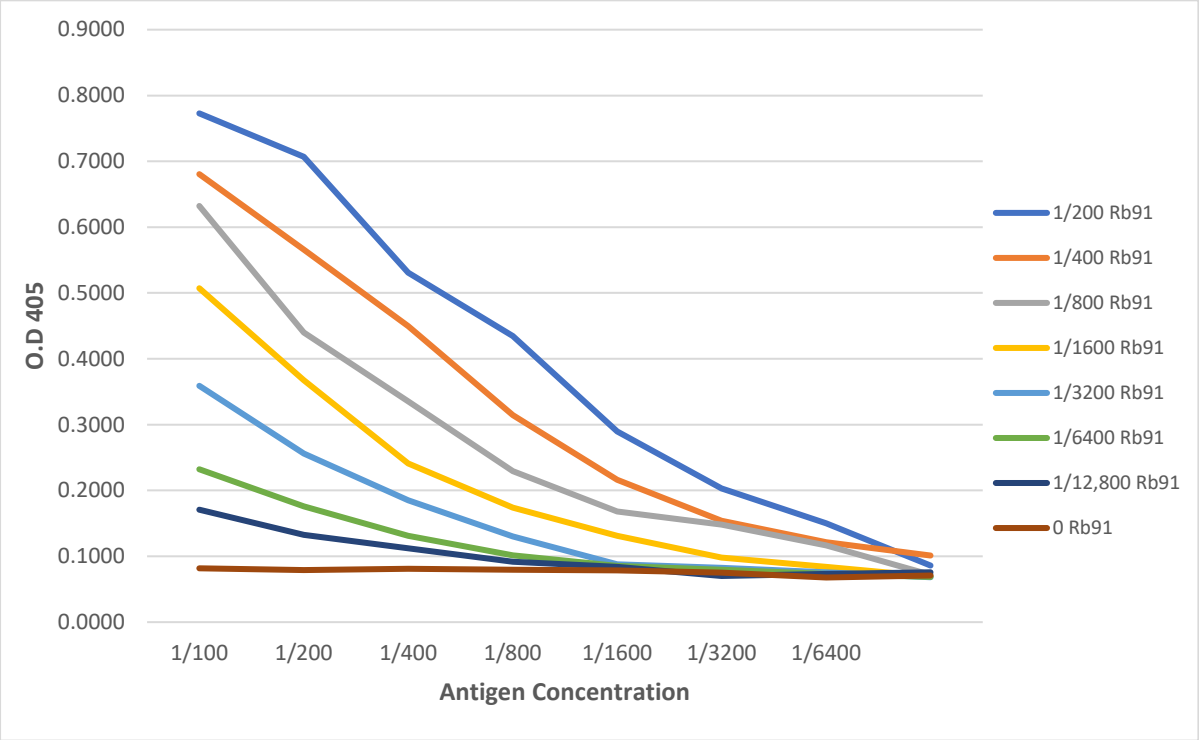


Figure 30: Results of Rb91 checkerboard titration showing serial dilution of antibody and the amount of antigen that can be detected. The blank wells gave OD values of 0.07 which shows this is the level of noise from a blank plate. For antibody Rb5px (peroxidase con conjugated) (Fig.31) a similar dilution-dependent activity was observed. For antibody dilutions 1/200 – 1/800 there was good recognition of antigen down to at least 1/800 antigen dilution (937 ng/mL protein concentration). Again, in order to conserve reagents, a working dilution of 1/500 was chosen for the capture ELISA system.

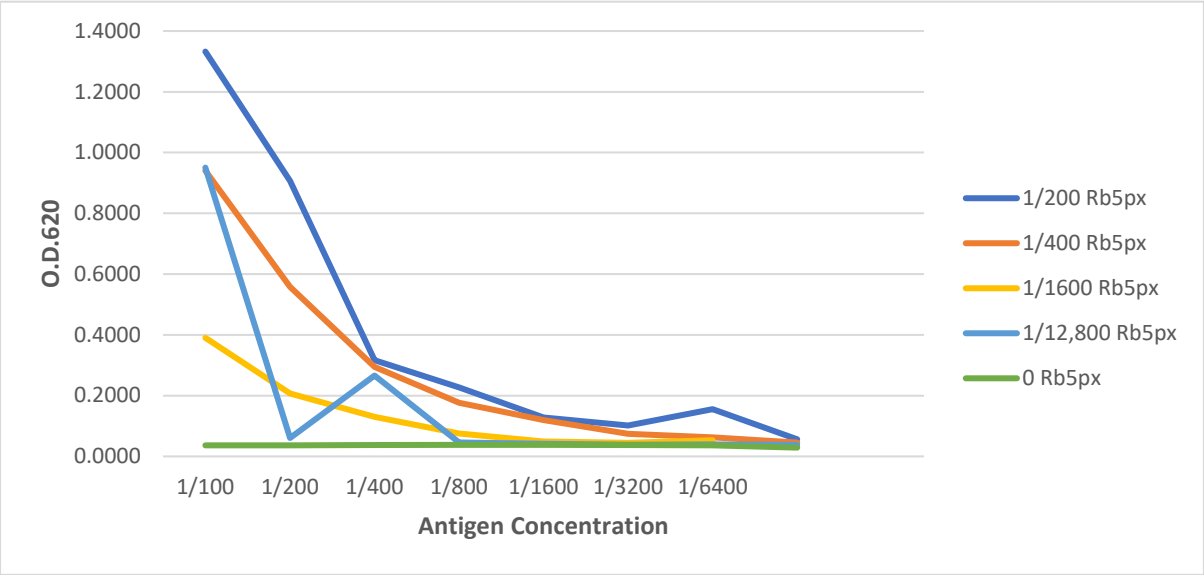


Figure 31: The results of Rb5px checkerboard titration showing serial dilutions of antibody and the amount of antigen that can be detected. The blank wells gave OD values of 0.04 which is the background noise.

To establish the performance of the capture antibody system using the established antibody dilutions, a serial dilution of EgP antigen was used from 1/100 to 1/6,400 with no antigen in the final wells as a control.

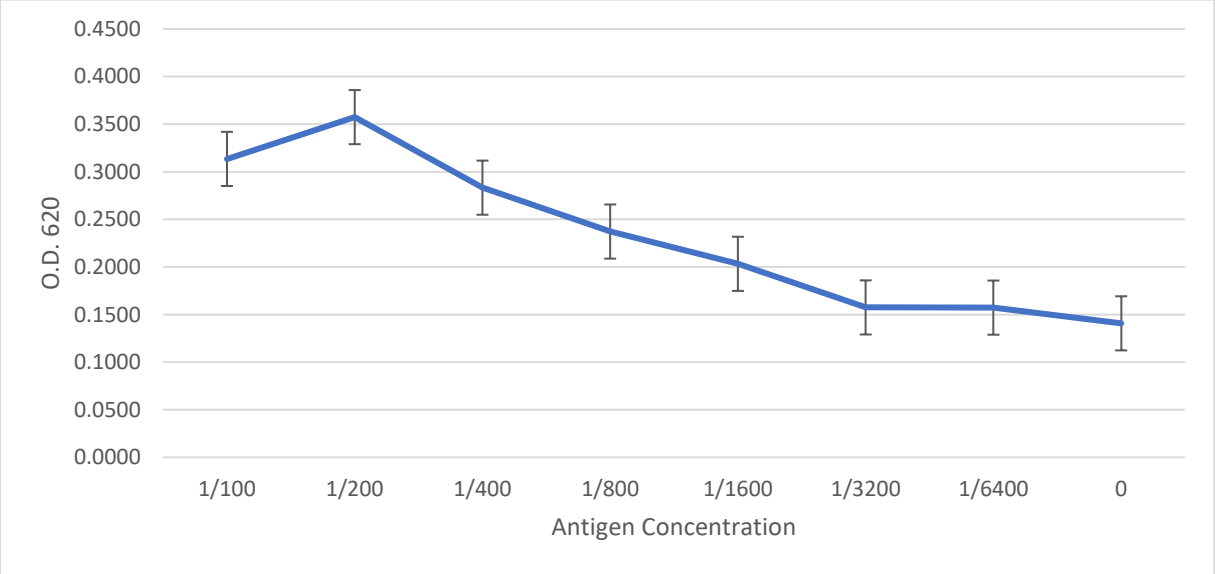


Figure 32: Serial dilution of EgP antigen to assess the performance of the chosen antibody concentrations. Blank wells gave O.D values of 0.03 which is the background noise. Error bars represent standard error.

Establishment of a positive/negative cut off value for copro-ELISA.

To establish a positive/negative cut off a bank of known positive controls collected by van Kesteren (2015) from Kyrgyzstan and 12 known negative samples from previous testing in the Falkland islands run in triplicate in the coproantigen system (Figure 33). The mean OD for negative samples was 0.114 with a standard deviation of 0.0326 giving a cut off value of 0.21 (mean + 3sd).

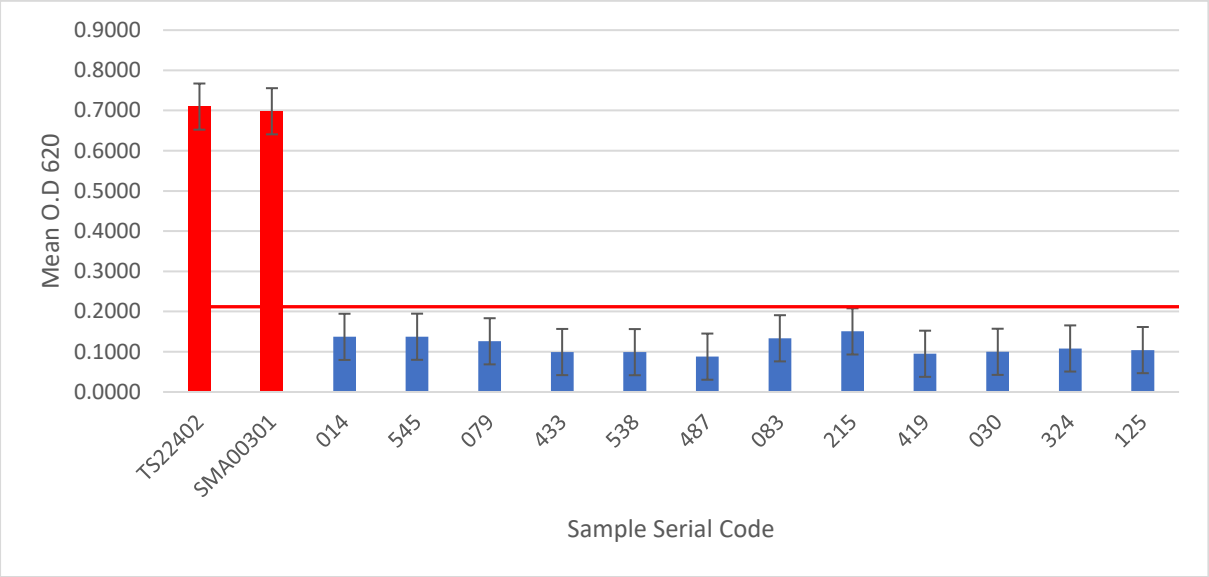


Figure 33: Mean O.D values of triplicate Copro-ELISA results from Negative samples collected and tested in 2012 to establish a positive/negative cut off. Including two positive controls. The mean OD value was 0.114 with a standard deviation of 0.0326 giving a cut off value of 0.21 (mean + 3sd). Error bars represent the standard error of the mean value.

Screening of dogs in the Falkland Islands by Copro-ELISA and Copro-PCR (11th June 2018 to 13th March 2020).

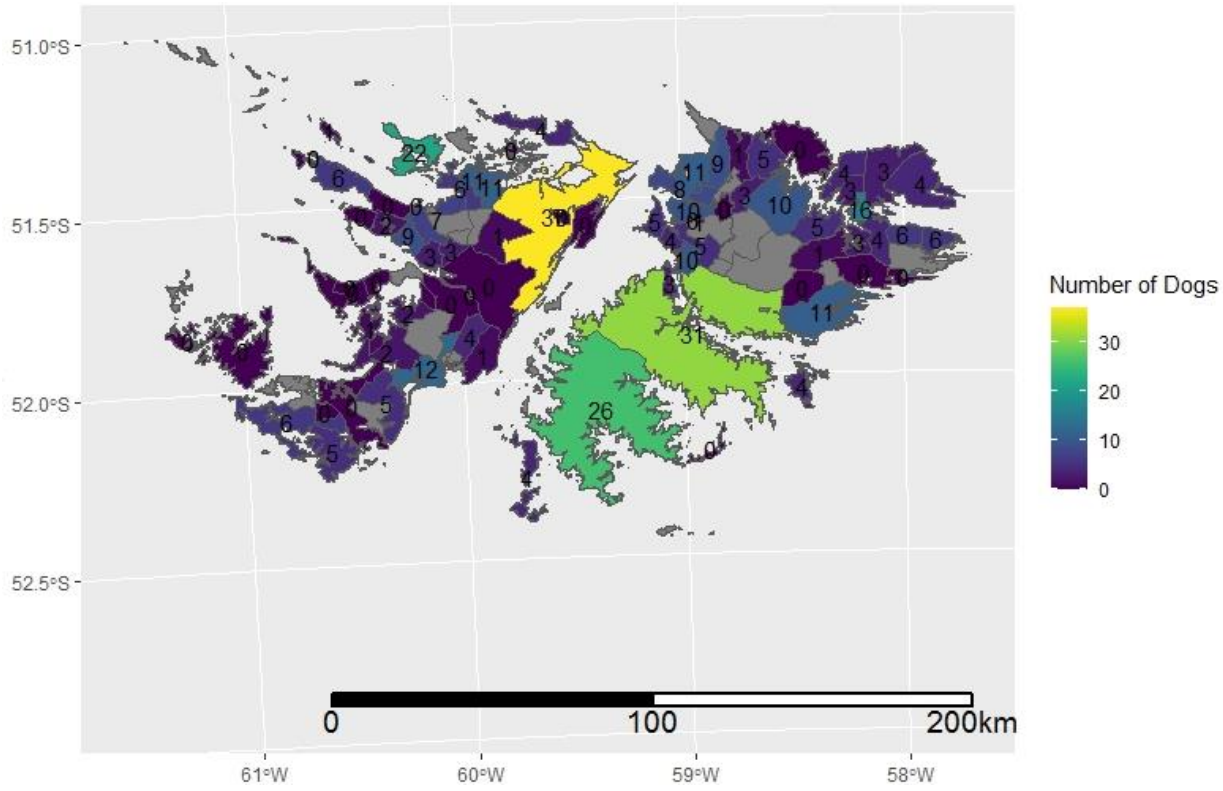
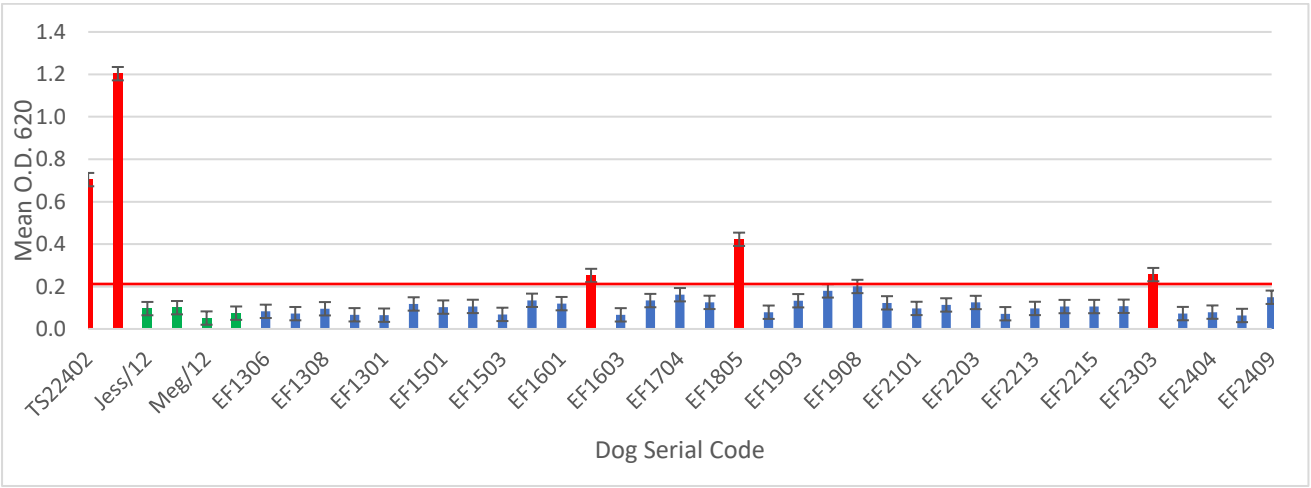


Figure 34: The number of dogs on each farm collected for Faecal sample analysis. Grey areas are unfarmed areas where no dogs are present.

Figure 34 shows a map of the Falkland Islands indicating the number of registered dogs on each farm. A total of 589 faecal samples were collected from the Falkland Islands from 12th March 2018 and 27th April 2018 and assayed by Copro-ELISA and PCR.



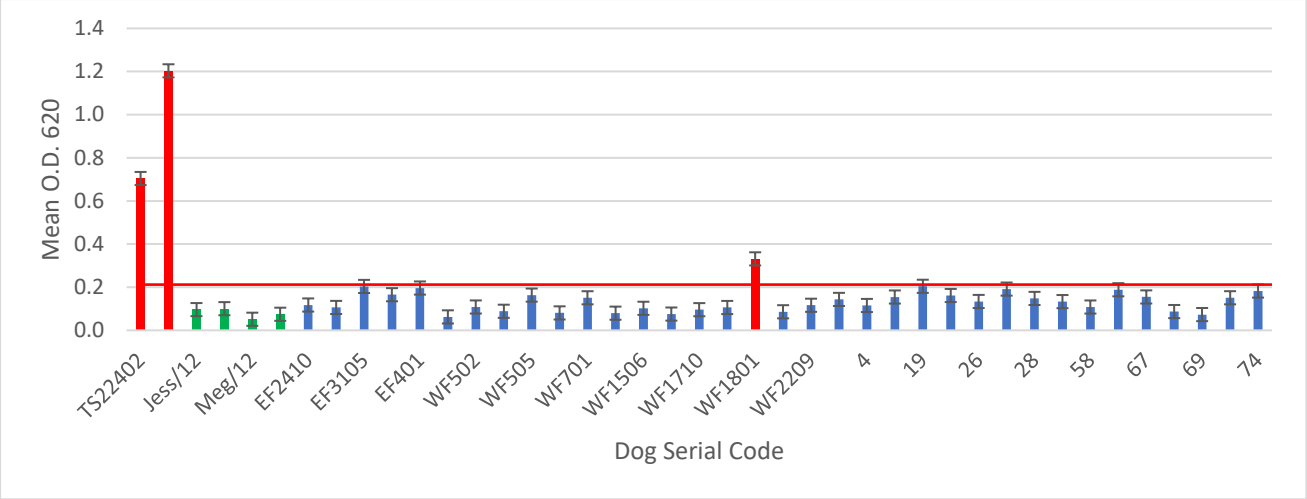


Figure 35: An example of mean O.D values of triplicate Copro-ELISA results from faecal samples re-tested after initial analysis, including four positive farm dogs (in red). Cut off 0.212 and two positive (first columns to the left in red) and 4 negative controls (in green). Error bars represent the standard error of the mean value. The blank wells gave OD values of 0.036 which is the background noise. See appendix II for results from all samples.

In sampling all 589 faecal samples, four were determined to be above the cut off value of 0.21 (EF 1805, EF1602, EF2303 WF1801) and additional 2 samples (122 and 123) were marginally over the cut-off (See figure 35). When all suspect samples were retested using the same method, these tested negative and only the original four samples tested positive. All positive samples had considerably lower optical densities to the two positive control samples.

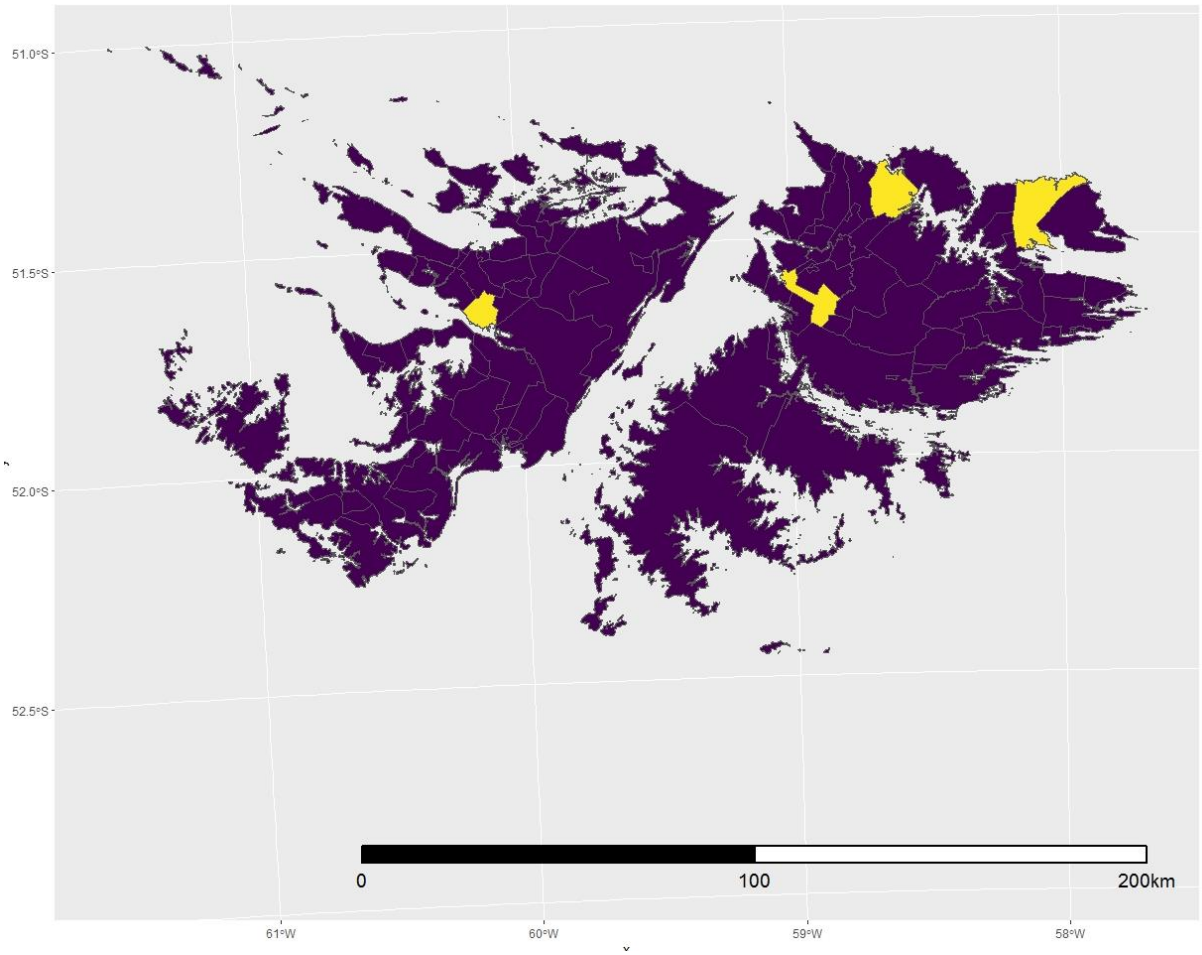


Figure 36: Location of Copro-ELISA positive dogs in the Falkland Islands in 2018.

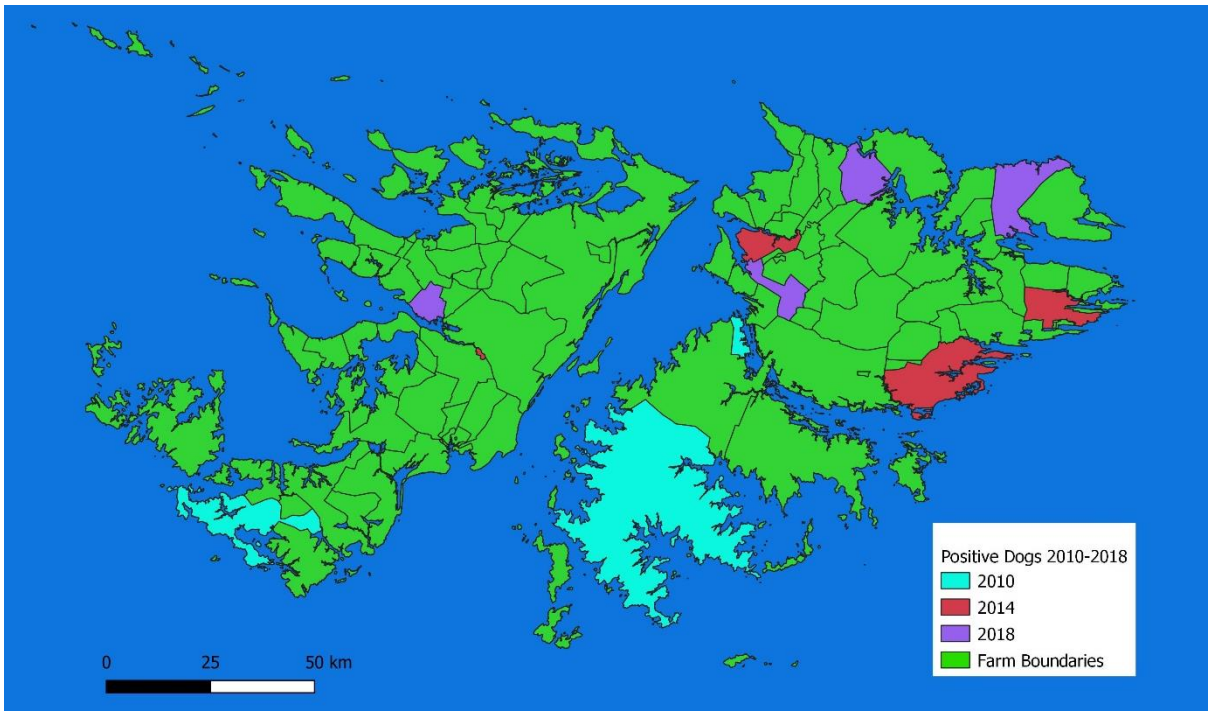


Figure 37: Locations of farms where dogs have tested Copro-PCR or Copro-ELISA positive between 2010 and 2018.

3.3.1.2 Faecal Sample Analysis-Copro-PCR

All samples tested using the described PCR were negative for both *Echinococcus* and general cestode specific DNA including those which were copro-ELISA positive. However, multiple samples showed faint bands of approximately 650bp in length as can be seen in Lane 19 of Figure 38. Upon sequencing, these bands were non-specific binding of primers to bacteria *Psychrobacter maritimus* (Accession number: MT594461.1 100% match with NCBI database) and other common gut microbiota such as Fusobacteria (Accession number: MN537499.1 99.44% match with NCBI database) and *Escherichia coli* (Accession number: MT629905.1 95.14% match with NCBI database). The gut bacteria is commonly found in dogs intestinal tract and play important roles in maintaining the metabolic functions, pathogen defence and supporting the immune system (Pilla & Suchodolski, 2020).

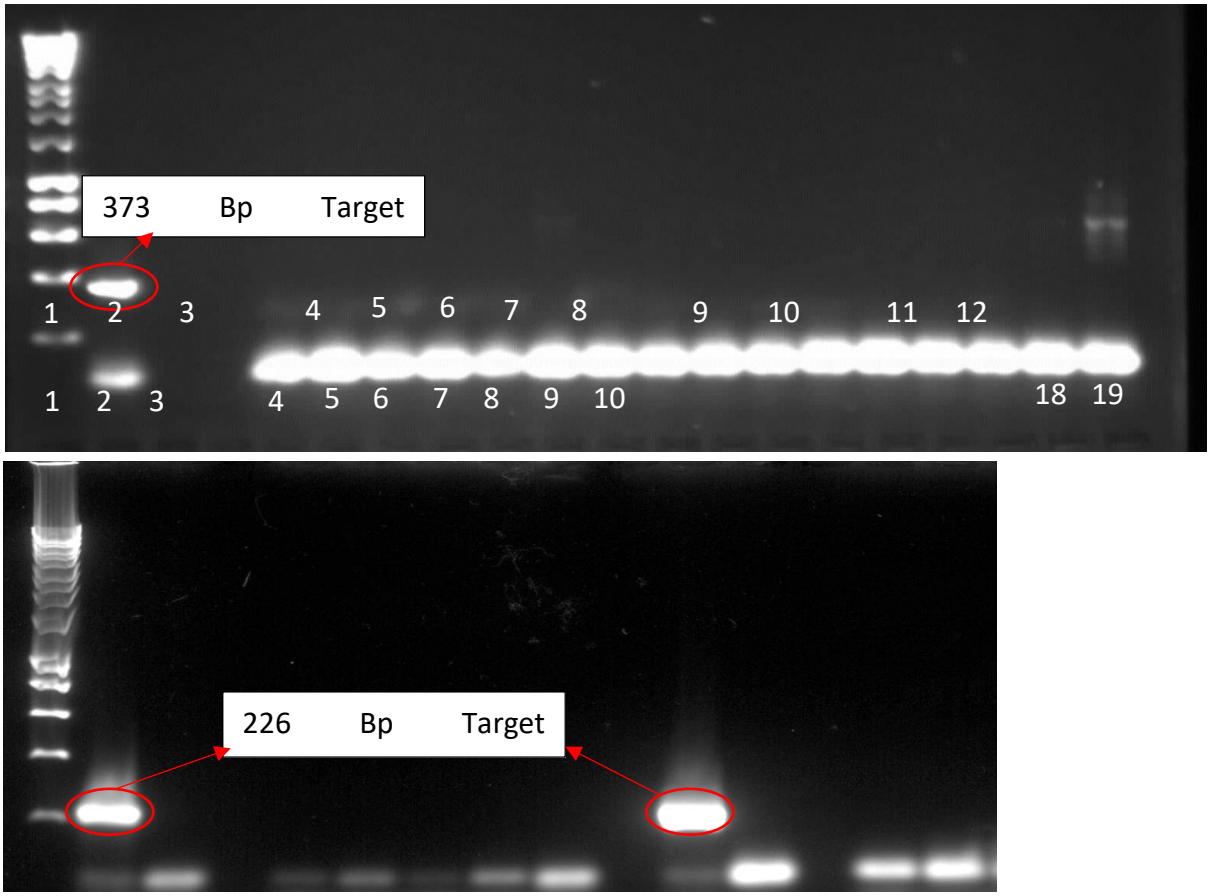


Figure 38: (top) Universal cestode primer PCR on Faecal samples collected from all working and pet dogs in the Falkland Islands in 2018. Lane 1: 1kB Hyperladder. Lane 2: Positive control (*E. granulosus* G1 genomic DNA). Lane 3: Negative Control (PCR Grade water). Lane 4-18: Faceal DNA extraction samples. Lane 19: Faecal DNA extraction sample with band of unknown size. (Bottom) *E. granulosus* G1 primer PCR on Copro-ELISA positive and Borderline samples. Lane 1: 1kB Hyperladder. Lane 2 and 9: Positive control (*E. granulosus* G1 genomic DNA). Lane 3 and 10: Negative Control (PCR Grade water). Lane 4-8 and 11 &12: Faecal DNA Exactions.

3.3.1.3 Faecal sample Microscopy

All copro-ELISA positive samples from first and second screening, were subjected to flotation and microscopy to identify any possible cestode eggs in the faecal sample.

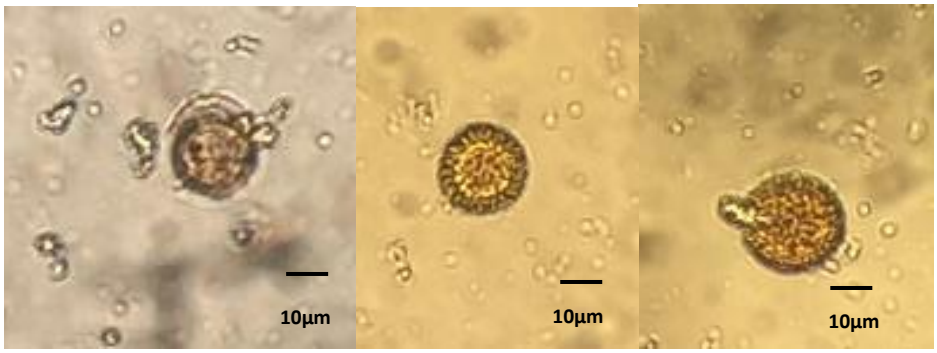


Figure 39: Suspected taeniid eggs discovered after faecal egg flotation and microscopy of PBS slurry remaining from Copro-ELISA preparation of Copro-ELISA positive.

One of the three positive Copro-ELISA samples had structures resembling taeniid eggs visible under x40 magnification (Figure 39). Based on size and shape, the structures observed were similar size to cestode eggs, 30µm in diameter, and appeared to have an outer layer of embryophoric blocks. However, oncospheral hooks could not be observed to conform identification. Nevertheless, the microscope slides were washed into an eppendorf with 70% ethanol and used in DNA extraction using the QIAamp® Fast DNA Stool kit (Qiagen, Hilden, Germany) previously used. Both the *E. granulosus* genotype 1 (G1) primers and the Universal Cestode primers failed to identify parasite specific DNA. The overall status of the of the four copro-ELISA positive samples in relation to previous history of Echinococcosis is shown in table 2.

Table 2: Status of Copro-ELISA positive samples, their history of CE in sheep population and current prevalence of *T. hydatigena*.

Reference number	Copro-ELISA O.D. Value	PCR Result	Presence of eggs	Previous farm history of positive dogs Y/N	Last recorded Presence of CE in sheep	Current prevalence of *T. hydatigena* in sheep
EF1602	0.2521	negative	Possible	No	2006	1.78% (2018)
EF1805	0.42207	negative	No	No	N/A	7.14% (2020)

EF2303	0.2562	negative	No	No	2020	6.19% (2020)
WF1801	0.3314	negative	No	No	2020	3.24% (2020)

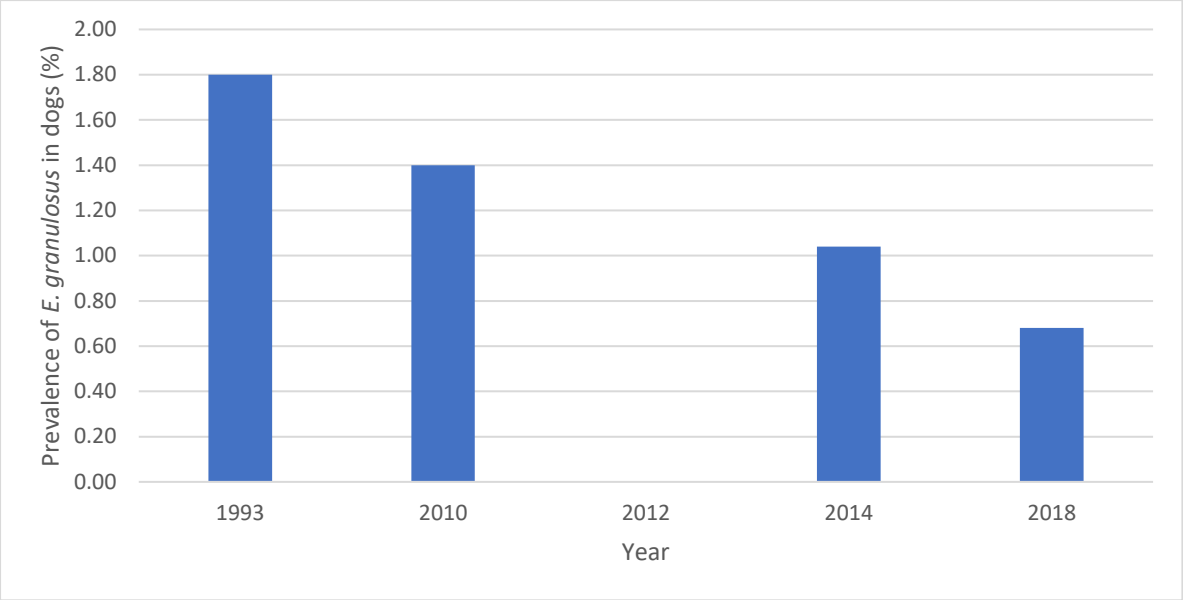


Figure 40: Prevalence of E. granulosus infection in dogs via coproantigen testing (1993, 2014 and 2018) and copro-PCR (2010).

When incorporating the present study into data previous screening surveys (Figure 40), the overall coproantigen prevalence has continued to drop from 1.80% in 1993 to 0.68% in 2018.

3.3.4 Fox sampling and analysis

Figure 41 shows the fox faecal samples screened using the same Copro-ELISA as the dog faecal samples, all fox scats were negative. The same samples were also tested using the same universal PCR primes and *E. granulosus* G1 primers, which were also negative.

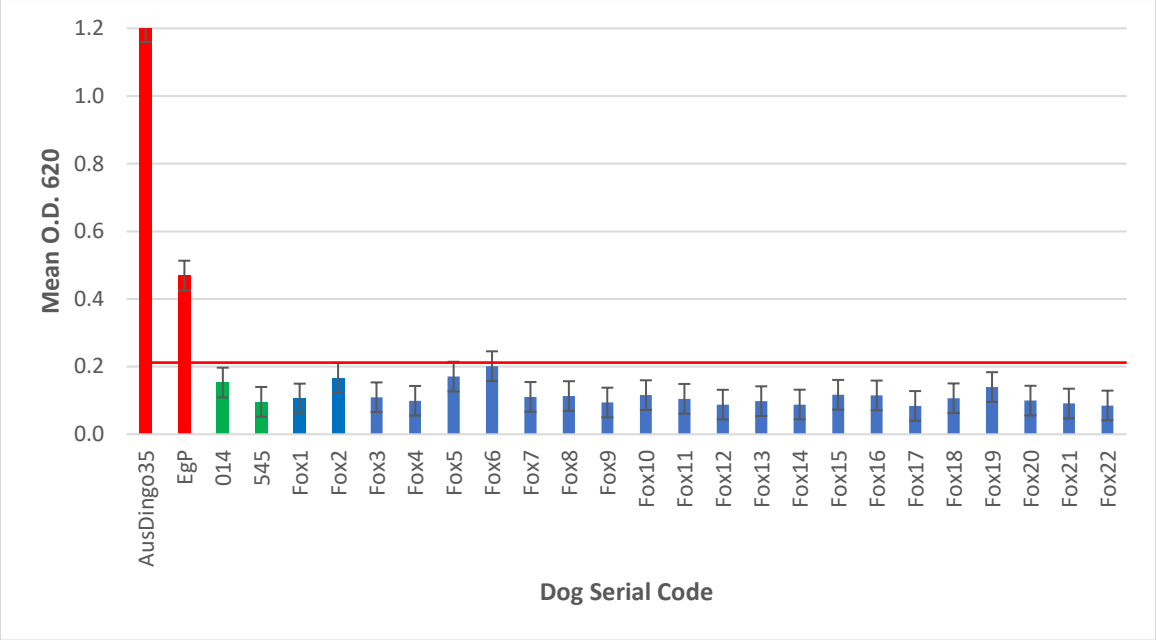


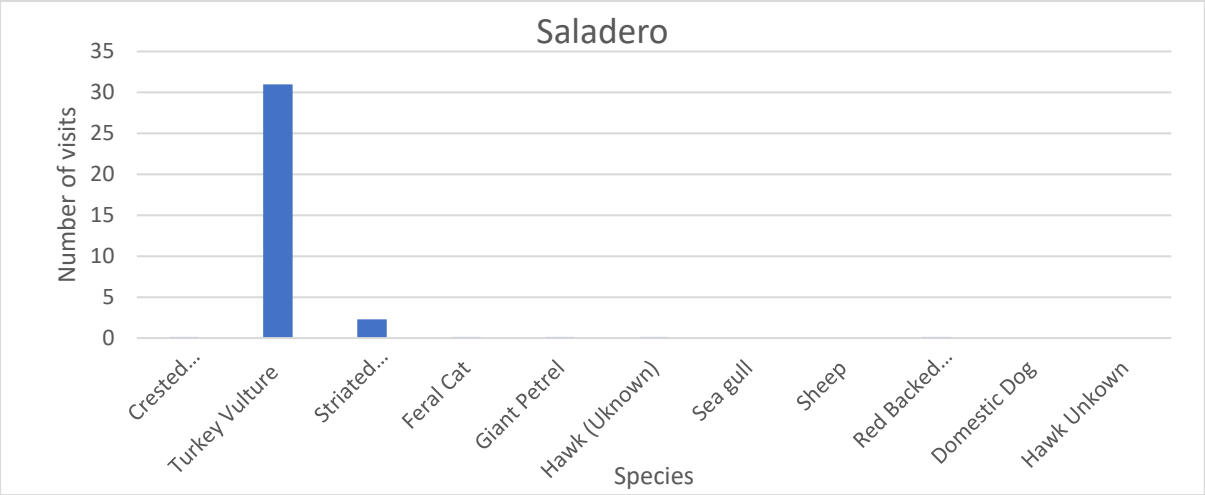
Figure 41: Mean O.D values of triplicate Copro-ELISA results from Fox faecal samples collected from Weddell Island. Cut off 0.212 and two positive and 4 negative controls. Error bars represent the standard error of the mean value. The blank wells gave OD values of 0.037 which is the background noise.

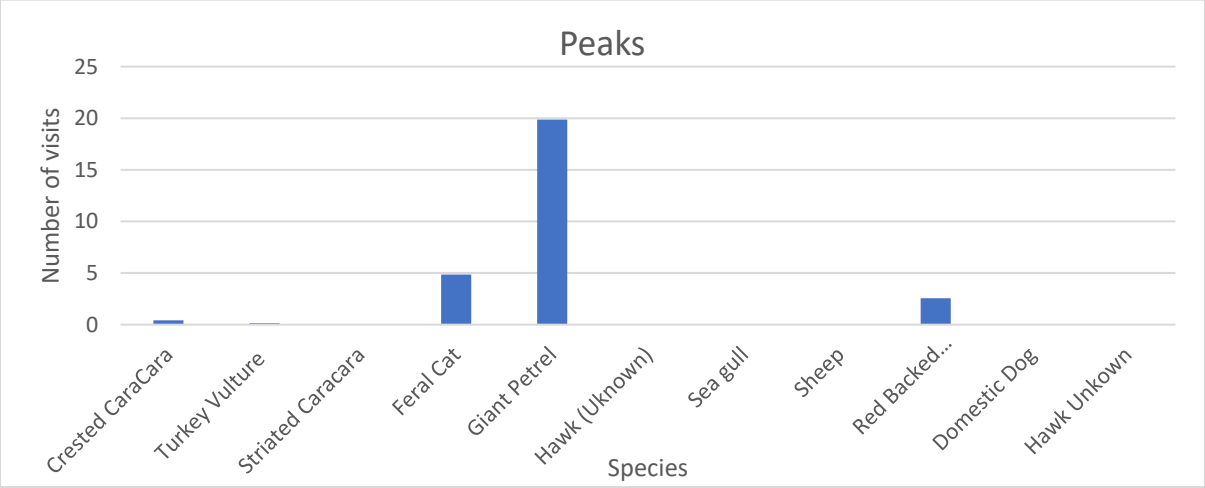
3.3.2 Camera Trap Surveillance of Cull Sites

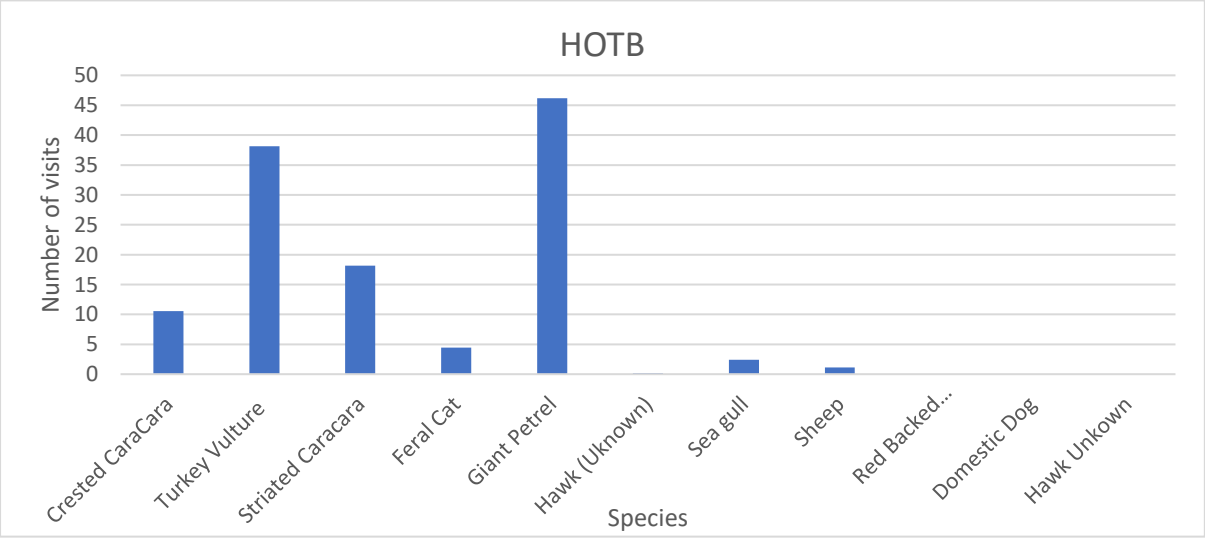
A total of four individual cull sites were surveyed using remote camera traps across four different farms, with two sites on West Falkland and two sites on East Falkland. The number of sheep left on each of the different cull sites ranged from eight to over 30 individuals and in three out of four examples, the animals were left completely intact. At one cull site, the fore legs were removed for dog meat, but the rest of the animal was intact, including offal.

Figure 42: The author and farmer (left) setting up remote camera traps on cull sites and the author collecting cameras, observing the level of decomposition.

In all locations, the first species to arrive at the cull site and feed on the carcasses were bird species, such as the Crested caracara (*Caracara plancus*), the Red backed hawk (*Geranoaetus polyosoma*), Turkey vultures (*Cathartes aura*) and Giant petrels (*Macronectes giganteus*).







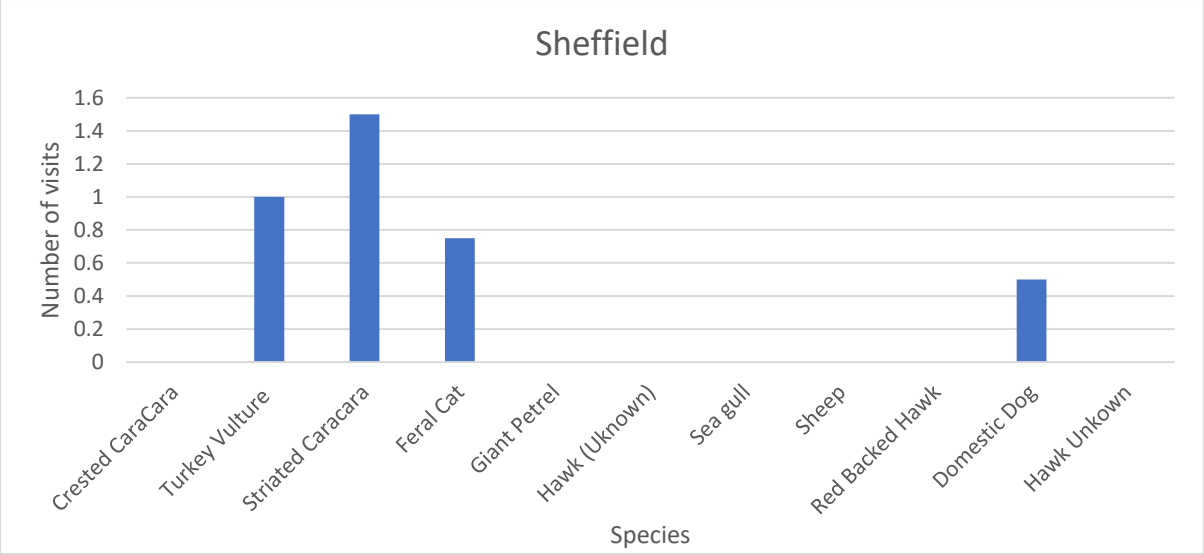


Figure 43: The average number of visitations of each scavenging species on each farm cull site.

The various bird species can be seen feeding on carcasses and in some cases removing material from the cull site (see figure 44). A total of seven different bird species were documented at the cull sites with the most common species being Turkey Vultures (*Cathartes aura*) and Giant Petrels (*Macronectes giganteus*).

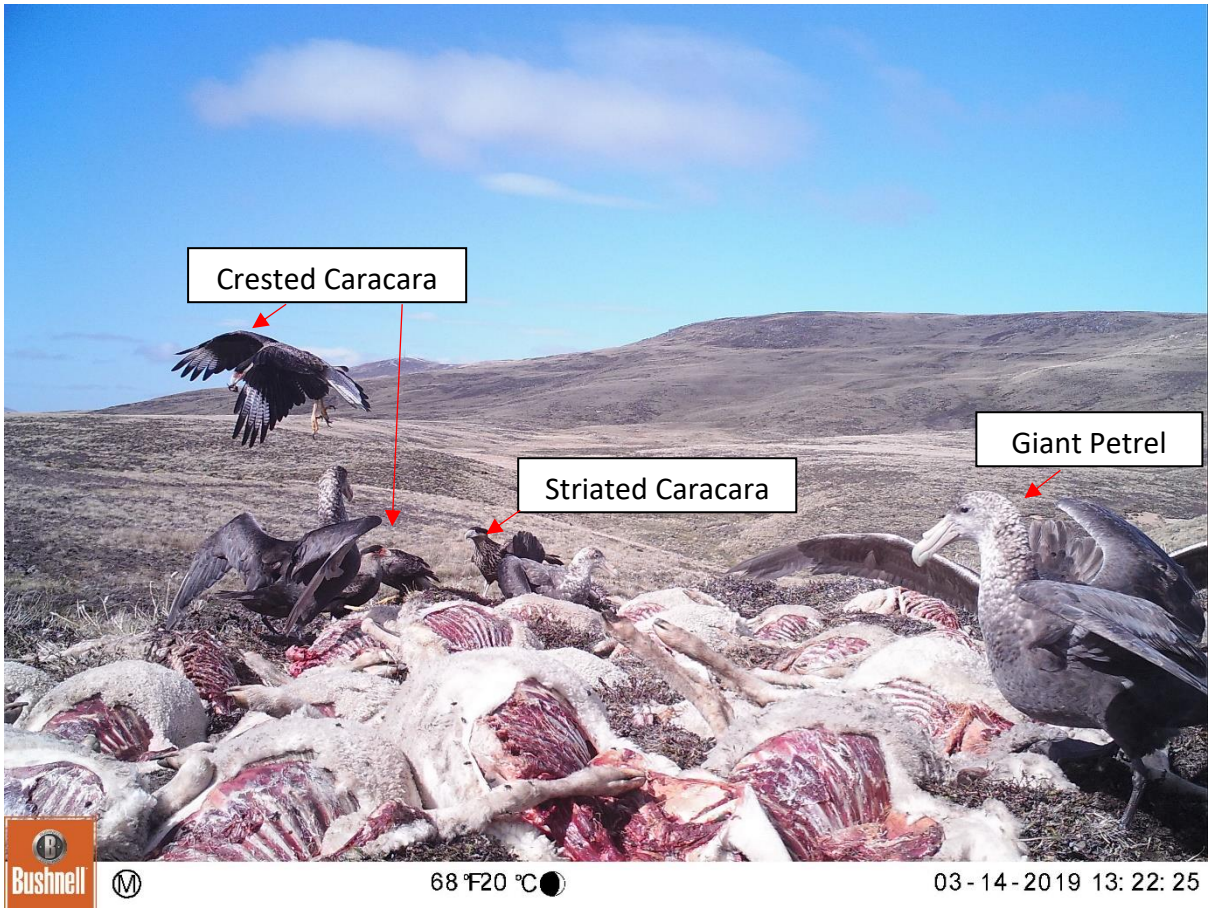


Figure 44: Multiple scavenging bird species feeding on culled sheep carcasses left on the pasture to decompose.

Feral cats (*Felis catus*) live all over the Falkland Islands, with farmers describing seeing them in nests in the grass. They were observed feeding on all the cull sites and were always the first non-avian species to arrive. One cull site was visited 31 times by multiple cats over a seven-day period and on another cull site, multiple cats were seen feeding at one time (see figure 45).

Figure 45: Feral cats feeding on carcasses left at cull sites.

On one occasion, camera traps at a single cull site caught images of a dog feeding on the carcasses on the fourth day the animals were left at the cull site (see figure 46). Identification of whether it was a single dog multiple times or multiple dogs feeding was not possible due to the quality of the images however, the evidence is clear that it was a dog from the farm that the cull site was on.

Figure 46: Camera trap images taken at night showing a dog feeding on sheep carcases.

3.3.3 Feral cats in the Falkland Islands, FIV and intestinal parasite burden.

Feral cats have been regularly documented feeding on sheep carcasses by farmers and this was also evident on the camera trap surveillance of cull sites above. Farmers were asked to send any cats that they had caught to the Department of Agriculture rather than disposing of them themselves for post-mortem for evidence of intestinal burden as well as testing for FIV by taking a cardiac puncture from each individual.

Table 3: Worm burden and FIV status of Feral cats dissected in the Falkland Islands.

Farm	Cestode	Nematode	FIV Result
Dunbar 1	8	0	-ve
Fitzroy	5	10	-ve

Fitzroy 1	4	3	+ve
Fitzroy 2	0	5	+ve
MPA 1	2	0	-ve
MPA 2	20	5	+ve
N. Arm 1	0	0	-ve
N. Arm 2	0	0	-ve
N. Arm 3	1	0	-ve
N. Arm 4	0	0	+ve
N. Arm 5	0	4	-ve
N. Arm 6	3	4	+ve
N. Arm 7	5	0	+ve
Stanley	0	0	-ve

A total of 14 cats were collected from five different locations, either private farmland, the military base, or strays in Stanley. The Veterinary Department in the Falklands has documented cases of FIV before and regularly in feral cats brought into the vets. Of 14 cats, six (42.9%) tested positive for FIV. In total, seven (50%) cats had tapeworm infection, with worm burden varying from a single worm to 20 individual worms. DNA from these cestodes were sequenced and confirmed via Blast search of Genbank database to have 100% homology with *Taenia taeniaformis* (Accession number: FJ597547.1. There was no evidence of any *E. granulosus* infections and all samples were Copro-ELISA and Copro-PCR negative when tested for *E. granulosus* G1. There was evidence of other parasites in the intestine of cats, with 50% of inspected individuals being infected with roundworm.

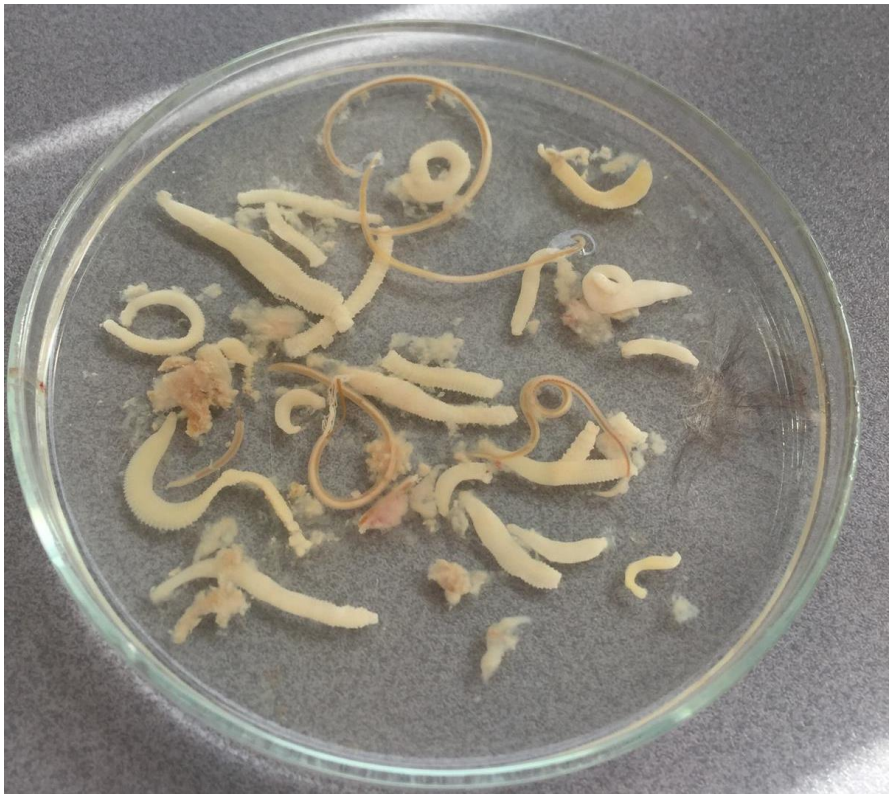


Figure 47: Mixed parasite infection from a feral cat dissected in the Falkland Islands containing *T. taeniaformis* and suspected *Toxocara cati*.

Based on visual inspection the roundworms were thought to be to be *Toxocara cati*. Size of worms ranged from three to five cm and were cylindrical in shape. Vets in the Falklands noted that previous cases of *Toxocara cati* have been seen in domestic cats, making it likely that this common cat parasite is what has been found in the dissections.

3.4 Discussion.

Upon testing of dogs in the Falklands, four were found to be copro-ELISA positive. Though not confirmed by copro-PCR, this evidence suggests that these dogs may have gained access to infective material on the farm. This is further supported by a dog being observed feeding on dead sheep culled on a farm, which shows that the dog was not properly restrained while not working, as well as gaining access to potentially infected offal from these older cull ewes, the most likely age group to be infected (Torgerson et al., 1998). The practice of culling older sheep and leaving them on the pasture uninspected creates a significant risk of transmission of *E. granulosus* to dogs if not properly restrained.

Due to the regular dosing of dogs in the Falklands with anthelminthics, the prevalence of *E. granulosus* in dogs was expected to be low. The next dosing was due the week following the collection of samples, so dogs had not been dosed for over a month prior to sample collection. The prevalence of *E. granulosus* in dogs based on copro-ELISA results was 0.68% with only four of 589 dogs tested being positive. However, none of these samples were positive for either *Echinococcus* specific or general taeniid copro-PCR. One of the samples had suspected *Taenia* spp. eggs present based on microscopy but again diagnosis could not be confirmed with PCR. Discrepancies between copro-ELISA, copro-PCR and egg/worm detection have been reported in other studies (Lahmar et al., 2007) and it is important to understand what is being detected in each case. Both Copro-PCR and microscopy are largely dependent on the presence of parasite eggs in faecal samples and will have higher sensitivities in dogs with mature worms and large worm burdens (Lahmar et al., 2007; Wang, Wang, Cai, Wang, Huang, Feng, Bai, Qin, Manguin, Gavotte, Wu, & Frutos, 2021). Sensitivities will be much lower (around 25-50%) in dogs with prepatent infections or low worm burdens although there is some evidence of PCR positive results in prepatent infections suggesting that the technique can detect DNA other than that in eggs (Abbasi et al., 2003; Lahmar et al., 2007; Naidich, McManus, Canova, Gutierrez, Zhang, Guarnera, & Rosenzvit, 2006).

With copro-ELISA it is molecules from the surface of the worms, somatic antigens or excretory/secretory products which are being detected. Many of these have been shown to be highly glycosylated molecules of good stability (Craig et al., 2015). The presence of eggs is not required, and the technique can therefore pick up pre-patent infections as shown by experimental infections (Jara, Rodriguez, Altamirano, Herrera, Verastegui, Gímenez-Lirola, Gilman, & Gavidia, 2019). In previous studies copro-ELISA sensitivities of 78-100% have been reported with worm burdens of less than 50 worms often producing false negative results (Craig et al., 2015).

Variation in sensitivities of coproantigen surveys can also arise through different approaches to establishing positive/negative cut off values. The method used in the current study defines an OD value of the mean + 3 x SD of a panel of “negative” samples. This is based on the assumption that the ODs of a negative panel will fall into a Gaussian (normal) distribution where 99.9% of the observations will lie within 3 x SD of the mean. The current normal panel was based on samples that had previously been taken from the Falkland Islands in 2012 and

tested negative by copro-ELISA and copro-PCR. With current dosing regimen these animals would be expected not to be harbouring any helminth parasites and background ODs would be low. Working with a panel of *Echinococcus* negative dogs which had not been exposed to a high level of dosing may result in a higher background and a higher cut off value due to the presence of other helminths.

In relation to the four samples which were coproantigen positive in the current study, it is likely these could represent dogs with a prepatent infection and/or low worm burden. With the current situation on the Falkland Islands in relation to frequent anthelminthic dosing, it is likely that these dogs had recently been treated with anthelmintics and therefore any infections would be relatively recent. However, because these samples could not be confirmed by PCR or egg microscopy they have been defined as "suspect" rather than "confirmed positive". The presence of hydatid cysts in sheep from one of the farms where a suspect dog came from provides circumstantial evidence that these may be real infections.

The dogs testing positive with copro-ELISA were from four separate farms, both on East and West Falklands, with two farms having a recent case of CE in a sheep (2019 and 2020, respectively). However, the others have had no recent cases. The current prevalence of *T. hydatigena* for each farm were EF1603= 1.78% (2018), EF1805= 7.14%, EF2303= 6.19% and WF1801=3.24%. The mean prevalence of *T. hydatigena* between 2011 and 2020 was 2.2%, meaning that three of the farms have a high prevalence of *T. hydatigena* which could indicate that this high prevalence is related to the presence of a positive dog on the farm. Previous testing was carried out via the University of Salford in 2010, 2012 and 2014 with eight (1.4%) Copro-PCR positive dogs in 2010, no ELISA positive dogs in 2012 (therefore no PCR was done) and six (1.04%) Copro-ELISA positive dogs in 2014, though in this survey ethanol-based samples were lost in transit from the Falkland Islands and the copro-PCR could not be performed. In the previous surveys, none of the current farms tested positive by copro-ELISA of copro-PCR and prior to 2019, the most recent CE infection in sheep was in 2006 on one farm and 1994 on another, suggesting that there are not regular infections occurring in both dogs and sheep.

Prior to the analyses done at the University of Salford, the last testing was done during a study into CE in the Falkland Islands from 1991 to 1993, which found a seroprevalence of *E. granulosus* of 3.5% in 908 dogs tested for anti-*E. granulosus* antibodies, and 1.8% of 464 dogs

were Copro-Antigen positive ((Reichel et al., 1996). During this time, no adult tapeworm was discovered when arecoline purgation was carried out on seropositive or Copro-Antigen positive dogs. It was described that this was expected as dogs were treated six-weekly with anthelmintic drugs, as well as the lack of accuracy in arecoline purgation (Reichel et al., 1996; Wachira et al., 1990). The current results do suggest that there is a possibility that some dogs (0.68%) may have had access to infected offal and are still contributing to the transmission of *E. granulosus* in the Falklands. The observation of dogs feeding at cull sites provides an obvious potential route of infection.

Due to the nature of farming in the Falkland Islands and the lower productivity and carrying capacity of the land, older and less productive sheep that have too poor condition to be taken to the abattoir are culled and left on the land. These animals are not eviscerated and no inspection for disease or illness occurs. As these are older animals, they are at greater risk of being infected with *E. granulosus* and if they are infected, likely to harbour large numbers of protoscoleces, so pose a higher risk of transmission (Torgerson et al., 1998). Camera trap photos showed that multiple species feed on these feed on these animals, the predominant group of species being birds. Though birds are not involved as hosts for *E. granulosus* and other canid taeniids it has been demonstrated that birds do not just feed at the cull site but may also carry pieces of the carcass elsewhere to feed or possibly for their young (see Figure 44). This removal of material could possibly contribute to mechanical transmission if they were to move some infected material and drop it in an area where dogs could access it and potentially become infected. Species of greater concern are those species that are known to be definitive hosts of cestode parasites. Feral cats are regularly seen across the entire Falkland Island archipelago, both close to farm settlements and further afield in the open pasture. Feral cats were documented feeding on all four of the cull sites observed and were the first non-avian species to arrive at the cull sites. Though described as poor hosts for *E. granulosus* where worms do not reach maturity, feral cats have been described as hosts for *T. hydatigena* (Borji et al., 2011). In the current study none of the post-mortem autopsied cats had *T. hydatigena* present and only contained *T. taeniaeformis,* a taeniid parasite with a cat-rodent lifecycle (Burlet, Deplazes, & Hegglin, 2011). Most of these cats came from farms, and were likely caught around the farm settlement, where their main diet is likely to comprise of rodents, reducing the likelihood of being infected with ovine infecting taeniids. The

positioning of traps near to cull sites may give a more accurate interpretation of the parasitic fauna of cats that feed on carcasses. It is acknowledged that the sample size of 14 is low and that the population size of cats is high, but the current study gave no indication that cats were involved in transmission *of E. granulosus* or any other taeniid cestode except for *T. taeniaeformis.*

The situation may be more complex by the fact that FIV was detected in six cats. This virus has been previously recorded in the Falklands and the immunosuppressive impact of this infection could lead to cats becoming infected with parasites that they do not normally harbour (Armua-Fernandez et al., 2014; Lawrence, 2002). Indeed, in several parts of the world, immunosuppressed cats have been found to act as intermediate hosts for *E. granulosus* with fertile hydatid cysts developing within the internal organs (Avila et al., 2021; Burgu, Vural, & Sarimehmetoğlu, 2004; McDonald & Campbell, 1963). As cats in the Falkland Islands have been diagnosed with FIV, this is also a possible risk that if a cat infected with CE was caught and consumed by a dog, transmission could occur (Armua-Fernandez et al., 2014; von der Ahe, 1967).

The most important species to feed on the carcasses were at a single farm where the working dogs from the property were documented at the cull site feeding. This is clear evidence that, given the chance, dogs have been feeding on carcasses left on pasture. This creates a huge risk of transmission should they come across a carcass of a sheep that has been infected with CE. This farm also had a dog test positive for *E. granulosus* by copro-ELISA, which is supported by the evidence of dogs having access to infected offal on the farm. This evidence also suggests that if rules are not followed and dogs can roam freely unsupervised, they will come across carcasses of dead sheep and feed on it, thus increasing the risk of transmission of multiple cestode parasites.

The fox population on the six islands in the Falkland archipelago that they inhabit is approximately 3000. There were difficulties in obtaining samples from these remote locations, and a sample size of only 22 samples is limiting in understanding the role foxes play in transmission of *E. granulosus*. There is also no evidence of cases of CE in the sheep population as the only farmed island, Weddell Island, has not supplied sheep to the abattoir recently to support the possibility of foxes causing infections in sheep. There were no positive faecal samples from the fox scats collected from Weddell, which is not unexpected due to the

small sample size. However, Patagonian foxes are known definitive hosts for both *E. granulosus* and *T. hydatigena* in South America and are known to scavenge on carrion as part of their varied diet (Muñoz-Pedreros et al., 2018; Zanini et al., 2006). Foxes do therefore have the potential to play a role in the transmission of cestode parasites if they were to come across infective material from sheep that die naturally on the farm. However, the contribution of infected foxes on Weddell Island to infection of sheep on East and West Falkland seems unlikely even though eggs can be transferred some distance by wind and insect vectors (See chapter 4).

Based on the evidence from the data collected in this chapter, we have discovered that despite the control measures in place, dogs are still having access to infected offal. though not proven definitively with PCR confirmed cases, the Copro-ELISA positive result suggests transmission from sheep to dogs via infected offal is continuing. From the small sample of other hosts investigated, these results did not suggest the involvement of any other definitive host being involved in the transmission of *E. granulosus* in the Falkland Islands.

Chapter 4. Prevalence of taeniid Metacestodes in sheep at slaughter.

4.1 Introduction

Human CE is prevalent in rural agricultural communities globally and is considered as a serious public health risk in many developing countries, often being most prevalent in poor communities with limited health facilities as well as communities' dependent on pastoralism (Deplazes et al., 2017). In areas where human CE is endemic there are accompanying high levels of infection in intermediate hosts (predominantly ungulates) where the metacestode stage of the parasite develops. Species that are most notably infected are livestock such as sheep, goats, pigs and cows (Eckert & Deplazes, 2004) although other livestock such as camels and horses can become infected. Intermediate hosts have a higher prevalence of infection than definitive hosts due to the biotic potential of an individual adult worm in a dog being able to produce large number of eggs that are then consumed by multiple ungulates, from a single definitive host (Torgerson & Heath, 2003). Intermediate hosts are not a direct risk of infection to humans but are a key driver in the transmission to dogs, continuing the life cycle. Surveillance of domestic intermediate hosts is a vital tool in understanding the transmission of cestode parasites such as *E. granulosus* because of the availability of regularly updated prevalence data produced at surveillance sites such as abattoirs or research projects in more rural environments.

Globally, human CE causes major risks to public health in rural and developing countries. In those regions where the prevalence of human cases is high, control measures are usually implemented to reduce the public health risk and prevent the transmission cycle continuing (Budke et al., 2006). The prevalence in humans is normally lower than that of domestic intermediate hosts, as humans are accidental hosts resulting from the consumption of eggs through unwashed vegetables or close interactions with dogs (Alvarez Rojas et al., 2018). In the Falklands CE is no longer a concern to public health, as a result of the eradication campaign, however, in the 1960s and 1970s, 11 human cases were diagnosed, which prompted the implementation of the control programme. A further 18 individuals were diagnosed through serology testing for *E. granulosus* in 1988, in a population of approximately 2,000, this is a very high prevalence which prompted the control programme in the first place

(Whitley, 1983). As described in chapter one, the prevalence in humans reduced to zero and the prevalence of CE in sheep reduced to below 1% (Reichel et al., 1996). Even so, cases are still seen annually in sheep slaughtered at the abattoir.

The communities most likely to have cases of CE are those agricultural communities where animal husbandry is a contributor to the economy. These regions not only have to face the health risks, but also the impact of CE on their livestock. Communities are impacted economically by the condemnation of offal and lack of production from infected individuals (Budke et al., 2006; Foreyt, Drew, Atkinson, & McCauley, 2009). In some areas losses in sheep with CE have been reported to approximate 7–10% of milk yield, 5–20% of meat or total carcass weight, and 10–40% of wool production (Battelli, 2009). The quantification of losses caused by infected viscera is influenced by both the legislative rules of each country (e.g. compulsory condemnation and destruction) and the number of animals slaughtered under veterinary supervision. Depending on the utilisation of viscera and on the total or partial condemnation of infected organs, the order of magnitude of losses can vary. For instance, in South America, it was estimated that the viscera of 2 million cattle and 3.5 million sheep are condemned every year, and that the cost of such condemnation amounts to US$ 6.3 million in Argentina and US$ 2.5 million in Chile (Battelli, 2009).

4.1.1 Typical prevalence of infection in intermediate hosts in countries where CE occurs.

The presence of *E. granulosus* in domestic livestock varies in different regions of the world. The most notable areas where high prevalence occurs are P.R. China/Tibet/Mongolia, Central Asia, Southern Europe, north and east Africa and South America. In various studies the prevalence of infection in domestic livestock in these areas is variable within the region and is influenced by livestock management, dog populations and ownership behaviour (Deplazes et al., 2017). Table x shows some typical examples of *E. granulosus sensu lato* prevalence in domestic livestock from endemic countries. In comparison recent annual Government zoonosis records in Great Britain, based on abattoir surveillance, report 1,315 bovine cases of hydatidosis, based on visible cysts (from 3,676,638 animals slaughtered – 0.03%), 23,596 cases in sheep (from 26,569,918 animals slaughtered – 0.08%) (Collins, 2019).

Table 4: Example prevalences of *E. granulosus* in domestic livestock. (Data taken from (Deplazes et al., 2017; Eckert et al., 2001)).

Region	**Prevalences in Sheep**	**Prevalences in cattle**	**Prevalences in pigs**	**Prevalences in other**
P.R. China	11-83%	3-81%	24%	78% Yaks
Kyrgyzstan/Kazakhstan	37-64%	24%	8 %	N/A
Portugal	8-30%	??	7-12%	N/A
Morocco	48-58%	37-43%	N/A	30% Camels
Kenya	16/43%	50%	N/A	N/A
Argentina	3-22%	15-19%	10%	6% Goats

Ungulates such as sheep and goats are most common intermediate hosts for CE, becoming infected when grazing in areas where dogs have defecated. Countries where agriculture is predominantly dependant on farming of these species often have high rates of infection of CE (Bortoletti, Gabriele, Seu, & Palmas, 1990). Due to the transmission cycle of *Echinococcus* species, they're over dispersed in definitive hosts, the biotic potential of the parasites and the persistence of eggs in the environment, prevalence in intermediate hosts are often higher than that of definitive host (Cabrera et al., 1995; Craig et al., 2015; Thevenet et al., 2005).

Close interaction between dogs and livestock is a key risk factor in transmission of CE, and the sharing of wells and other water sources could result in the contamination and then consumption of eggs by intermediate hosts when drinking in the dry arid climate where water is in short supply (Kia, Ouma, Mulambalah, & Okoth, 2019). Kenya is also surrounded by other highly endemic countries such as Sudan (44.6% prevalence in domestic camels (Elmahdi, Ali, Magzoub, Ibrahim, Saad, & Romig, 2004)) and Ethiopia (8-30% in sheep (Assefa, Mulate, Nazir, & Alemayehu, 2015)) where grazing across borders and sale of livestock result in continued maintenance of transmission (Abakar, Mahdi, Mohammed, & Malik, 2016; Dinkel, Njoroge, Zimmermann, Wälz, Zeyhle, Elmahdi, Mackenstedt, & Romig, 2004; Kia et al., 2019). North Africa is also highly endemic for CE, with countries such as Morocco and Tunisia having prevalences of over 40% of domestic livestock. Most of the slaughter in these countries occurs

in rural, unhygienic abattoirs with minimal slaughter protocols and restrictions, leading to high risk of transmission to the large dog populations. (Azlaf & Dakkak, 2006).

Central Asian countries such as Kyrgyzstan Kazakhstan, and Mongolia, along with various provinces of China such as Xinjiang, Sichuan, and Tibet, have high levels of nomadic herdsmen dependent on pastoralism as a way of life. As with other countries with high prevalences of CE in livestock, close association between dogs and sheep result in high endemicity. Regular practice is for dogs to roam freely with the grazing livestock, and are regularly fed offal directly from slaughtered animals, creating ideal conditions for continued transmission between infected dogs and livestock (Yuan, Wu, Zeng, Liu, Xu, Gao, Li, Li, Huang, Yu, & Sun, 2017). In Mongolian and Tibetan regions, where religions such as Buddhism are common, believe in allowing older livestock to die naturally, as well as not killing stray dogs and feeding and adopting them. This results in large number of older animals, that are more likely to be infected (Torgerson et al., 1998) as well as more free roaming dogs to have access to the carcasses. (Yang et al., 2009).

Despite efforts to control CE across South America, *E. granulosus* persists in countries such as Argentina, albeit at a low level. The sporadic nature of control programmes and the large and rural populations of sheep and dogs and regular local slaughter and unregulated disposal of offal has resulted in the continued transmission of *E. granulosus*. The conditions in areas of South America also allow for the prolonged survival of eggs in the environment, that means due to the sporadic nature of control measures, the transmission cycle is not broken for a long enough period and livestock continue to become infected over time (Thevenet et al., 2005).

4.1.2 Diagnosis of CE in livestock.

Studies estimating the prevalence of *E. granulosus* in livestock are largely based on either meat inspection records at abattoirs or specific culls. The use of immunodiagnostic approaches to detect antibodies against hydatid cyst fluid antigens has been shown to have limited success with animals showing variable levels of antibody production based on age and cyst numbers. With ELISA approaches sensitivities of between 65% and 89% have been reported with hydatid cyst fluid antigens and specificities of up to 90% (Craig et al., 2015) with some cross reaction with *T. hydatigena* cases. In addition, the time constraints of applying

immunodiagnostic tests for large scale screening make it a less favourable approach to meat inspection.

Post-mortem meat inspection can also produce variable results and detection of cysts is dependent on their age, size, and location. Detection of infection in young animals is therefore more difficult and is likely to be underestimated. Infections begin as an oncosphere (around 20μm in size) hatches and the hexacanth larvae invades the tissues and starts to develop into a hydatid cyst (Williams & Colli, 1970). In the very early stages cysts will appear as small white lesions which may be missed or mis-identified as either other taeniid infections, granulomas, tumours or caseous lymphadenitis (Eckert et al., 2001). The growth rate of hydatid cysts is thought to be around 1 cm per year (Torgerson, Ziadinov, Aknazarov, Nurgaziev, & Deplazes, 2009), and it is therefore likely that identification of hydatid cysts would be most reliable from one year post infection onwards. Detection of infection in young animals is therefore more difficult and is likely to be underestimated, and the WHO recommends that where age-dependent prevalence is being studied in young animals, it is essential to thinly slice both the liver and lungs at about 2 mm thickness and submit all lesions for staining and microscopy. Material fixed in formalin can be processed by conventional staining methods for histological examination (Eckert et al., 2001). However, it is unlikely that this often happens in practice. Once animals are older hydatid cysts with a characteristic structure are more likely to be identified correctly although this will be dependent on the experience of the meat inspector. There may also be considerable variation on the time spent on each carcass and the tissues that are examined carefully. The recording of appropriate data from abattoirs is also of key importance in surveillance of disease.

The World Organisation for Animal Health (OIE) defines surveillance as “the systematic, ongoing collection, collation and analysis of information relating to animal health and the timely dissemination of information so that action can be taken” (World Organisation for Animal Health, 2019). These data used in control programmes to monitor the impact of control measures over the eradication campaigns. These data become even more important towards the end of the campaign to focus control efforts. In New Zealand, when cases of CE in sheep were reduced to low levels, infections were able to be restricted to a collection of farms. Restrictions on animal movements and sales were then implemented to prevent the

potential spread of CE by farmers unknowingly selling infected sheep to other farms (Pharo, 2002).

4.1.3. *Taenia hydatigena* and *Taenis ovis* infections in livestock.

Both *T. hydatigena* and *T.ovis* have similar life cycles to *E. granulosus* in that the adult worm occurs in the intestine of dogs and sheep and other ungulates are infected by eating eggs from contaminating dog faeces on pastures and dogs are infected by eating raw internal organs from sheep. Within the sheep, both parasites occur as cysticerci which are small cysts containing a single scolex. *Taenia ovis* (*Cysticercus ovis*) is approximately 6-10mm in diameter and occurs in the skeletal and cardiac muscles causing sheep measles (Zheng, 2016). *Taenia hydatigena* (*Cysticercus tenuicollis* or bladderworm) is larger at 1-2cm in diameter and occurs in the abdominal cavity, intestinal mesenteries, and liver tissue (DeWolf et al., 2012; Scala et al., 2016). As adult parasites in dogs, both worms are considerably larger that *E. granulosus*. Adult *T. hydatigena* grow up to about one meter in size whilst *T. ovis* can reach lengths of around 1.5m. Both worms can produce and shed on average two proglottids containing 20,000-50,000 eggs making their biotic potential much greater than *E. granulosus* (Gemmell, Lawson, & Roberts, 1987; Gregory, 1976). The presence of either parasite in sheep is an indication that dogs or some other host is contaminating the environment and is an indicator that control approaches for Echinococcosis may be incomplete. The prevalence of *T. hydatigena* at higher prevalences is also documented as Typical prevalences of *T. hydatigena* in sheep can range from 4% to 52% (Braae, Saarnak, Mukaratirwa, Devleesschauwer, Magnussen, & Johansen, 2015; Scala et al., 2016) and 2%-24% for *T. ovis* (Hajipour et al., 2020). The higher prevalence of the latter two parasites is reflection of their hyperendemic nature within an environment and has been observed in the greater effort needed for their control when compared to *E. granulosus*. As described, this is as a result of the greater proliferation of eggs from the adult worm, allowing for greater dispersal and therefore ingestion by intermediate hosts (Gemmell, Lawson, & Roberts, 1986c; Gemmell, Lawson, Roberts, Kerin, & Mason, 1986d; Torgerson & Heath, 2003; Torgerson, Pilkington, Gulland, & Gemmell, 1995).

4.1.4 *E. granulosus* and other Taeniid infections in Sheep in the Falkland Islands

Echinococcus granulosus has been endemic in the Falkland Islands for many years (see Chapter 1), with a small number of sheep being discovered with infections at the abattoir annually. *Taenia hydatigena* has also been found in sheep in the Falklands, though it is not known when it was first introduced. The prevalence of *T. hydatigena* has followed a similar trend over time to that of *E. granulosus*, reducing significantly from the implementation of control measures to combat Hydatid disease. However, while CE dropped to 1.8% by 1983, *T. hydatigena* remained high at 10.1%. In recent years, the prevalence of *T. hydatigena* has decreased to 3.17%, 2.37%, 1.64% and 1.89% in consecutive years from 2015 to 2018, with the prevalence increasing in 2019 to 6.04%.

The objectives of this chapter are (i) to establish how meat inspection at the main abattoir in Stanley is carried out and how the relevant data are recorded and (ii) to evaluate the slaughter data for individual years from 2006 to 2020 in relation to prevalence of infection of *E. granulosus*, *T. hydatigena* and *T. ovis* in three age categories of sheep slaughtered at the abattoir. These are new season lambs (NSL), yearling lambs (YLL) and adult sheep (adult).

4.2. Methods

4.2.1 Observations at Stanley abattoir and the meat inspection process

All livestock intended for the export or local commercial markets are slaughtered at the Sand Bay abattoir situated just outside the capital of Stanley. Here, all animals are inspected by an EU registered meat hygiene inspector (MHI) as well as a vet from the Falkland Island Government Department of Agriculture. All ailments and/or diseases discovered in animals are recorded and a disease report is produced for all sheep slaughtered. During the export season, animals are slaughtered every weekday, whereas outside of the export season they are usually slaughtered once a week. The sheep slaughter line runs at approximately 80 sheep per hour, brought up to the slaughterhouse floor by a conveyer from the lairage. Once an animal is slaughtered, its head is removed immediately and disposed of, and the carcass is hung onto the processing line. The skin is removed at this point the stomach and intestines are separated from the pluck (liver, lungs, and heart) and sent down a chute past the MHI

post where inspection takes place. The pluck is left attached to the carcass on the processing line which passes the other side of the MHI post. The MHI therefore has just under one minute to inspect the stomach, intestines, pluck, and carcass, although they can stop the line should they require more time to investigate something more closely. Each carcass and offal are inspected visually, and the organs are palpated. Any conditions identified are marked with a colour coded tag and the information is then recorded on a computer at the weighing station. From here, all carcases and pluck are moved into a chilling room prior to butchery and preparation for export or the local market. All offal not destined for commercial sale is disposed of on the same day as the animal is slaughtered. Offal is loaded into the back of lorries and driven to a disposal site 3-4km away, where they are deposited into the sea.

4.2.2 Abattoir recording of data.

Once the carcass of a sheep has passed the MHI post and any conditions have been recorded at the weighing station, the information is compiled into a disease summary report (Figure 48).

Disease Summary

Farm and Mob		Type	Kill Date	No. Killed	Condition	Totals	% Condition	% Farm
2 Sisters								
	317	Sheep Lamb	08/04/2013	42				
			Total Killed:	**42**	**Total Condemned:**	**0**		
BELONGA US								
	92	Sheep Lamb	06/02/2013	103	SCL-Caseous Lymphadinitis	1	100.00	0.97
			Total Killed:	**103**	**Total Condemned:**	**0**		
Bleaker Is								
	134	Sheep Lamb	18/02/2013	51	SPP-Pleurisy / Pneumonia	12	48.00	4.18
	135	Sheep Yearling	19/02/2013	90	SCT-Cystericus Tenuicollis	1	4.00	0.35
	136	Sheep Mutton	19/02/2013	146	SCL-Caseous Lymphadinitis	1	4.00	0.35
					SCO-Contamination	1	4.00	0.35
					SCT-Cystericus Tenuicollis	8	32.00	2.79
					SPC-Pericarditis	1	4.00	0.35
					SPP-Pleurisy / Pneumonia	1	4.00	0.35
			Total Killed:	**287**	**Total Condemned:**	**1**		
Blue Beach								
	20	Beef All	14/01/2013	4				
	50	Sheep Mutton	23/01/2013	99	SCL-Caseous Lymphadinitis	7	7.53	0.70

Figure 48: An example of a Disease Summary report produced by the Sand Bay Abattoir.

4.2.2 Identification of suspect parasitic material.

When suspected hydatid cysts are discovered, in either sheep or cattle, the farm of origin is documented before the cysts are taken to the laboratory at the Department of Agriculture for microscopy. To confirm whether the cyst is Hydatid or not, the cyst fluid is extracted, and

the germinal layer is scraped with a scalpel and spread on a microscope slide. The presence of multiple protoscoleces, with visible structures such as hooks, and suckers confirms that the cyst is caused by *E. granulosus*. Once confirmed, cyst material is put into 70% ethanol and sent to the University of Salford for PCR analysis. If protoscoleces are not found, but the cyst is thought be a hydatid cyst, the sample is also sent to the University of Salford in the same fashion. After confirmation at the lab in the Falklands, the farm of origin is contacted to get information about the animal that was positive, asking for information on the age of the animal, whether it was born on their farm or purchased from another, and any other information the farmer can provide. A total of 16 hydatid cysts were confirmed in the Falkland Islands by microscopy and transported to the University of Salford for molecular analysis (five in 2018, five in 2019, and six in 2020). A total of 25 *T. hydatigena* cysts were imported as well for sequencing.

4.2.3 DNA Extraction from Suspected Cyst material

From an individual cyst, 25mg of tissue is cut into small segments and put into a 1.5ml microcentrifuge tube, then 180µl of ATL Buffer and 20µl Proteinase K is added and mixed by vortexing. The sample is then incubated at 56°C until the tissue is completely lysed (1-3 hours). 200µl of AL Buffer is added before incubating again at 56°C for 10 mins. 200µl is then added and mixed using a vortex before the entire solution is added to a DNeasy Mini spin column with a 2ml collection tube. The spin column was centrifuged at 8000 rpm for 1 minute and the flow through discarded and put into a new 2ml collection tube. Then 500µl of AW1 buffer is added to the spin column and again centrifuged at 8000 rpm for one minute and the flow through discarded. The spin column is again placed in a new 2ml collection tube and 500µl of AW2 Buffer is added and centrifuged at 14,000 rpm for 3 minutes. Finally, the spin column is added to a 1.5ml microcentrifuge tube and 200µl of AE Buffer is added to elute the DNA. The spin column is left at ambient temperature for 1 minute then centrifuged at 8000 rpm for 1 minute, leaving the eluted DNA in the 1.5ml microcentrifuge tube.

4.2.4 PCR on Cyst material

Both cysts caused by *E. granulosus* and *T. hydatigena* were collected from the Sand Bay abattoir for DNA analysis. Once DNA was extracted, hydatid cysts caused by *E. granulosus* were analysed using *E. granulosus* G1 primers and bladder cysts caused by *T. hydatigena* were

analysed using Universal Cestode primers (Boufana et al., 2013; von Nickisch-Rosenegk et al., 1999). The protocol to be used for PCR analysis on the DNA extracted from cyst material collected in the Falkland Islands was designed by Boufana et al. (2013) using the adaptations described in Chapter 3 (section 3.2.3). Briefly, 1µl of Template DNA was added to the reaction mix and used the same PCR cycle of an initial incubation period of 5 minutes at 94°C for 1 cycle, 36 cycles consisting of 30s at 94°C, 50s at 62°C, and 30s at 72°C amplifying a *E. granulosus*-specific 226 bp fragment. The same was the case for the universal primers identifying *T. hydatigena* cysts with the PCR cycles of 5 minutes at 94°C for 1 cycle, 40 cycles of 30 seconds at 94°C, 1 minute at 55°C, and 30 seconds at 72°C to amplify a fragment of DNA 314 bp in length. To confirm species and genotype, all positive PCR DNA was sequenced (Source Bioscience, UK) and analysed using a BLASTn search of the NCBI Genbank database.

4.2.5 Data collection and processing

There is a single licensed abattoir in the Falkland Islands, the Sand Bay abattoir. This abattoir produces annual disease summary reports based on the findings of the Meat Hygiene Inspector and/or veterinarian present at the time of slaughter, containing the information shown in Table 5. As these reports were compiled in PDF format and the data were not otherwise directly available, the author manually inputted all available report data for sheep (excluding pigs and cattle) between 2006 and 2020 into an Excel spreadsheet.

Table 5: Contents of annual disease summary reports produced by the abattoir upon inspection of slaughtered animals.

Farm supplying sheep to abattoir	Ownership/Receipt of animals
Lot Number	Identification of group that are slaughtered
Total number of sheep slaughtered that year	Total number of sheep slaughtered during the Export Season
Date of Slaughter	dd/mm/yyyy
Age of Sheep. Divided into 3 categories	New Season Lambs (NSL)- Less than 12 months old
Age category data only available from 2011 onwards	Yearling Lambs (YLL)- 12-18 Months old
	Mutton (Sheep)- 18 months and older
Number of Sheep in a lot	Total of individuals slaughtered in the lot

Condition	The number and type of all diseases and/or ailments observed at inspection
Total Condemned	The total of carcasses removed from commercial sale due to their condition

As the disease summary reports did not contain cases of CE (which were only confirmed once microscopic analysis has been completed by the Department of Agriculture and maintained in a separate data set), the author then added these reports to the dataset.

For each cestode disease, lots were split by age category, and each was classified according to whether at least one infected animal was identified at slaughter and the proportion of these animals was estimated. Data were aggregated over all lots and the proportion of infected animals was estimated. Data were checked for inconsistencies in total lot size using R version 4.1.1 (R Core Team, 2021) and the Tidyverse package (Wickham, Averick, Bryan, Chang, McGowan, François, Grolemund, Hayes, Henry, Hester, Kuhn, Pedersen, Miller, Bache, Müller, Ooms, Robinson, Seidel, Spinu, & Yutani, 2019). For each farm, estimates of the total number of dogs were obtained from veterinary records, estimates of the total number of sheep were obtained from farming statistics produced by the Department of Agriculture (Department of Agriculture, 2020), and the total area of land belonging to the farm was estimated from farm boundary data provided by records from the Department of Agriculture, using the st_area function in the sf package (Pebesma, 2018). From these, the number of dogs per sheep and the number of dogs per km^2 on each farm were estimated.

4.2.6 Statistical analyses of data

Due to the low prevalence of CE in the Falklands, it was decided that there was insufficient data on *E. granulosus* infection for robust statistical analysis of spatial and temporal trends. As *T. hydatigena* is found at a higher prevalence, and because of the similarities in lifecycle, it was decided that this data would be used for statistical analysis.

In order to identify which farm-level factors were associated with a higher risk of infection with *T. hydatigena*, mixed effects logistic regression models were created using the glme function in the lme4 package (Bates, Mächler, Bolker, & Walker, 2015), including the overall status of the age-specific lot (i.e. the presence or absence of detected infected animals in the

lot) as the outcome and the farm as a random effect. Models were compared using an information theoretic approach (Burnham & Anderson, 2002) by comparing the corrected version of Akaike's information criterion (AICc) values using the AICcmodavg package (Mazerolle, 2020). An initial investigation was conducted in order to identify the baseline model structure. Three models were created, each of which included the random intercept, one with the number of animals in each age-specific lot, and one with an interaction between the number of animals and the age class of the lot. As the model containing the interaction term gave the lowest AICc value (table 6), the dataset was split by age class and model comparison was then performed for each of the three resultant datasets (new season lambs, yearling lambs, and sheep).

Table 6: Primary models in order of lowest AICc score and the variable included in each model (green).

Model	Farm-Random Effect	Total Slaughtered	Total slaughtered: Age class interaction	AICc	Delta AICc
m3				662.69	0
m2				890.24	227.55
m1				1099.92	437.23

For each age group, a number of models were created, based upon knowledge of the likely factors influencing cestode transmission:

- M1: Random effect only
- M2: M1 plus linear effect of total number slaughtered
- M3: M2 plus linear effect of number of dogs
- M4: M2 plus presence/absence of dogs
- M5: M2 plus linear effect of number of dogs per head of sheep
- M6: M2 plus linear effect of number of dogs per m^2 of farm
- M7: M2 plus linear effect of log of number of dogs per head of sheep
- M8: M2 plus linear effect of square root of number of dogs per head of sheep
- M9: M2 plus linear effect of log of number of dogs per m^2 of farm
- M10: M2 plus linear effect of square root of number of dogs per m^2 of farm

These were then ranked in order of AICc for each age group, with the model with the lowest AICc estimate selected as the "best" model. For this model, odds ratios (ORs) confidence intervals, and p-values were estimated for all variables (table 7).

Temporal trends in infection status were considered separately. Data were consolidated at the farm level for both sheep and lambs (NSLs and YLLs combined) for each year and the proportion of detected infections with each parasite was identified for each. Spearman's Rank correlation analysis was used to identify whether there were monotonic trends in the prevalence of *T. hydatigena* infection over time. An increase over time would be suggestive of a systematic failure of control leading to ongoing transmission, whereas intermittent infection events would not necessarily show such a pattern. Spearman's rank correlation wase also used to identify whether the farm-level prevalences of each taeniid parasite (*E. granulosus, T. hydatigena* and *T. ovis*) were correlated with each other, which could be suggestive of a shared definitive host.

4.3 Results.

4.3.1 Confirmation of parasite material.

Organs containing suspected hydatid cysts are either brought to the Department of Agriculture whole, or the segment containing the cyst is cut away by the MHI and that is then delivered to the Department of Agriculture. Figure 49 shows a whole liver containing a hydatid cyst, as well as multiple daughter cysts from an adult sheep.

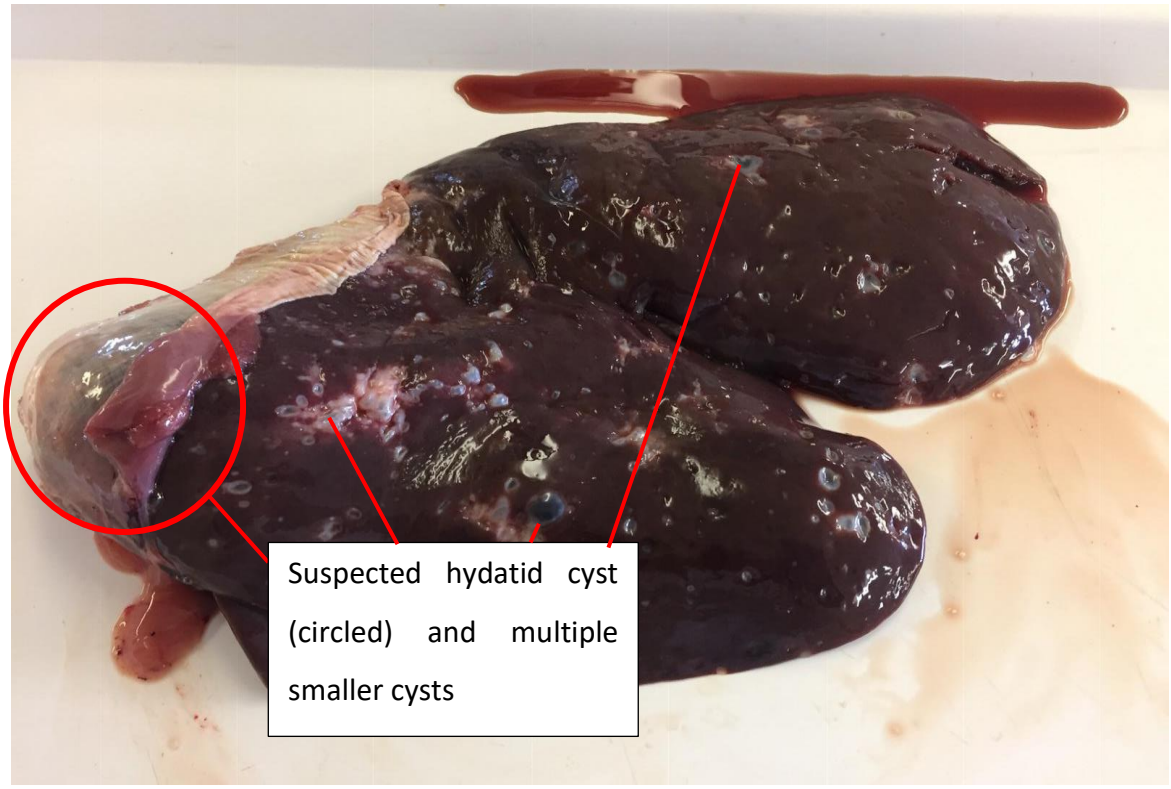


Figure 49: A liver containing a large hydatid cyst and multiple smaller cysts discovered in an adult sheep at the Sand Bay Abattoir.

The cyst fluid is extracted from the suspected hydatid cyst and the germinal layer is scraped onto a microscope slide. Figure 50 shows a brood capsule containing invaginated protoscoleces and evaginated protoscoleces from a suspected hydatid cyst.

C. Hooks

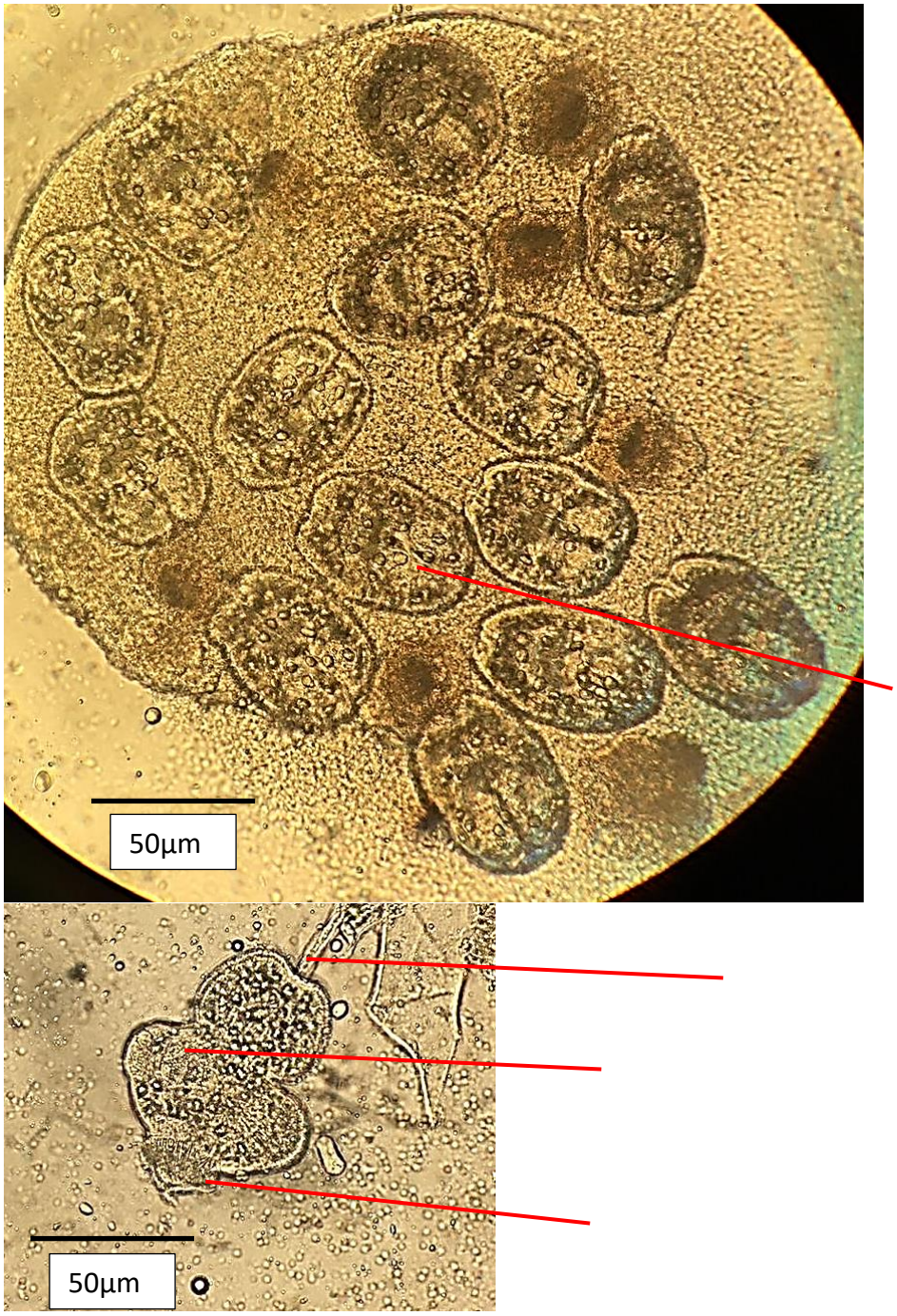


Figure 50: Invaginated (above) with visible hooks (A) and evaginated protoscoleces containing attachment stalk (B), suckers (C) and scolex (D) scraped from the germinal layer of a suspected hydatid cyst. Microscope wasset at x40 magnification.

Following DNA extraction from the Germinal layer of Hydatid cysts confirmed by the presence of protoscoleces under a microscope, PCR analysis using *E. granulosus* G1 primers was used to confirm the diagnosis.

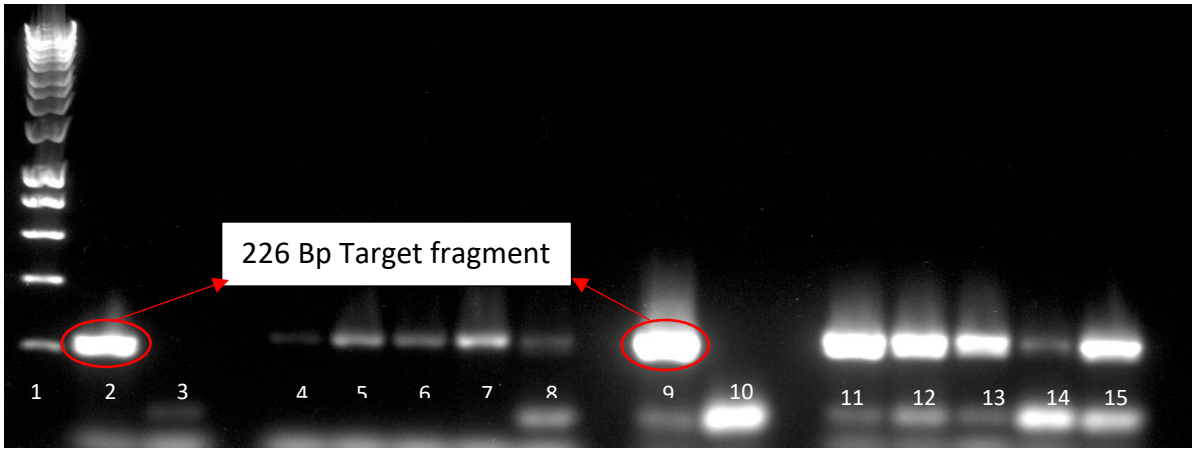


Figure 51: *E. granulosus* G1 PCR results from 2018 and 2019 Hydatid cysts. Lane 1- 1kb Hyperladder. Lane 2 & 9- *E. granulosus* G1 Positive control. Lane 3 & 10 - PCR grade water Negative controls. Lane 4-8- 2018 Hydatid Cyst samples. Lane 11-15- 2019 Hydatid Cysts samples.

A total of five ovine Hydatid cysts were confirmed from five different farms in both 2018 and 2019 (Figure 51). In 2020, a further five cysts were confirmed from four different farms. All the cysts confirmed by PCR were sequenced using *E. granulosus* specific primers Eg1F81 and Eg1R83 and analysed via BLAST search of the NCBI Genbank database and found to have 100% homology with *E. granulosus* Sequence ID: LC476714.1 (mitochondrial nad1 gene for NADH dehydrogenase subunit I).

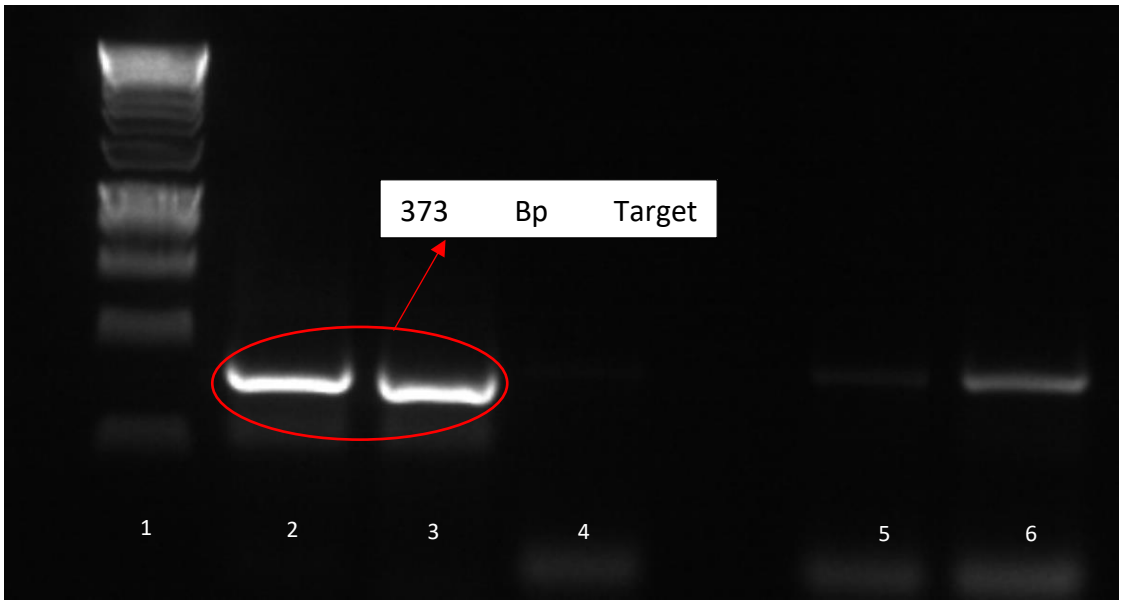


Figure 52: Universal Cestode PCR results from a Bovine Hydatid Cyst with protoscoleces present at microscopy, collected at slaughter from the Sand Bay Abattoir. Lane 1- 1kb Hyperladder. Lane 2 & 3- *E. granulosus* G1 and *T. solium* positive controls. Lane 4- PCR grade water negative control. Lane 5-6- Repeated samples from Bovine Hydatid Cyst.

In 2019, a single hydatid cyst was found in a cow from West Falklands. The cyst was confirmed to be taeniid using Universal Cestode PCR primers (Figure 52), and the DNA was sequenced

to confirm the species. The sequenced DNA was analysed via BLAST search of the NCBI Genbank database and confirmed to have 100% homology with *E. granulosus* strain G1 isolate UKR-BT2-2 small subunit ribosomal RNA gene Sequence ID: MT396431.1.

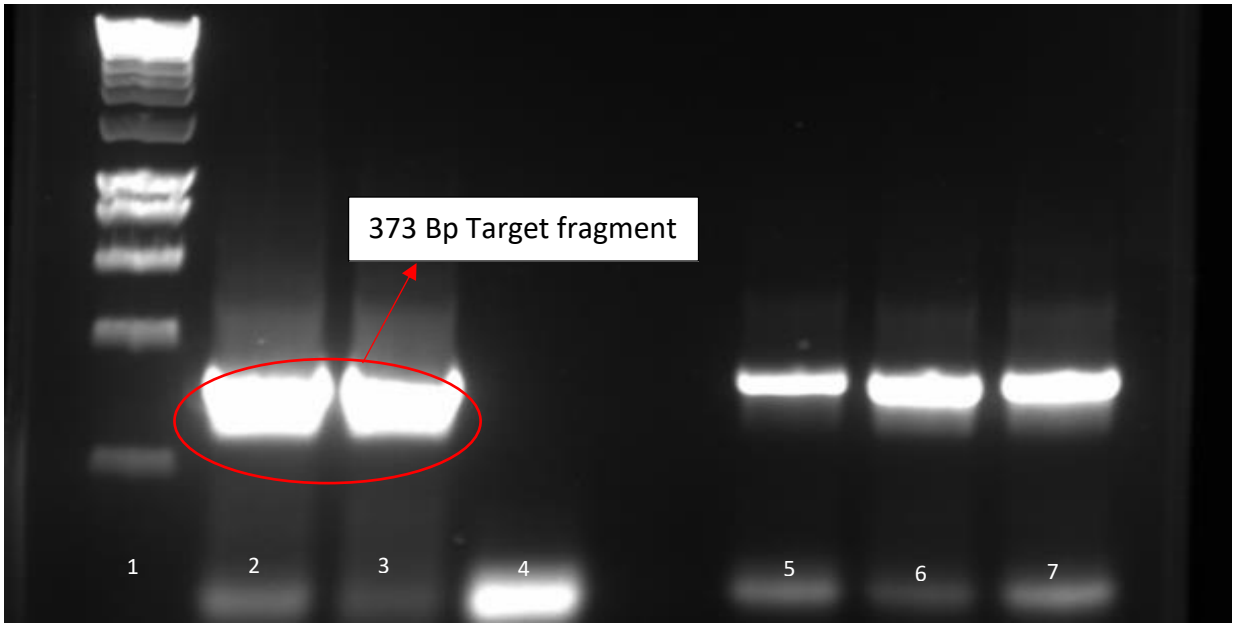


Figure 53: Universal Cestode PCR results from *T. hydatigena* cysticerci collected at slaughter from the Sand Bay Abattoir. Lane 1- 1kb Hyperladder. Lane 2 & 3- *E. granulosus* G1 and *T. solium* positive controls. Lane 4- PCR grade Water Negative Control. Lane 5-7- *T. hydatigena* samples.

A selection of *T. hydatigena* cysticerci were collected from the abattoir to confirm their species, after positive PCR using Universal Cestode primers, the DNA was sequenced and analysed via BLAST search of the NCBI Genbank database and confirmed with 100% homology to *T. hydatigena* isolate AM.Suli-19 small subunit ribosomal RNA gene, Sequence ID: MK858251.1.

4.3.2 Changes in Prevalence of Taeniid infections over time in animals slaughtered at the Sand Bay abattoir

A total of 86 individual farms supplied animals to the abattoir over the 15-year period considered here, excluding five lots for which animals from more than one farm were transported to the abattoir together for logistical reasons, making it impossible to identify the source farm.

A total of 650,247 sheep were slaughtered from 86 farms, with a mean annual slaughter of 43,350 (range 30,029-57,798). Over this period, 36 sheep were diagnosed with *E. granulosus* infection at the abattoir (0.0055%), 14,186 sheep were diagnosed with *T. hydatigena* (2.2%), and 465 sheep were diagnosed with *T. ovis* (0.072%). Table 7 summarises the prevalence of

each taeniid infection, number infected, as well as number of infections per 10,000, as this is a useful comparison for farmers to make based on the size of their farm.

Table 7: Annual prevalence of Taeniid infections in sheep of all ages between 2006-2020

Year	Total of sheep slaughtered	Total *Taenia hydatigena* (%)	Total *Echinococcus granulosus* (%)	Total *Taenia ovis* (%)
2006	30808	520 (1.69)	5 (0.02)	95 (0.31)
2007	34212	1446 (4.23)	3 (0.01)	153 (0.35)
2008	22139	489 (2.21)	1 (<0.01)	0 (0)
2009	23543	214 (0.91)	0 (0)	0 (0)
2010	23373	425 (1.82)	1 (<0.01)	0 (0)
2011	37722	276 (0.73)	1 (<0.01)	0 (0)
2012	45987	190 (0.41)	0 (0)	0 (0)
2013	57670	1452 (2.52)	0 (0)	97 (0.17)
2014	55718	557 (1.00)	4 (0.01)	4 (0.01)
2015	51321	1658 (3.23)	4 (0.01)	46 (0.09)
2016	43847	1072 (2.44)	5 (0.01)	54 (0.12)
2017	43243	723 (1.67)	2 (<0.01)	7 (0.02)
2018	47166	850 (1.80)	5 (0.01)	7 (0.01)
2019	44665	2688 (6.02)	5 (0.01)	2 (<0.01)
2020	44202	1749 (3.96)	5 (0.01)	0 (0)

Table 8: Prevalence of Taeniid infections in sheep and lambs annually.

Year	Type	Total animals slaughtered	Total *Taenia hydatigena* (%)	Total *Echinococcus granulosus* (%)	Total *Taenia ovis* (%)
2011	NSLamb	13964	26 (0.19)	0 (0)	0 (0)
2011	YLLamb	6170	70 (1.13)	0 (0)	0 (0)
2011	Sheep	17588	180 (1.02)	1 (0.01)	0 (0)
2012	NSLamb	16022	19 (0.12)	0 (0)	0 (0)
2012	YLLamb	6813	16 (0.23)	0 (0)	0 (0)
2012	Sheep	23152	155 (0.67)	0 (0)	0 (0)
2013	NSLamb	17613	18 (0.10)	0 (0)	13 (0.07)
2013	YLLamb	10091	123 (1.22)	0 (0)	11 (0.11)
2013	Sheep	29966	1311 (4.37)	0 (0)	73 (0.24)

2014	NSLamb	16044	33 (0.21)	0 (0)	0 (0)
2014	YLLamb	12433	84 (0.68)	0 (0)	2 (0.02)
2014	Sheep	27241	440 (1.62)	4 (0.01)	2 (0.01)
2015	NSLamb	6571	24 (0.37)	0 (0)	0 (0)
2015	YLLamb	12716	148 (1.16)	0 (0)	9 (0.07)
2015	Sheep	32034	1486 (4.64)	4 (0.01)	37 (0.12)
2016	NSLamb	3424	21 (0.61)	0 (0)	0 (0)
2016	YLLamb	10343	74 (0.72)	0 (0)	1 (0.01)
2016	Sheep	30040	977 (3.25)	5 (0.02)	53 (0.18)
2017	NSLamb	3793	14 (0.37)	0 (0)	0 (0)
2017	YLLamb	9182	56 (0.61)	0 (0)	1 (0.01)
2017	Sheep	30268	653 (2.16)	2 (0.01)	6 (0.02)
2018	NSLamb	2893	13 (0.45)	0 (0)	0 (0)
2018	YLLamb	10347	43 (0.42)	0 (0)	1 (0.01)
2018	Sheep	33926	794 (2.34)	5 (0.01)	6 (0.02)
2019	NSLamb	1381	1 (0.07)	0 (0)	0 (0)
2019	YLLamb	6811	28 (0.41)	0 (0)	0 (0)
2019	Sheep	36473	2659 (7.29%)	5 (0.01)	2 (0.01)
2020	NSLamb	678	0 (0)	0 (0)	0 (0)
2020	YLLamb	2995	10 (0.33)	0 (0)	0 (0)
2020	Sheep	40529	1739 (4.29)	5 (0.01)	0 (0)

4.3.2.1 *Echinococcus granulosus*

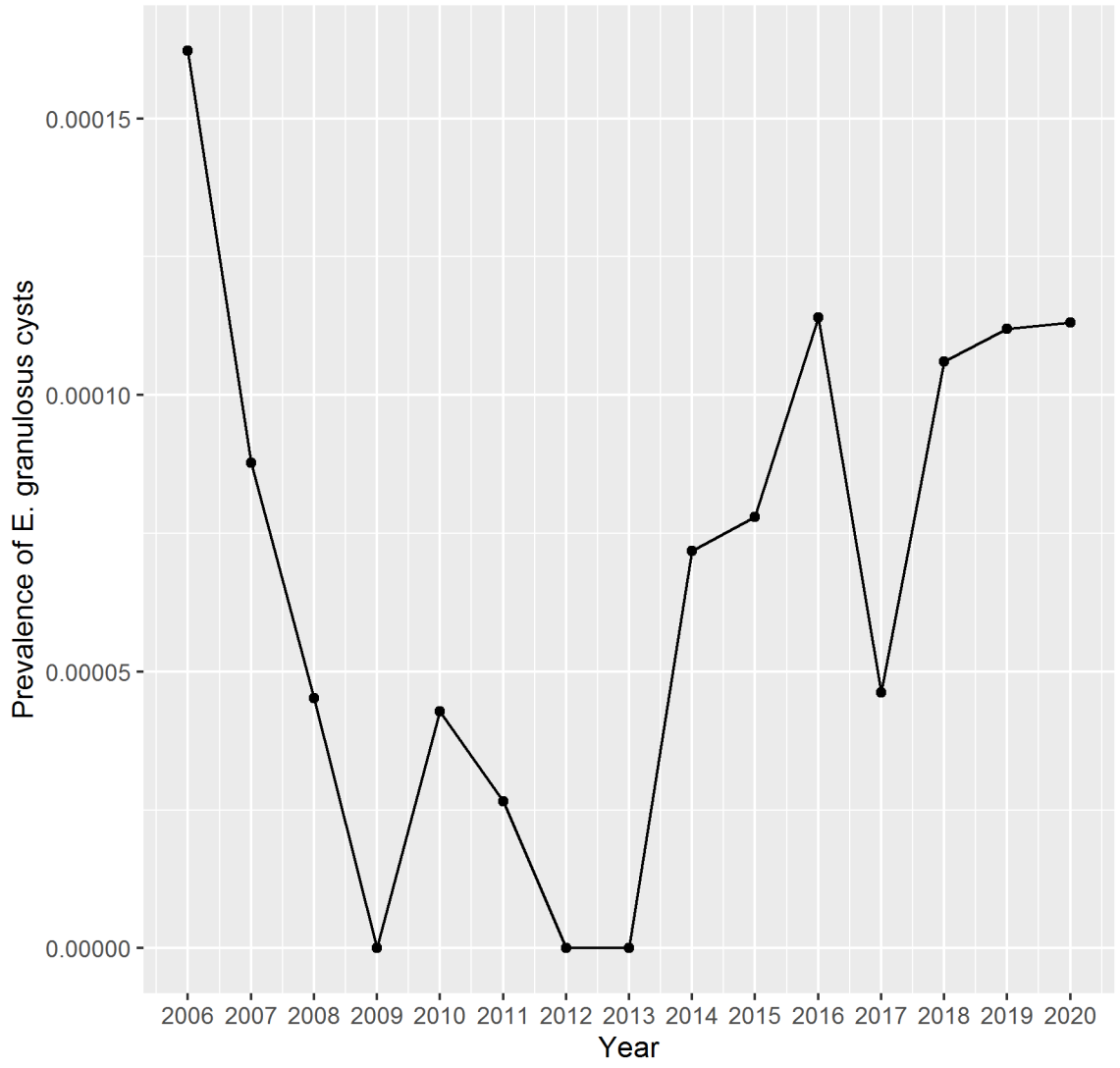
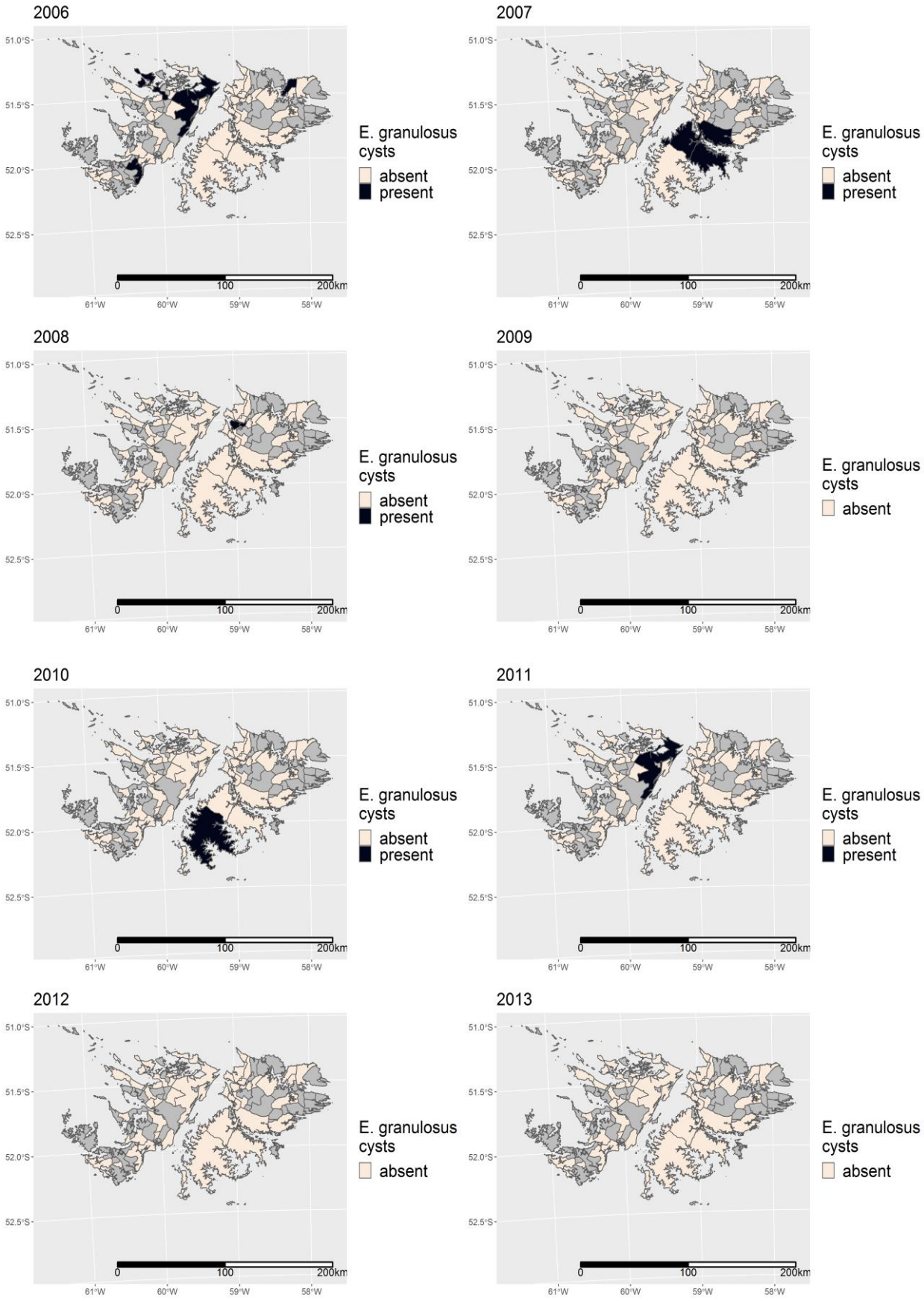


Figure 54: Annual Prevalence of *E. granulosus* in all sheep across the Falkland Islands, slaughtered between 2006-2020.

Over the data period, a total of 41 sheep were found to contain hydatid cysts, giving a mean prevalence of 0.007% (range 0.000-0.016%), with all infections being discovered in older sheep. From 2006 to 2013, the prevalence decreased to almost zero with only one sheep being diagnosed annually with CE in the years of 2008, 2010, and 2011 and no sheep in 2007, 2009, 2012, and 2013. The prevalence increased in 2014, with four (0.007%) sheep diagnosed with CE (Figure 54). Prevalence in sheep remained above 0.003% for the remainder for the study period.


2006
2007
2008
2009
2010
2011
2012
2013
E. granulosus cysts
absent
present
51.0°S
51.5°S
52.0°S
52.5°S
0
100
200km
61°W
60°W
59°W
58°W

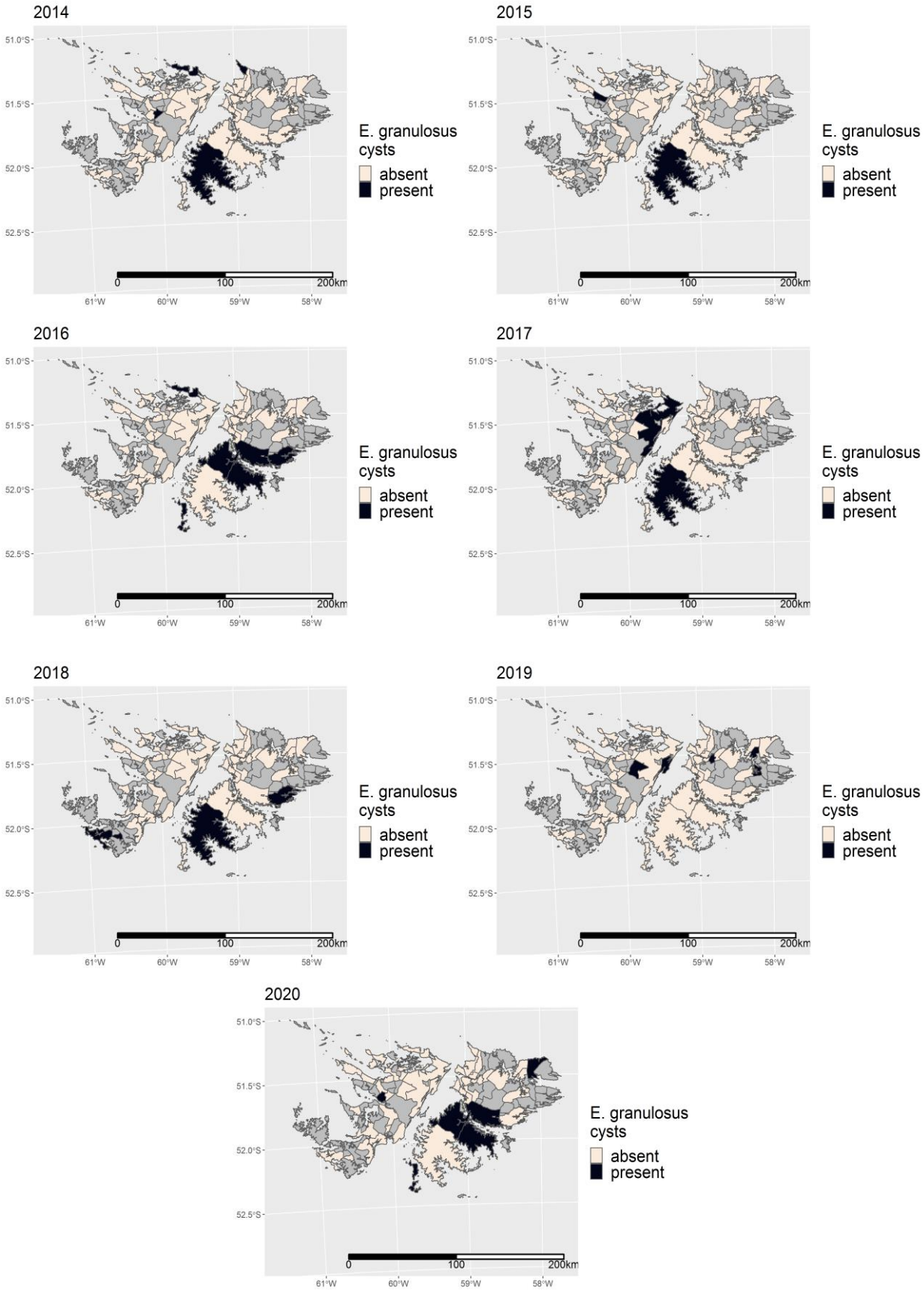


Figure 55: The number and location of Hydatid Cysts in sheep identified between 2006-2020. Black areas represent farms where infected sheep are present, cream areas represent farms have no infected sheep and grey areas are farms that do not supply sheep to the abattoir.

The location of farms where sheep were observed with CE varied over the time period. Figure 55 shows the farms with infected sheep. In 2006 four farms with infected sheep were present on West Falkland and only one on East Falkland. From 2014-2020 the farms recording CE in sheep were more extensive with farms in the southern half of East Falkland and some farms on West Falkland showing evidence of infected sheep.

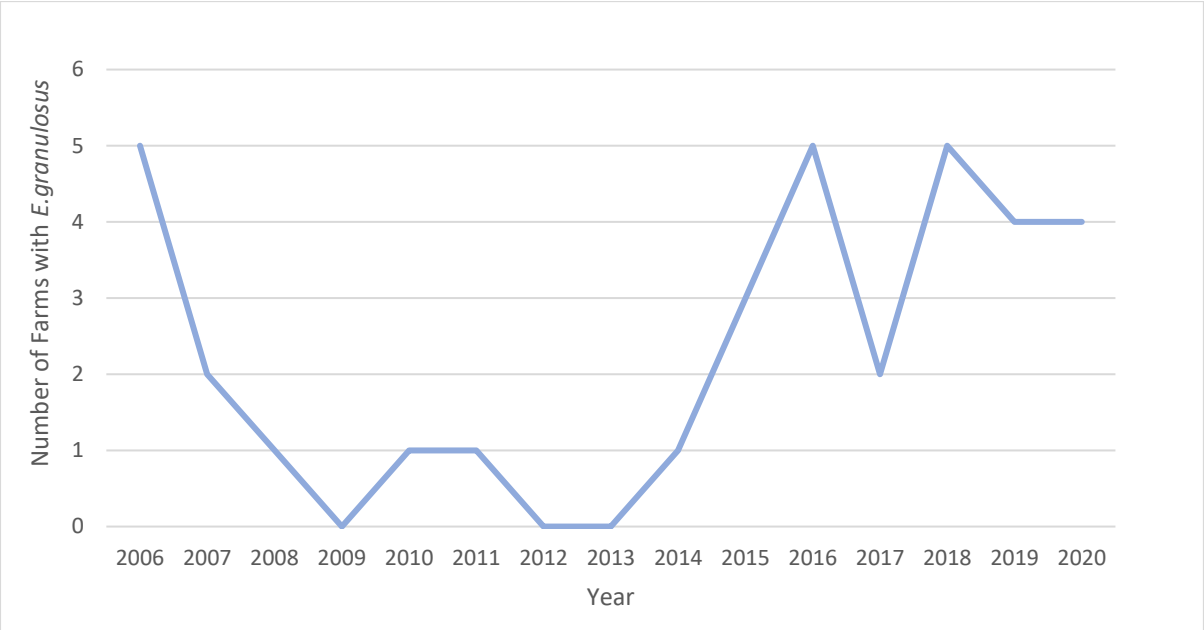


Figure 56: Numbers of farms with *E.granulosus* in sheep each year from 2006-2020

Figure 56 shows that between 2009 and 2013 only two farms reported infected sheep but between 2015 and 2020 the number of farms with CE in sheep rose to 16 of which 12 had not reported any CE since at least 2006. During this later period, two farms (Goose Green and North Arm) had CE infected sheep in more than two years. However, these are two of the largest farms on the islands sending most sheep to the abattoir (40.44% in 2019).

4.3.2.2 *Taenia ovis*

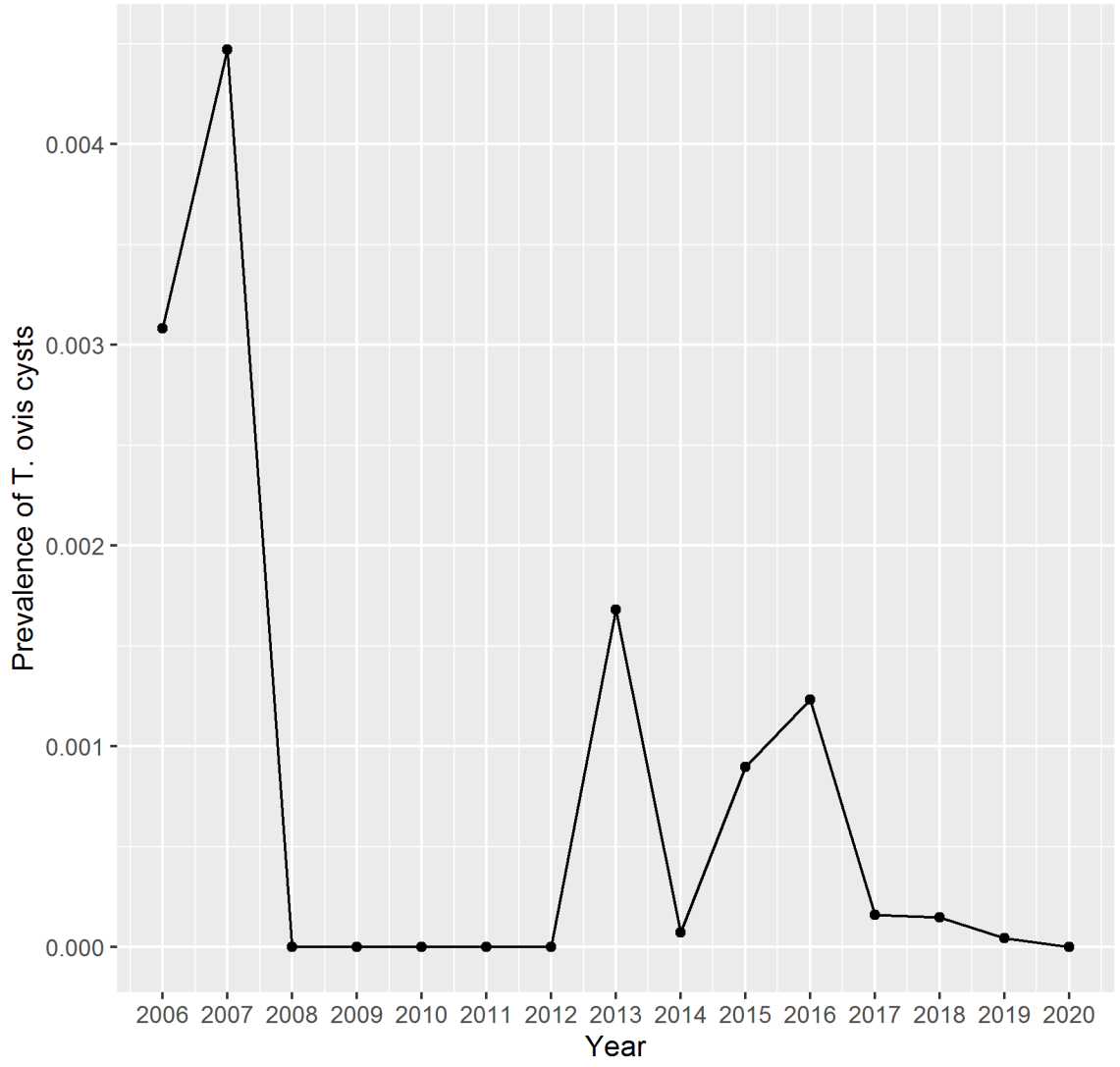


Figure 57: Annual prevalence of *T. ovis* in all sheep on the Falkland Islands slaughtered between 2006-2020.

Taenia ovis was identified in 465 sheep at the abattoir during the data period (Mean prevalence = 0.079%, Range 0.000-0.447%). Figure 57 shows that in 2007 the parasite was present in 33 farms but was not recorded again until 2013 when 97 (0.168%) sheep were identified across 18 farms. The prevalence decreased again in 2014 (0.007%) before another increase in prevalence to 0.090% in 2015 and 0.123% in 2016 across 26 farms, before decreasing again, reaching a prevalence of zero in 2020.

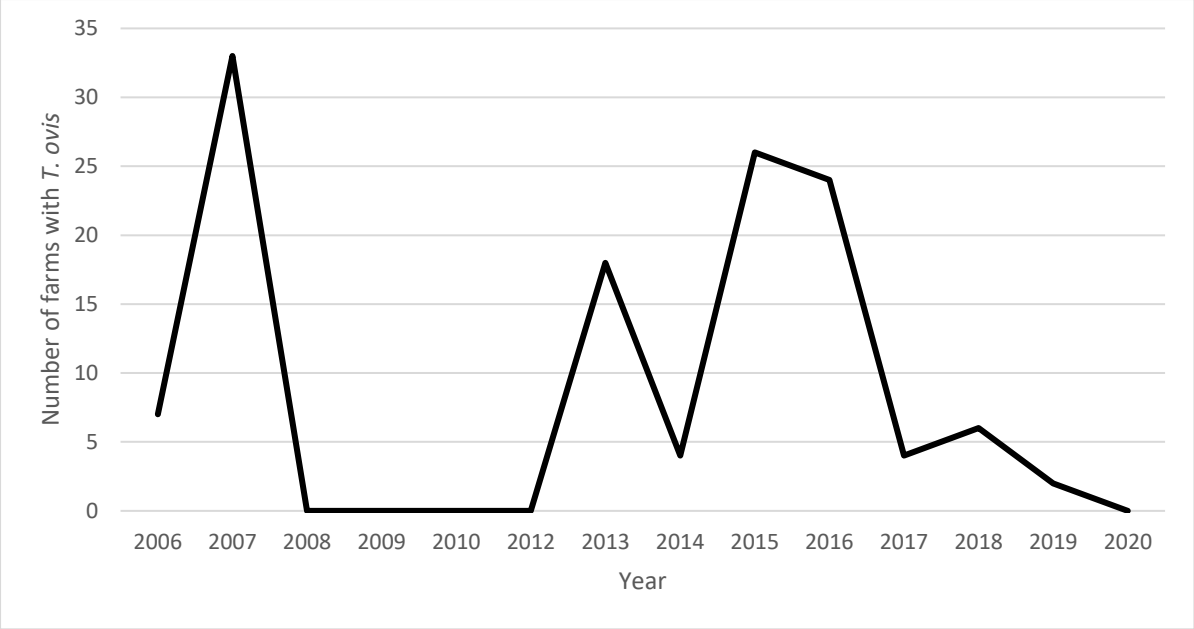


Figure 58: Numbers of farms with *T. ovis* in sheep each year from 2006-2020.

In the period after 2013 *T. ovis* was only found in lambs on ten farms. Of these farms, five were in East Falkland, and one island farm close to East Falklands (Figure 58). From these farms, three regularly send NSLs to the abattoir and showed a peak of infection in lambs in 2013 with a subsequent follow up peak in YLLs one or two years later, as can be seen in figures 59, 60, 61.

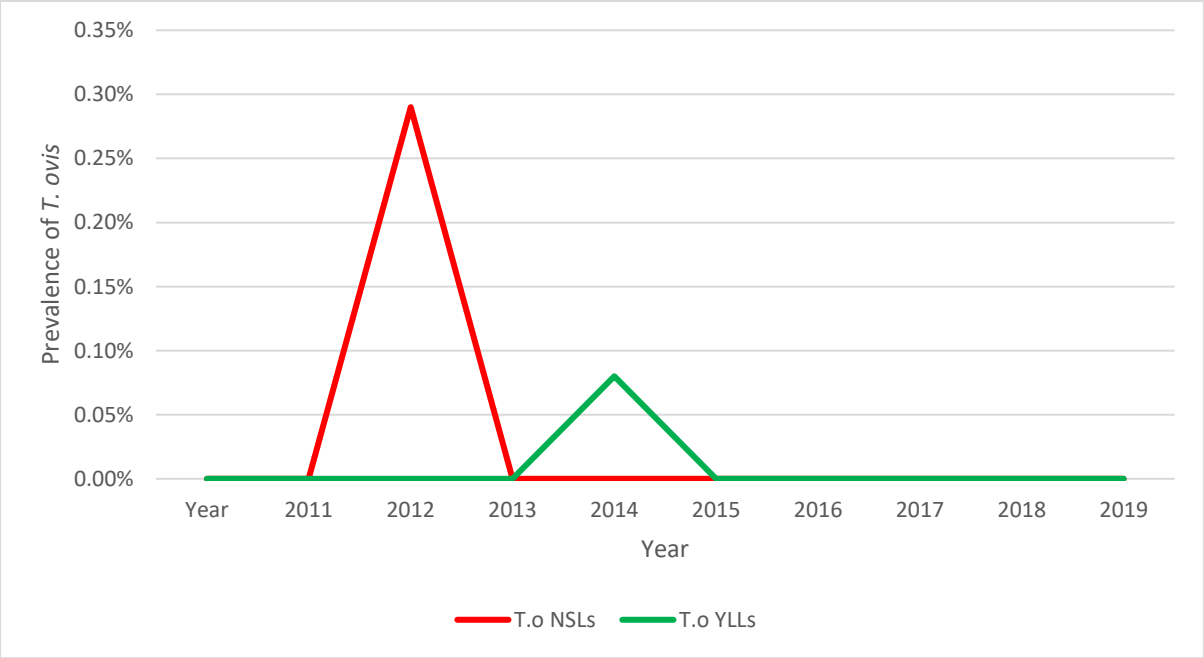


Figure 59: Prevalence of *T. ovis* in new season lambs and yearling lambs on farm EF7 between 2011-2020.

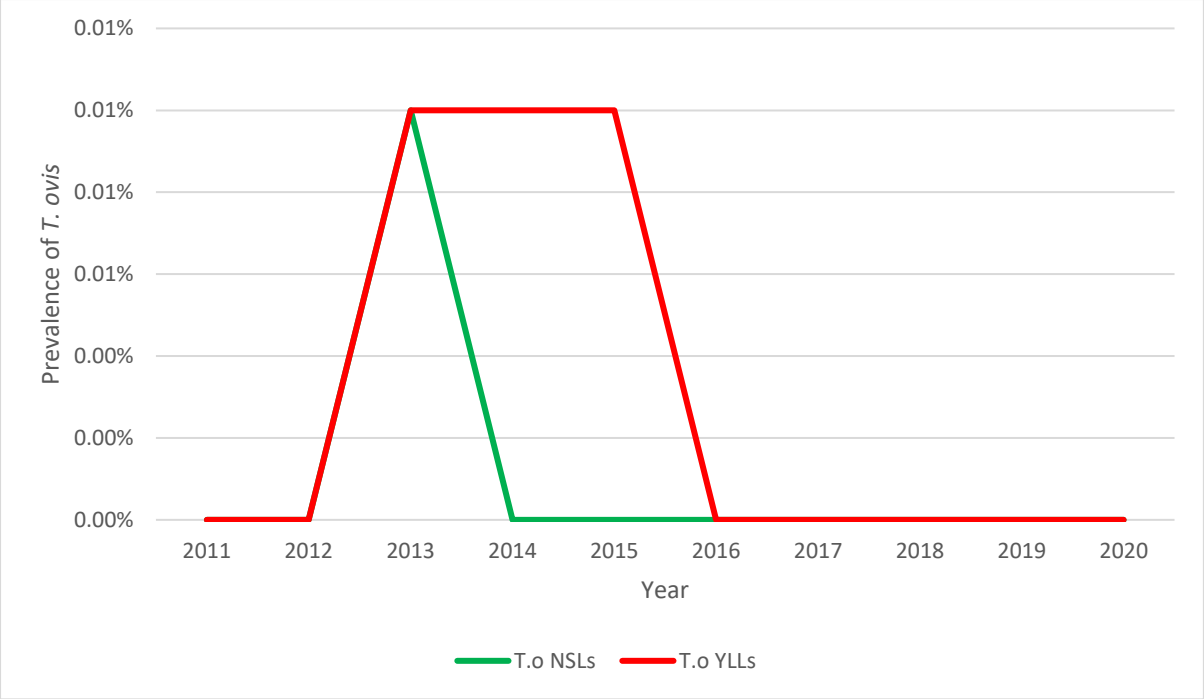


Figure 60: Prevalence of *T. ovis* in new season lambs and yearling lambs on farm EF22 between 2011-2020.

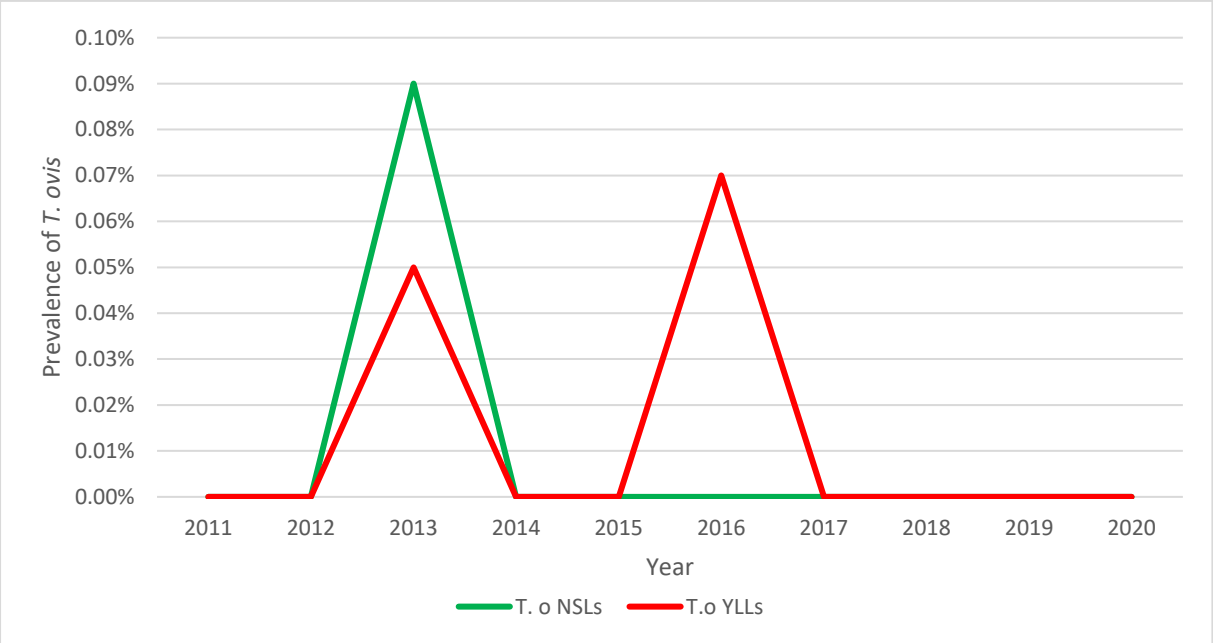


Figure 61: Prevalence of T. ovis in new season lambs and yearling lambs on the EF Island farm between 2011-2020.

This peak was also seen in the two farms sending just YLLs regularly to the abattoir over the data period. With large increase in prevalence in 2013 and subsequent years (figure 62, 63).

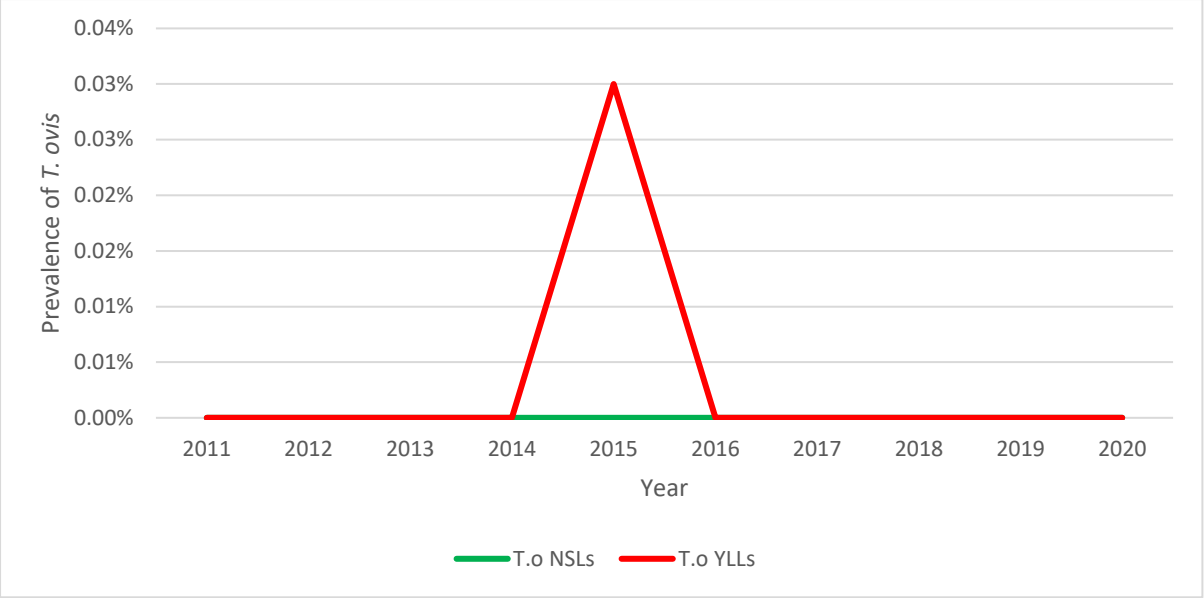


Figure 62: Prevalence of *T. ovis* in new season lambs and yearling lambs on the farm EF9 between 2011-2020.

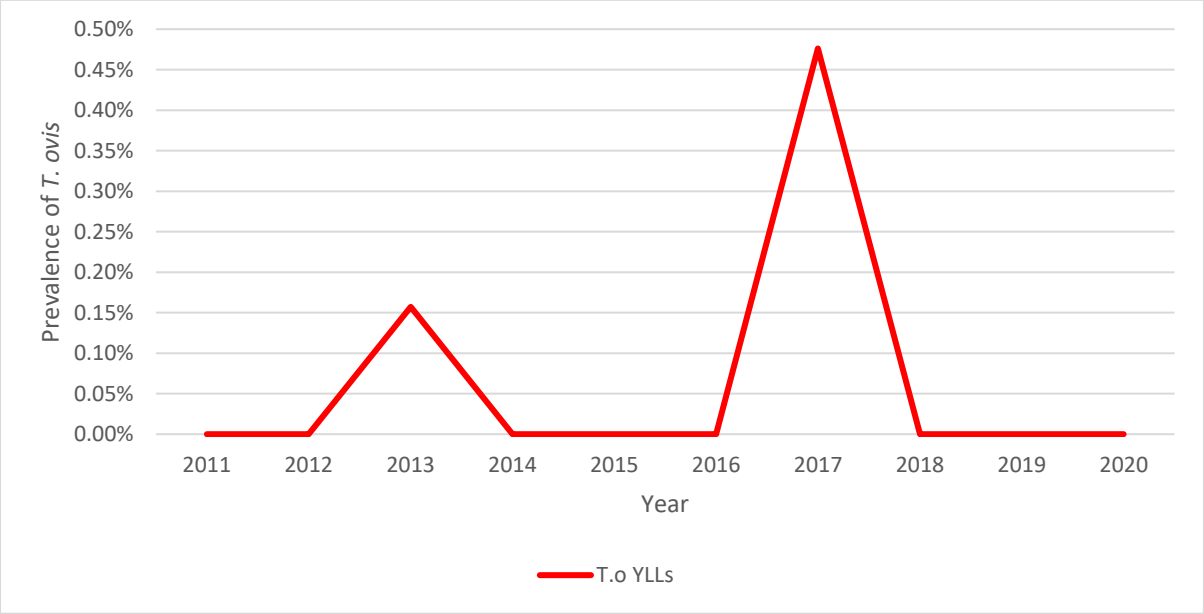


Figure 63: Prevalence of *T. ovis* in yearling lambs only on the farm EF14 between 2011-2020.

Each of these farms also had a major peak of infection in sheep in 2007. These data indicate sporadic infection of lambs simultaneously in six farms in 2013.

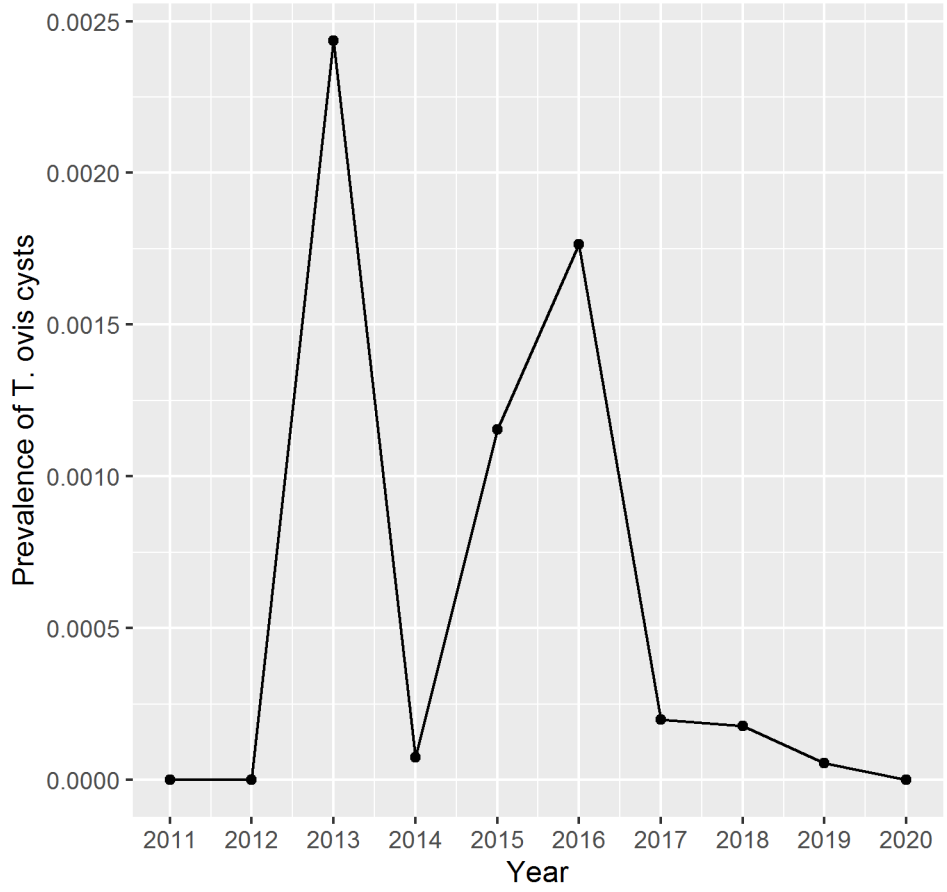


Figure 64: Annual Prevalence of *T. ovis* in adult sheep between 2011-2020

Taenia ovis was found in lower prevalences than *T. hydatigena*, remaining below 0.250% throughout the observation period in all sheep slaughtered. In adult sheep, zero cases were discovered in 2011 and 2012, however, there was a large increase in prevalence from 2012 to 2013, increasing to 0.244%. Prevalence immediately decreased to 0.007% before increasing once more to 0.116% in 2015 and 0.176% in 2016. In contrast to *T. hydatigena* in adult sheep, *T. ovis* decreased steadily from this point eventually reaching zero in 2020.

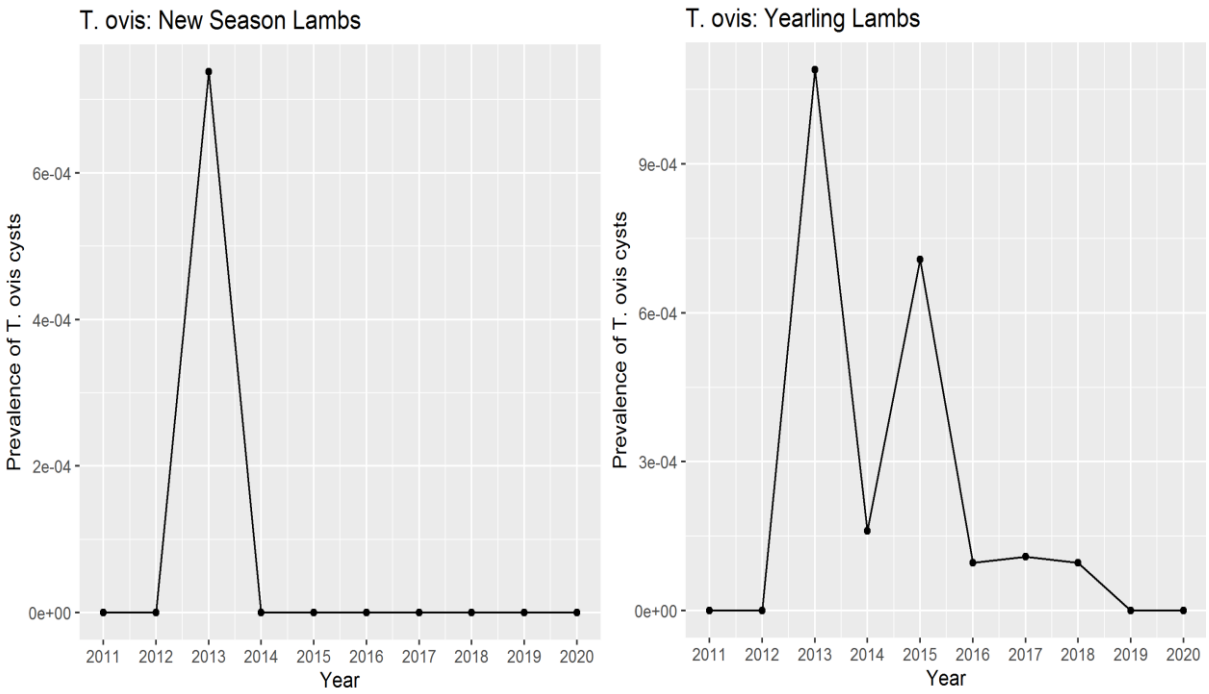


Figure 65: Annual Prevalence of *T. ovis* in new season and yearling Lambs between 2011-2020.

In NSLs, *T. ovis* was only discovered in 2013, with a prevalence of 0.074%. All other years no infections in NSLs. As with NSLs, no cases of *T. ovis* were discovered in YLLs until 2013, when the prevalence increased to 0.109%. The prevalence decreased to 0.016% before increasing again in 2015 to 0.071%. As with *T. ovis* in adult sheep, the prevalence declined in 2016 to 0.010%, and continued steadily down until 2020 where the prevalence was once again zero.

4.3.2.3 *Taenia hydatigena.*

Taenia hydatigena was identified in sheep at higher prevalences than *E. granulosus* and was present on many farms at some point. The mean prevalence between 2006-2020 was 2.30% (range 0.40-6.018%). As with *E. granulosus,* the prevalence of *T. hydatigena* appeared to be decreasing from 2006 to 2012, where there was then an increase in prevalence until 2019 when prevalence peaked at 6.018% of sheep slaughtered being infected with *T. hydatigena* (see figure 66).

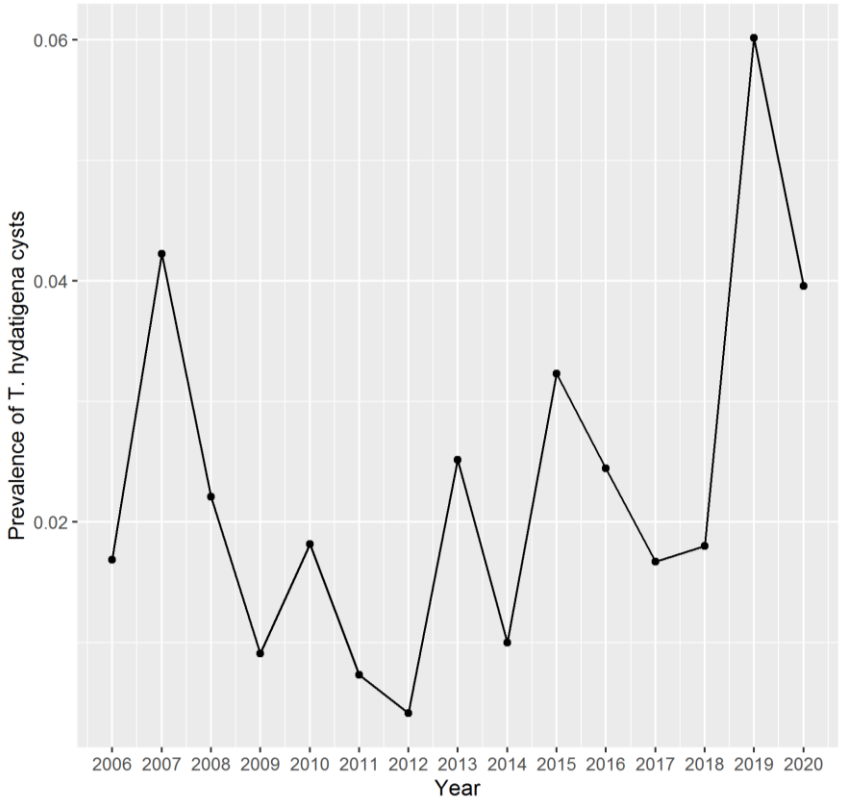


Figure 66: The annual prevalence of *T. hydatigena* in all sheep on the Falkland Islands slaughtered at the abattoir between 2006-2020.

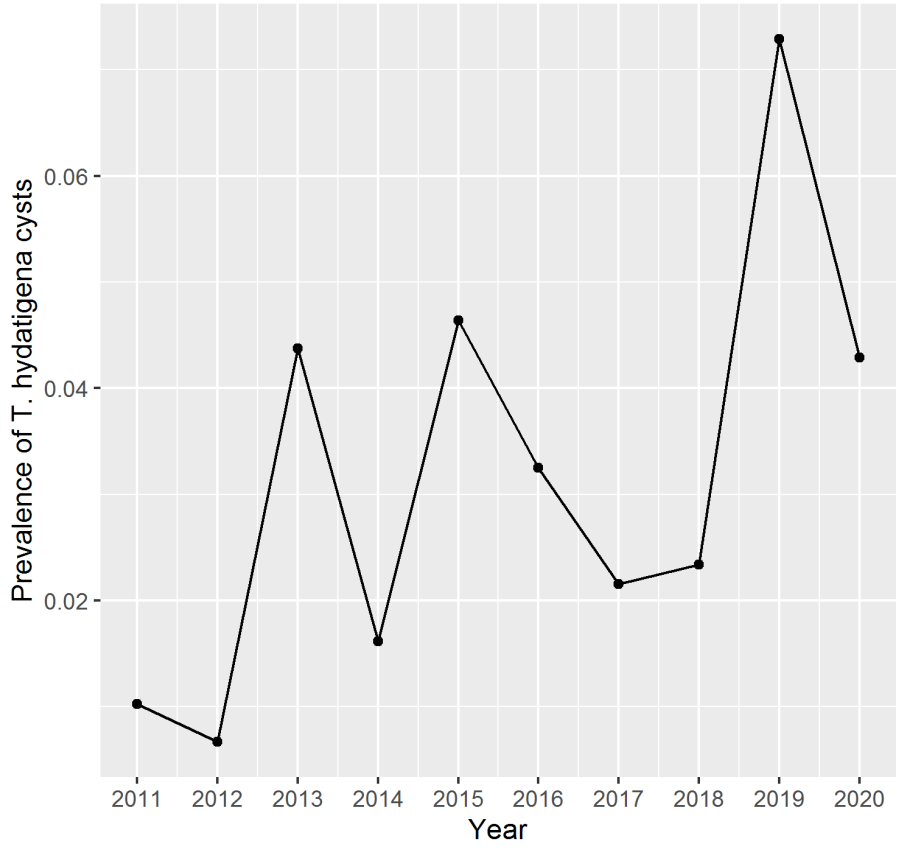


Figure 67: Annual prevalence of *T. hydatigena* in adult sheep from 2011-2020.

Most *T. hydatigena* infection was in adult sheep, with the temporal trend in prevalence in adult sheep has been increasing from 1.023% in 2011 to 7.290% in 2019 (figure 67), following the same trend as the data of all sheep over this period (figure 68). Adult sheep were found to have the highest prevalence of *T. hydatigena* infection with a mean prevalence of 1.447%.

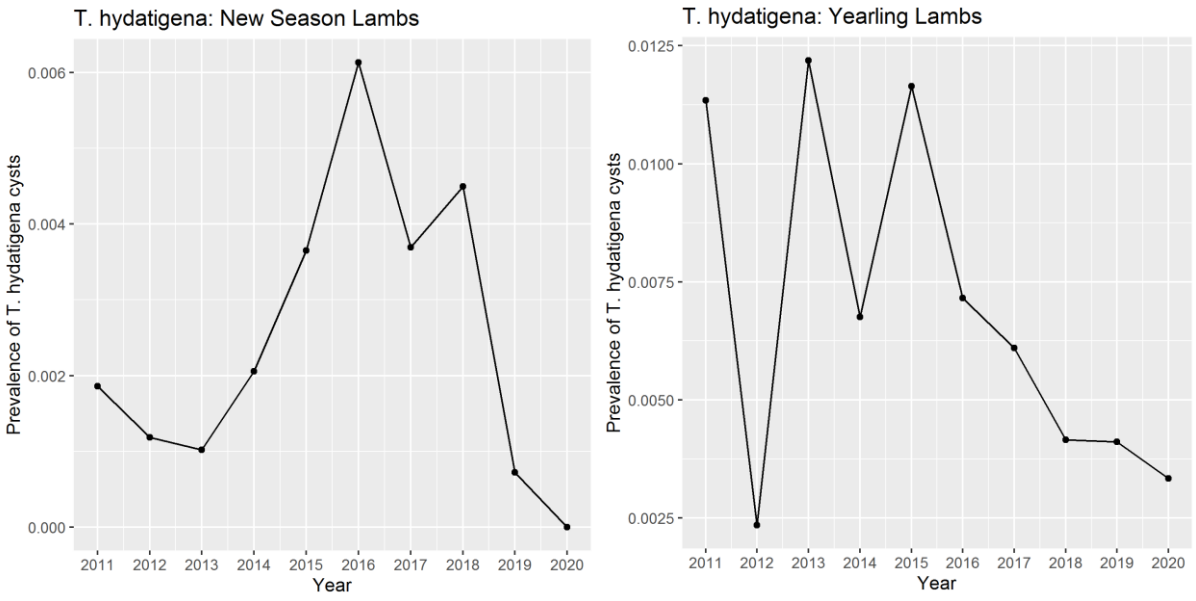
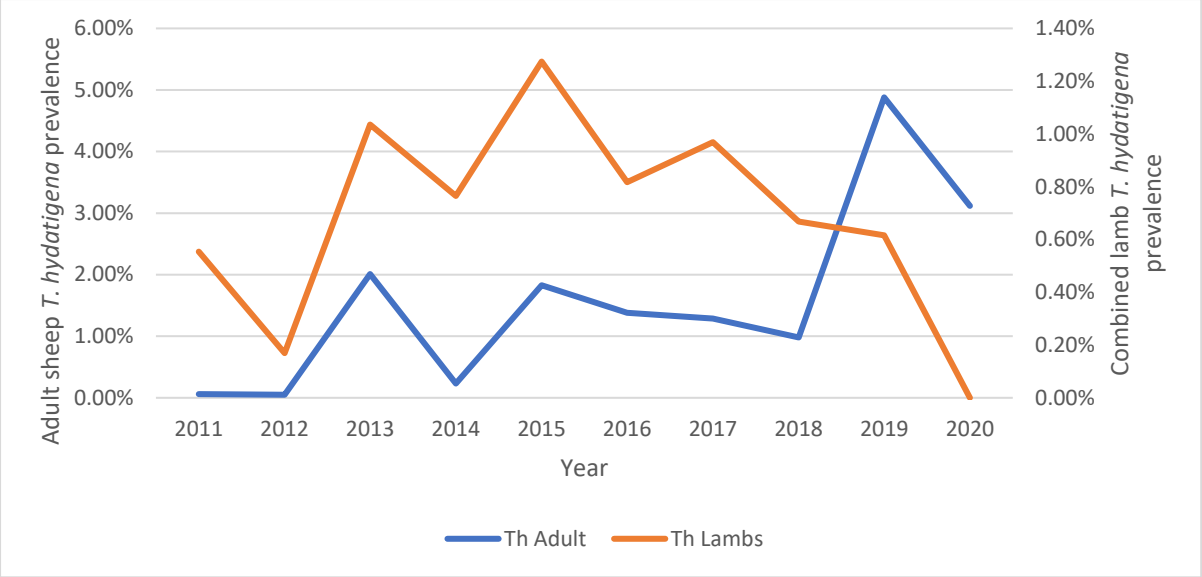


Figure 68: Annual prevalence of *T. hydatigena* in new season (left) and yearling (right) Lambs from 2011-2020.

As seen in figure 68, the prevalence of *T. hydatigena* in NSLs and YLLs decreases from 2011 (0.18% NSLs and 1.1% YLLS) to 2012, reaching as low as 0.1%, but from 2013 there was a large increase in prevalence over the following three years, peaking in 2016 at 0.61%. The prevalence in YLLs fluctuated over these years between 0.2% and 1.2% (Figure 68). In both NSLs and YLLss, the prevalence generally decreases following the peak prevalence in 2015-2016to a prevalence in 2020 of zero amongst NSLs and 0.3% amongst YLLs.

In relation to prevalence levels across all farms, figure 69 and 70 shows example of where these peak and troughs can be seen at farm level.



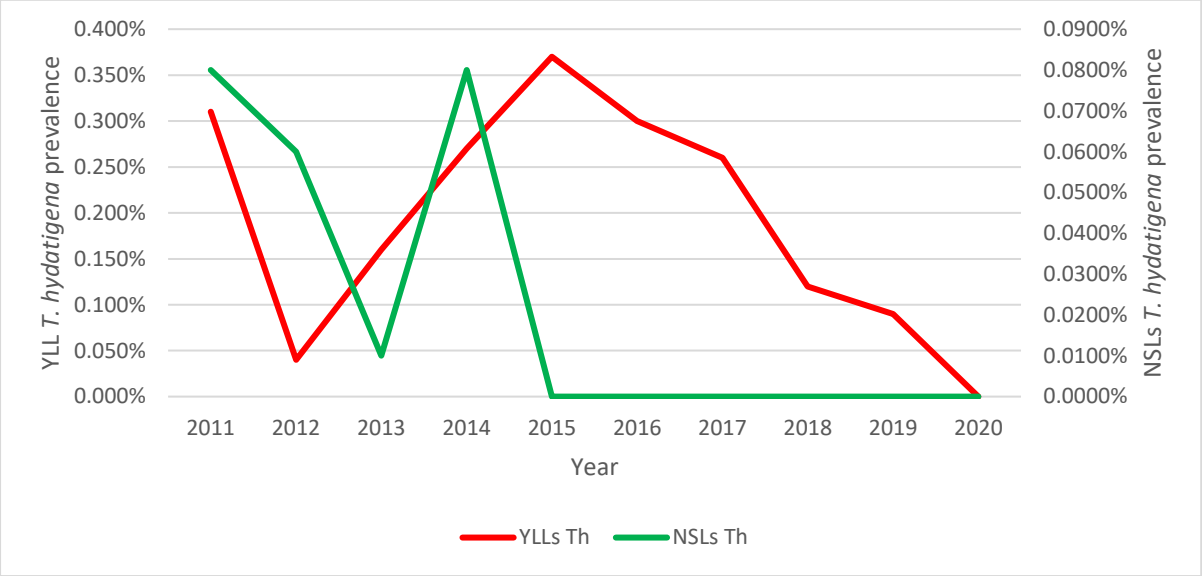


Figure 69: Farm EF22's *T. hydatigena* prevalence in NSLs and YLLs and the same prevalence compared between adult sheep and lambs annually.

In NSLs, prevalence of *T. hydatigena* peaks in 2014 at 0.10%. In YLLs, a similar peak is observed a year later in 2015 at 0.370%. Prevalence in NSLs decreased quickly in 2015 whereas the decrease in YLLs was a steadier decline over a five-year period. A similar peak and time lag was observed in combined lamb prevalence and adult sheep prevalence. The peak in lamb prevalence can be observed in 2013 (1.035%) and 2015 (1.274%), but compared to adult prevalence of *T. hydatigena,* there is a time lag before a peak is seen in adults in 2019 (4.88%).

Trends described above can also be seen on farms that consistently supplied sheep to the abattoir annually. Where peaks in NSL prevalence can be seen, a peak in YLLs can be observed the following years. Figure 70 shows a peak in *T. hydatigena* prevalence in NSLs in 2012

(0.40%), then in 2013 and 2014, prevalence in YLLs increases peaking at 0.090%. A similar time lag in prevalence can be observed from a peak in lamb prevalence (2013 and 2014) that is followed by a peak in adult prevalence in the following years.

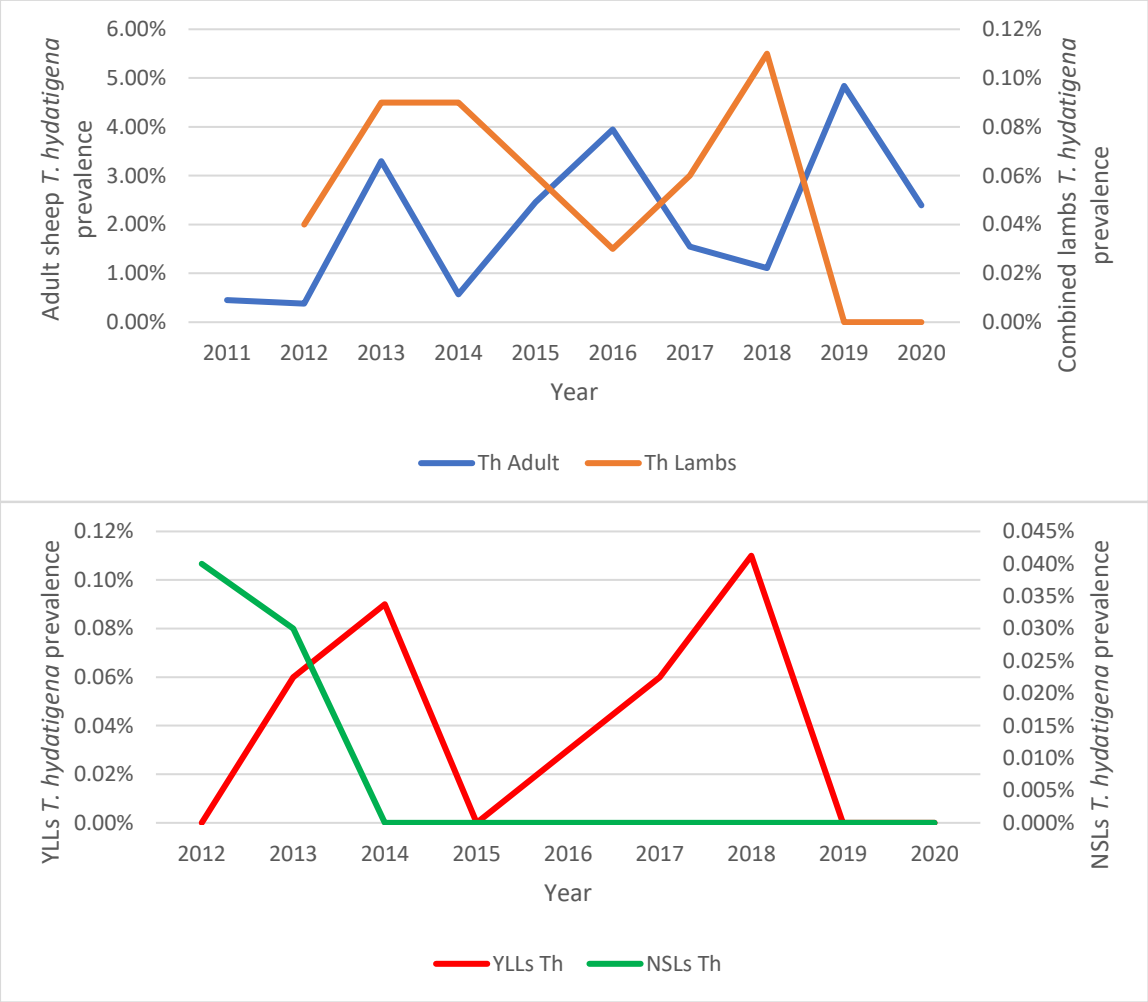


Figure 70: Farm WF14's *T. hydatigena* prevalence in NSLs and YLLs as well as adult sheep prevalence compared to combined lamb prevalence (no lambs were slaughtered in 2011 from this farm).

The farm used as an example in figure 69, this trend can be seen twice. With the described peak in lamb prevalence in 2013 and 2014 and adult prevalence peaking in 2016, but also when lamb prevalence peaks in 2018 (0.110%) and adult prevalence peaks in 2019 (4.840%) the following year.

4.3.2.4 Taeniid infections in Farms with Coproantigen positive dogs.

In 2010 and 2014 there were nine dogs on farms that have tested positive for copro-PCR (2010) and copro-ELISA (2014) (eight working dogs and one pet dog on farm). In 2010 there were five dogs on three farms that tested copro-PCR positive. One farm does not supply sheep to the abattoir, though the other two do. Farm EF22, which had three coproPCR positive dogs

in 2010, had cases of CE detected in adult sheep at slaughter in 2014, 2015, 2017 and 2018. The prevalence of *T. hydatigena* in this farm can be seen in figure 71. The farm had a high prevalence of *T. hydatigena* in 2011 in NSLs (0.8%) and YLLs (0.31%), before the prevalence decreased to 0.04% in YLLs in 2012 and 0.06% in NSLs in 2013. In adult sheep on the same farm, the year following the dogs testing positive, prevalence of *T. hydatigena* was 0.06%. This increased to 2.01% in 2013 before decreasing once again to 0.23% in 2014. There was also. In 2015 there was also an increase in *T. hydatigena* prevalence in YLLs as well as an increase in prevalence in adult sheep (0.21% increase from 2013 in YLLs and 1.6% increase in adult sheep from 2014).

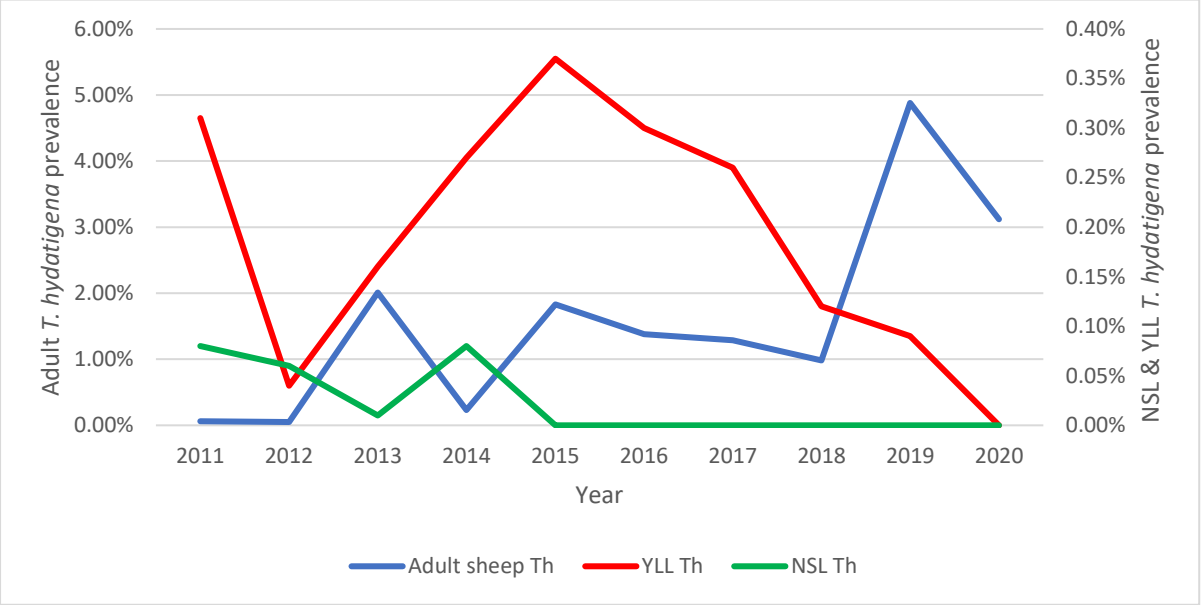


Figure 71: The annual prevalence of *T. hydatigena* in adult sheep, NSLs and YLLs on farm EF22 that had *E. granulosus* positive dogs in 2010.

Figure 72 shows the prevalence of *T. ovis* on the same farm which also increased after this time period in all age groups of sheep. There was a steep increase in prevalence from zero to 0.01% in NSLs and YLLs and 0.23% in adult sheep in 2013 before decreasing to zero once again in 2014 in NSLs and adult sheep, and to zero in 2016 in YLLs.

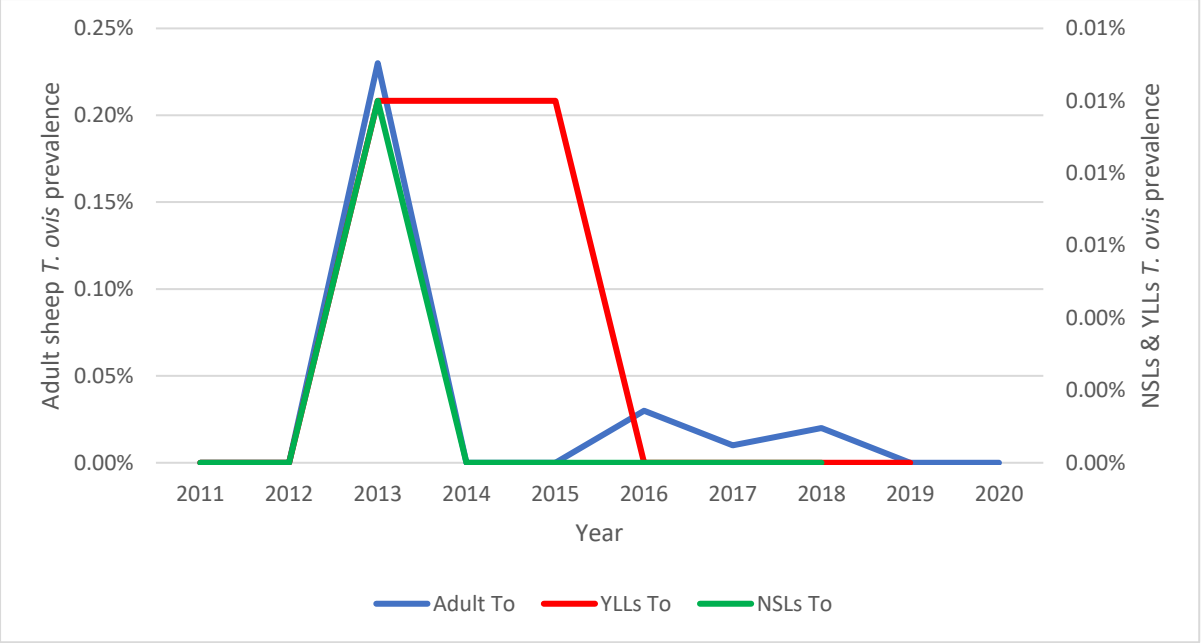


Figure 72: The annual prevalence of *T. ovis* in adult sheep NSLs and YLLs on farm EF22 with *E. granulosus* positive dogs in 2010.

The other farm (WF15) that had a positive dog in 2010 also had a case of CE in an adult sheep detected at slaughter in 2018. Although this farm only supplies adult sheep to the abattoir, similar trends in *T. hydatigena* prevalence to those observed in farm EF22 were seen after the positive dog in 2010 (see figure 73).

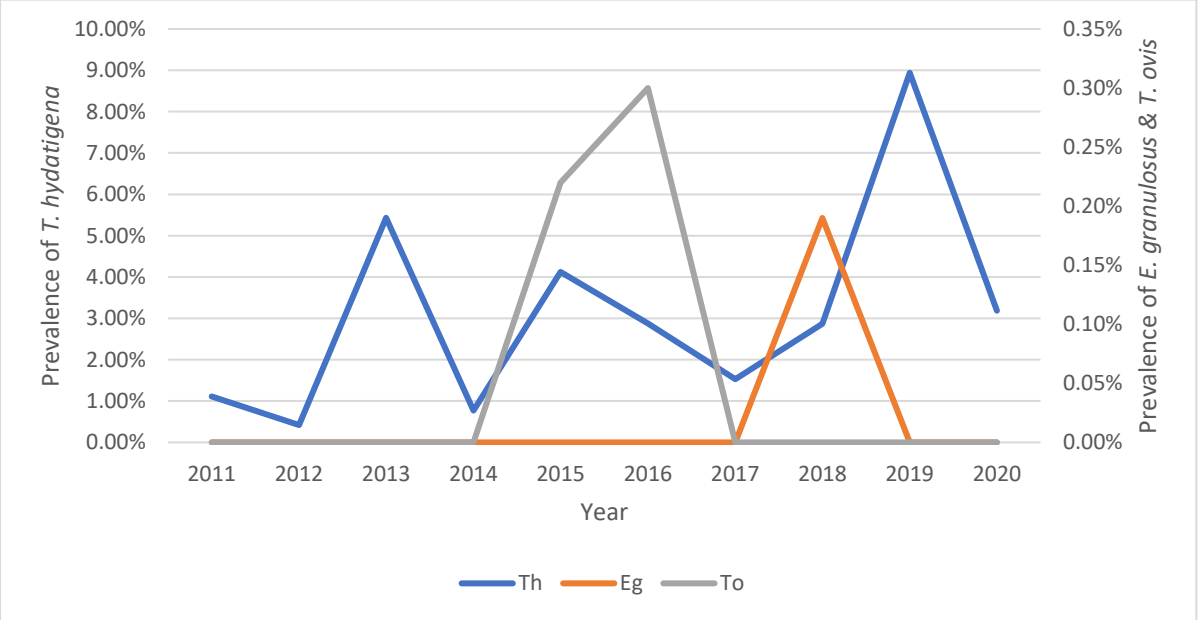


Figure 73: The annual prevalence of *T. hydatigena, E. granulosus* and *T. ovis* on farm WF15 with a positive dog in 2010.

On this farm the prevalence of *T. hydatigena* increased in 2013 to 5.430%, which was followed by an increase in *T. ovis* in 2015 and 2016 to a prevalence of 0.30%. As with the other farm with *E. granulosus* positive dogs, this farm also had a case of CE in an adult sheep in 2018.

In 2014, four dogs that live on farms tested copro-ELISA positive for *E. granulosus* (three working dogs and one pet dog owned by the farmer). One farm, (EF7) had two positive dogs, andand a case of CE in 2016 and 2018. Figure 74 shows the farm's *T. hydatigena* prevalence in all age groups of sheep.

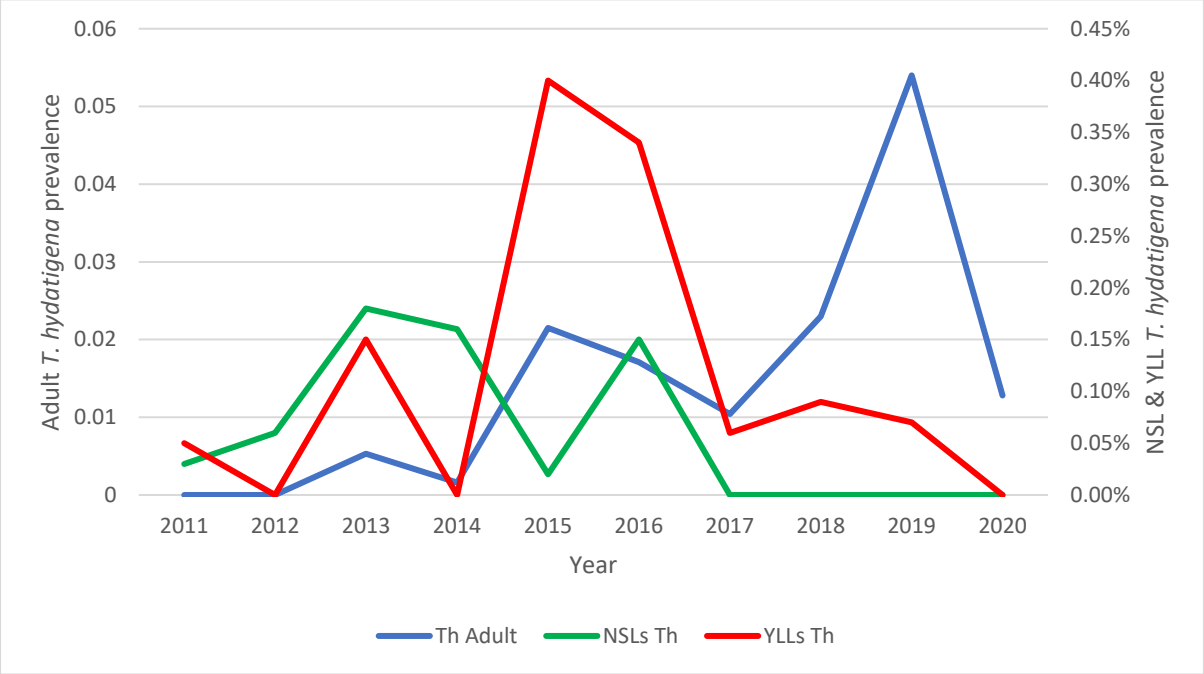


Figure 74: The annual prevalence of *T. hydatigena* in adult sheep, NSLs and YLLs on farm EF7 with copro-ELISA positive dogs in 2014.

The prevalence of *T. hydatigena* increased in both adult sheep and YLLs following 2014, from 0.160% to 2.150% in adult sheep and zero to 0.040% in YLLs. A similar increase was seen in the prevalence of *T. ovis*, increasing in both YLLs and adult sheep in 2015.

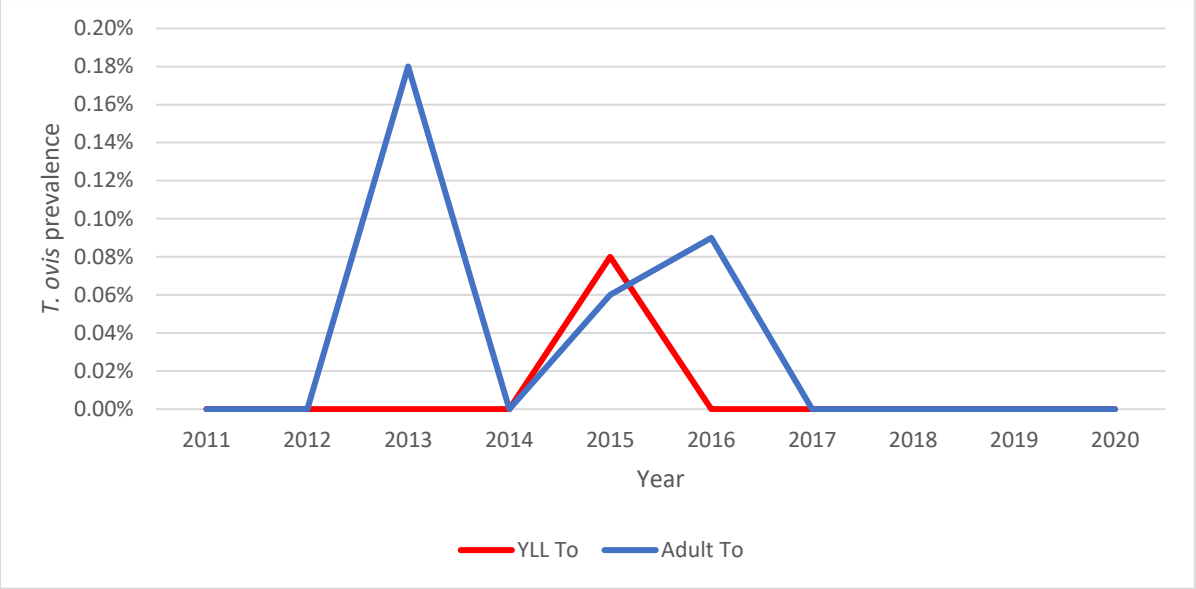


Figure 75: The annual prevalence of *T. ovis* in adult sheep and YLLs on Farm EF7 with copro-ELISA positive dogs in 2014

Figure 75 shows the increase in *T. ovis* in 2015 in YLLs from zero to 0.080% and zero to 0.090% in adult sheep. The other farm (EF1) with a working dog who tested positive had no cases of CE identified in subsequent years, although similar trends in both *T. hydatigena* and *T. ovis* prevalence were observed (figure 76).

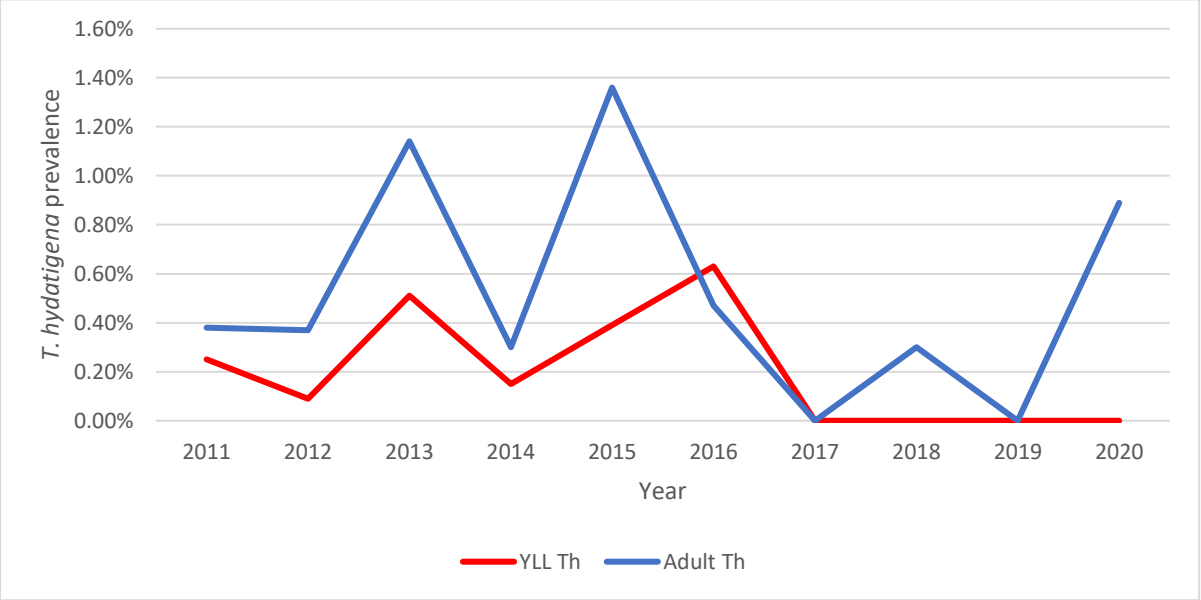


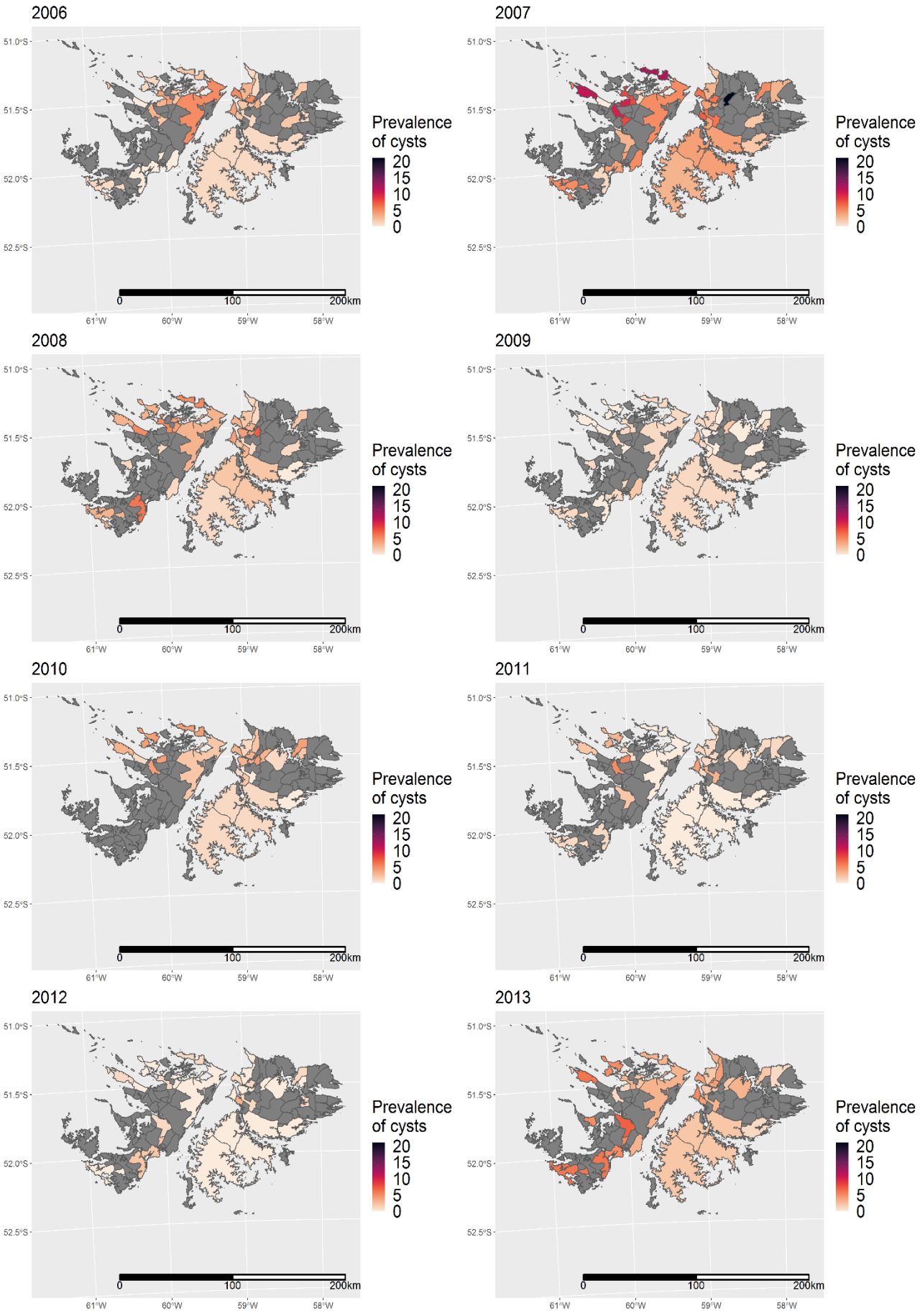
Figure 76: The annual prevalence of *T. hydatigena* in adult sheep and YLLs on farm EF1 with copro-ELISA positive dogs in 2014.

As with the previous farm, there is an increase in prevalence of *T. hydatigena* in 2015. Increasing to 0.390% in YLLs and 1.360% in adult sheep. The same farm had a small increase in *T. ovis* in adult sheep, rising from zero to 0.160% before returning to zero in 2017.

The final farm that had a positive pet dog is a small holding and only supplied sheep to the abattoir three times over the time period data was collected. The farm supplied NSLs in 2017 and 2018, and adult sheep in 2019. No cases of CE were identified in these sheep, but the prevalence of *T. hydatigena* was 1.04% and 1.45% in NSLs respectively and 9.92% in adult sheep.

4.3.2.5 *Taenia hydatigena* infection on a farm-by-farm basis.

The prevalence of infection varies between farms in a year-by-year basis (Figure 77). It is difficult to compare these farms annually however as not every farm supply sheep to the abattoir every year. In 2006 a total of 41 farms with *T. hydatigena* were focused on the northern parts of both East and west Falkland. By 2012 the number of farms had dropped to 34 farms largely in the south of West Falkland and the northwest of East Falkland. In 2019 there is a more extensive distribution of *T. hydatigena* across 46 farms in several areas of both East and West Falkland. Several of the farms in East Falkland are very large and therefore it is difficult to interpret exactly where infection may be acquired.


2006
2007
2008
2009
2010
2011
2012
2013
Prevalence of cysts
20
15
10
5
0
51.0°S
51.5°S
52.0°S
52.5°S
61°W
60°W
59°W
58°W
0
100
200km

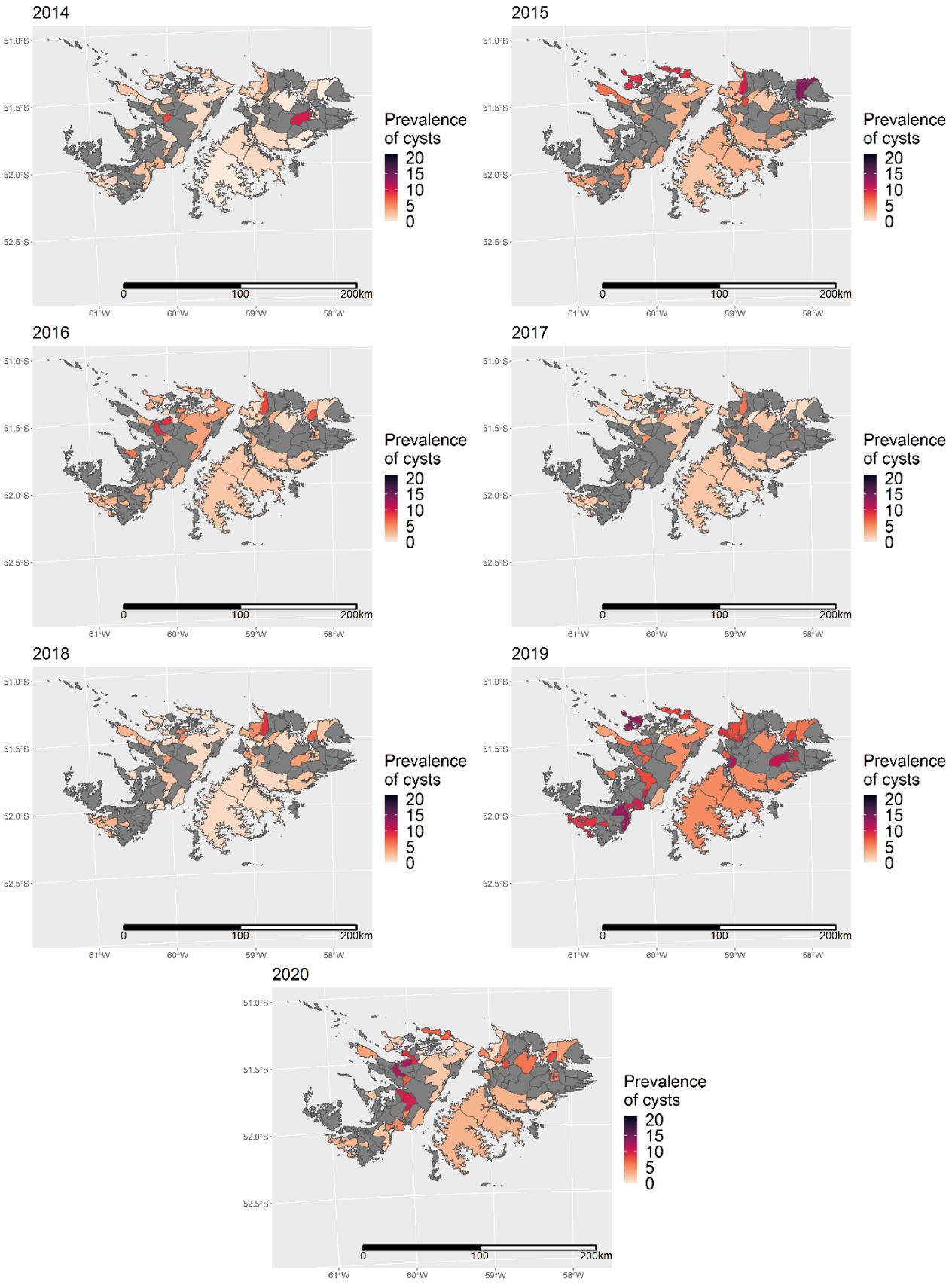


Figure 77: Annual farm prevalence of *T. hydatigena* between 2006-2020. Coloured areas represent the prevalence of *T. hydatigena* on farms that provided sheep to the abattoir. Grey areas are farms that do not supply sheep to the abattoir.

4.3.3 Temporal trends in the prevalence of *T. hydatigena* on farms in sheep and lambs

The relationship between the prevalence of *T. hydatigena* and the year was analysed to identify any changes in prevalence between 2011 and 2020. This analysis was repeated for both adult sheep and lambs and for each individual farm. To account for multiple comparisons, the p-value for significance was set using the Bonferroni correction as $(0.05 \div 68 = 0.000735)$ in adult sheep and $(0.05 \div 40 = 0.00125)$. For adult sheep, a total of 48 farms had a positive correlation coefficient (coefficient estimate range=0.0167-1), indicating an increase in prevalence over time. However, none of these associations were significant (Bonferroni corrected p value=0.0007). A total of 18 farms had a negative correlation coefficient association, though none of which were significant.

For lambs, a total of 17 farms had a positive correlation coefficient (coefficient estimate range=0.0317-1), however none of these correlations were significant (Bonferroni corrected p value=0.001). A total of 23 farms had a negative correlation coefficient (coefficient estimate range=-0.0325-1), once again, none of these associations were significant.

4.3.4 Correlation of taeniid parasites in sheep and lambs.

Correlation coefficients were estimated in order to identify whether the prevalences of each of the three taeniid species found in sheep in the Falkland Islands on individual farms in sheep of different ages were related to each other. As *E. granulosus* has only been discovered in adult sheep over the time period, only adult sheep were analysed for its correlations with the other two parasites.

The prevalence of *Echinococcus granulosus* and *T. hydatigena* had a significant positive correlation (Correlation estimate= 0.102, p=0.026) in adult sheep. A weaker, nonsignificant, positive correlation was observed between *E. granulosus* and *T. ovis* (Correlation estimate= 0.062, p=0.179). A stronger, highly significant correlation was observed between *T. hydatigena* and *T. ovis* (Correlation estimate= 0.164, p= <0.001). This was also observed amongst YLLs (Correlation estimate= 0.218, p=0.001), but not amongst NSLs (Correlation estimate= 0.045, p=0.496).

4.3.4 Factors affecting *T. hydatigena* infection in sheep

Generalised linear mixed-effect models were used to investigate key risk factors that influence *T. hydatigena* infections on farms. Initial analysis identified that these models should include farm as a random effect and that the effect of the number of animals in the lot varied for the different age groups (see table 6). It was decided to stratify the data by age rather than include interaction terms in a single model.

A number of different models were applied to the stratified data and were compared using AICc. Table 9 shows these secondary models and the new parameters used to establish key risk factors of *T. hydatigena* infection

Table 9: Secondary models in order of lowest AICc score for Sheep, NSLamb and YLLamb age classes and new variables included in each model.

Model	Farm-Random Effect	Dogs on farm Y/N	Number of Dogs	Dog per head of sheep	Dogs per m2	Log(Dogs per head of sheep)	Log(Dog per M2)	Sqrt(dogs per head of sheep)	Sqrt(Dogs per m2)	Total Slaughtered	AICc	Delta AICc
Sheep												
m8a								■		■	181.57	0
m5a				■						■	181.58	0.01
m7a						■				■	181.65	0.08
m6a					■					■	187.04	5.47
m4a		■								■	187.68	6.11
m3a			■							■	188.34	6.77
m2a										■	212.49	30.92
m9a							■			■	244.38	62.82
m10a									■	■	248.08	66.52
m1a	■										285.34	103.77
NSLambs												
m5b				■						■	206.01	0
m6b					■					■	206.07	0.06
m7b						■				■	206.28	0.27
m8b								■		■	208.2	2.19
m3b			■							■	213.43	7.41
m4b		■								■	213.43	7.41
m9b							■			■	243.76	37.75
m2b										■	244.04	38.02
m10b									■	■	245.26	39.24
m1b	■										281.39	75.38
YLLambs												
m8c								■		■	188.41	0
m7c						■				■	189.15	0.75
m5c				■						■	189.58	1.17
m4c		■								■	191.34	2.93
m6c					■					■	194.1	5.7
m3c			■							■	194.36	5.95
m2c										■	210.19	21.79
m10c									■	■	243.25	54.84
m9c							■			■	244.17	55.76
m1c	■										262.04	73.63

In all age categories, the number of dogs per head of sheep and total sheep slaughtered were the variables that when included in models were considered the best models based off lowest AICc score. In order to improve the confidence intervals (CIs) of the models, some of the variables were modified to use their log or square root. From the AICc ranking, the best model was the square root of the dogs per head of sheep and total slaughtered for adult sheep and YLLs, and was the third best model for NSLs, with a delta AIC of 2.19, making it suitable to be used. It was established that this model ranked highest across all age categories, best representing the data. Table 10 shows the regression analysis results for each age class of adult sheep NSLambs and YLLambs.

Table 10: Results from logistic regression identifying the key risk factors associated with *T. hydatigena* infections in adult sheep, NSLambs and YLLambs.

Parameter	Infected	Uninfected	Odds Ratio	2.5-95% CI	*P*-value
Sheep	**427**	**44**			
Sqrt(Dogs per head of sheep)			0.70	3.72	0.671
Total sheep slaughtered			1.01	1.02	1.48E-07
NSLambs	**70**	**157**			
Sqrt(Dogs per head of sheep)			4.03	31.88	0.15251
Total sheep slaughtered			1.00	1.00	0.00142
YLLambs	**177**	**81**			
Sqrt(Dogs per head of sheep)			5.13	25.52	0.040473
Total sheep slaughtered			1.01	1.01	1.87E-05

Table 10 shows that the total number of sheep slaughtered is significantly associated with an increased risk of *T. hydatigena* infection in all age classes ($p<0.05$), as would be expected (increasing the number of sheep slaughtered by a farm increases the chance of discovering sheep infected with *T. hydatigena*). Although there was no significant effect of the dogs per head of sheep term for adults or NSLambs, this was a significant predictor of infection amongst YLLambs (OR 5.13; $p=0.04$). Although exact interpretation is difficult due to the transformation, this shows that yearling lambs from farms with higher numbers of dogs per head of sheep were more likely to be found to be infected with *T. hydatigena* at slaughter.

4.4 Discussion

As described in Chapter 3, there is clear evidence that *E. granulosus* has been circulating in dogs in the Falkland Islands in recent years, albeit at a low level. The focus of this chapter is the presence of *E. granulosus* in intermediate hosts – in particular, sheep. Although detection of infection is more challenging in living intermediate hosts, the fact that many of these animals are slaughtered in an abattoir and inspected for infection by trained inspectors, means that data are readily available for inspection and analysis. There remains clear evidence that sheep in the Falkland Islands continue to be infected with *E. granulosus*, though as with dogs, at a low level (Figure 54). The finding that this infection is spread across a number of different farms (Figures 55 and 56) suggests that this is a widespread issue. Although levels of infection were too low to undertake robust statistical analysis, the suggestion of an increase in prevalence – at both the individual animal and the farm level – in recent years is worthy of further investigation. Of the three farms with confirmed *E. granulosus* in their dogs in 2010, two were found to subsequently have hydatid cysts in their sheep, with the status of the remaining farm being unknown as they did not sent sheep to the abattoir in subsequent years. Similarly, one of the three farms with infected dogs in 2014 subsequently had cases of CE identified in sheep. These results strongly suggest that *E. granulosus* is being spread between dogs and sheep, despite the current intensive control schemes in place. The lack of detection of hydatid cysts in all these farms may reflect a failure of detection in the abattoir, a failure to send infected animals to the abattoir, or a lack of transmission resulting from the relatively low biotic potential of *E. granulosus* in comparison with other taeniid cestodes. These issues are therefore explored further here.

On average, over 43,000 sheep are processed annually at the Sand Bay abattoir, which accounts for approximately 9% of the sheep population. These animals have been supplied to the abattoir from 86 farms over the period where abattoir data has been collected, with 48 farms on average supplying sheep annually. All carcasses are inspected, and records made of any disease or ailment present before the carcass is processed for sale. The focus of this project is primarily on the taeniid infection *E. granulosus, T. hydatigena* and *T. ovis.* Though all are endemic to the Falkland Islands, *E. granulosus* and *T. ovis* are found in very low prevalence, which makes robust data analysis difficult. *Taenia hydatigena* on the other hand is more prevalent, being identified in over 2% of sheep slaughtered annually. Due to their

similarities in life cycle, using the prevalence data for *T. hydatigena* allows for more meaningful analysis, acting as a proxy for *E. granulosus* (Miran et al., 2017). *Taenia hydatigena* also has a higher biotic potential, which explains its higher prevalence, but also results in greater proliferation of eggs, which can be reflected in the prevalence data, making trends more profound (Phythian et al., 2018b; Torgerson & Heath, 2003). This approach was supported by correlation analysis between these parasites, as there is a positive association between *E. granulosus* and *T. hydatigena* ($p=0.026$) in adult sheep in the Falklands. *Taenia ovis* was decided to not be a good option, as it also is found in low prevelences (though slightly higher than *E. granulosus*) and only a weak nonsignificant positive association with *E. granulosus*.

The presence of *T. hydatigena* in sheep indicates that dogs have had access to infective material at some point and have not been successfully treated with praziquantel for some time. Over the observation period, the prevalence of *T. hydatigena* fluctuated in all age categories of sheep slaughtered. The lowest prevalence was found in NSLs and the highest was found in adult sheep, which is to be expected as the probability of infection of taeniid parasites increases with age (Torgerson et al., 1998). Though in lower prevalences in lambs, these infections provide a more accurate timeframe of when infection occurred, and when sheep are most likely to have grazed on contaminated pasture because lambs would be uninfected at birth and become infected within their lifetime. *Taenia hydatigena* infection in lambs began to increase from 2012, gradually in NSL and more abruptly in YLLs. The difference in this increase is likely be due to the length of time grazing on pasture contaminated with taeniid eggs. If the environment was contaminated around this period by an infected dog, it would be expected that the older animals, in this case YLLs, would have spent more time over the past year grazing compared to NSLs, which will have only grazed for a short period after being weaned from the mother. This increases the chance of becoming infected with viable cestode eggs, as well as allowing enough time for cysticerci to develop (Sweatman & Plummer, 1957; Torgerson et al., 1998). In adult sheep, the prevalence is reflective of the sheep population, but does not provide accurate evidence of when infections could have occurred.

To analyse the temporal changes of *T. hydatigena* in sheep and lambs, the monotonic association between prevalence each year was observed. This was to assess where continued

increase in prevalence could suggest farms where lack of control of the parasite may be occurring and to help focus measures to reduce transmission in those areas. A total of 48 farms had a slight positive correlation of increasing prevalence in adult sheep throughout the observed period of data, however none of these associations were significant. As the prevalence on farms is not growing continuously, it suggests that the causes of continued infection in taeniids is likely down to spontaneous infection events causing an increase in contamination of pasture with eggs, and their ability to survive for an extended period of time resulting in spikes in prevalence and then a slow decline (Thevenet et al., 2005). This is unlikely to be as a result of continued failure in control measures because if dogs were regularly becoming infected with taeniid tapeworm, eggs would be contaminating pasture at a much higher rate, which would result in the prevalence in sheep to continue to increase year on year. The same analysis in lambs showed no significant correlations on any farms, but did show two farms negative correlations in lambs, suggesting that lamb infections have continually been reducing on these farms. This analysis on lamb abattoir data is less accurate, as lambs are slaughtered less at the abattoir and some farms do not send lambs every year. This makes analysis more difficult due to the lack of data, as well as the lower prevalences of infections found in lambs.

Globally, there are several major risk factors associated with taeniid transmission, including dog ownership, home slaughter of livestock and feeding dogs offal (Ghatee, Nikaein, Taylor, Karamian, Alidadi, Kanannejad, Sehatpour, Zarei, & Pouladfar, 2020; Yuan et al., 2017). In the Falklands, the number of dogs owned, per head of sheep was positively associated with *T. hydatigena* infection in sheep. The ratio of dogs to sheep on farms was significantly associated with *T. hydatigena* infection in YLLs (p= 0.04). This suggests that the more dogs owned relative to the number of sheep, will increase the risk of infections of *T. hydatigena* and through the similarities of their life cycles, infections of *E. granulosus* infections in sheep are likely to increase as well. The number of animals slaughters was significantly associated in all age categories, which was most significant in adult sheep (p<0.001). This is expected as adult sheep are most likely age category to be infected with taeniid metacestodes (Torgerson et al., 1998), and therefore increasing the number slaughtered at the abattoir and inspected by MHIs increases the likelihood of discovering infections. As seen in chapter two, a questionnaire survey was carried out with questions relating to potential risk factors. This was

not used in this analysis as the response rate was low among farms that supplied animals to the abattoir, with only 13 farms who responded regularly sending sheep to the abattoir regularly. Therefore, running analysis on only these farms would introduce significant bias to the results of risk factor analysis.

The prevalence of *E. granulosus* across the Falkland Islands was low throughout the period where data was analysed, increasing slightly towards the end of this study. This was also the case for *T. ovis,* however there were spikes in prevalence in 2013 and 2015, while *T. hydatigena* remained at a higher prevalence over the same period, fluctuating year on year as well as between farms. Infections in all sheep slaughtered followed a similar trend of higher prevalences in 2006/7 followed by decreasing in prevalence in the early years of the observation period. The prevalenceof *T. ovis* and *T. hydatigena* increase sharply in 2013 in both age categories of lambs, as well as in adult sheep, before decreasing after several years. This sudden increase and decrease in NSLs and YLLs could represent a spontaneous event such as the point at which an infected dog contaminated the environment, and then also represent the length of time that eggs were able to survive and remain viable, which is known to be over three years (Thevenet et al., 2005). This was especially evident on a collection of farms localised in East Falklands. On three farms, the prevalence in NSLs increased sharply to from zero to 0.29%, 0.01% and 0.09% respectively before decreasing once again. This suggests an influx of viable eggs from an infected dog contaminating the environment on which these lambs grazed at this point in time. This is further supported by a similar spike in YLLs at the same time and in subsequent years on the same farms and similar farms that only supplied YLLs to the abattoir. This is impossible to confirm as there was no regular testing of the dog population at the time and will never be known. The time lag of infection between NSLs, YLLs and adult sheep displays the importance of long-term analysis of abattoir data to monitor trends of prevalence and so focus control measures to target areas where prevalence is high or increasing, as well as monitor where disease is decreasing and analyse what is being done to encourage this.

Sudden increases in prevalence of taeniids have occurred in recent years, which is likely caused by the presence of positive dogs in the area. When the dog population was tested for *E. granulosus* in 2010 and 2014, a total of nine dogs across six farms tested positive. The prevalence of *T. hydatigena* and *T. ovis* on both farms in 2010 increase in the five years

following PCR confirmed positive dogs, and both farms had cases of CE during the period of time data was collected (2014, 2015, 2017, 2018 on one farm and 2018 on the other). Only one of the farms regularly sent lambs to the abattoir, and on that farm in 2011, *T. hydatigena* infections in YLL were 0.31%, which is high compared to other years. There was also a sharp increase in *T. hydatigena* and *T. ovis* in lambs as well as adult sheep in 2013. *Taenia ovis* prevalence decreased soon after, however *T. hydatigena* continued to increase in lambs reaching a peak in 2015 (0.37%) before decreasing steadily over the next five years. This increase and slow decrease could be as a result of the introduction of infective taeniid eggs into the environment, and then the residual effect of their prolonged survival, which is over three years (Sánchez Thevenet, Basualdo, & Alvarez, 2010; Thevenet et al., 2005). On farm WF15, that also had positive dogs in 2010, similar trends were seen, though as the farm rarely sends lambs to the abattoir, the trends were delayed due to the time lag in infection of intermediate host and identification of infection at the abattoir. There was an increase in prevalence in 2013, as with the previous farm, however, increases in *T. ovis* were observed in 2016, and a case of CE was discovered in 2018. This delay is likely to be as a result of the time lag in infections being discovered in older sheep, as the infections could have occurred when the sheep were lambs, but not discovered until the sheep was slaughtered at an older age. To observe spikes in other locations where taeniid infections are endemic is difficult due to parasites such as *E. granulosus* and *T. hydatigena* being discovered at a higher prevalence, meaning that peaks of infection are not as prominent due to the continued infection pressure on intermediate hosts (Torgerson et al., 1998), however in countries such as Ethiopia, the prevalence of *T. hydatigena* was significantly higher in older sheep (p<0.01), which was attributed to the longer time of exposure time to infective eggs by older animals, as well as the challenge in identifying cysts in younger animals that may not have had as much time to develop (Gessese et al., 2015). As described in chapter one, Tasmania is endemic for *E. granulosus,* and control programmes have been implemented to reduce the prevalence of taeniid infections, successfully achieving so. Prevalence in Tasmania has been shown to be below 1% in sheep similar to the Falkland Islands. In a study observing seven years of abattoir data (2007-2013) found that for the first three years of the study, no cases of CE were discovered in sheep, however in 2010, 12 cases (0.04%) of CE were discovered in Tasmanian sheep. For the following two years, the prevalence decreased to 0.02%, 0.01% and then returned to zero in 2013. This survey shows similar results to the data analysed in the Falkland

Islands, with a spike in infection of *E. granulosus* in adult sheep, before decreasing over the following few years. A similar spike was seen in cases of *T. ovis* at the same time, though in higher prevalences. Increasing from 2.8% in 2010 to 4.1% in 2011, before decreasing to 2.8% then 1.2% in 2013 (Phythian, Jackson, Bell, Citer, Barwell, & Windsor, 2018a). These results show similar spikes in infection as in the Falklands and are likely caused by the dispersal and ingestion of eggs from infected dogs. In 2014, 7.8% of dogs in Northern Tasmania were copro-PCR positive and 0.98% were copro-PCR positive for *E. granulosus*. These results show that continued transmission of *E. granulosus* is still occurring in Tasmania, despite control measures and is likely caused by the sporadic infection of dogs causing the observed spike in cases in sheep, which is enough to maintain *E. granulosus* at low levels on the island (Jenkins, Lievaart, Boufana, Lett, Bradshaw, & Armua-Fernandez, 2014). This is likely the case in the Falklands, with such low levels of infection in sheep caused by the sporadic infection and proliferation of eggs by a small number of dogs.

In 2014 in the Falklands, three working dogs from two farms tested copro-ELISA positive, with a small farm that had a pet dog test copro-ELISA positive as well. Farm EF7 regularly sends lambs and adult sheep to the abattoir and saw an increase in *T. hydatigena* in YLLs and adult sheep in 2015 and in YLLs in 2016. NSLs prevalence was higher in 2014 than in any other years following the presence of a positive dog, showing the potential immediate impact of an infective dog within the areas well as the residual effect described above. There was also an increase in *T. ovis* infection. In YLLs, the prevalence peaked in 2015, and in adults this prevalence peaked in 2016. As described, this farm had cases of CE in adult sheep in 2016 and 2018. Though there is no PCR confirmation of positive dogs due to samples being lost in transit, the increased prevalence, and cases of CE in sheep on this farm support the likelihood of an infected dog contaminating the pasture. The second farm, EF1, supplies less sheep to the abattoir, however there was still an increase in in prevalence in both YLLs and adult sheep in 2015. The third farm that had a copro-ELISA positive pet dog only provided sheep to the abattoir three times, once in 2017, 2018 and 2019. The first two years, 96 and 69 NSLs were slaughtered respectively, and both years had a *T. hydatigena* prevalence of 1.04% and 1.45%, which is very high for NSLs. In 2019, 121 adult sheep were slaughtered, and their prevalence was 9.92%, which again is very high. Though there is no link to infections from the year that there was an ELISA positive dog on the farm, the high prevalences suggest that there is regular

contamination of pasture on the farm. There were no cases of CE in sheep, however, the neighbouring farm that has no dogs, and hasn't for over ten years, had a case of CE in 2018. Due to the known dispersal of taeniid eggs over hundreds of metres, and some suggestions up to ten kilometres (Jansen, Dorny, Gabriël, Dermauw, Johansen, & Trevisan, 2021), it is possible that the infection on the neighbouring farm that has no dogs could be linked to the infected dog in 2014.

The three taeniid species that are found in sheep in the Falkland Islands share a definitive host. To look at correlations between these parasites and look at whether increases in one result in increases in another, potentially suggesting a single definitive host causing infection. In adult sheep, there was no significant correlation between *E. granulosus* and *T. ovis,* however, there was between *E. granulosus* and *T. hydatigena* ($p=0.026$) and a highly significant correlation between *T. hydatigena* and *T. ovis* ($p=<0.001$). The observations that prevalences of these parasites occur in peaks or spikes in indicative of "cysticercosis storms" which have been reported in other areas (Eichenberger, Karvountzis, Ziadinov, & Deplazes, 2011) where prevalences are generally low and protective immunity in sheep has not been established (Gemmell, Lawson, Roberts, & Griffin, 1990). Such outbreaks of *T. ovis* have also been linked to scavenging of sheep carcases by dogs (DeWolf et al., 2012). The correlation between both *E. granulosus* and *T. ovis* with *T. hydatigena* in adult sheep suggests the same definitive host occurring sporadically. As cats are poor hosts for *E. granulosus* (Konyaev et al., 2012) and *T. ovis* (Borji et al., 2011) it is likely that these would be dogs, as these are the only other known definitive host in the Falklands. No *E. granulosus* infections were discovered in lambs, though there was a positive correlation between *T. ovis* and *T. hydatigena* in YLLs ($p=0.001$), which again suggests the same definitive host. There was no correlation in NSLs which is likely due to the low levels of infection found in NSLs as they have spent the least amount of time grazing and so are less likely to have picked up infections. This co-infection with multiple taeniid species has been seen in other regions where these parasites are endemic. In Iran, *E. granulosus* was found at a prevalence of 10.8%, with 6.6% of those infected, also harbouring other taeniid parasites including *T. hydatigena* and *T. ovis* (Beiromvand, Rafiei, Razmjou, & Maraghi, 2018). In Tanzania, taeniid infection is common in sheep and goats slaughtered at abattoirs. In 2013, 71% of sheep and goats were infected with metacestodes of both *T. hydatigena* and *E. granulosus* in the Ngorogoro region, of these

11.7% had mixed infection of both these parasites. Definitive hosts are known to also be coinfected with such parasites, so contamination of grazing land by a single definitive host is possible. Intestinal parasite studies have found that coinfection of taeniid species in dogs as high as 5.6% (Beiromvand et al., 2018) in endemic regions which supports the evidence that dogs are the definitive host for multiple taeniid cestodes in the Falklands.

The slaughter process in the Falkland Islands is like many abattoirs and is aimed at being as efficient as possible. This does however create the possibility of missing diseases. The separation of the stomach and intestines down a separate pathway to the carcass means that if there is *T. hydatigena* cysts attached to these organs, which they commonly are, they could be missed. This can lead to under-reporting of infection and reduce the accuracy of the data. Though not a disease that will result in the carcass being condemned, or a risk to human health, in the face of a control programme, accurate data on all cestode parasites is vital in understanding their continued transmission. It has been mentioned by the trained professionals on the line that these diseases could be missed as they are not being looked for on every carcass, but if the inspector has time, they will also inspect the stomach and intestines on the secondary shoot. Another concern with this, is that the stomach and intestines are then deposited out to sea. If *T. hydatigena* infections have been missed, they then could potentially wash up on a beach nearby where dogs could gain access to them. This creates a major risk of transmission, and potentially could explain why in 2010, two pet dogs in Stanley were copro-PCR positive for *E. granulosus* and in 2014, two more pet dogs in Stanley were copro-ELISA positive. An improved inspection system of multiple trained individuals inspecting or allowing for both the pluck and stomach and intestines to be inspected properly would ensure that no infective material is missed, improving the accuracy of the data, and preventing infective material reaching the sea and potentially definitive hosts.

To gain a further insight into the true prevalence of taeniid parasites in the Falklands, improved surveillance in all settings where animals are slaughtered is vital. Home slaughter regularly occurs in the Falklands; however the majority send sheep to the abattoir and only slaughter enough required for home consumption, dog meat and culling. In 2020, 20,350 sheep were slaughtered on farms, which accounts for 4% of the sheep population, however, this accounts for approximately 47% of the sheep slaughtered annually. In this project, the

impact of home slaughter was not analysed due to lack of information on the numbers of animals slaughtered on individual farms, with only limited questionnaire response (see chapter 2) providing information on a small number of farms, this information couldn't be used in this analysis it would introduce bias only analysing the farms that completed the questionnaire. This is an important area of investigation moving forward, as animals slaughtered on farms are not inspected by trained staff, and no data is collected on the disease status of these individuals. This means that the prevalence estimates created from the abattoir data is likely underestimating the true prevalence of taeniid infections in the Falklands. Also, there are farms that do not supply animals to the abattoir, and only slaughter animals for use on their farm. These animals are again not inspected and there is no disease data collected, therefore not representing the infection status of these farms and animals. This is important information that, if possible, to collect disease data on home slaughtered animals would help support control measures and provide a wider understanding of prevalence data on farms that don't supply animals to the abattoir. This is also a likely source of infective material as animals killed for dog meat or culling purposes are older animals, most likely to be carrying taeniid infections (Torgerson et al., 1998). Gaining an understanding of the true prevalence of taeniid infections in the Falklands by inspecting sheep slaughtered on farms is important in improving surveillance on farms as well as the abattoir to furthering the efforts of the control programme.

A major difficulty that had to be overcome in this project was the way in which data has been collected and stored over the years. When collating the disease summary reports into a centralised database, it became clear there were discrepancies in the recording of CE cases. When a suspected hydatid cyst is discovered at the abattoir, it is sent to the laboratory at the Department of Agriculture for microscopy. Once confirmed, it is recorded in a separate data set and the farmer from which the sheep came is contacted, however the positive diagnosis is not always reported in the disease summary report. To ensure the accuracy of the final data base, the author had to search through the data to find where CE was and wasn't reported in the disease summary reports and add it from the secondary data base from the Department of Agriculture. The lack of a centralised data base in a program with statistical analysis capabilities means that the data is unusable for analysis. The created dataset can be built upon to continue the work carried out in this chapter and support the control programme.

Control of hydatid disease in the Falkland Islands has reduced in a similar fashion to countries such as New Zealand, where successful control measures have reduced the prevalence significantly to where discovery of hydatid cysts is rare. However, unlike New Zealand, there are still cases annually, and the control programme is still focused on targeting the adult parasite rather than surveillance and monitoring of the sheep population. In New Zealand, from 1990 when cases were very unusual, the control programme developed into a surveillance and trace back system whereby any infections in sheep that were discovered were investigated in isolation, implementing restrictions on movement of animals, and monitoring of all animals within the age range of the infected sheep, with movement only allowed for animals going to slaughter. Dosing of dogs was localised on farms that had infections and the focus of the entire control programme looked at widespread surveillance and localised strict controls on infected farms (Pharo, 2002). The Falklands still enforces dog dosing across all farms and pet dogs, along with abattoir surveillance, with limited follow up of diagnosis at the abattoir. Like New Zealand, the continued surveillance at the abattoir is necessary to continue for many years to come, due to the survival of taeniid eggs in the environment as well as the age at which sheep are slaughtered continuing the life cycle for longer. Unlike New Zealand, the risk of re-infection from live animal imports is acknowledged, the Falkland Islands do not regularly import live animals and therefore can focus on the eradication on local farms and adopt a similar system of monitoring abattoir data, as well as using localised control measures when infections are discovered. These control measures will be in place for the foreseeable future, to prevent the rise in infections should a dog become infected once more. This was the case in Cyprus, after the control programme was stopped in Northern Cyprus after five years, cases began to increase sporadically across the islands and the control measures had to be re-introduced years later (Economides & Christofi, 2000; Economides et al., 1998). With the continued monitoring at the abattoir, and improved on farm monitoring at home slaughter, further steps towards eradication of taeniid tapeworm can become a reality.

Chapter 5. Environmental Contamination with Taeniid Eggs

5.1 Introduction

The Falkland Islands is a lowly populated area comprising two main islands, East and West Falkland and 776 small islands with an overall area of 4,700 square miles. The highest point is Mount Usborne (705m or 2,313 ft) whilst the remainder on the topography consists chiefly of low undulating ground, a mixture of bare rock, pasture and peat bog, with many shallow freshwater tarns, and small streams running in the valleys. There are no native tree species, although multiple species of bushes can be found, such as European Gorse (*Ulex europaeus*) and Diddle-dee (*Empetrum rubrum*). The grazing area for sheep on each farm is extensive and the potential area for exposure to parasite eggs is therefore very large. The potential for infection will depend on the presence of eggs on pasture, their ability to survive in the environment and how they are dispersed from original deposition sites.

The biotic potential of taeniid cestodes is extremely high due to the number of proglottids which are produced and the numbers of eggs per proglottid. For instance, *Taenia hydatigena* has been estimated to release an average of two proglottids per day (equivalent to around 76,000 eggs per day), whilst *T. ovis* can release three proglottids per day (equivalent to produce 261,000 eggs per day) (Gemmell et al., 1987). Conversely, *Echinococcus* spp. is a much smaller worm releasing a single gravid proglottid. Estimates of egg productivity in these species are around 820 per proglottid, with gravid proglottids being released every 14 days (82 eggs per day) (Gemmell et al., 1986c; Mohammadzadeh, Sadjjadi, Rahimi, & Shams, 2012; Wachira, Macpherson, & Gathuma, 1991). However, worm burdens for *Echinococcus* spp. are normally in the hundreds or even thousands (mean = 202, Australia, New Zealand) meaning the one infected dog could potentially be releasing more than 12,000 eggs per day (Gemmell, 1990).

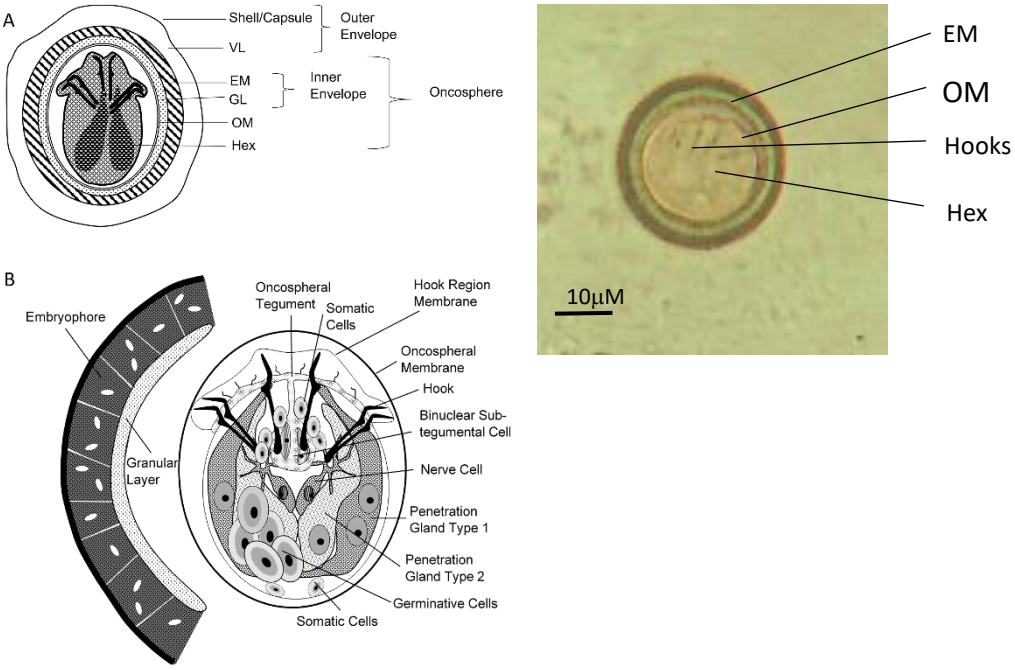


Figure 78: General description of the egg and oncosphere of Echinococcus spp. (A) Schematic diagram of an oncosphere illustrating the structure and bilateral symmetry in the pattern of hooks and cellular organization of the hexacanth embryo. VL: vitelline layer; EM: embryophore; GL: granular layer; OM: oncospheral membrane; Hex: hexacanth embryo. (B) Cellular organization of the oncosphere. Oncospheres are approximately 25 × 30 μM. (from Vuitton et al. (2020))

The eggs (figure 78) are approximately 30 μM in diameter and possess a thick embryophore composed of keratinised protein blocks. This is very resistant and enables the eggs to survive in the environment for long periods of time.

5.1.1 Egg survival in different environmental conditions.

The survival of taeniid eggs in the environment varies between species and different environmental conditions. A range of laboratory and field studies have been carried out to examine the effects of temperature and humidity on egg survival as judged either by *in vitro* hatching and activation assessment or infectivity in relation to experimental infections. In general eggs are more susceptible to low humidity (<34%) than temperature fluctuation (Jansen et al., 2021). However relatively short periods of exposure (two hours) to extremely high temperatures (60^{o}C+) can bring about quick killing of larvae (Federer, Armua-Fernandez, Hoby, Wenker, & Deplazes, 2015). Several studies with *E. multilocularis* eggs have shown that survival at low temperatures is high and that eggs can withstand freezing. Under laboratory conditions , (Veit, Bilger, Schad, Schäfer, Frank, & Lucius, 1995) showed a high sensitivity to

elevated temperatures and to desiccation. At 45 °C and 85–95% relative humidity, infectivity was lost after three hours as well as after four-hour exposure to 43 °C suspended in water. Exposure to 27% relative humidity at 25 °C as well as exposure to 15% relative humidity at 43 °C resulted in a total loss of infectivity within 48 and two hours, respectively. Temperatures of 4 °C and of –18 °C were well tolerated (478 days and 240 days survival, respectively), whereas exposure to –83 °C and to –196 °C quickly killed off the eggs (within 48 h and 20 h, respectively). Under natural conditions in south west Germany eggs of *E. multilocularis* showed good infectivity even after 252 days indicating long term survival in a climate where ground temperature extremes ranged from -7.5^{o}C to 40^{o}C (Veit et al., 1995). Similarly, good egg survival and hatching was observed up to 279 days in *T. hydatigena and T. ovis* eggs stored in water at 7^{o}C (Gemmell, 1977).

Studies on *E. granulosus* in Kenya have shown that eggs can lose viability within days at temperatures exceeding 30^{o}C in a hot arid climate, with eggs remaining viable for several weeks in cooler regions with temperatures of around 20^{o}C (Wachira et al., 1991). In contrast other studies in Patagonia, Argentina showed that *E. granulosus* eggs maintained in an arid climate were capable of infecting sheep 41 months later showing a much greater environmental persistence (Thevenet et al., 2005).

It is clear that in suitable climatic conditions with temperatures in the range of 7-25^{o}C taeniid eggs can potentially be infectious for years (Jansen et al., 2021). However, the micro-conditions relating to moisture and temperature variations may be important in determining the survival of individual eggs. The climate in the Falkland Islands is cold maritime: winter is cold and snowy, while summer is cool and windy, with significant rainfall. Relative humidity is high, and average daily temperature of the warmest months, January and February, is about 10 °C with highs around 14 °C while the average of the coldest months, namely June, July and August, is about 2 °C, with highs around 3/4 °C. The highest recorded temperature is 26 °C, while the lowest is -11 °C (climate-data.org, 2021). This climate fits within the parameters discussed above and is very likely to allow for the long-term survival of taeniid eggs in the environment. The size of the eggs and their high environmental persistence means that there is a high likelihood that they could be dispersed considerable distances from initial faecal deposition sites thus creating a wider area for potential infection of livestock.

5.1.2 Dispersal of eggs in the environment

When taeniid eggs are deposited in dog faeces, several factors can aid in their dispersal over a larger area (Sánchez Thevenet et al., 2019). Firstly, the shed proglottids are motile and can move some distance from the faecal mass. With the larger taeniid species this can be several centimetres but with *E. granulosus* (figure 79) it is likely to be less.

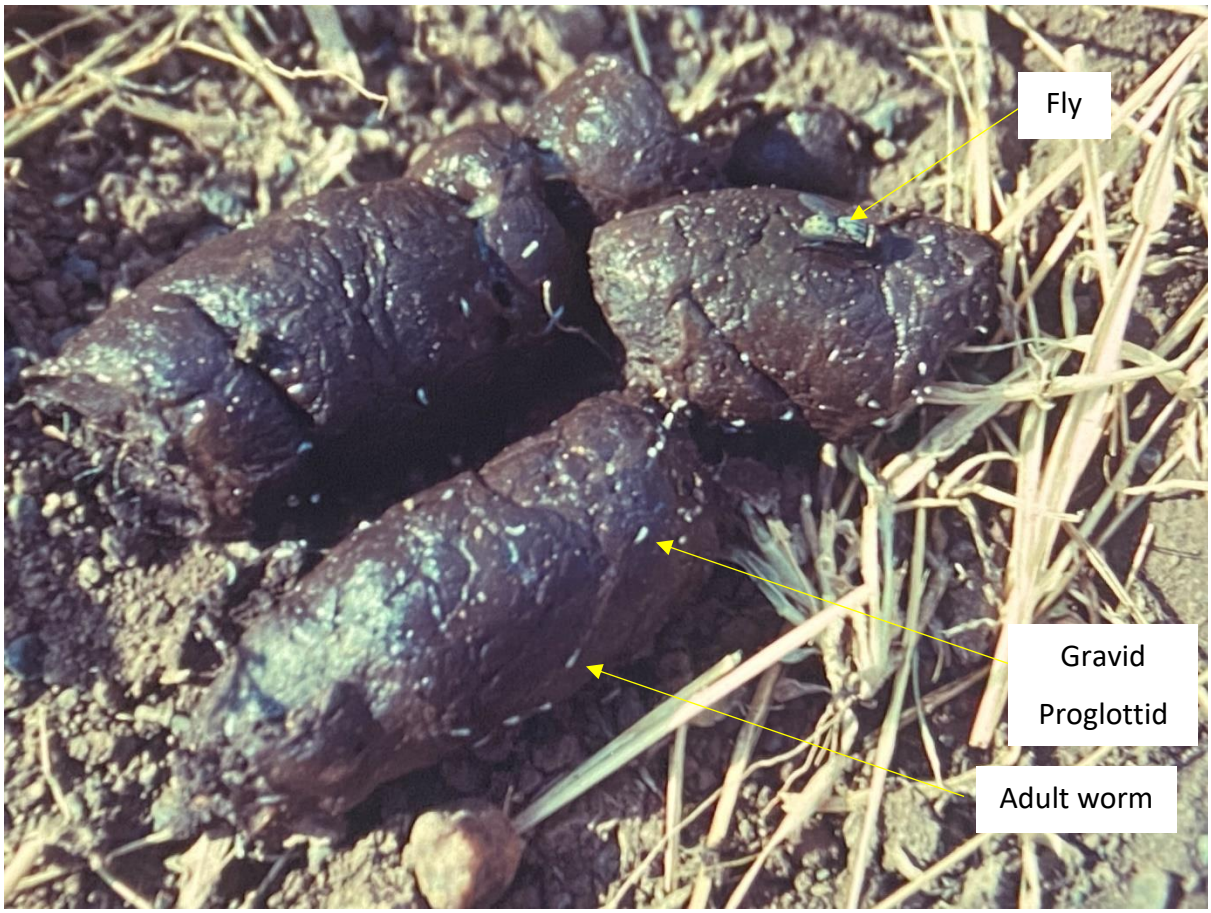


Figure 79: Dog faecal deposit from Turkana, Kenya with motile proglottids on the surface (from M.T. Rogan, original).

Second, livestock and other animals can step in faecal masses and carry faecal material on their hooves for considerable distances. Thirdly, as the faecal material dries out, eggs become incorporated in the soil and dust and can be carried by the wind and in surface water (Gemmell et al., 1987; Sánchez Thevenet et al., 2019).

An additional route of egg dispersal may also involve biological agents. In a long study in New Zealand known as the Styx field trial, Gemmell (1968) employed sentinel lambs placed at different distances from confined areas, where infected dogs had been maintained. These studies showed that *T. hydatigena* eggs were dispersed up to 80m over ten days, and although the majority of the eggs remained within a radius of 180m from the deposition site after a

month, they could still be dispersed up to a distance of ten kilometres or over 30,000ha within three months, possible involving transport by insects (Gemmell, 1997). The role blowflies play as mechanical carriers of *T. hydatigena* faeces was demonstrated in a series of experiments (Lawson & Gemmell, 1985). Under laboratory conditions, *Calliphora quadrimaculata*, *C. hortona* and *C. stygia* ingested up to 5000 *T. hydatigena* eggs per fly from the faeces surface. Most of the eggs were discharged within two days, and 38–48 % of them lost the embryophores during the gut transit (Lawson & Gemmell, 1985). In a field trial carried out in southern New Zealand, 25 % of field-collected flies, belonging to the species *Hybopygia varia*, *C. quadrimaculata*, *C. hortona* and *C. stygia*, carried *T. hydatigena* eggs in their gut after feeding for at least three minutes on naturally infected dog faeces. When the flies above were fed to healthy lambs, they successfully transmitted infection by the eggs carried in their body (Lawson & Gemmell, 1985). The role of insects in dispersing taeniid eggs has recently been reviewed by Benelli, Wassermann, and Brattig (2021) and indicates a relevant role of houseflies (Muscidae), blowflies (Calliphoridae), dung beetles (Scarabaeoidea), darkling beetles (Tenebrionidae), ground beetles (Carabidae) and skin beetles (Dermestidae) in the spread of taeniid eggs in the environment.

In other studies on St Kilda, a remote island off the coast of Scotland where no dogs are present, *T. hydatigena* had been found in Soay sheep and it has been proposed that eggs could disperse over 60km as a consequence of the transfer of viable eggs by birds (Torgerson et al., 1995). Some taeniid eggs are known to survive transit through the intestine of sea birds such as gulls (Crewe & Crewe, 1969). Contamination of fruit, vegetables and mushrooms with *E. multilocularis* has been shown to occur and this could be an additional route of dispersal via animal consumption (Torgerson, 2016).

5.1.3 Detection of eggs in the environment.

To establish risk factors for environmental contamination, a number of studies have been carried out to detect the presence of *Echinococcus* spp. eggs in environmental samples. Early studies were based on microscopy as a detection method (Craig, Macpherson, Watson-Jones, & Nelson, 1988) but more recent work has focused on PCR to detect *Echinococcus* spp. eggs. Eggs have been detected in soil, water and vegetation (Alvarez Rojas et al., 2018; Federer et al., 2016; Lass, Szostakowska, Myjak, & Korzeniewski, 2015; Matsuo & Kamiya, 2005; Shaikenov et al., 2004; Umhang, Bastien, Renault, Faisse, Caillot, Boucher, Hormaz, Poulle, &

Boué, 2017). In these studies samples were obtained either from collecting washes from fruit and vegetation or by soil sedimentation and concentration of eggs by flotation. The sensitivity of this approach is variable but increases with egg density.

Most studies involving the detection of *Echinococcus* eggs in soil have focused on establishing the presence or absence in various samples, rather than establishing dispersal of eggs. However, in a recent study in Patagonia, Argentina, Sánchez Thevenet et al. (2019) set up an experimental study over a five-year period within an enclosure housing two dogs harbouring a worm burden ranging from 100 to 1000 mature adult *E. granulosus*. Faecal deposits from infected dogs were left undisturbed for five years and water and soil samples were taken at different time intervals for assessment by both microscopy and a copro-ELISA to detect parasite antigen in soil. Results showed that parasite eggs were detected up to 41 months later in faeces from infected dogs, soil and sediment, and coproantigen tests remained positive for up to 70 months in faeces. Overall, parasite eggs were found within a maximum distance of 115m from the contaminated dog faeces deposition site. This study has shown for the first time that specific parasite antigens could also be detected in soil and that these were robust, being detectable for several years. Patagonia has a similar climate to the Falkland Islands and this approach could be of benefit in establishing environmental contamination in the Falkland Islands and create another method of surveillance to help move the control programme from the attacking based methods currently in place to a more consolidatory phase focussing on surveillance of both intermediate and definitive hosts. The objective of this chapter was, therefore, to establish whether the copro-antigen test used for dog diagnosis could be used to establish soil contamination in an environment where parasite eggs are likely to be very low in number.

5.2 Methods of detection from environmental samples

5.2.1 Soil Sample Collection and preparation for Analysis

As there was insufficient time to sample all farms with dogs during the initial sampling, 41 soil samples were taken from 22 farms where high numbers of *Cystericus tenuicollis* (metacestode

stage of *T. hydatigena*) had been detected in sheep slaughtered at the Sand Bay abattoir. Surrounding farms with land bordering the chosen farms were also sampled. Control samples were also taken from an area within Stanley rarely frequented by dogs. Soil was collected by using a 7.5cm soil probe, collecting a minimum of 200g from areas immediately below or surrounding the dog kennels and shearing shed of each farm and transferred into individual plastic bags to be brought back to the lab. Samples were dried on brown paper for 24 hours at 25°C in an oven (Horiuchi & Uga, 2016; Sánchez Thevenet et al., 2019). Dried material was then stored in clean plastic bags for use in microscopy and ELISA.

Subsequent sampling of soil samples from the remaining farms was done by members of the Department of Agriculture during visits to the unsampled farms and processed by the lab technician in the department of Agriculture following the same protocol described in section 5.2.2. In total, 83 samples were taken from 54 farms (old kennels on three farms that do not have dogs were also sampled).

5.2.2 Soil analysis for the presence of eggs, coproantigens and DNA.

For microscopy, a faecal flotation protocol was used in the same way as described in Chapter 3. For the ELISA, the soil was prepared using the same protocol as described for the faecal samples, initially adding 10ml of 0.3% Phosphate Buffered Saline (PBS) Tween with 10% formalin (Fisher Scientific, Loughborough, UK) to the soil sample then continuing as described In Chapter 3. Flotation and PCR testing of samples was also done as described in Chapter 3.

5.3 Results

5.3.1 Standardisation of the coproELISA

To establish a positive/negative cut-off with the copro-ELISA capture antibody system, the same process used for the faecal samples in chapter 3, with known positive controls collected by van Kesteren (2015) from Kyrgyzstan, and another bank of 12 negative samples previously tested from the Falkland Islands run in triplicate (figure 80). The mean optical density (OD) value for negative samples was 0.0895 with a standard deviation of 0.0082 giving a cut-off 0.133 (mean + 3sd).

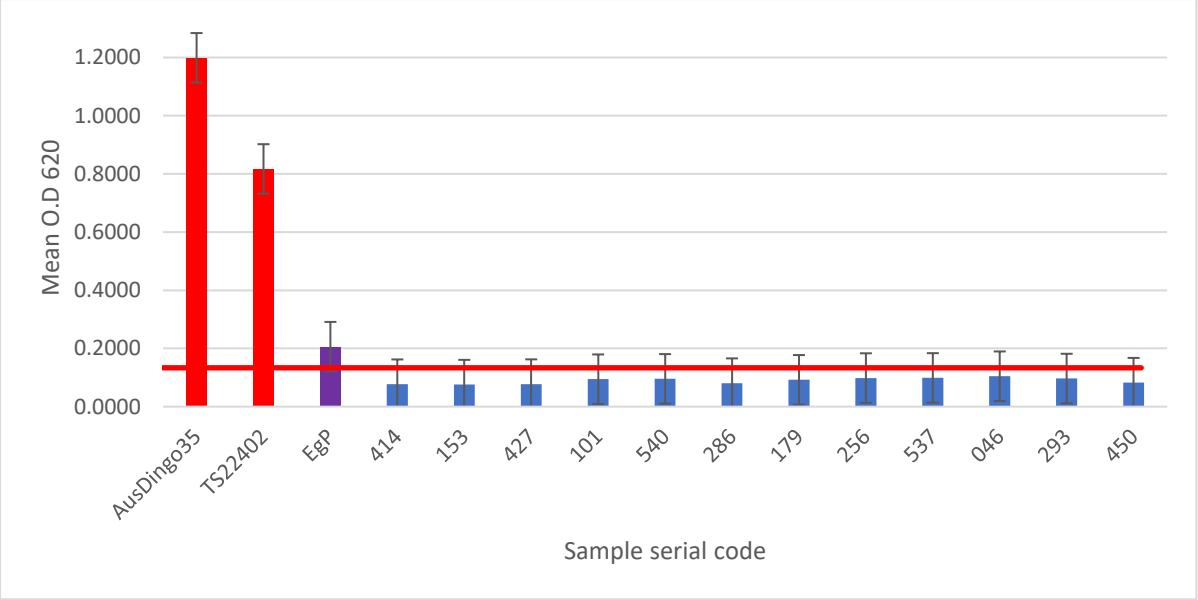


Figure 80: Mean O.D values of triplicate Copro-ELISA results from negative samples collected and tested in 2012 to establish a positive/negative cut off. Including three positive controls, two (in red) faecal positive controls and one (in purple) of soil spiked with *E. granulosus* protoscolex antigen. The mean OD value was 0.0895 with a standard deviation of 0.0082 giving a cut-off of 0.114 (mean + 3sd). Error bars represent the standard error of the mean value.

5.3.2 Soil sample analysis

A total of 82 soil samples were collected between 13th March 2019 and 17th September 2020 and assayed using the same copro-ELISA as the dog and fox faecal samples in 2018.

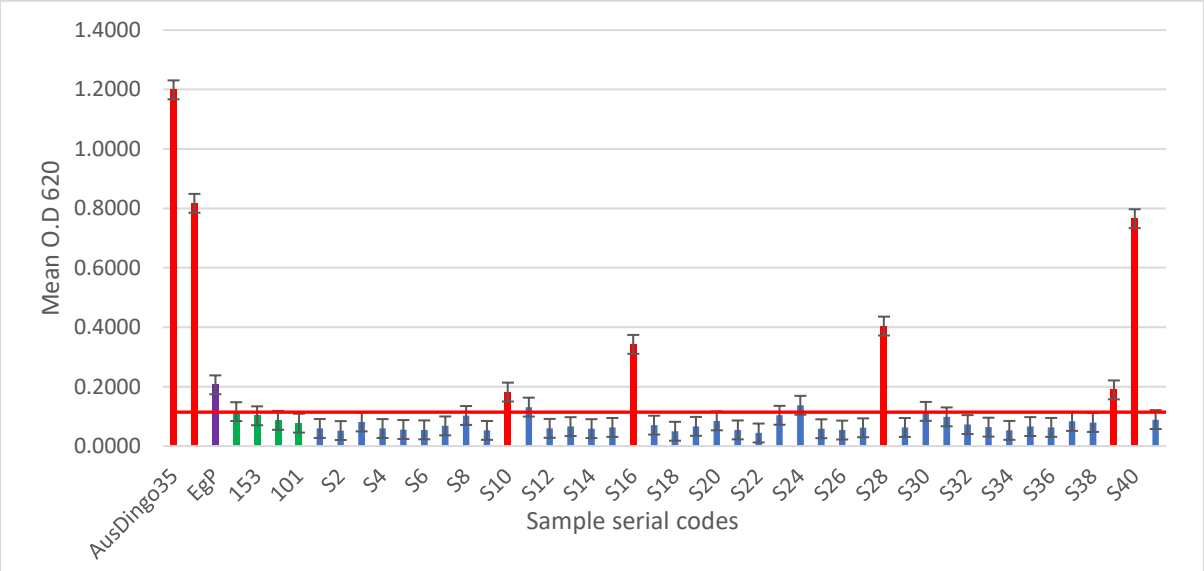


Figure 81: An example of mean O. D values of triplicate soil ELISA results from soil samples collected from farms in the Falklands. Figure shows five positive farm locations (in red). Cut off 0.114 and three positive (first two columns to the left in red were positive faecal samples and in purple was soil spiked with *E. granulosus* protoscolex antigen) and 4 negative controls (in green). Error bars represent the standard error of the mean value. The blank wells gave OD values of 0.033 which is the background noise. See appendix III-a for results from all samples.

When analysing the 82 samples, six were determined to be above the cut off value of 0.133 (S10, S16, S28, S39, S40 and S48) (figure 81). Three of the six positive samples were close to the O.D value of the faecal controls, and considerably higher than the spiked control sample. The other three positive samples were similar to the spiked control sample (mean O.D 0.21). Figure 82 shows the locations of all the farms sampled and the positive farms.

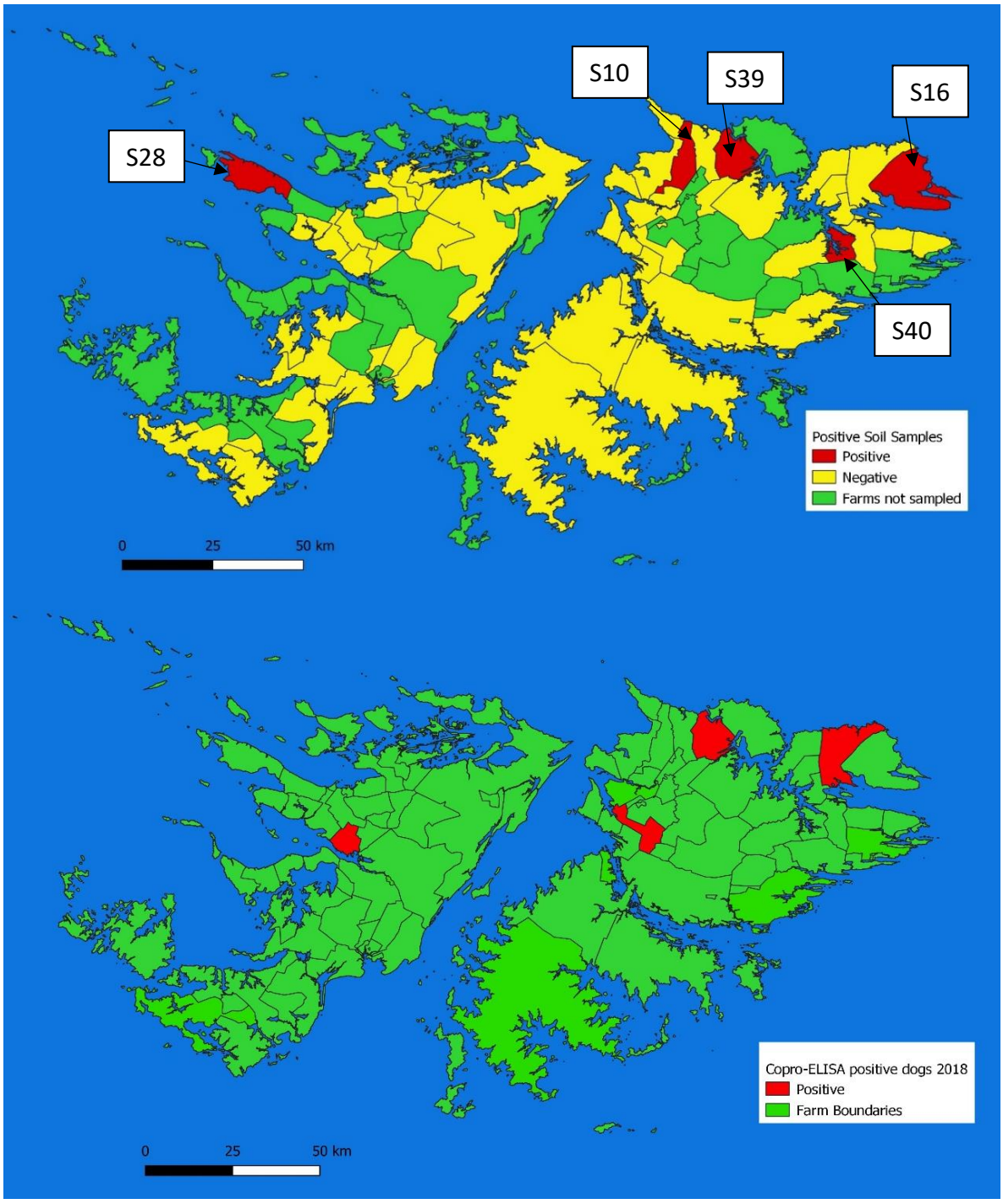


Figure 82: The locations of all the farms where soil samples (top) were collected (yellow) and the coproantigen positive farms (red). Green areas are farms not sampled and the locations (bottom) of copro-ELISA positive dogs in 2018

Microscopy on all the positive and borderline samples showed no signs of taeniid eggs after flotation, and figure 83 shows that all positive soil samples were also PCR negative when tested using the prescribed PCR (see chapter 3) using universal cestode primers.

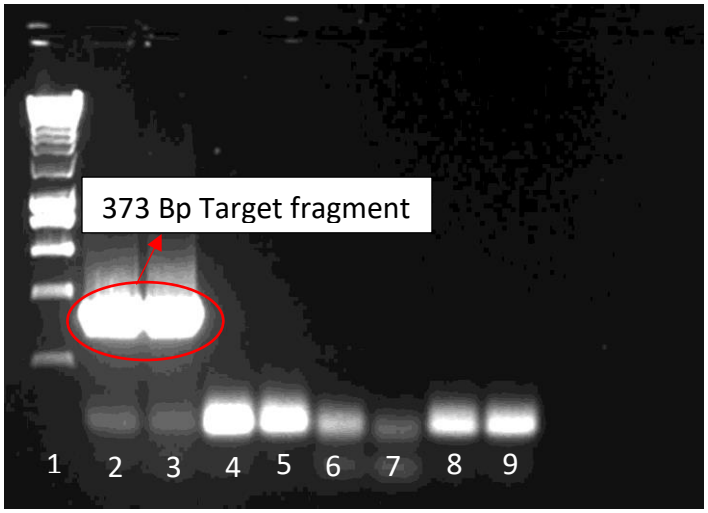


Figure 83: Universal cestode primer PCR on ELISA positive soil samples collected from areas around dog kennels on farms in the Falkland Islands in 2019 and 2020. Lane 1: 1kB Hyperladder. Lane 2 and 3: Positive control (*E. granulosus* G1 genomic DNA, *T. solium* genomic DNA). Lane 4: Negative Control (PCR Grade water). Lane 5-9: Soil DNA extraction samples.

One of the positive soil samples, S39 also had a positive dog in 2018 and a high prevalence of *Taenia hydatigena* (7.14%), while another sample from farm S40 had a sheep discovered with Cystic Echinococcosis (CE) in 2019 by the farmer during home slaughter which was confirmed by microscopy and PCR. No other positive samples had any recent history of CE in sheep or positive dogs. Of the six positive samples, only two have a *T. hydatigena* prevalence above 1% (S28=1.37% and S40=7.14%)

Table 11: Status of ELISA positive soil samples, their history of positive dogs and CE infection in sheep population and their current prevalence of *T. hydatigena.*

Reference number	Copro-ELISA O.D. Value	Presence of eggs	Previous farm history of positive dogs (Y/N)	Last recorded Presence of CE in sheep	Current prevalence of *T. hydatigena* in sheep (%)
S10	0.1818	No	No	N/A	0.66
S16	0.3423	No	No	N/A	0.31
S28	0.4039	No	No	N/A	1.37

S39	0.1891	No	Yes-2018	N/A	7.14
S40	0.7653	No	No	2019	0.46
S48	0.2182	No	No	N/A	N/A

5.4 Discussion

The current pilot study has shown that the coproantigen system used to test dog faecal samples was also capable of detecting material in soil samples from a small number of farms.

Use of similar techniques in Argentina has shown evidence of antigen in samples 41 months after infected dogs had been removed from the sampled area, and in one case 70 months after (Sánchez Thevenet et al., 2019). Due to the low prevalence of infection in the Falkland Islands and no PCR or egg confirmed positive dogs during this project, it is not possible to confirm the specificity of these positive reactions. However, of the farms with positive environmental samples, one (S39) also had a history of dogs testing positive in the last coproantigen screening in 2018. Also, this farm had a high recent prevalence of *T. hydatigena* (7.14%) indicating the likely occurrence of infected dogs in recent years. Another one of the positive farms had a recent history of CE in sheep, with S40 having a sheep discovered with a hydatid cyst in 2019 when the farmer was slaughtering animals on the farm. All farms except one had evidence of *T. hydatigena* in sheep with two of the positive farms having prevalences of this infection at greater than 1%. Due to the regular dosing of dogs, these positive results could be the result of prepatent infections and so there is the possibility that no eggs could be contaminating the area sampled at the time the sample was collected.

It could be expected that farms with positive dogs in the most recent screening (2018) would have positive soil samples. However, three of the farms with positive dogs did not have a positive soil sample. This may relate to the sampling method, whereby some kennels are raised, and faecal matter drops through slats onto the ground, whereas some are concrete floors and faecal material is washed down a channel into drains making the chances of

sampling the correct area where faecal material will be and therefore coproantigens more difficult. The sampling technique clearly needs some refining. Samples were taken from as close to the dog kennels as possible. In cases where concrete floors were in kennels, the drainage area where the kennels were cleaned was sampled. In larger dog runs, samples were taken in a rough transect across the area. To improve the sampling technique and allow for more analysis to be conducted, a more focused sampling approach should be used. On farms where dogs remain in smaller kennels, faecal deposits are more concentrated. It was reported by Sánchez Thevenet et al. (2019) that dogs during the original pilot study defecated in a particular area of the plots they were in, which is where most of the positive soil samples were found. Using a rationalised approach to each of the different styles of kennels/runs or having prior knowledge from farmers of areas where dogs more regularly defecate could increase the likelihood of sampling an area where dogs have defecated.

The current study was designed as a starting point to establish whether coproantigens could be used as an indicator of environmental contamination and the results are encouraging. Further progression once a standardised and optimised approach has been developed could be to sample at set distances at different angles from the kennels. As stated, coproantigens were found in the areas where dogs regularly defecate. However, egg dispersal is known to occur over considerable distances (Gemmell, 1968; Sánchez Thevenet et al., 2019). By taking samples at set distances from a kennel site, perhaps in the direction of prevailing winds or regular routes of dogs from the kennels, the distance of egg dispersal could be observed. This could potentially be linked to the distance of livestock pens and races from the kennels, which due to the potential intensity of eggs within the area, is a likely area where sheep could become infected. In addition, with the longevity of antigen persistence in the environment as shown by Sánchez Thevenet et al. (2019) sampling of areas around dog kennels could be a useful indicator of historic dog infection rather than current infection. In practice a better approach may be to develop a system which could detect *T. hydatigena* antigens as this is more prevalent in sheep and is an indicator of the breakdown of control measures for dogs.

In locations where farms have tested positive for *Echinococcus* antigens, it is important to continue a focused surveillance of abattoir findings as well as home slaughter monitoring of offal to monitor the prevalence of CE in sheep as well as other taeniids such as *T. hydatigena* and *T. ovis.* This continued surveillance is important for the development of a consolidation

phase of control whereby the focus of control is on surveillance of abattoir data from farms. Positive environmental samples from farms suggest there could be eggs contaminating the pasture, and therefore create a risk of infection being passed onto sheep. Observing the prevalence of taeniid infections in sheep over the following years will indicate whether the positive samples are as a result of prepatent infection or mature adult tape worm producing eggs and contaminating the environment. These farms must be observed to ensure practices abide by the laws in place and that the control measures are being adhered to strictly to reduce the chance of infection occurring again, as the risk of transmission is higher should infections be mature.

As coproantigens can be discovered in samples for a long time (up to 70 months (Sánchez Thevenet et al., 2019)) it is difficult to estimate an exact period of when contamination occurred, along with the survival of eggs for over three years (Thevenet et al., 2005) the time at which an infected dog contaminated the area is harder to establish. Nevertheless, the positive result suggests that dogs on these farms have come into contact with infective material at some point and so could be contaminating the pasture sheep graze on. This further supports the need for focussed surveillance of abattoir findings from these farms specifically in the future to monitor the potential impact on taeniid prevalence.

Monitoring the impact of a positive dog on a farm by observing abattoir data over a number of years is vital for control to analyse the dynamics of infection in sheep. Monitoring can further inform the impact of non-adherence to existing control protocols on the prevalence of CE in the Falkland Islands and determine whether transmission occurs between sheep and dogs and potentially identify key areas of the control programme that have the biggest impact on the transmission or preventing the transmission of cestode parasites.

Chapter 6. Estimating the reproduction number for *E. granulosus* in the Falkland Islands

6.1 Introduction

6.1.1 The basic reproduction number, R_0

The central questions in the current thesis are how *E. granulosus* is persisting in the Falkland Islands and what can be done to control it. These are ultimately questions about parasite stability, which can be quantified using the basic reproduction number (R_0) and the net reproduction number (R). The aim of this chapter is to estimate these parameters for *E. granulosus* in the Falklands.

R_0 is most commonly considered for microparasites such as bacteria and viruses, for which it can be described as the expected number of secondary cases produced by an infected individual within a susceptible population over the duration of its infective period (Diekmann et al., 1990). It is a threshold quantity – which can help to identify whether a pathogen is expected to invade and persist in a population made up of susceptible individuals or not. More specifically, if the R_0 value is greater than one, the pathogen would be expected to increase exponentially following initial entry into a susceptible population, as each infected individual is potentially infecting more than one susceptible individual within the population. The opposite is true if R_0 is less than one: if each infected individual is infecting 'less than one other individual', the prevalence of infection would be expected to exponentially decrease in size.

Although R_0 does not indicate how rapidly this growth or decline proceeds, it can be used to give an indication of the effort required for control measures to eradicate a disease, with higher values of R_0 requiring greater control effort (Heesterbeek & Dietz, 1996). To better understand this, a related measure, the net reproduction number (R), can be defined. This has a similar interpretation to R_0 but incorporates the effect of density dependent constraints (such as immunity) in a population. In the case of an endemic disease in a steady state (i.e. for which the prevalence is not changing over time), R would be expected to be one. In situations where this is maintained with minimal impact of density dependent constraints, R_0 would be expected to be similar to R. However, if R_0 is considerably greater than one, the

pathogen may be in a hyperendemic state (Gemmell, Lawson, & Roberts, 1986b), being strongly regulated by density dependant constraints. In these cases, as control efforts reduce the impact of these constraints, R may be maintained or even increased – making control more challenging.

It is also important to note that estimates of R_0 are not fixed for a given pathogen but will vary depending on the population in question. These estimates are often based on several key basic parameters such as the length of time an individual is infectious for, likelihood of infection when an individual comes into contact with another host or vector and the rate of contact between the infective individual and susceptible others (Delamater, Street, Leslie, Yang, & Jacobsen, 2019).

A number of different ways of estimating R_0 have been described. Many of these are based upon the specification of an underlying mathematical model of pathogen transmission (Heffernan, Smith, & Wahl, 2005), but it may in some cases be possible to base estimates on the rate of pathogen spread (r), given some knowledge of the generation time between successive infections (Wallinga & Lipsitch, 2007). The mathematical models used to estimate R_0 are based upon an understanding of the processes that make up the biological system being modelled. Mechanistic models such as these differ from "phenomenological" models as they explicitly consider the biological mechanisms that support disease transmission. These parameters may vary from estimations of the time taken for a given biological process to occur to larger more complex parameters such as the probability of contact between hosts and the force of infection of a pathogen (Lessler & Cummings, 2016). Establishing this kind of model is challenging, as identifying initially the important biological processes that need to be included within the model requires a good understanding of the lifecycle and transmission of the disease and which aspects of this can be excluded for the sake of model tractability. Once a model has been developed, the difficulty comes in parameterising the model. In some cases, parameter estimates can be established by fitting the model to real-world data. In cases where this is not possible data from published epidemiological literature and/or expert opinion may be used. And if even this is not possible, sensitivity analysis can be used to observe the parameter through a range of values and monitor the model output based on this range (Wu, Dhingra, Gambhir, & Remais, 2013).

6.1.2 Difficulties in R_0 estimation for macroparasites

As with many parasites, conventional interpretations of R_0 for *E. granulosus* are more challenging due to the fact that parasite generally does not multiply within the host (meaning that classifying hosts as "infected" or "uninfected" ignores a lot of the variety in true infectious potential) and due to the complexity within the life cycle due to the various life stages and the times in which these take to be completed (Anderson & May, 1992; Birhan, Munganga, & Hassan, 2020; Heesterbeek & Roberts, 1995). In contrast to the interpretation of R_0 estimations for a pathogen such as a bacterium or virus in which substantial within-host multiplication of the agent takes place, the R_0 for macroparasites such as helminth worms is defined as "the average number of offspring that are produced throughout the reproductive lifespan of a mature parasite and that survive to reproductive maturity in the absence of density-dependent constraints on population growth" (Anderson & May, 1992). This interpretation is similar to the reproduction number estimated for free living species.

As a result of the complexities of R_0 calculation for macroparasites, many of the generic analytic methods used for microparasites are not appropriate. Instead, more ad-hoc methods tailored to the organism in question are commonly used (Anderson & May, 1992). Alternatively, mathematical methods based upon the next generation matrix of a structured population can be used (Heesterbeek & Roberts, 1995), with the resultant quantity sometimes defined as Q0 (despite being mathematically equal to R_0).

6.1.3 Estimating the R_0 for *E. granulosus*

Echinococcus granulosus has an indirect lifecycle with three developmental life stages: egg, metacestode and adult. Two of these three stages are within hosts, and the egg stage is environmental. Accounting for environmental contamination adds another level of complexity to R_0 calculation, as it allows transmission to be indirect. Indirect transmission is when a pathogen is acquired from the environment by means of grazing on contaminated pasture/feed, vectors such as biting insects or animal to person contact. Various pathogens such as *Bacillus anthracis*, survive in the environment for extended periods. In the case of *B. anthracis*, spores can reside in the environment where an infected individual has died, creating an area of contaminated environment (Blackburn, Ganz, Ponciano, Turner, Ryan, Kamath, Cizauskas, Kausrud, Holt, Stenseth, & Getz, 2019). For *E. granulosus,* this is represented by the persistence and dispersal of infected eggs.

Transmission models have been used to understand parasites such as *E. granulosus* for decades looking at population dynamics, transmission dynamics and lifecycles, as well as simulate and investigate control programmes with the aim of reducing prevalence and in some cases eradicate these parasites from populations. Early modelling techniques used biological parameters (estimated in Gemmell et al. (1986c)) to describe the basic transmission dynamics of *E. granulosus* in a dog-sheep cycle (Roberts, Lawson, & Gemmell, 1986). These studies were used to identify the stability of the parasite and other taeniid parasites within a population, and how that stability is influenced by prescribed control measures (Gemmell et al., 1987). These studies showed that the effort needed to reduce *E. granulosus* from endemic state to extinction steady state is only enough to reduce *T. hydatigena* and *T. ovis* from a hyperendemic to an endemic state. This resulted in some cases in a counterintuitive increase in infection burden following the start of effective control measures. These results highlight the differences within epidemiologically similar parasites and different requirements for eradication (Gemmell et al., 1987).

Many studies have been conducted using models to investigate the transmission dynamics and effective control measures for *E. granulosus* in various regions (Cabrera et al., 1995; Torgerson, 2003; Ziadinov, Mathis, Trachsel, Rysmukhambetova, Abdyjaparov, Kuttubaev, Deplazes, & Torgerson, 2008). Wang, Zhang, Jin, Ma, Teng, and Wang (2013) developed a deterministic model to study transmission dynamics of *E. granulosus* in the Xinjiang region of China and showed how these influence R_0. Observing the destabilisation of these dynamics and the changes in R_0.

The aim of this chapter is to develop a mechanically informed compartmental model of the life cycle of *E. granulosus* to observe key processes supporting the parasites transmission, as well as identifying the impact of these processes in isolation.

6.2 Methods

6.2.1 Basic model structure

Using R statistical software version 4.1.1 (R Core Team, 2021), an *E. granulosus* life cycle model was developed to estimate the number of secondary adult tapeworms caused by a single *E. granulosus* infection in a dog. The model is specific to the Falkland Islands and encapsulates the different processes which allow completion of the parasite lifecycle, including biological and environmental factors as well as farming practices. This was used to

estimate R_0 and is shown in Figure 84. Although not shown in the figure, the mathematical calculations involved in the calculation of R_0 are described below.

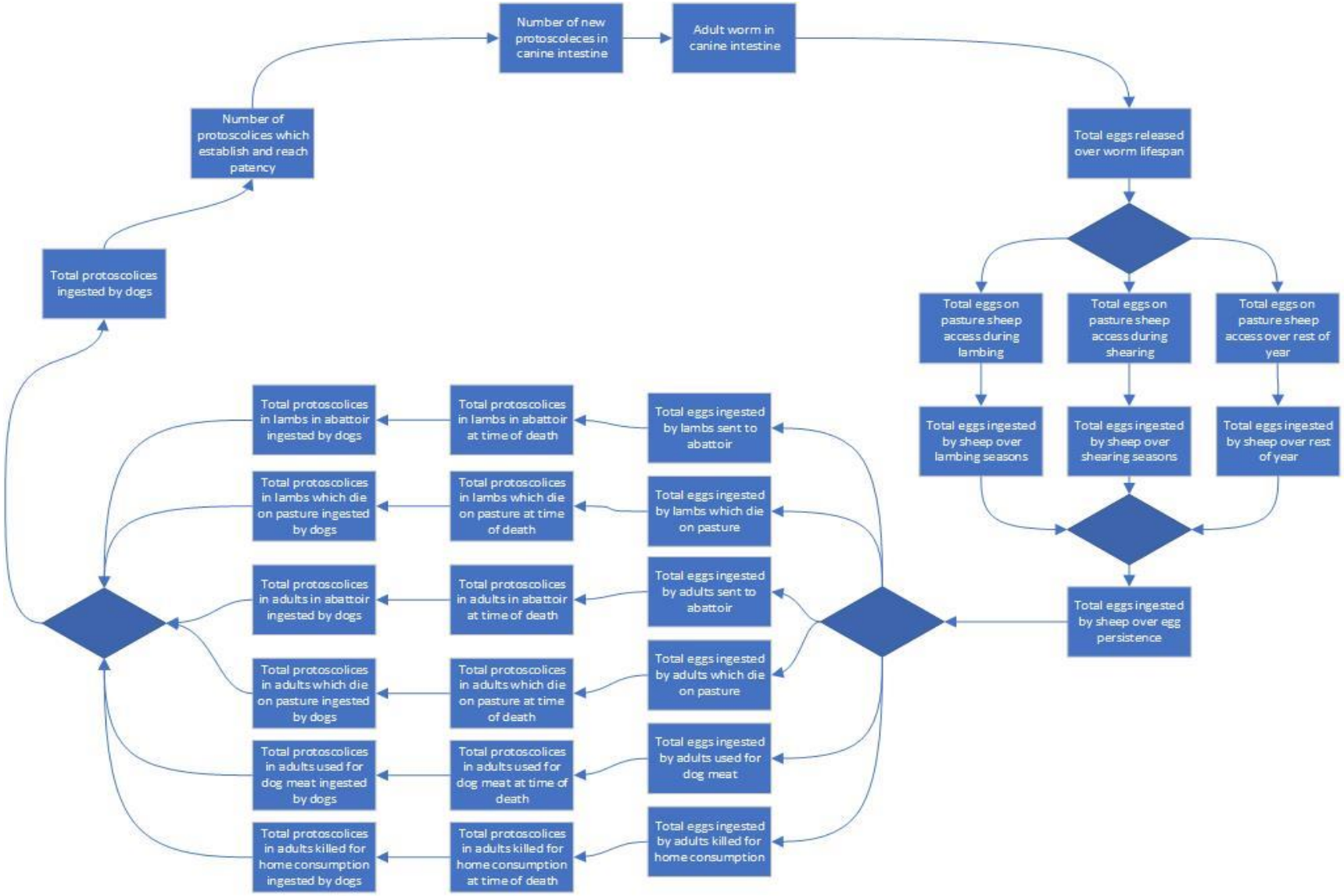


Figure 84: Basic flow diagram of the life cycle of *E. granulosus* from a single adult worm in a canine intestine to the number of secondary adult worms.

6.2.2 Calculation of R_0

Number of eggs released by adult worm over its lifetime.

Assuming that an adult worm in an adult dog survives for a length of time determined by the mean worm lifespan (μ_A) and the mean dog lifespan (d_A), the total rate of worm death can be calculated as the sum of the reciprocals of these durations. The adult worm lifespan (and therefore the duration of egg production) is the reciprocal of this:

Duration of egg production = $1 \div \left((\frac{1}{\mu A}) + (\frac{1}{dA}) \right)$

Over this time, it is assumed that a given number of eggs (λ) are expelled on average each day, meaning that the total egg production over the duration of egg production is:

Total egg production = $\text{Duration of egg production} \times \lambda$

Total number of eggs deposited on pasture grazed by sheep

It was assumed that the eggs released by dogs are deposited randomly onto three pasture "zones". These zones may overlap with each other and are defined by the likely presence of sheep at certain (non-overlapping) periods of the year ("seasons"):

- Pasture used by sheep during the shearing season (s)
- Pasture used by sheep during the lambing season (l)
- Pasture used by sheep during the rest of the year (r)

Based on farmer questionnaires (see appendix IIII-a), estimates were created of the probability that dogs defaecate on each of these pasture zones during each season (based upon an estimate of the frequency of defaecation on pasture and an assumption that dogs defaecate once per day on average): θ_s, θ_l, and θ_r. If it is assumed that the shearing season is S_s days long, that the lambing season is S_l days long, (and therefore that the remainder of the year represents S_r=365-(S_s+ S_l) days), the total number of eggs released on each pasture zone can be estimated as the product of the total egg production, the probability of dog defaecation on the pasture (θ), and the proportion of the year which the season in question represents $(\frac{S}{365})$.

Total number of eggs ingested by sheep

To consider the number of eggs ingested by sheep, estimates were made of the probability that the location of an egg on grazed pasture is grazed by sheep over the course of the season, the probability of ingestion if this occurs (θ), and the length of time an egg remains viable on pasture. It was assumed assume here that each zone is completely grazed once by sheep over the course of a season, regardless of its size or the number of sheep. Since there is only one of each season per year, the probability that an egg on grazed pasture is not ingested over a period of E years of egg persistence can be calculated as:

Probability egg not ingested = $(1-\theta)^E$

And therefore, the probability of ingestion can be calculated as:

Probability of egg ingestion = 1 - Probability egg not ingested

The number of eggs ingested over the course of each season is then the product of the probability of egg ingestion and the number of eggs deposited on pasture.

Total number of protoscoleces ingested by dogs

Because new protoscoleces are formed inside a mature hydatid cyst over time, the number of protoscoleces inside an infected sheep at death (i.e. the time that dogs can first become infected) will depend on the age of the cyst at the time of death. This will itself depend on the age of the sheep both at the time of first infection and at the time of death. Whilst the former is not easily estimable for the Falkland Islands situation, the latter will depend on the sheep itself. Additionally, the type of death would be expected to affect the probability of dogs accessing infectious offal. Bearing all of this in mind, the sheep population was split into the following six mutually exclusive sheep types:

- Animals slaughtered in the abattoir:
    - Animals which will ultimately be slaughtered at the abattoir as lambs (mean age at death: A_a)
    - Animals which will ultimately be slaughtered at the abattoir as adult sheep (mean age at death: A_b)
- Adult sheep slaughtered on farm:
    - Animals which will ultimately be slaughtered on farm for home consumption (mean age at death: A_e)

    - Animals which will ultimately be slaughtered on farm for dog meat (mean age at death: A_f)
- Animals which die on pasture:
    - Lambs which will ultimately die on pasture (mean age at death: A_c)
    - Adult sheep which will ultimately die on pasture (mean age at death: A_d)
-

The proportion of the total sheep population in each of these categories at any one time (accounting for their different expected lifespans) was obtained by expert opinion. The total number of encysted eggs in each group was then estimated as the product of this proportion, the total number of eggs ingested by sheep, and the probability that an egg encysts following ingestion (τ).

To estimate the number of protoscoleces in each animal at the time of death, the age at first infection (Φ) needs to be defined. From this, the cyst age at death was estimated as the difference between Φ and the age at death (A). The number of protoscoleces at this cyst age was predicted by fitting a logistic curve to data on the mean number of protoscoleces per cyst in animals of different ages, as shown in Table 12 (Torgerson et al., 2009).

Table 12: The number of protoscoleces per sheep at a given age (Torgerson et al., 2009).

Age of Sheep	Number of Protoscoleces
1	215
2	870
3	2035
4	3061
5	8192
6 and older	12603

The nls() function in R was used to fit a logistic curve to these data to allow predictions of protoscolex numbers. It was assumed that the estimate for sheep aged six and older related to sheep aged six years only, and that the animal age was equal to the cyst age. The predictions from this model are shown in Figure 85.

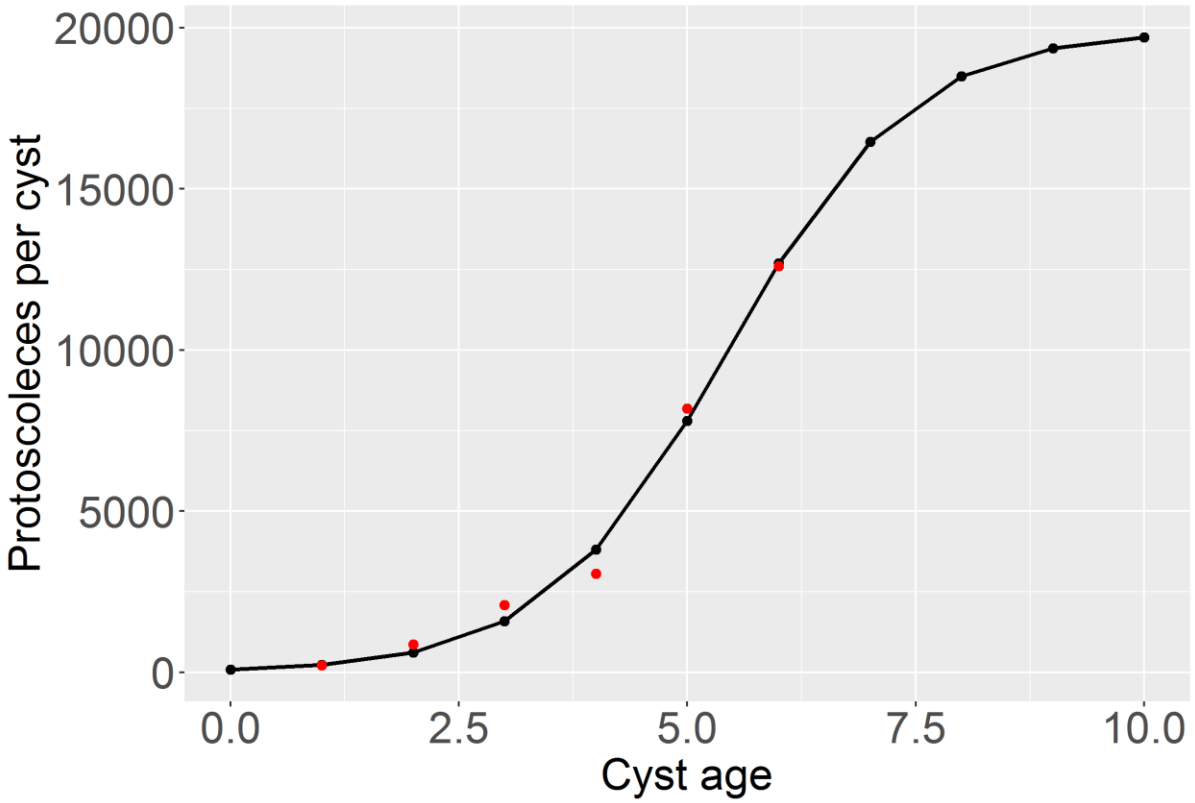


Figure 85: Predicted numbers of protoscoleces per cyst as a function of cyst age (black) and data from Torgerson et al. (2009) seen in table 12 (red).

The product of the number of encysted eggs and the number of protoscoleces which develop over the lifespan of the sheep type in question gives an estimate of the total number of protoscoleces in sheep of this type which are available to be passed on to dogs.

Finally, the number of protoscoleces which establish in dogs was estimated as the product of the total number of protoscoleces in each sheep type, the probability of offal of this sheep type being ingested by dogs, and the probability of ingested protoscoleces establishing in dogs, summed over all sheep types. As all animals slaughtered at the abattoir are inspected, it was assumed that their offal can never become accessible to dogs and is not consumed, with the parameter remaining at zero. However, animals that die naturally on the pasture are not inspected and should a dog come across it whilst gathering or if left unsupervised, there is a chance of offal being ingested. Finally, although animals that are killed on farm for home consumption or dog meat must have their offal disposed of by farmers so that dogs cannot access them, this is not always achieved. For example, animals being left in open cull sites which dogs can access, or offal being dumped onto the beach, which has been documented

to in the Falkland Islands to be associated with copro-antigen and seropositive dogs (Reichel et al., 1996).

The number of adult tapeworms which ultimately develop from these protoscoleces was then estimated as the product of the number of established protoscoleces and the probability of an established protoscolex reaching patency. This probability will depend partly on the biology of the parasite itself, but also on the frequency of dosing with praziquantel (measured as the inverse of the dosing interval in weeks, (υ). Assuming that the efficacy of praziquantel (i.e. the probability of removing infection) is κ, the expected number of doses over the duration of a prepatent period of ν weeks is the product of the dosing frequency and the prepatent period duration (or $\left(\frac{\nu}{\upsilon}\right)$). Assuming that the dosing interval is no lower than the prepatent period, the probability that these doses effectively remove infection is then estimated as the product of κ and $\left(\frac{\nu}{\upsilon}\right)$ or 1.0 (whichever is lower).

6.2.3 Model parameterisation

Due to the low prevalence of *E. granulosus* infection in dogs and sheep in the Falkland Islands and the presence of an intensive control scheme, it was not possible to estimate model parameters from real-world data. Instead, estimates were taken from published literature and expert opinion. Table 13 lists the parameters and their description that are used in the model.

Table 13: List of model parameters, their descriptions and estimations, and the source of the information.

Parameter	Parameter Estimate	Description	Source
λ	82.3	The mean daily egg production by a single adult worm	(Mohammadzadeh et al., 2012)
μ_A	0.75 years	Expected lifespan of adult worm	(Ziadinov et al., 2008)
d_A	12.5 years	Expected Lifespan of Definitive Host	(Wang et al., 2013) Supported by veterinarians in the Falklands
S_a	4 months	Length of shearing season	
S_b	1.5 months	Length of Lambing season	
S_c	6.5 months	Length of Winter	
B_s	0.08292727	Probability of dogs defecating on grazed pasture during shearing	Questionnaire data estimating the frequency of dogs defecating on pasture
B_l	0.05869099	Probability of dogs defecating on grazed pasture during lambing	Questionnaire data estimating the

			frequency of dogs defecating on pasture
B_r	0.05869099	Probability of dogs defecating on grazed pasture during winter	Questionnaire data estimating the frequency of dogs defecating on pasture
θ	0.1	The probability that an egg is ingested by an intermediate host if the pasture is grazed	Estimation
E	3.4167 years	Length of survival of eggs in the environment	(Thevenet et al., 2005)
N_{Ma}	0.07	Probability that a lamb ingests eggs from pasture that is slaughtered at the abattoir	Estimates based on age of animal and their likelihood of ingestion over lifespan
N_{Mb}	0.15	Probability that an adult sheep ingests eggs from pasture and is slaughtered at the abattoir	
N_{Mc}	0.03	Probability that a lamb ingests eggs from pasture and dies naturally on pasture	
N_{Md}	0.4	Probability that an adult sheep ingests eggs from pasture and dies naturally on pasture	
N_{Me}	0.15	Probability that sheep killed for home consumption ingest eggs from pasture	
N_{Mf}	0.2	Probability that sheep killed for dog meat ingest eggs from pasture	
τ	0.0033	Probability that eggs ingested encyst	(Roberts et al., 1986)
A_a	1	Age of lambs slaughtered at the abattoir	Estimated by veterinarians in the Falkland Islands.
A_b	5.6	Age of adult sheep slaughtered at the abattoir	
A_c	1	Age of lambs that die naturally on pasture	
A_d	10	Age of adult sheep that die naturally on pasture	
A_e	3	Age of sheep killed for home consumption	
A_f	6	Age of sheep killed for dog meat	
Φ	0, 2, 4, 6, 8 Years of age	Age at first infection	Sensitivity analysis
ω_a	215	Total Protoscoleces in lambs slaughtered at the abattoir	(Torgerson et al., 2009)
ω_b	8192	Total Protoscoleces in adult sheep slaughtered at the abattoir	(Torgerson et al., 2009)
ω_c	215	Total Protoscoleces in lambs that die naturally	(Torgerson et al., 2009)
ω_d	12603	Total Protoscoleces in adult sheep that die naturally	(Torgerson et al., 2009)
ω_e	2095	Total Protoscoleces in sheep killed for home consumption	(Torgerson et al., 2009)
ω_f	12603	Total Protoscoleces in sheep killed for dog meat	(Torgerson et al., 2009)
ψ_{Na}	0	Probability of definitive host ingesting the offal of a lamb slaughtered at the abattoir	
ψ_{Nb}	0	Probability of definitive host ingesting the offal of an adult sheep slaughtered at the abattoir	

ψ_{Nc}	0.2	Probability of definitive host ingesting the offal of a lamb that dies naturally on pasture	
ψ_{Nd}	0.2	Probability of definitive host ingesting the offal of an adult sheep that dies naturally on pasture	
ψ_{Ne}	0.1	Probability of definitive host ingesting the offal of a sheep killed for home consumption	
ψ_{Nf}	0.1	Probability of definitive host ingesting the offal of a sheep killed for dog meat	
ρ	1	Maximum probability of ingesting offal	
I	1	Proportion of protoscoleces ingested if definitive host ingests offal	(Bosch, Hagen-Plantinga, & Hendriks, 2015; Stahler, Smith, & Guernsey, 2006)
ε	0.047	Proportion of protoscoleces that establish in definitive host	(Gemmell et al., 1986c)
σ_p	0.004	Proportion of established protoscoleces that develop into an adult worm	Parameter to estimate the effect of dog dosing on transmission

6.2.4 Uncertain parameters

Some parameters were not possible to estimate with any degree of certainty from expert opinion or scientific literature and were therefore explored as a form of sensitivity analysis. These parameters are shown in Table 14 and described in more detail below.

Table 14: Uncertain parameters influencing the life cycle of *E. granulosus*.

Parameter	**Estimates used**	**Description**
θ	1e-06, 1e-05, 1e-04, 1e-03, 1e-02, 1e-01, 1	Rate of ingestion of eggs on pasture grazed by sheep (per year)
Φ	0, 2, 4, 6, 8	Age at first infection
ρ	1e-06, 1e-05, 1e-04, 1e-03, 1e-02, 1e-01, 1	Maximum probability of ingesting offal

The rate of ingestion of eggs on pasture (θ) would be expected to be affected by the likelihood of sheep grazing on the exact portion of pasture the eggs are present (which will be affected by sheep grazing densities) as well as the probability of ingestion if this occurs (which will be affected by sheep grazing habits and egg location). In order to capture the duration of egg persistence explicitly in the model, this parameter describes the rate of ingestion per year, based on the assumption that stocking density of sheep is such that the total pasture area is grazed completely once over the course of the season (regardless of the

total pasture area or the length of the growing season), and that there is a single season per year.

The age of first infection (Φ)needs to be accounted for because multiple protoscoleces of *E. granulosus* develop within cysts over time. This means that sheep infected at a younger age will develop more protoscoleces within their cysts than comparable sheep infected later.

Because feeding of offal to dogs is not permitted in the Falkland Islands, the probability of definitive host ingesting protoscoleces is largely dependent upon the ability of dogs to scavenge dead sheep and the probability of ingestion of infective offal if this does take place. The former of these will vary for different sheep types, depending largely on whether they are slaughtered or die on-pasture. To account for this variation, we specify the relative probabilities for different sheep types and then vary the absolute estimate using the parameter ρ.

Estimates of both Θ and ρ were varied on a log scale between 0.00001 and 1 due to the very limited knowledge of the true value. The estimate for Φ was varied between 0 and 10, to represent the range of potential ages of first infection.

6.2.5 Evaluation of control strategies

As well as exploring uncertain parameters, a sensitivity analysis approach was used to explore the impact of different expected scenarios. In the current analysis, the effect of praziquantel dosing was considered, which is currently mandated (every five weeks) but is unsupervised, which is known to have a lower performance than supervised dosing (Buishi, Walters, Guildea, Craig, & Palmer, 2005). We considered two forms of dosing failure:

- No administration of praziquantel (either due to a lack of dosing, or dogs not ingesting administered tables). For this, the efficacy of praziquantel was reduced to zero, to represent the effect of complete failure to dose dogs.
- Underdosing. This was modelled by reducing the efficacy of praziquantel from 99.6% to 88.6%, representing the efficacy reduction of 11% resulting from a reduction in praziquantel dose from 5mg/kg to 2.5 mg/kg (Gemmell et al., 1977). Again, this assumes that this is the case for all dogs.

6.3 Results

6.3.1 Evaluating environmental parameters and control methods.

Figure 86 shows the results of sensitivity analysis. The effect of varying the probability of sheep ingesting taeniid eggs from the pasture they graze (x axis), the age of first infection (columns), the probability of a dog ingesting offal (y axis) and the percentage failure of dog dosing (rows) on the estimate of R_0 (colour scale) is shown. Areas in green represent R_0 estimates lower than one, whereas those in red represent R_0 estimates greater than one.

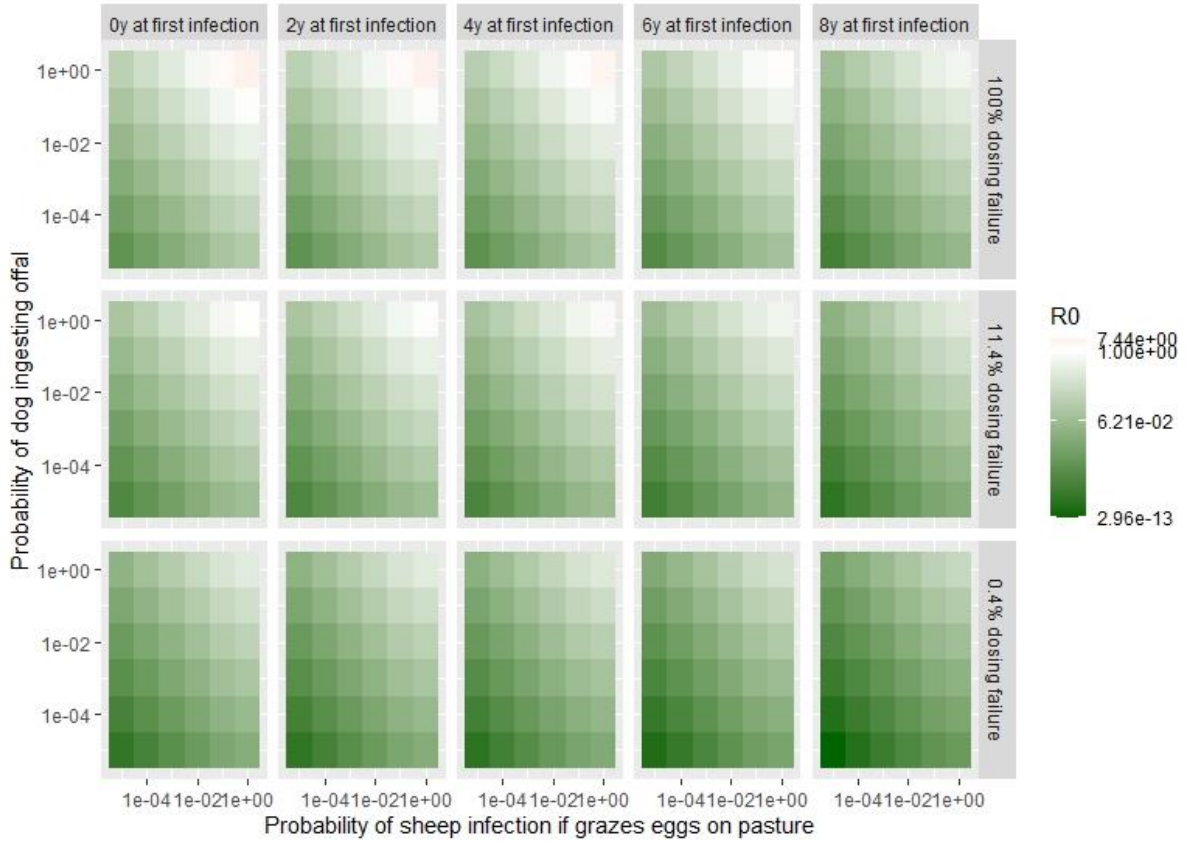


Figure 86: Hotspot map of sensitivity analysis of parameters (x axis) probability of sheep ingesting taeniid eggs from the pasture they graze, the age of first infection, (y axis) the probability of a dog ingesting offal and the percentage failure of dog dosing.

Of the 540 different scenarios analysed in Figure 86, seven have an R_0 estimate greater than one, as shown in Table 15.

Table 15: Scenarios where model identifies parameter combinations that cause R_0 to increase above one.

Probability of egg ingestion	**Age at first infection**	**Probability of ingestion of offal**	**Dosing failure**	R_0 **Estimate**
0.1	0	1	100% dosing failure	2.248
1	0	1	100% dosing failure	7.436

0.1	2	1	100% dosing failure	2.012
1	2	1	100% dosing failure	6.654
0.1	4	1	100% dosing failure	1.358
1	4	1	100% dosing failure	4.492
1	6	1	100% dosing failure	1.342

R_0 estimates above one were only seen in cases of complete dosing failure and ingestion of offal by dogs, with relatively high probabilities of egg ingestion by sheep. The scenarios that result in the greatest increase in R_0 occur when probability of ingestion of eggs by intermediate host and ingestion of offal by the definitive host increase above 0.010 and 0.100 respectively when there is complete failure of dosing. The highest R_0 estimate results from these parameter estimates occurring and the intermediate host becoming infected at less than one year of age (7.436), decreasing as the age at first infection increases until eight years old at first infection, where the R_0 estimate does not reach above one under any of the conditions, though increases to its highest estimate (0.217) when the probability of both ingestion parameters is one, and 100% dosing failure occurs.

In the scenarios where dosing has a failure of just 0.4% (representing the baseline rate efficacy of praziquantel with 100% dosing compliance), the R_0 estimate does not reach close to one for any probabilities of ingestion or even in the earliest age of first infection. Even in the scenarios where parameters brought about the highest estimates under 100% dosing failure, the R_0 estimate is only 0.03. When the percentage failure is increased to 11.4% (representing routine underdosing), the R_0 estimate increases in the same fashion as previously described, however still does not increase above one.

6.4 Discussion

In this project, a mechanistic approach has been used to create a compartmental model of the lifecycle of *E. granulosus*. As the low prevalence of infection with *E. granulosus* in the Falkland Islands and the presence of an intensive control scheme make conventional parameterisation efforts challenging, this model was used to explore the limits of parasite

stability rather than to accurately predict the current situation in the Falkland Islands. Although Figure 86 shows that perpetuation of infection would not be expected in the majority of scenarios (as shown by an R_0 estimate of below 1), it was possible in some cases. These scenarios are associated with the following three processes occurring together:

- High probabilities of ingestion of eggs on pasture by sheep, at a young age.
- High probabilities of ingestion of offal by dogs.
- Missed doses of praziquantel.

Although this series of events is unlikely to be common throughout the population as a whole, it is plausible that it is met intermittently, allowing continued spread of *E. granulosus*.

Evidence from the described model suggest that transmission through the conventional dog-sheep lifecycle is still a possibility in the Falkland Islands, with scenarios of specific parameter estimates have shown that the R_0 can increase above one. This is also supported by the evidence in previous chapters where multiple sheep have been identified annually to be infected with CE, as well as dogs being identified as coproELISA positive. Testing prior to this study also found PCR confirmed positive dogs have been discovered in recent years (2010). There is also the possibility that infection is occurring through failure of the praziquantel dosing regimen. This could be through dogs regurgitating the tablets after they were administered and so the drug isn't absorbed, or the potential for resistance reducing the efficacy of the drug. This was not possible to investigate within this study, however, would be interesting to investigate further.

For many years, the prevalence of *E. granulosus* has remained below 1% in sheep, with the number of cases not exceeding five a year since 2005. Early predictions taken from the data were that the R_0 estimate would be just below one, as the prevalence has not increased and remained stable for over ten years, decreasing in prevalence prior to this, with CE in sheep remaining endemic in the Falkland Islands. However, due to the continued discovery of small numbers of infected sheep and dogs, there must be occasions where the R_0 for *E. granulosus* is increasing above one. The overall low prevalences of *E. granulosus* in the Falklands support the results from the model, finding that the majority of R_0 estimates were lower than one, with a very low number (seven) of scenarios where the R_0 estimate can increase above one, potentially causing an increase in infections and resulting in low levels of infection continuing to be discovered annually. However, this model cannot account for the prevalences of other

taeniid parasites such as *T. hydatigena* and *T. ovis* as these parasites have different life cycle parameters due to their differences in size and biotic potential (Torgerson & Heath, 2003). Though if this was an area of further interest for the Falkland Islands, the parameters could be changed to account for these other parasites.

This variability is considered in the current model in the form of the sensitivity analyses but will be explored further in future work. Although most of the time the prevalence is shrinking or remaining stable, the model shows that there remains the potential in certain settings for perpetuating transmission to occur and the R_0 to increase above one. In particular, this could occur due to a combination of high probabilities of sheep ingestion of eggs at an early age, high probabilities of canine ingestion of sheep offal, and dog dosing failure. This shows the value of creating a mechanistic model of transmission, allowing key areas of the lifecycle to be analysed and explored to better understand these "critical control points".

The model shows that if the probability of sheep ingesting eggs increases, potentially through an area of contaminated pasture being grazed more intensely or a higher concentration of eggs being on the environment. The likelihood of ingestion of eggs is increased by situations where dogs and sheep are brought closer together. Throughout the year, different seasonal activities such as gathering for shearing or lambing, result in high-risk period where the interaction of dogs and sheep are increased for these events. The association between dogs and sheep is a global risk factor for increased infections of *E. granulosus* (Buishi et al., 2005; Hu, Wu, Guan, Wang, Wang, Cai, & Huang, 2014; Oba, Ejobi, Omadang, Chamai, Okwi, Othieno, Inangolet, & Ocaido, 2016), and this increase in interaction raises the probability of sheep ingesting eggs which as described in the model, will increase the R_0.

Another key element of the model is the age at first infection. If a lamb becomes infected with *E. granulosus* eggs within its first year of life, when it is slaughtered as an adult, the number of protoscoleces that develop over its lifetime will be more than an older animal that becomes infected at the age of four for example, if that sheep is slaughtered at six, the protoscoleces will have only had two years of growth and so less development of protoscoleces, assuming the animals age is equal to the age of the cyst (Torgerson 2009). This increase in protoscoleces development increases the biotic potential of the metacestode stage of the parasite, allowing more adult tapeworm to develop if consumed by a definitive host (Torgerson & Heath, 2003). Evidence of lambs becoming infected with taeniid eggs from identification of infection at the

abattoir shows that lambs are exposed to such infections in the Falklands, and so potentially could be infected with *E. granulosus* eggs should they be on the pasture they are grazing. As shown in the model, this will produce a greater R_0 estimate the younger the age at first infection. It could be possible to reduce the chance of lambs becoming infected by reducing their contact with pasture where dogs are more likely to have defecated. For example, post lambing, ewes and their lambs may be kept in pasture closer to the settlement. This is likely to have more eggs on the pasture as dogs spend most of their time in the settlement or nearby to it, resulting in a greater amount of defecation occurring around these areas. If the land allowed as well as the labour to be able to check on stock further from the settlement, moving younger stock further from the settlement could potentially reduce the chance of lambs ingesting eggs while grazing from a younger age, which would reduce the R_0 as seen in the model.

The likelihood of dogs ingesting offal is an associated risk of the incorrect disposal of offal. Farms regularly slaughter animals for home consumption, dog meat, and culling of older animals. In the first two cases, offal should be disposed of in dog proof containers and stored for 28 days, which if not followed carries fines of up to £500. However, there is limited inspections carried out on slaughter practices and offal disposal. Results from the questionnaire survey in chapter two showed that some farms are still disposing of offal on beaches, which can allow dogs to access it. As seen in the model, if the probability a dog ingests offal, the R_0 increases, and transmission continues. In the case of where animals are culled, they are not inspected prior to being left on the pasture. As observed in chapter three, dogs will actively feed on these carcasses. As these are older animals, they are a greater risk of being infected with taeniid metacestodes, therefore increases the risk of transmission to dogs (Torgerson et al., 1998). There is also the possibility of dogs discovering animals that have died of natural causes throughout the year, where it would be impossible for farmers to find and dispose of their offal. By increasing the probability of dogs ingesting offal through these practices where offal is inadequately disposed of, the R_0 for *E. granulosus* is increased, as seen in the model. This is a direct controllable parameter which can be reduced through stricter adherence to the control measures already in place to further reduce the R_0 estimate and the taeniid parasites as a whole in the Falklands.

Dogs in the Falklands are dosed with praziquantel every five weeks as part of the control programme in place. Varied dosing success rates were observed in the model, with the dosing programme showing clear success in reducing R_0 and keeping the estimate below one. In the scenario where dosing efficacy was reduced through under dosing, the results showed that this was unlikely to be increasing R_0 above one, so is not thought to be a major contributing factor, though should the other probabilities of ingestion of eggs and offal be high, this does increase the R_0 estimate to close to one (0.848). The main issue arises if dosing is missed entirely. This results in any ingested protoscoleces that develop into adult worm to reach patency, significantly increasing the R_0. In the worst-case scenario, where probability of ingestion of eggs and offal are at their highest, the R_0 estimate could potentially reach above seven. Complete failure of dosing could occur through the regurgitation of tablets administered or resistance of *E. granulosus* in the Falklands. These possibilities were not investigated within this project, though could be looked into in the future, should it be necessary.

From the model, seven scenarios based on these three key model components resulted R_0 increasing above one, suggesting continued transmission of *E. granulosus* in the Falklands. These elements show key areas where control measures must be focused in order to reduce and consistently maintain R_0 below one. It is important to note, that R_0 in this model is not represented by a time unit, rather a generation interval that takes into account each stage of the life cycle, in this case the lifespan of the adult worm in a dog (nine months (Ziadinov et al., 2008)), the length of time eggs can survive on pasture (3.4 years (Thevenet et al., 2005)) as well as the lifespan of the intermediate host (sheep) which can vary, however this shows the length of time that the effort to maintain R_0 below one must be maintained, without any infectious outbreaks, for to successfully eradicate *E. granulosus*.

The objective of this model is to provide a simplified representation of the complex lifecycle of *E. granulosus* and make it as unique to the situation in the Falkland Islands as possible. This model does have some limitations, though it has been attempted to reduce these where possible. R_0, as described is an estimate of secondary infections in a susceptible population, assuming the entire population is susceptible. A more appropriate term is the effective reproduction number (R or Reff). This is similar to R_0, though it does not assume susceptibility of the entire population, allowing for the possibility of immunity (Delamater et al., 2019). In

the case of *E. granulosus* in the Falklands, the R_0 estimate, and the R number are likely to be similar, as there is little density dependant constraints on the spread of the parasite such as immunity or limited space for intermediate hosts. However, in the case of *T. hydatigena,* which is at a higher prevalence in the Falkland Islands and sheep could develop a level of immunity through previous exposure (Gemmell et al., 1990). This is something that, should *T. hydatigena* be investigated, the effective reproduction number should be considered.

The model has been developed by using single point estimates for each parameter, and therefore produces a single R_0 estimate for any given set of parameter values. This does not take into account the influence of stochasticity in *E. granulosus* transmission, especially in the face of intensive control measures. To account for this issue, the model could be developed such that the single point estimates for parameter values shown in Table 13 are replaced with distributions (potentially obtained from expert knowledge elicitation (European Food Safety Authority, 2014)). By using a Monte Carlo simulation approach with stochastic selection of individual parameter values, estimates of the full potential distribution of R_0 could be captured. By considering this variation in R_0, the interpretation can be moved away from a single "best guess" towards an estimate of the probability of R_0 exceeding one (and therefore allowing continued transmission). The model could then be used to explore the effect of changing the parameter distributions to observe whether R_0 can be brought consistently below one.

Spatial variation in *E. granulosus* infection has been previously found in dogs (Mastin et al., 2011) and sheep (Bosco, Alves, Cociancic, Amadesi, Pepe, Morgoglione, Maurelli, Ferrer-Miranda, Santoro, Nascimento Ramos, Rinaldi, & Cringoli, 2021), and is likely to play an important role in both transmission and control. This approach could be further expanded to consider spatial variation in R_0, which would be expected to be a particular issue for the dog to sheep and sheep to dog transmission stages. By dividing the landscape into grid cells and estimating R_0 for each, potential high-risk areas could be identified. This could include hypothetical hotspots of egg-contaminated pasture, accounting for the potential dispersal of eggs (which have been reported to move 115m from their deposition site (Sánchez Thevenet et al., 2019)), but also areas where canine access of sheep offal is more likely. More explicit consideration of spatial factors could also help to improve the parameterisation of some of the less certain parameters described above.

Another important feature of the *E. granulosus* transmission cycle is the presence of overdispersion in parasite numbers in both definitive and intermediate hosts, meaning that a small number of infected individuals contain a majority of individual parasites (Craig et al., 2015). As a result, these individual hosts can be responsible for a large proportion of the transmission, with important implications for parasite stability (Churcher, Ferguson, & Basáñez, 2005). Although the current model does not explicitly account for overdispersion, this could be captured to some degree when parameterising the stochastic and spatial formulations described above. Mathematical methods capable of capturing overdispersion are also available (Arakala, Hoover, Marshall, Sokolow, De Leo, Rohr, Remais, & Gambhir, 2018), which may have particular advantages for exploring the limits of stability in the presence of a control scheme. These would be worthy of future exploration within the context of *E. granulosus* in the Falkland Islands and other areas where eradication is a goal of control.

In summary, a framework has been developed which is able to capture the main biological processes of importance to spread of *E. granulosus* in the Falkland Islands. Although it was not possible to use field data in the model, this model identifies key parameters which may impact on R_0, and thus provides a tool to allow the Falkland Island government and Department of Agriculture to improve data collection around these parameters. It also allows the exploration of different circumstances that can either increase or decrease the R_0 estimate, to best advise policies and control measures moving forward in the campaign to eradicate *E. granulosus* from the Falkland Islands.

Chapter 7: Major findings in the investigation into the continued transmission of *Echinococcus granulosus* and other cestodes in the Falkland Islands.

The aim of this project was to explore the current situation in relation to Cystic Echinococcosis in the Falkland Islands; to evaluate whether transmission of the parasite between hosts is still occurring and to provide advice on how to proceed with the current control programme. The outcomes are best summarised by a series of questions.

1. Is transmission of *E. granulosus* to dogs still occurring?

Within the last ten years prior to the commencement of this study, the entire dog population in the Falkland Islands has been tested using copro-analysis techniques three times, in 2010, 2012 and 2014. A total of eight dogs were Copro-PCR positive in 2010 and six dogs were copro-ELISA positive in 2014, showing that in recent history, dogs have still become infected with *E. granulosus*. In this project, four dogs on different farms were suspected of being positive through copro-ELISA testing (though not confirmed by PCR), which suggests the possibility that a small number of dogs may still be getting infected. In addition to these, five farms showed evidence of the presence of *Echinococcus* coproantigens in the soil near to dog kennels. One of these farms also had a coproantigen positive dog.

For dogs to test positive, they must consume infected offal. Current laws in the Falkland Islands ban the feeding of offal to dogs, and so dogs must be getting access to offal by other means. Offal should be adequately disposed of in dog proof containers and stored for a minimum of 28 days before being discarded. In the early 1990s, methods of disposal primarily involved burying, burning, long term storage or dumping into the sea. During a study conducted at the time, 36% of the farms with copro-positive dogs deposited their offal from slaughter straight into the sea (Reichel et al., 1996). During the current study, a questionnaire found that 9% (2 of 23, see chapter 2.3.2) of farms who completed the survey still deposited untreated offal into the sea, with one of these farms having a copro-ELISA positive dog. This evidence shows that depositing offal into the sea is not a suitable method of disposal and risks the possibility of dogs gaining access to infective material, though offal washing back up on the beach or them accessing it at the deposition site. In addition, the generally higher,

more widespread prevalence of *Taenia hydatigena* in sheep indicates that dogs are also becoming infected with adult worms of this parasite whose larval stages are more likely to occur in intestinal mesentries of sheep. At slaughter on farms, stomachs and intestines are often dumped directly into the sea and would be a source of infection to dogs if consumed.

Further evidence of dogs accessing potentially infective material is through cull sites. Farmers in the Falklands cull older sheep that are no longer productive for wool and these animals are deposited on mass on the pasture in a cull site and left to breakdown. The carcasses are left whole, and no inspection of the offal or carcass takes place. As these are older animals, they are more likely to be infected with taeniid cestodes, so this practice increases the risk of transmission to dogs. In chapter 3.3.2, evidence of dogs feeding on these cull sites shows how dogs can encounter these carcasses, increasing the risk of transmission. The farm where the dog was seen feeding at a cull site also had a copro-ELISA positive dog in 2018 and two sheep with PCR confirmed hydatid cysts in 2020. Any dog which had been infected with *E. granulosus* is also likely to be infected with other taeniid cestodes. Between 2011 and 2020 the mean prevalence of *T. hydatigena* was 2.2% in sheep slaughtered at the abattoir. Of the four farms where Echionococcus copro-ELISA positive dogs were found, three of these farms had a *T. hydatigena* prevalence higher than the mean, ranging from 3.24% to 7.14%. As there were no Echinococcus PCR confirmed cases, it cannot be said for sure that these increases are as a result of the copro-ELSIA positive dogs, however it is evidence that control measures are not being adhered to sufficiently and transmission of cestode parasites is continuing via dogs on these farms. For these dogs to be able to transmit to sheep they must also have mature worms which are producing eggs. If the prescribed schedule for dosing with praziquantel every five weeks is adhered to and is successful production of adult worms should not be possible. The presence of potentially infected dogs and the widespread occurrence of larval cestodes in sheep indicated that in at least some cases dogs are not being dosed or that the dosing is ineffective. The current project did not investigate dosing on a farm-by-farm basis but is it is possible that this could be ineffective through dogs regurgitating tablets or inappropriate dosage in a dog weight basis.

These observations from the various aspects of the study focusing on definitive host infection show clear areas where control measures around offal disposal and dog control are not being adhered to, and the evidence suggests that infection is occurring in these areas as a result.

2. Is there an alternative hose for adult taeniid worms?

The population of feral cats on the Falkland Islands is large and there is clear evidence that these are frequent feeders at cull sites. However, in the small number of cats examined at autopsy there was no evidence of any taeniid infections other than *T. taeniaeformis* (transmitted from mice). Cats have been reported to show occasional low-level infection with *T. hydatigena* and *T. ovis* in other studies but *E. granulosus* and *T. ovis* do not develop well in cats (Borji et al., 2011; Hajipour et al., 2020; Konyaev et al., 2012). It is therefore unlikely that the distribution patterns of three larval cestode species is sheep could be explained by the presence of infected cats.

The presence of Patagonian foxes on Weddell Island was also considered to be a possibility for definitive hosts. In the current study none of the fox scats tested showed any evidence of taeniid infection. In addition, the distribution of infection of all three cestode species in sheep was not any higher in the farms on West Falkland nearest Weddell Island to indicate that this could be the source of any infections.

3. Is transmission of *E. granulosus* to sheep still occurring?

The presence of *E. granulosus* in sheep is still evident in the Falkland Islands, with two to five infected animals being discovered annually since 2014. Prior to this, no sheep were discovered with cysts at the abattoir in 2012 and 2013, and only one sheep was found infected in 2010 and 2011. The prevalence is very low however with a slight increase in recent years, with a total of 28 infected sheep identified at the abattoir since 2015. The parasite has only been detected on older sheep around six years + and if it is assumed that these were infected in the early stages of life (Torgerson et al., 1998) then the most recent infections could have taken place around 2013/15. As eggs can survive for several years in a climate similar to the Falklands (Sánchez Thevenet et al. (2019). The deposition of eggs from infected dogs in the environment may have taken place before this time. Results from previous dog studies have indicated that copro-positive dogs were identified in 2010 and 2014 and if truly positive for *Echinococcus* worms, could have been responsible for contamination of the environment and the infection of sheep.

In terms of comparison of the abattoir infection levels of the other taeniid parasites in adult sheep, it appears that all three species show higher prevalences in 2006/7 and 2013 onwards with much lower levels in the period between 2008 and 2012. This is evident both prevalence data and number of farms showing infections. Data on infection levels in lambs has only been available since 2011 but also shows distinct peaks for *T. hydatigena* and *T. ovis* in 2013/2015 accompanied a rise in adult infections with *T. hydatigena*. There was no evidence of *E. granulosus* infection in lambs although it is acknowledged that this would be very difficult to detect in young animals die to the size of the developing cysts. In individual farms consistently supplying sheep to the abattoir, there were slight differences in the timing of the infection peaks, but all generally occurred within 1-2 years of each other. The presence of infection peaks indicates the likelihood sporadic infection of a small number of dogs rather than frequent presence of infected definitive hosts. As described in chapter six, it is likely that increases in prevalence of *E. granulosus* in sheep could be related to the unlikely scenarios involving the presence of an infected dog, that result in the R_0 estimate increasing above one. This also suggests that another definitive host acting outside of the control programme, is not responsible because if this was the case, for example feral cats, then the prevalence would remain constant over time due to the regulatory effects of density dependant processes acting on the parasite population (Churcher, Filipe, & Basáñez, 2006).

Infection peaks or outbreaks of taeniid parasites such as *T. ovis* have been reported previously in other countries particularly when infection levels are generally low. In such situations an endemic state of taeniid infection characterised by infrequent exposure to the parasite implies that the infection cannot be controlled by naturally acquired immunity. This may result in cysticercosis 'storms' with sudden high infection rates (Eichenberger et al., 2011).

In terms of identifying farms where individual dogs could have been responsible for contaminating the environment with *T. hydatigena* eggs, the mapping analysis provided no clear indication of "hot spots" of sheep infection. The fact that some farms are very large means that the exact points where sheep are becoming infected would not be known. However, the presence of infected farms which are separated by great distances infers that either more than one dog in different locations, at any time point, is infected and/or that egg dispersal form one contaminated farm to others can occur relatively quickly and over

substantial distances. Wind has been known to play a part in dispersal of eggs, and the semi-arid windy climate of the Falkland Islands is likely to aid this dispersal. Studies have shown egg dispersal of over 10km in a short period of time (Lawson & Gemmell, 1983) and in some cases up to 60km (Torgerson et al., 1995). Dispersal by mechanical movement by humans, vehicles, birds and insects could also potentially occur in the Falklands, as it has been described elsewhere that these mechanisms play a role in transmission (Benelli et al., 2021). With the long-term survival of taeniid eggs and the potential for long distances of dispersal in the Falklands, these conditions may support the prolonged endemicity of taeniid infections in the Falkland Islands.

4. What are the risk factors which may be responsible for continued transmission of *E. granulosus* and other taeniid parasites?

As stated previously the present study has indicated that, on some farms, the Government regulations for control of Echinococcosis are not being fully upheld. In particular, inappropriate disposal of offal by dumping on the seashore was evident in a small number of farms. In addition, there was evidence that some dogs were not always under the control of the owners and were getting access to sheep carcases at cull sites. Cull sites in themselves represent a risk in that they are contain older animals which are more likely to be infected with these parasites. Scavenging by birds and cats is likely to increase the area over which potentially infective material could be spread and be accessed by dogs.

The risk of maintaining infection in sheep is dependent on the existence of egg producing adult worms in dogs. The longer they are infected, the greater the contamination of the environment would be. The continued presence of *T. hydatigena* in particular indicates that some dogs have not being appropriately dosed with praziquantel. Indications from analysis of *T. hydatigena* data suggests that the number of dogs per head of sheep on a farm was a risk factor positively correlated with infection of *T. hydatigena* in sheep. This means that the higher the number of dogs compared to the number of sheep on a farm, the increased likelihood of sheep being infected with *T. hydatigena.* This is an area where farmers must decide on the requirement of multiple dogs on their farm and whether this is worth the potential risk, as well as the extra labour of housing and dosing the dogs.

5. What recommendations for further control of taeniid parasites in the Falkland Islands could be made?

(i) Controlling dogs and their access to offal.

Over the course of this project, it has become evident that dogs are still involved in transmission of taeniid parasites in the Falklands. Dogs are still accessing infected offal, resulting in them becoming infected adult tapeworm. This is a key area where control measures are not being observed. As described, farms are still depositing offal including stomach and intestines into the sea. Previous studies have described this as being a risk factor associated with copro-antigen infected dogs and one of two farms in the current study disposing of offal like this had a copro-ELISA positive dog, and sheep infected with CE. This is a specific example of where the control programme needs updating to state not only how offal should be disposed, but a list of ways how offal should not be disposed of, eg. disposal on seashores.

There is also evidence of dogs accessing culled animals that have been left on the pasture. The practice of culling older animals in this way has been done for many years to manage stock numbers over winter. This is an important practice to maintain the health of the sheep population and ensure that the land can support those put out to pasture, however, this is also a major risk of transmission due to the older animals that have been killed being more likely to be infected with taeniid parasites and so leaving them uninspected and exposed on the pasture increases the chance of dogs coming into contact with them. Evidence in chapter 3.3.2 shows that given the opportunity, dogs will feed on these carcasses. This evidence exposes an example of where dogs have not been properly secured while not working, and so shows the risk of dogs becoming infected. As stated, culling is an important practice and part of Falkland's farming, however greater controls around this practice should be brought in to prevent the transmission of taeniids potentially infecting these sheep. This would include reducing the number of cull sites and the number of animals which are left there. Increasing the number of old animals sent to the abattoir would reduce the risk of dogs feeding on them. In addition, if animals are to be left at cull sites, then offal should be removed and disposed of appropriately beforehand.

(ii) Monitoring the dog population and dosing regimen.

As described, it is likely that dogs are becoming infected occasionally because of a combination of failures in the control programme happening simultaneously, resulting in transmission of *E. granulosus*. To monitor these occurrences, the dog population could/should be monitored on a more regular basis. Analysing the entire dog population by copro-analysis is a time consuming and labour-intensive task, as well as being a costly undertaking. A solution to this would be to monitor dogs on farms with a history of infected dogs and/or CE infections in sheep, as well as surrounding farms. Regular copro-analysis of dogs working on farms where recent CE infections in sheep have been identified will monitor areas where the risk of infection in dogs is higher. This could also be extended to neighbouring farms as well, as dispersal of eggs could result in infections in sheep beyond the targeted farms borders. A more focused approach to control at this stage of the eradication campaign can help isolate localised outbreaks, in a similar fashion to the techniques used in New Zealand. This involved restricting the movements of sheep from infected farms to only the abattoir, as well as the testing and dosing of dogs on these farms (Pharo, 2002). The development of Copro-ELISA techniques for soil samples also gives an alternative approach for identifying farms with infected dogs. The current study shows that the technique is feasible but it may be more useful to identify farms where dogs are infected with *T. hydatigena* as this is much more prevalent that *E. granulosus*.

Dogs are dosed every five weeks in the Falklands, under a very intensive regimen. During the New Zealand campaign, dog dosing was continued for almost 20 years before moving to a more targeted approach. Anthelmintic treatments have been in place in the Falklands for close to 60 years, with the only change in the frequency of dosing coming in 2010, reducing the interval from six weekly to five weekly dosing. In previous successful eradication campaigns like New Zealand, the frequency of dosing was reduced when the infections in dogs reduced significantly. Once the prevalence in dogs reached 0.01%, the control programme focussed dog treatments on farms where dogs still tested positive and where sheep were still being diagnosed with CE. This reduced the number of dogs being dosed by 70% allowing further resource to be applied in more surveillance based practices (Lawson, 1994). Following more regular testing of dogs, a similar approach could be taken in the

Falklands. Focussed dosing and/or reducing the frequency of dosing across the Falklands, helping move the eradication campaign towards a surveillance-based approach rather than the blanket intense dosing campaign currently in place. For dogs to become infected and produce eggs, the 5 weekly dosing must not have been effective or missed. From the results in chapter 6 the resulting effects on R_0 of a reduction in efficacy of praziquantel have been modelled and with an 11% reduction in efficacy the value of R_0 could rise from 0.34 to 0.82. In such a situation it may be more productive to have dosing of dogs carried out under veterinary supervision but at less frequent intervals (e.g. every three months) to ensure that dogs are dosed correctly at least in those farms with a history of Echinococcosis.

(iii) Slaughter surveillance and data collection

A vital aspect of any disease control programme is the accurate monitoring of the prevalence of disease. In the Falkland Islands, the only monitoring of taeniid infections in sheep occurs at the abattoir, however one third of all the animals slaughtered in the Falklands happen on farms. In 2020, approximately 20,350 sheep were slaughtered on farms for various reasons, however there is no disease data collected on these animals. This leaves a large gap in the prevalence data collected on taeniid infections in the Falklands, and as these animals are likely to be older animals, their risk of being infected is higher than those of a younger age slaughtered at the abattoir for meat (Torgerson et al., 1998). In a move towards a surveillance-based focus of control, data needs to be collected on all animals slaughtered, to get a more accurate prevalence of taeniid infections. This is especially important as cases of CE in sheep are so low that missing one or two cases can make a large difference especially when trying to focus control measures in a more localised approach. This would also require more education for farmers in knowing what to look for when inspecting offal and recording their findings. There is an obvious problem with this approach as it would rely of farmers to give an accurate and honest report back. However, the incorporation of community-based education sessions could emphasise the importance of honest reporting.

As described in chapter 4, the Sand Bay abattoir is an EU registered abattoir with up-to-date processing facilities and all animals slaughtered are inspected by a registered meat hygiene inspector and/or veterinarian. Following discussions with veterinarians and MHIs, it has been

mentioned that inspections are focussed on the offal still attached to the carcass, the inspection of stomach and intestines that is removed from the carcass down a different shoot is inspected when possible if there is time. As *T. hydatigena* is often found in the omentum, mesentery, and peritoneum of infected sheep (Scala et al., 2016) and therefore, with less focus on inspecting the stomach and intestines, cases could be missed, underestimating the prevalence of *T. hydatigena*. As shown in chapter 4.3, using *T. hydatigena* as a proxy for *E. granulosus* in data analysis, trends of infection were able to be observed in this similar parasite. If the prevalence is underrepresented, this may result in important trends not being discovered when analysing the data and restrict the use of such data.

Disease data is collated into annual reports by the abattoir, which were used to create the database for statistical analysis. As stated previously, this data is vital in monitoring infections and calculating prevalences both on a farm level and for the Falklands as a whole. Though a large database was able to be collated from multiple years of records, there were some difficulties in collating a combined database. Only the final ten years were broken down into age categories of animals slaughtered. Maintaining records on the disease status of animals at different ages is important in understanding the transmission dynamics of taeniid parasites, as infections in lambs suggest more recent contamination of pasture. It is vitally important that meat inspectors can accurately identify taeniid cysts in lambs. This is extremely difficult in the case if *E. granulosus* and ideally any suspect lesions should be submitted for molecular diagnosis by PCR. Sheep brought to the abattoir are separated and slaughtered in groups, described as lots. In multiple cases, the lot numbers did not add up to the total number of animals slaughtered. Small inaccuracies such as these make it difficult to trust the validity of the data, as these differences in the number of sheep killed in lots and the total number of animals slaughtered on the farm changes the prevalence estimates and so makes the data less reliable.

In the occasions where sheep are identified with CE at the abattoir, the cysts are sent to the Department of Agriculture for confirmation by microscopy. They are then added to a separate database held by the Department of Agriculture. While combining such data sets, it was apparent that in some cases, the abattoir adds these cases of CE to its report and sometimes does not. It was also apparent that when comparing both data sets, there were differences in the number of cases of CE identified. Reports showing different farms with cases of CE to

those confirmed by the Department of Agriculture and vice versa. This further reduces the reliability of the data as the differences between the data sets means an accurate number of cases cannot be established and so prevalence estimates are also less accurate. From this project, a database of all the taeniid infections in sheep over the last ten years for age separated slaughters and five years before that for all animals has now been created. This can be added to as a central database for taeniid infections diagnosed at the abattoir and can be expanded in future years to allow for accurate data to be stored and used for future analysis, which is vital when moving towards a surveillance-based approach.

Overall, the current study shows that the occurrence of *E. granulosus* in sheep at the abattoir is very low and likely to be confined to older sheep. However, nothing is known about the infection status of those sheep which are killed on farms. If similar prevalences occur in these animals as those taken to the abattoir, then it can be assumed that the overall numbers of infected sheep across the islands is relatively small. These scenarios, explained in chapter six, where transmission is occurring and pushing the R_0 estimate above one, can reset the clock on the time and effort required to drive *E. granulosus* to extinction in the Falkland Islands. The current study did not conclusively identify any infected dogs but did identify four suspect dogs which were coproantigen positive. It is, therefore, important to maintain close surveillance on infection levels in sheep over the next seven or eight years to establish if any of these suspect dogs could have contaminated the environment with eggs. If no sheep show any infection after this period, then complete eradication is likely.

Chapter 8: Future work and the global relevance of the project as a whole.

Throughout this project, it is evident that the control programme in place in the Falkland Islands has been successful in reducing the prevalence of *E. granulosus* in both the sheep and dog populations. However, the continued presence of a small number of infected sheep and possible infections in dogs is of concern. In addition, greater levels of infection in lambs and sheep with *Taenia ovis* and *T. hydatigena* indicates that viable eggs of these parasites are present in the environment and therefore that some dogs are getting access to infected sheep offal/ carcases in recent years. Future work could be done to provide further information on how dogs may gain access to such material and in particular the importance of animals which

are slaughtered on farms rather than the abattoir. There are a significant number of farms which only carry out home slaughter and information on the infection status of these animals is therefore unknown (32% of sheep were slaughtered on farms in 2020). Possible studies are as follows:

i) Collection of more detailed data on home slaughter activity. This would include, gaining further information through questionnaires or interviews on the number of animals slaughtered on farms and their individual uses (culling, home consumption, dog meat etc) as well as the frequency and seasonality of slaughter would be valuable to improve the surveillance and monitoring of disease. It would be particularly important for farm workers to examine and record the presence of taeniid larvae in the offal and carcases to the best of their ability. This would include the heart and intestines as well as liver and lungs. By gaining more information on the number of animals slaughtered on farms and the number of taeniid infections discovered, further correlation analysis could be carried out investigating the relationship between the number or frequency of slaughter and the prevalence of taeniid infection.

ii) Further investigations on disposal of sheep carcases/offal. Official legislation states that for all sheep that are slaughtered on farms, livers and lungs must be disposed of by burning or long-term storage in sealed containers. As *T. hydatigena* and *T.ovis* can occur in other tissues it is important to establish how organs such as intestines and hearts are disposed of. Establishing if these are accessible by dogs may help to explain the persistence of these parasites. In addition, it would be useful to establish the survivability of *T. hydatigena* and *T. ovis* cysticerci in sea water. This could be established by laboratory simulation using evagination and flame cell activity as measures of viability.

iii) It is important to establish the infection status of old animals deposited at cull sites. This would need to be carried out at targeted farms which have little information from the abattoir and be carried out by negotiation with farmers and veterinary/abattoir staff.

By utilising the extra information on frequency and seasonality of slaughter, it can also be used to focus dog dosing. Current measures implement dosing at five weekly intervals, which

is very intensive. In other control programmes such as Iceland, New Zealand and Cyprus, dosing was reduced after an initial intensive phase to less frequent and more focused dosing regimens. These countries focused dosing to periods where dogs and sheep interacted a lot or there was a major risk of transmission, such as following the slaughter season or after a sheep had been identified with hydatid cysts at abattoir inspection (Beard, 1973; Economides & Christofi, 2000; Lawson, 1994). By understanding potentially high-risk periods throughout the year, as well as high risk areas, the dosing programme could be reduced in the Falkland Islands and allow for more focussed dosing to be carried out by trained individuals.

Within the current project attempts were made to establish whether other definitive hosts *for E. granulosus* could be present in the Falkland Islands. The most likely of these would be the Patagonian Fox which is largely restricted to Weddell Island and surrounding smaller islands. Although the present copro-analysis and previous fox culls in 2007 have not indicated the presence of *E. granulosus*, it would be useful to gain a more solid confirmation that they are not involved.

iv) Weddell Island. It would be useful to carry out a more detailed study on Weddell Island to establish the size of the fox population through scat distribution mapping and coprological analysis. This could be supplemented by fox culls if possible.

v) Information on farming practice and prevalence of taeniid parasites in sheep for Weddell Island is limited to one year (2020). It would be important to determine if *E. granulosus* has ever been found by farmers in their sheep by interview. In 2020 approximately 5% of culled sheep had *T. hydatigena* yet there are currently no dogs on the island. A further understanding of the dog population over time would also help to understand egg contamination of the environment.

In relation to monitoring infection in dogs, the current study utilises assays which are highly specific for *E. granulosus*. The observed prevalence of this parasite is very low, and it may be more informative to be able to detect taeniid worms in general in dogs in order to understand more about routes of infection.

vi) Capture antibody coproantigen detection systems which are less specific could be developed by raising antisera in rabbits against *T. hydatigena* and *T. ovis* cysticerci obtained from the abattoir.

To continue the surveillance of infections in the intermediate host, the use of environmental sampling shown in chapter five would be a very useful tool. Being able to detect potential infections from farms without sampling and testing every dog would reduce the required labour, but also show potential hotspots of infection in intermediate host by identifying farms where dogs have potentially been infected. By taking soil samples from around kennel sites at more regular intervals could be a useful tool to monitor definitive host infections. Though it does not identify a time when a dog is infected, it will identify potentially at-risk farms and expose possible farms where control measures are not being strictly adhered to. The current study has shown the potential for this idea, but the approach needs further refinement.

vii) In farms where soil samples gave a positive reaction, it would be important to understand more about the spatial distribution of antigen in the immediate vicinity of the dog kennels. Transects utilising samples taken at different distances from the kennels could be used to define the validity of the results obtained and establish a strategy for routine sampling across different farms.

8.1 Global Relevance

As described in previous chapters, CE is globally distributed and predominantly impacts rural agricultural communities. The significant contribution that this thesis makes to understanding epidemiology and transmission lies, in particular, in the evaluation of infection levels in sheep over time and the monitoring of three related parasite species to provide an understanding of transmission mechanisms. It provides direct evidence of the longevity of parasite egg survival and the potential extent of egg distribution. To help reduce *E. granulosus* prevalence on a more global scale, control and surveillance measures used in successful campaigns described in this thesis must be used, but also evidence from this project has shown that some other tools may be useful in the surveillance of taeniid parasites. Using environmental sampling to identify previous infection on farms could be a tool to improve understanding of areas where definitive host infection is still occurring and help focus

measures such as dog dosing in these areas. In order to achieve a successful control campaign of parasites such as *E. granulosus*, traceability of infection in both hosts is vital. By identifying definitive host infection, it allows predictions of where hotspots of intermediate host infection may occur. It is also important, however, to have accurate and traceable information on the slaughter of animals and being able to identify a region or farm where infections are coming from. In the Falkland Islands, this has been useful as in the majority of cases, infected sheep can at the very least be traced to the farm from which they were sent to the abattoir, and in some cases, if this is not the farm of birth, the original farm can also be traced. This information is useful in isolating locations where infections in intermediate hosts are continuing and could be used in similar ways to what was done in New Zealand, where farms were quarantined, and animals were only allowed to be transferred to abattoirs for thorough inspection to take place. In countries where this traceability is possible, even to an area of farms, it can help focus control measures and attempt to isolate outbreaks of infection to prevent hyperendemic infection occurring and derailing the success of a control programme already in place. As seen in various infectious disease control programmes, contact tracing is vital in reducing spread of disease and so the ability of tracing the source of infective animals is a vital element of control of CE globally.

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Appendices

I. Appendix I: Chapter 2 material

Appendix I-a. Livestock management and dog ownership questionnaire administered to farmers in 2018.

Livestock Management & Dog Ownership Questionnaire

5. Serial Number:…… 2. Name of Farm:…… 3. Name of Farmer:……

4. Location of Farm (Circle one): East Falklands- [North] [South] [East] [West] [Central]

West Falklands- [North] [South] [East] [West] [Central]

Dog Ownership:

5. Do you own a dog? a. [Y]

b. [N]

6. How many dogs do you own? a. [1]

b. [2]

c. [3]

d. [4]

e. [More than 4 (Please specify)]

7. What is your dog's role? a. [Pet]

b. [Working animal]

c. [Both]

8. Where do you keep your dog? a. [In the house]

b. [Locked in Kennel]

c. [Tied up outside]

d. [Free roaming on property]

9. What do you feed your dog? a. [Processed dog food]

b. [Raw meat]

c. [Raw offal]

d. [Other (Please specify)]

Livestock Management:

10. Do you have Seasonal pastures? a. [Y]

b. [N]

11. How do you rotate these pastures? (Please explain)

12. What water sources do your sheep use to drink? a. [Natural streams/rivers]

b. [Drinking Troughs]

c. [Available standing water]

d. [All of the above]

Hydatid Control:

13. Where is the slaughter of your animals conducted? a. [Abattoir]

b. [Home Slaughter]

c. [Both]

14. Do you home slaughtered animals for use on your farm? a. [Y]

b. [N]

15. How many animals do you home slaughter per year? a. [0-20]

b. [21-40]

c. [41-60]

d. [61-80]

e. [More than 80 (please specify)]

16. What time of year do you home slaughter animals? a. [Spring]

b. [Summer]

c. [Autumn]

d. [Winter]

17. What do you do with offal? a. [Incinerate and bury]

b. [Throw in the oil drums]

c. [Boil]

d. [Feed to dogs]

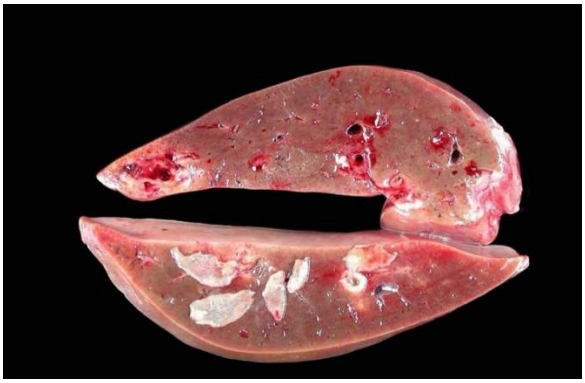
e. [Freeze]

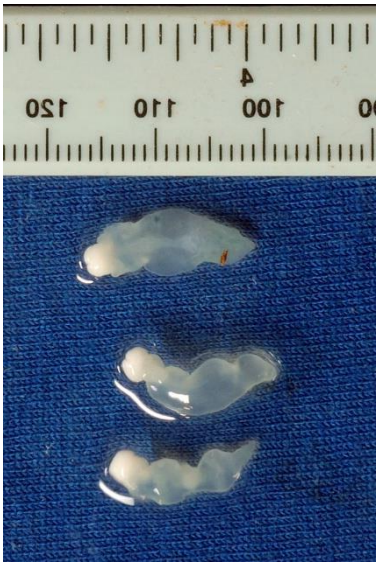
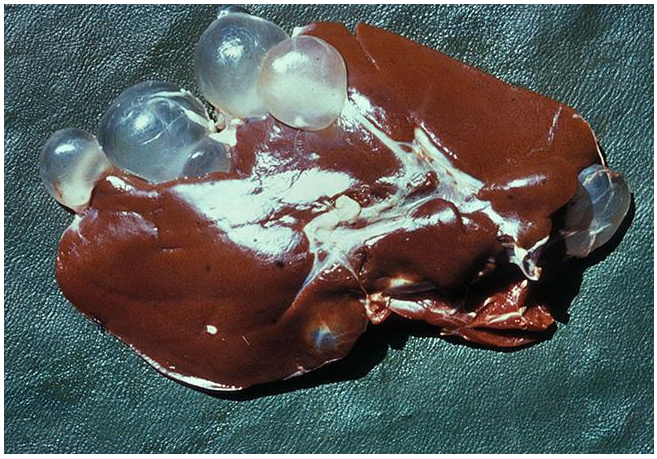
f. [Combination of above (Please explain)]

18. Do you know the difference between Hydatid cysts and *other Taeniid cestode* cysts? a.[Y]

b. [N]

19. Please identify the Hydatid cysts.

a.  b. 

c.  d. 

20. Have you seen cysts in any of the following? A. [Home slaughtered animals]

b. [Geese]

c. [Other (Please specify)]

21. Do you have a rodent population on your farm? a. [Y]

b. [N]

20. Do you have any questions, observations or comments you would like to make to the PhD student?

Please feel free to contact Dom via email d.west2@edu.salford.ac.uk

II. Appendix II: Chapter 3 material

Appendix II-a. O.D values for all samples of farm dogs.

Serial Code	Triplicate O.D value			Mean O.D value
EF101	0.1081	0.1033	0.1173	0.1096
EF102	0.1064	0.0934	0.1090	0.1029
EF103	0.1398	0.1315	0.1420	0.1378
EF104	0.0952	0.0936	0.1415	0.1101
EF105	0.0963	0.1073	0.1251	0.1096
EF106	0.0997	0.0914	0.1274	0.1062

EF107	0.1096	0.7866	0.6831	0.5264
EF108	0.0917	0.1215	0.1010	0.1047
EF109	0.0888	0.1222	0.0943	0.1018
EF110	0.2223	0.1120	0.1015	0.1453
EF201	0.1691	0.1392	0.1578	0.1554
EF202	0.0985	0.0964	0.0956	0.0968
EF203	0.0528	0.0467	0.0583	0.0526
EF204	0.0469	0.083	0.0442	0.0580
EF205	0.0695	0.0734	0.0836	0.0755
EF206	0.1348	0.151	0.1435	0.1431
EF207	0.077	0.098	0.1162	0.0971
EF208	0.0824	0.1104	0.0825	0.0918
EF209	0.1202	0.0995	0.1031	0.1076
EF210	0.1424	0.1622	0.1196	0.1414
EF211	0.082	0.0738	0.0855	0.0804
EF212	0.0753	0.0676	0.0761	0.0730
EF213	0.7286	0.2158	0.162	0.3688
EF214	0.1146	0.1074	0.1556	0.1259
EF215	0.1014	0.0859	0.0747	0.0873
EF216	0.0869	0.0832	0.0751	0.0817
EF302	0.0962	0.1039	0.0914	0.0972
EF401	0.925	0.1161	0.1123	0.3845
EF501	0.0717	0.0687	0.0962	0.0789
EF502	0.1422	0.1455	0.136	0.1412
EF503	0.0537	0.0647	0.0727	0.0637
EF601	0.0628	0.0967	0.074	0.0778
EF602	0.9114	0.0742	0.1534	0.3797
EF603	0.0988	0.0951	0.1279	0.1073
EF604	0.0694	0.0651	0.0877	0.0741
EF605	0.1117	0.1002	0.1021	0.1047
EF702	0.0863	0.0711	0.9684	0.3753
EF703	0.1057	0.0942	0.0712	0.0904
EF704	0.0806	0.0681	0.0651	0.0713
EF705	0.0707	0.8406	0.0522	0.3212
EF706	0.0666	0.0544	0.054	0.0583
EF707	0.0639	0.0619	0.0565	0.0608
EF708	1.1201	0.104	0.809	0.6777
EF709	0.0548	0.4982	0.0563	0.2031
EF710	0.0816	0.0765	0.0726	0.0769
EF711	0.0654	0.8531	0.0682	0.3289
EF712	0.0705	0.0707	0.071	0.0707
EF801	0.0846	0.0832	0.0683	0.0787
EF802	0.62	0.6692	0.0548	0.4480
EF901	0.0564	0.3378	0.0586	0.1509
EF902	0.0534	0.2816	0.0533	0.1294

EF903	0.0549	0.052	0.6988	0.2686
EF904	0.0776	0.0775	0.0797	0.0783
EF905	0.0522	0.0554	0.0538	0.0538
EF906	0.0522	0.6101	0.0603	0.2409
EF907	0.0706	0.3134	0.0704	0.1515
EF908	0.0531	0.2114	0.0611	0.1085
EF909	0.0541	0.0539	0.0695	0.0592
EF910	0.0604	0.0597	0.0698	0.0633
EF911	0.0609	0.0601	0.065	0.0620
EF912	0.0486	0.0564	0.2297	0.1116
EF913	0.0708	0.0595	1.1411	0.4238
EF914	0.0899	0.1035	1.0412	0.4115
EF915	0.0891	0.1787	0.0667	0.1115
EF916	0.0752	0.0693	0.066	0.0702
EF917	0.0639	0.1152	0.0669	0.0820
EF918	0.9334	0.9988	0.0658	0.6660
EF919	0.9328	1.6111	0.0588	0.8676
EF920	0.1869	0.0759	0.0781	0.1136
EF921	0.8302	0.0535	0.0547	0.3128
EF922	0.694	0.2009	0.055	0.3166
EF923	0.0559	0.5112	0.5028	0.3566
EF924	0.0382	0.0391	0.0493	0.0422
EF925	0.0396	0.5895	0.4004	0.3432
EF926	0.0542	0.4171	0.3093	0.2602
EF927	0.0551	0.0511	0.2227	0.1096
EF928	0.0559	0.4241	0.0567	0.1789
EF929	0.2898	0.0512	0.2171	0.1860
EF930	0.8467	0.0438	0.0571	0.3159
EF931	0.0507	0.0498	0.0387	0.0464
EF1001	0.5076	0.0425	0.0426	0.1976
EF1101	0.3523	0.0557	0.0579	0.1553
EF1102	0.4651	0.0791	0.0797	0.2080
EF1103	0.0651	0.7269	0.1213	0.3044
EF1104	0.0681	0.0795	0.0776	0.0751
EF1201	0.0751	0.0933	0.0933	0.0872
EF1202	0.0765	0.0728	0.0745	0.0746
EF1203	0.0640	0.0780	0.0622	0.0681
EF1301	0.0802	0.5061	0.8863	0.4909
EF1302	0.0705	0.6758	0.0589	0.2684
EF1303	0.9222	0.0773	0.0731	0.3575
EF1304	1.0312	0.0972	0.0951	0.4078
EF1305	0.1473	0.0783	0.0762	0.1006
EF1306	0.8485	0.0710	0.0777	0.3324
EF1307	0.7403	0.0571	0.0602	0.2859
EF1308	0.5478	0.0504	0.0479	0.2154

EF1309	0.0864	0.8919	0.0609	0.3464
EF1310	0.0657	0.7209	0.0619	0.2828
EF1401	0.1247	0.1089	0.1285	0.1207
EF1402	0.8587	0.1095	0.1408	0.3697
EF1403	0.7668	0.6294	0.1268	0.5077
EF1501	0.5576	0.0613	0.0685	0.2291
EF1502	0.9382	0.0890	0.1708	0.3993
EF1503	0.5443	0.0749	0.1013	0.2402
EF1504	0.1514	0.1608	0.2148	0.1757
EF1601	0.1062	0.2722	0.1081	0.1622
EF1602	0.4202	0.3257	0.3043	0.3501
EF1603	0.8140	0.0523	0.0533	0.3065
EF1604	0.1002	0.1439	0.1317	0.1253
EF1605	0.0651	0.0622	0.0626	0.0633
EF1701	0.0989	0.0788	0.0922	0.0900
EF1702	0.0883	0.0912	0.068	0.0825
EF1703	0.1588	0.1189	0.4969	0.2582
EF1704	0.1485	0.3453	0.1777	0.2238
EF1705	0.3272	0.1896	0.1159	0.2109
EF1706	0.1621	0.1287	0.1275	0.1394
EF1801	0.1609	0.1714	0.1672	0.1665
EF1802	0.3021	0.5752	0.2335	0.3703
EF1803	0.5647	0.4747	0.6541	0.5645
EF1804	0.2491	0.306	0.4483	0.3345
EF1805	0.9325	0.7678	0.7262	0.8088
EF1901	0.0887	0.0941	0.0833	0.0887
EF1902	0.0746	0.9764	0.0571	0.3694
EF1903	0.5573	0.0947	0.1353	0.2624
EF1904	0.5327	0.2838	0.0611	0.2925
EF1905	0.0645	0.1	0.0528	0.0724
EF1906	0.8409	0.0927	0.5664	0.5000
EF1907	0.077	0.0771	0.0729	0.0757
EF1908	0.152	0.1674	0.6748	0.3314
EF1909	0.264	0.1652	0.7088	0.3793
EF2001	0.097	0.1718	0.1265	0.1318
EF2002	0.1205	0.0636	0.3379	0.1740
EF2003	0.0635	0.0642	0.0568	0.0615
EF2004	0.0652	0.0648	0.0555	0.0618
EF2101	0.0616	0.5325	0.0607	0.2183
EF2102	0.0922	0.0975	0.0923	0.0940
EF2103	0.0675	0.066	0.0919	0.0751
EF2104	0.0666	0.0669	0.0626	0.0654
EF2105	0.0596	0.0601	0.0644	0.0614
EF2106	0.0588	0.0602	0.0575	0.0588
EF2201	0.0616	0.0622	0.3868	0.1702

EF2202	0.059	0.0615	0.0637	0.0614
EF2203	0.0651	0.0642	0.7606	0.2966
EF2204	0.0661	0.0717	0.0645	0.0674
EF2206	0.1093	0.094	0.8607	0.3547
EF2207	0.0572	0.0645	0.057	0.0596
EF2208	0.0912	0.0853	0.078	0.0848
EF2209	0.0728	0.0635	0.0951	0.0771
EF2210	0.0795	0.0549	0.0595	0.0646
EF2211	0.0814	0.0594	0.0714	0.0707
EF2212	0.083	0.0744	0.1324	0.0966
EF2213	0.0623	0.0621	0.9369	0.3538
EF2214	0.0556	0.0582	0.4005	0.1714
EF2215	0.0643	0.064	0.6854	0.2712
EF2216	0.0678	0.0699	0.2681	0.1353
EF2217	0.0756	0.0744	0.0878	0.0793
EF2218	0.0626	0.0509	0.0542	0.0559
EF2219	0.057	0.064	0.0531	0.0580
EF2220	0.0578	0.0599	0.0562	0.0580
EF2221	0.0595	0.058	0.0538	0.0571
EF2222	0.0592	0.0613	0.0571	0.0592
EF2223	0.0599	0.0485	0.06	0.0561
EF2224	0.0561	0.0578	0.0512	0.0550
EF2225	0.059	0.0591	0.0553	0.0578
EF2226	0.0527	0.0506	0.0546	0.0526
EF2227	0.0536	0.0549	0.0759	0.0615
EF2301	0.0447	0.0513	0.0418	0.0459
EF2302	0.0464	0.0433	0.0445	0.0447
EF2303	0.7662	0.0684	0.4872	0.4406
EF2401	0.3016	0.1794	0.4109	0.2973
EF2402	0.5242	0.0605	0.6268	0.4038
EF2403	0.2921	0.5313	0.0509	0.2914
EF2404	0.0469	0.0733	0.7742	0.2981
EF2405	0.0524	0.0557	0.0644	0.0575
EF2406	0.4074	0.0566	0.0595	0.1745
EF2407	0.054	0.0531	0.0553	0.0541
EF2408	0.072	0.0677	0.3789	0.1729
EF2409	0.0507	0.0502	0.2773	0.1261
EF2410	0.0503	0.0673	0.5929	0.2368
EF2501	0.0507	0.065	0.1902	0.1020
EF2502	0.0961	0.0963	0.0913	0.0946
EF2503	0.0978	0.0906	0.0847	0.0910
EF2504	0.1446	0.1371	0.1397	0.1405
EF2505	0.1096	0.1164	0.1021	0.1094
EF2506	0.2239	0.2449	0.1846	0.2178
EF2507	0.1446	0.169	0.1398	0.1511

EF2508	0.0741	0.0664	0.0765	0.0723
EF2601	0.1511	0.1403	0.1372	0.1429
EF2602	0.0919	0.1121	0.1008	0.1016
EF2603	0.154	0.1542	0.1684	0.1589
EF2604	0.1138	0.1379	0.145	0.1322
EF2702	0.0948	0.0994	0.1098	0.1013
EF2801	0.0541	0.055	0.0571	0.0554
EF2802	0.1242	0.1417	0.0816	0.1158
EF2803	0.1116	0.1067	0.0977	0.1053
EF2901	0.1051	0.1137	0.0977	0.1055
EF2902	0.1215	0.0713	0.5595	0.2508
EF2903	0.1117	0.0993	0.1138	0.1083
EF2904	0.0485	0.0468	0.0733	0.0562
EF2905	0.0717	0.0682	0.0753	0.0717
EF2906	0.1184	0.1135	0.1473	0.1264
EF2907	0.1151	0.1234	0.1429	0.1271
EF2908	0.135	0.1381	0.1346	0.1359
EF2909	0.1261	0.1473	0.124	0.1325
EF2910	0.0470	0.0499	0.0513	0.0494
EF2911	0.0600	0.0632	0.0787	0.0673
EF3001	0.0494	0.0534	0.0565	0.0531
EF3002	0.0579	0.0602	0.0673	0.0618
EF3003	0.0559	0.0598	0.0648	0.0602
EF3004	0.1206	0.0689	0.0931	0.0942
EF3005	0.0675	0.0577	0.0671	0.0641
EF3006	0.0510	0.0482	0.0514	0.0502
Ef3007	0.0717	0.0685	0.0716	0.0706
EF3101	0.1563	0.1587	0.1621	0.1590
EF3102	0.1311	0.1452	0.1383	0.1382
EF3103	0.0956	0.0994	0.1050	0.1000
EF3104	0.1074	0.0896	0.1368	0.1113
EF3105	0.2908	0.1822	0.2079	0.2270
EF3201	0.9858	0.1003	0.1005	0.3955
EF3202	0.1320	0.1165	0.0932	0.1139
WF101	0.0597	0.0538	0.0543	0.0559
WF102	0.0655	0.073	0.0729	0.0705
WF103	0.0786	0.0755	0.0704	0.0748
WF104	0.0769	0.0747	0.0653	0.0723
WF105	0.0586	0.0616	0.0524	0.0575
WF201	0.0499	0.0581	0.0501	0.0527
WF301	0.176	0.0553	0.0623	0.0979
WF302	0.0615	0.0584	0.0646	0.0615
WF401	0.0697	0.4103	0.0876	0.1892
WF402	0.3974	0.363	0.0667	0.2757
WF501	0.1965	0.0996	0.0716	0.1226

WF502	0.2094	1.0733	0.1252	0.4693
WF503	0.5057	0.5882	0.0455	0.3798
WF504	0.3909	0.0505	0.3146	0.2520
WF505	0.076	0.5035	0.0713	0.2169
WF506	0.1949	0.4068	0.0652	0.2223
WF601	0.1296	0.061	0.0574	0.0827
WF701	0.2106	0.1893	1.0776	0.4925
WF801	0.0526	0.0482	0.0502	0.0503
WF802	0.0507	0.051	0.0536	0.0518
WF803	0.0528	0.0503	0.0422	0.0484
WF805	0.0603	0.0602	0.0613	0.0606
WF806	0.0586	0.0518	0.0461	0.0522
WF807	0.0545	0.0512	0.0528	0.0528
WF901	0.1263	0.1351	0.1471	0.1362
WF902	0.0826	0.1191	0.0865	0.0961
WF903	0.0731	0.0666	0.0828	0.0742
WF904	0.0673	0.0678	0.0846	0.0732
WF1001	0.0654	0.0705	0.0797	0.0719
WF1002	0.0792	0.0790	0.0817	0.0800
WF1102	0.0709	0.0700	0.0774	0.0728
WF1103	0.0806	0.0696	0.0741	0.0748
WF1104	0.0746	0.0537	0.0642	0.0642
WF1105	0.0652	0.0631	0.0581	0.0621
WF1106	0.0660	0.0547	0.0670	0.0626
WF1107	0.0597	0.0578	0.0589	0.0588
WF1108	0.0703	0.0729	0.0698	0.0710
WF1109	0.0696	0.0798	0.0832	0.0775
WF1110	0.0534	0.0510	0.0566	0.0537
WF1111	0.0532	0.0589	0.0636	0.0586
WF1112	0.0709	0.0771	0.0653	0.0711
WF1201	0.0597	0.0628	0.0629	0.0618
WF1202	0.0808	0.1107	0.1103	0.1006
WF1203	0.0927	0.0803	0.0906	0.0879
WF1204	0.0734	0.0657	0.0740	0.0710
WF1205	0.0690	0.0678	0.0694	0.0687
WF1206	0.0780	0.0730	0.0703	0.0738
WF1207	0.0768	0.0844	0.0747	0.0786
WF1301	0.0776	0.0837	0.0797	0.0803
WF1302	0.0711	0.0523	0.0555	0.0596
WF1303	0.0918	0.0949	0.0883	0.0917
WF1304	0.0556	0.0530	0.0527	0.0538
WF1305	0.0676	0.1450	0.0710	0.0945
WF1401	0.0669	0.0652	0.0600	0.0640
WF1402	0.0613	0.0604	0.0614	0.0610
WF1403	0.0645	0.0564	0.0609	0.0606

WF1404	0.0568	0.0611	0.0617	0.0599
WF1405	0.0520	0.0566	0.0485	0.0524
WF1406	0.0532	0.1459	0.0603	0.0865
WF1407	0.0551	0.0580	0.0609	0.0580
WF1409	0.0616	0.0595	0.0566	0.0592
WF1410	0.1185	0.0895	0.0850	0.0977
WF1411	0.0586	0.0570	0.0572	0.0576
WF1413	0.6053	0.0612	0.0603	0.2423
WF1414	0.0573	0.0572	0.0576	0.0574
WF1419	0.0593	0.0620	0.0567	0.0593
WF1433	0.0477	0.0421	0.0449	0.0449
WF1415	0.0620	0.0643	0.0714	0.0659
WF1416	0.0767	0.0649	0.0697	0.0704
WF1417	0.0695	0.0790	0.0684	0.0723
WF1418	0.0610	0.0612	0.0568	0.0597
WF1420	0.0487	0.0471	0.0418	0.0459
WF1421	0.0631	0.0596	0.0566	0.0598
WF1422	0.0501	0.0494	0.0504	0.0500
WF1425	0.0512	0.0443	0.0459	0.0471
WF1429	0.0582	0.0506	0.0407	0.0498
WF1430	0.0531	0.0554	0.0514	0.0533
WF1431	0.0581	0.0547	0.0545	0.0558
WF1443	0.0541	0.0443	0.0560	0.0515
WF1432	0.0519	0.0394	0.0401	0.0438
WF1434	0.0480	0.0406	0.0481	0.0456
WF1435	0.0517	0.0489	0.0489	0.0498
WF1426	0.0495	0.0491	0.0468	0.0485
WF1412	0.0625	0.0566	0.0571	0.0587
WF1438	0.0522	0.0452	0.0436	0.0470
WF1439	0.0498	0.0547	0.0510	0.0518
WF1440	0.0686	0.0596	0.0585	0.0622
WF1441	0.0459	0.0477	0.0510	0.0482
WF1442	0.0579	0.0539	0.0530	0.0549
WF1501	0.0457	0.0426	0.0454	0.0446
WF1503	0.0521	0.0459	0.0584	0.0521
WF1504	0.0589	0.0556	0.0645	0.0597
WF1505	0.0763	0.0526	0.0503	0.0597
WF1506	0.0516	0.0530	0.2691	0.1246
WF1507	0.0573	0.0514	0.0559	0.0549
WF1601	0.0498	0.0533	0.0518	0.0516
WF1602	0.0497	0.0510	0.0498	0.0502
WF1603	0.0479	0.0477	0.0480	0.0479
WF1604	0.0499	0.0507	0.0536	0.0514
WF1605	0.0562	0.0578	0.0587	0.0576
WF1606	0.0556	0.0554	0.0545	0.0552

WF1607	0.0496	0.0516	0.0499	0.0504
WF1608	0.0543	0.0530	0.0538	0.0537
WF1609	0.0465	0.0501	0.0493	0.0486
WF1610	0.0515	0.0521	0.0437	0.0491
WF1611	0.0397	0.0508	0.0507	0.0471
WF1612	0.0437	0.0570	0.1301	0.0769
WF1701	0.0525	0.0417	0.0505	0.0482
WF1702	0.0513	0.0522	0.0511	0.0515
WF1703	0.0487	0.0485	0.0492	0.0488
WF1704	0.0545	0.0617	0.0498	0.0553
WF1705	0.0514	0.0570	0.1239	0.0774
WF1706	0.0470	0.0507	0.0487	0.0488
WF1707	0.0545	0.0403	0.1412	0.0787
WF1708	0.0528	0.0521	1.3297	0.4782
WF1709	0.0495	0.0817	0.0508	0.0607
WF1710	0.0512	0.0567	0.8502	0.3194
WF1711	0.2495	0.0508	0.0505	0.1169
WF1801	0.0646	0.0626	0.5363	0.2212
WF1802	0.0390	0.0469	0.0498	0.0452
WF1803	0.0508	0.0559	0.0520	0.0529
WF1901	0.0765	0.0612	0.0889	0.0755
WF2001	0.0651	0.0586	0.0699	0.0645
WF2002	0.0591	0.0555	0.0626	0.0591
WF2003	0.0525	0.0478	0.0594	0.0532
WF2101	0.0496	0.0530	0.0488	0.0505
WF2102	0.0532	0.0579	0.0475	0.0529
WF2103	0.0764	0.0885	0.0759	0.0803
WF2104	0.1144	0.1217	0.1152	0.1171
WF2105	0.0559	0.0575	0.0612	0.0582
WF2106	0.0783	0.0771	0.0768	0.0774
WF2201	0.0534	0.0503	0.0613	0.0550
WF2203	0.0459	0.0527	0.0575	0.0520
WF2204	0.0472	0.0556	0.0571	0.0533
WF2205	0.0471	0.0554	0.0561	0.0529
WF2206	0.0522	0.0559	0.0427	0.0503
WF2207	0.0615	0.0619	0.0455	0.0563
WF2208	0.0569	0.0604	1.0561	0.3911
WF2209	0.0922	0.0660	1.0178	0.3920

Appendix II-b. O.D values for all samples of pet dogs.

Serial Code		Triplicate O.D value			Mean O.D value
1	/18	0.1924	0.0715	0.6744	0.3128
2	/18	0.0732	0.0605	0.0658	0.0665

3	/18	0.7312	0.0595	0.2289	0.3399
4	/18	0.5592	0.0497	0.1482	0.2524
5	/18	0.0528	0.0541	0.0563	0.0544
6	/18	0.0594	0.0534	0.0606	0.0578
7	/18	0.0702	0.0664	0.0662	0.0676
8	/18	0.0615	0.0565	0.0554	0.0578
9	/18	0.0703	0.0692	0.0724	0.0706
10	/18	0.0713	0.0584	0.0636	0.0644
11	/18	0.0594	0.0519	0.0496	0.0536
12	/18	0.0718	0.0680	0.0644	0.0681
13	/18	0.0840	0.0673	0.0718	0.0744
14	/18	0.0715	0.0737	0.0753	0.0735
15	/18	0.0601	0.0605	0.0645	0.0617
16	/18	0.0617	0.0568	0.0604	0.0596
17	/18	0.0574	0.0595	0.0578	0.0582
18	/18	0.0627	0.0615	0.6102	0.2448
19	/18	0.0679	0.0849	1.3209	0.4912
20	/18	0.0530	0.0700	0.4373	0.1868
21	/18	0.0650	0.0721	0.1936	0.1102
22	/18	0.0629	0.0708	0.5300	0.2212
23	/18	0.0695	0.0557	0.0635	0.0629
24	/18	0.0831	0.0538	0.0566	0.0645
25	/18	0.4000	0.0702	0.0677	0.1793
26	/18	0.2533	0.0638	0.0619	0.1263
27	/18	0.3834	0.0692	0.0710	0.1745
28	/18	1.0128	0.0533	0.0579	0.3747
29	/18	0.0432	0.0432	0.0418	0.0427
30	/18	0.1486	0.1577	0.1119	0.1394
31	/18	0.2641	0.1854	0.1644	0.2046
32	/18	0.3335	0.2841	0.2956	0.3044
33	/18	0.0719	0.0581	0.0566	0.0622
34	/18	0.0552	0.0508	0.0470	0.0510
35	/18	0.0390	0.0355	0.0401	0.0382
36	/18	0.0430	0.0511	0.0438	0.0460
37	/18	0.0431	0.0452	0.0486	0.0456
38	/18	0.0520	0.0772	0.0781	0.0691
39	/18	0.0549	0.0697	0.0658	0.0635
40	/18	0.0838	0.0847	0.0744	0.0810
41	/18	0.0328	0.0315	0.0368	0.0337
42	/18	0.0400	0.0409	0.0396	0.0402
43	/18	0.0397	0.0443	0.2708	0.1183
44	/18	0.0569	0.0560	0.0439	0.0523

45	/18	0.0678	0.0617	0.0518	0.0604
46	/18	0.0558	0.0511	0.0550	0.0540
47	/18	0.0837	0.0828	0.0968	0.0878
48	/18	0.1137	0.1158	0.1328	0.1208
49	/18	0.1169	0.1092	0.1049	0.1103
50	/18	0.2113	0.2024	0.1770	0.1969
51	/18	0.1458	0.1244	0.1144	0.1282
52	/18	0.1944	0.1655	0.1319	0.1639
53	/18	0.1179	0.1328	0.1542	0.1350
54	/18	0.0942	0.0938	0.5599	0.2493
55	/18	0.0982	0.0967	0.1049	0.0999
56	/18	0.1438	0.1307	0.1645	0.1463
57	/18	0.1063	0.0976	0.1392	0.1144
58	/18	0.0896	0.0855	0.6089	0.2613
59	/18	0.1905	0.1761	0.2951	0.2206
60	/18	0.1215	0.1261	0.1338	0.1271
61	/18	0.1184	0.1313	0.1330	0.1276
62	/18	0.1701	0.1766	0.1655	0.1707
63	/18	0.1432	0.1519	0.1588	0.1513
64	/18	0.1216	0.1265	0.1410	0.1297
65	/18	0.1890	0.1912	0.1595	0.1799
66	/18	0.1442	0.1451	0.1505	0.1466
67	/18	0.1743	0.1716	0.2326	0.1928
68	/18	0.1235	0.1259	0.7632	0.3375
69	/18	0.0925	0.0961	0.2524	0.1470
70	/18	0.1418	0.1355	0.1640	0.1471
71	/18	0.1167	0.1154	0.1349	0.1223
72	/18	0.1402	0.1312	0.1267	0.1327
73	/18	0.1393	0.1141	0.4445	0.2326
74	/18	0.1618	0.1362	0.3976	0.2319
75	/18	0.1316	0.0692	0.0634	0.0881
76	/18	0.0798	0.0645	0.0618	0.0687
77	/18	0.1054	0.0902	0.1490	0.1149
78	/18	0.1028	0.1059	0.1230	0.1106
79	/18	0.1418	0.1656	0.1207	0.1427
80	/18	0.1242	0.1213	0.1400	0.1285
81	/18	0.1455	0.1058	0.1697	0.1403
82	/18	0.0676	0.0720	0.0790	0.0729
83	/18	0.0774	0.0805	0.0731	0.0770
84	/18	0.1246	0.1267	0.1372	0.1295
85	/18	0.1093	0.1207	0.2529	0.1610
86	/18	0.4560	0.1148	0.1105	0.2271

87	/18	0.0809	0.0860	0.0817	0.0829
88	/18	0.2724	0.1036	0.1119	0.1626
89	/18	0.1170	0.1064	0.1217	0.1150
90	/18	0.1048	0.0981	0.0988	0.1006
91	/18	0.1073	0.1159	0.1197	0.1143
92	/18	0.3921	0.1066	0.1060	0.2016
93	/18	0.1179	0.1089	0.1137	0.1135
94	/18	0.1169	0.2908	0.1023	0.1700
95	/18	0.1688	0.1905	0.1831	0.1808
96	/18	0.2062	0.1605	0.1723	0.1797
97	/18	0.1890	0.1735	0.1857	0.1827
98	/18	0.1990	0.1835	0.1818	0.1881
99	/18	0.1877	0.1794	0.1490	0.1720
100	/18	0.1992	0.1960	0.1652	0.1868
101	/18	0.1614	0.1684	0.1725	0.1674
102	/18	0.1490	0.1752	0.1527	0.1590
103	/18	0.1317	0.1508	0.1284	0.1370
104	/18	0.1438	0.1386	0.1364	0.1396
105	/18	0.1628	0.1697	0.1666	0.1664
106	/18	0.1612	0.1508	0.1468	0.1529
107	/18	0.1878	0.1773	0.1839	0.1830
108	/18	0.1968	0.2005	0.1945	0.1973
109	/18	0.1836	0.1931	0.1625	0.1797
110	/18	0.1625	0.1866	0.1766	0.1752
111	/18	0.1085	0.1365	0.1337	0.1262
112	/18	0.1490	0.1389	0.1387	0.1422
113	/18	0.1262	0.1227	0.1205	0.1231
114	/18	0.1321	0.1287	0.1290	0.1299
115	/18	0.1569	0.1572	0.1666	0.1602
116	/18	0.2489	0.2482	0.2492	0.2488
117	/18	0.2139	0.2092	0.2128	0.2120
118	/18	0.1208	0.1178	0.1010	0.1132
119	/18	0.1474	0.1505	0.1462	0.1480
120	/18	0.1043	0.1035	0.1020	0.1033
121	/18	0.1418	0.1457	0.1396	0.1424
122	/18	0.2474	0.2459	0.2398	0.2444
123	/18	0.2477	0.2689	0.2530	0.2565
124	/18	0.2252	0.2281	0.2344	0.2292
125	/18	0.1683	0.1640	0.1894	0.1739
126	/18	0.1817	0.1745	0.3620	0.2394
127	/18	0.1986	0.1777	0.2218	0.1994
128	/18	0.2005	0.1983	0.1998	0.1995

129	/18	0.2076	0.1948	0.2099	0.2041
130	/18	0.2217	0.2417	0.4974	0.3203
131	/18	0.3343	0.1694	0.8691	0.4576
132	/18	0.1847	0.3092	0.8952	0.4630
133	/18	0.1697	0.1573	0.1835	0.1702
134	/18	0.1777	0.1748	0.1894	0.1806
135	/18	0.3828	0.1592	0.1225	0.2215
136	/18	0.1989	0.2015	0.2022	0.2009
137	/18	0.1988	0.9604	0.1900	0.4497
138	/18	0.2321	0.2209	0.2408	0.2313
139	/18	0.2781	0.1328	0.1293	0.1801
140	/18	0.0758	0.0849	0.0734	0.0780
141	/18	0.1680	0.5985	0.1497	0.3054
142	/18	0.1516	0.3590	0.1044	0.2050
143	/18	0.0957	0.0917	0.0942	0.0939
144	/18	0.0784	0.0680	0.0677	0.0714
145	/18	0.1126	0.0998	0.1047	0.1057
146	/18	0.1035	0.1008	0.1070	0.1038
147	/18	0.1526	0.1383	0.1371	0.1427
148	/18	0.1849	0.1354	0.1419	0.1541
149	/18	0.0852	0.0855	0.0809	0.0839
150	/18	0.0833	0.0896	0.0789	0.0839
151	/18	0.1431	0.1268	0.1254	0.1318
152	/18	0.1920	0.1813	0.1900	0.1878
153	/18	0.0674	0.0550	0.0640	0.0621
154	/18	0.0747	0.0670	0.0630	0.0682
155	/18	0.0747	0.0694	0.3820	0.1754
156	/18	0.0860	0.0735	0.3470	0.1688
157	/18	0.1183	0.0800	0.0723	0.0902
158	/18	0.0975	0.0904	0.2089	0.1323
159	/18	0.0584	0.0827	0.8018	0.3143
160	/18	0.5366	0.0975	0.8747	0.5029
161	/18	0.5644	0.1154	0.8241	0.5013
162	/18	0.4116	0.3372	0.0807	0.2765
163	/18	0.4572	0.1488	0.0810	0.2290
164	/18	0.6243	0.5808	0.0955	0.4335
165	/18	0.2829	0.0740	0.3272	0.2280
166	/18	0.1394	0.2521	0.1602	0.1839
167	/18	0.0762	0.0984	0.0921	0.0889
168	/18	0.2738	0.1202	0.7470	0.3803
169	/18	0.1744	0.1377	0.9856	0.4326
170	/18	0.1918	0.9510	0.3667	0.5032

171	/18	0.1678	0.4471	0.0717	0.2289
172	/18	0.7161	0.0653	0.0643	0.2819
173	/18	0.1061	0.2231	0.0831	0.1374
174	/18	0.1115	0.1930	0.0940	0.1328
175	/18	0.0868	0.4253	0.0839	0.1987
176	/18	0.0885	0.3447	0.0765	0.1699
177	/18	0.0888	0.0855	0.0771	0.0838
178	/18	0.0900	0.0872	0.0743	0.0838
179	/18	0.0829	0.0786	0.0752	0.0789
180	/18	0.0873	0.0747	0.0710	0.0777
181	/18	0.0907	0.0857	0.0816	0.0860
182	/18	0.0695	0.0602	0.0622	0.0640
183	/18	0.1579	0.0864	0.5553	0.2665
184	/18	0.4110	0.0612	0.3591	0.2771
185	/18	0.1218	0.0807	0.3344	0.1790
186	/18	0.0809	0.0637	0.2191	0.1212
187	/18	0.0728	0.5601	0.0651	0.2327
188	/18	0.1545	0.7188	0.0721	0.3151
189	/18	0.3684	0.0467	0.3034	0.2395
190	/18	0.6559	0.0619	0.2322	0.3167
191	/18	0.0821	0.0746	0.2204	0.1257
192	/18	0.0698	0.0671	0.0772	0.0714
193	/18	0.0985	0.1028	0.0864	0.0959
194	/18	0.1408	0.0897	0.0874	0.1060
195	/18	0.0533	0.0521	0.2617	0.1224
196	/18	0.0620	0.0583	0.4643	0.1949
197	/18	0.1292	0.0580	0.0567	0.0813
198	/18	0.0732	0.0719	0.2087	0.1179
199	/18	0.0892	0.0809	0.1704	0.1135
200	/18	0.0775	0.0754	0.1358	0.0962
201	/18	0.1070	0.1173	0.1143	0.1129
202	/18	0.1146	0.1069	0.0912	0.1042
203	/18	0.0905	0.0844	0.0798	0.0849
204	/18	0.1307	0.1242	0.1006	0.1185
205	/18	0.0794	0.0774	0.0802	0.0790
206	/18	0.0994	0.0935	0.1030	0.0986
207	/18	0.1106	0.1158	0.1216	0.1160
208	/18	0.0640	0.0682	0.0646	0.0656
209	/18	0.0643	0.0609	0.0607	0.0620
210	/18	0.1462	0.1391	0.1305	0.1386
211	/18	0.1111	0.0939	0.0935	0.0995
212	/18	0.1100	0.0962	0.1040	0.1034

213	/18	0.1167	0.1199	0.1136	0.1167
214	/18	0.0625	0.0562	0.0478	0.0555
215	/18	0.0579	0.0544	0.0546	0.0556
216	/18	0.0737	0.0629	0.0722	0.0696
217	/18	0.0956	0.0922	0.0928	0.0935
218	/18	0.1432	0.1352	0.1457	0.1414
219	/18	0.0926	0.0977	0.4906	0.2270
220	/18	0.0472	0.0609	0.7028	0.2703
221	/18	0.0667	0.0656	0.0653	0.0659
222	/18	0.0760	0.0768	0.0918	0.0815
223	/18	0.0693	0.0790	0.0835	0.0773
224	/18	0.1093	0.1108	0.4622	0.2274
225	/18	0.1271	0.1052	0.1041	0.1121
226	/18	0.0800	0.0752	0.0755	0.0769
227	/18	0.0771	0.0736	0.0684	0.0730
228	/18	0.1026	0.1024	0.0955	0.1002
229	/18	0.0627	0.0557	0.0553	0.0579

Appendix II-c. O.D values for all samples of retested samples.

Serial Code	Triplicate O.D value			Mean O.D value
EF213	0.2041	0.1945	0.1849	0.1945
EF602	0.1486	0.1360	0.1387	0.1411
EF702	0.1088	0.1123	0.1027	0.1079
EF705	0.0673	0.0658	0.0626	0.0652
EF708	0.1292	0.1274	0.1266	0.1277
EF709	0.0931	0.0925	0.0899	0.0918
EF711	0.0803	0.0844	0.0810	0.0819
EF802	0.0995	0.0957	0.0937	0.0963
EF901	0.0963	0.0995	0.0970	0.0976
EF902	0.0624	0.0594	0.0602	0.0607
EF903	0.0671	0.0777	0.0671	0.0706
EF906	0.0846	0.0882	0.0852	0.0860
EF907	0.1130	0.1238	0.1207	0.1192
EF912	0.0908	0.0963	0.0981	0.0951
EF913	0.0602	0.0687	0.0705	0.0665
EF914	0.0622	0.0675	0.0733	0.0677
EF918	0.1064	0.1000	0.0897	0.0987
EF922	0.0761	0.0753	0.0671	0.0728
EF926	0.0941	0.0959	0.0801	0.0900
EF927	0.0711	0.0654	0.0609	0.0658
EF928	0.1217	0.1144	0.1092	0.1151
EF930	0.0604	0.0616	0.0608	0.0609

EF1001	0.0667	0.0731	0.0714	0.0704
EF1101	0.0689	0.0691	0.0681	0.0687
EF1103	0.0984	0.1045	0.0910	0.0980
EF1302	0.0894	0.0955	0.0920	0.0923
EF1303	0.0861	0.0816	0.0851	0.0843
EF1304	0.1058	0.1043	0.0974	0.1025
EF1306	0.0850	0.0822	0.0822	0.0831
EF1307	0.0721	0.0735	0.0710	0.0722
EF1308	0.0992	0.0960	0.0906	0.0953
EF1309	0.0683	0.0681	0.0643	0.0669
EF1301	0.0618	0.0670	0.0661	0.0650
EF1402	0.1178	0.1178	0.1182	0.1179
EF1501	0.1003	0.1041	0.1047	0.1030
EF1502	0.1700	0.0766	0.0724	0.1063
EF1503	0.0685	0.0672	0.0706	0.0688
EF1504	0.1382	0.1286	0.1387	0.1352
EF1601	0.1163	0.1233	0.1186	0.1194
EF1602	0.2332	0.3001	0.2229	0.2521
EF1603	0.0679	0.0702	0.0616	0.0666
EF1703	0.1343	0.1280	0.1394	0.1339
EF1704	0.1622	0.1610	0.1612	0.1615
EF1705	0.1350	0.1193	0.1216	0.1253
EF1805	0.3122	0.5254	0.4304	0.4227
EF1902	0.0761	0.0807	0.0799	0.0789
EF1903	0.1201	0.1408	0.1382	0.1330
EF1908	0.1638	0.1863	0.1885	0.1795
EF1908	0.1843	0.2096	0.2071	0.2003
EF2002	0.1183	0.1255	0.1256	0.1231
EF2101	0.0939	0.0946	0.1020	0.0968
EF2201	0.1117	0.1158	0.1123	0.1133
EF2203	0.1208	0.1253	0.1285	0.1249
EF2206	0.0712	0.0739	0.0703	0.0718
EF2213	0.1015	0.0926	0.0962	0.0968
EF2214	0.0789	0.1595	0.0785	0.1056
EF2215	0.1021	0.1053	0.1096	0.1057
EF2216	0.1013	0.1105	0.1090	0.1069
EF2303	0.2557	0.2651	0.2478	0.2562
EF2401	0.0728	0.0738	0.0710	0.0725
EF2404	0.1018	0.0716	0.0649	0.0794
EF2406	0.0610	0.0650	0.0647	0.0636
EF2409	0.3179	0.0668	0.0636	0.1494
EF2410	0.1133	0.1217	0.1174	0.1175
EF2902	0.0696	0.0700	0.1785	0.1060
EF3105	0.1885	0.1926	0.2292	0.2034
EF3201	0.1262	0.2314	0.1378	0.1651

EF401	0.2003	0.1887	0.1994	0.1961
WF401	0.0555	0.0652	0.0661	0.0623
WF502	0.0979	0.1048	0.1219	0.1082
WF504	0.0809	0.0839	0.1004	0.0884
WF505	0.1548	0.1541	0.1809	0.1633
WF506	0.0776	0.0773	0.0871	0.0807
WF701	0.1404	0.1520	0.1603	0.1509
WF1413	0.0783	0.0826	0.0775	0.0795
WF1506	0.1026	0.1030	0.1007	0.1021
WF1708	0.0747	0.0777	0.0736	0.0753
WF1710	0.0930	0.0948	0.0993	0.0957
WF1711	0.1023	0.1085	0.1065	0.1058
WF1801	0.3267	0.3439	0.3235	0.3314
WF2208	0.0851	0.0854	0.0875	0.0860
WF2209	0.1122	0.1197	0.1167	0.1162
1	0.1193	0.1815	0.1290	0.1433
4	0.1027	0.1206	0.1223	0.1152
18	0.1270	0.2122	0.1242	0.1545
19	0.1940	0.1742	0.2443	0.2042
25	0.1558	0.1587	0.1699	0.1615
26	0.1330	0.1355	0.1329	0.1338
27	0.2509	0.1609	0.1619	0.1912
28	0.1519	0.1485	0.1433	0.1479
54	0.2494	0.0762	0.0739	0.1332
58	0.1315	0.0958	0.0970	0.1081
59	0.2003	0.1827	0.1811	0.1880
67	0.1393	0.1454	0.1798	0.1548
68	0.0891	0.0824	0.0895	0.0870
69	0.0754	0.0645	0.0792	0.0730
73	0.1465	0.1536	0.1529	0.1510
74	0.1974	0.1756	0.1737	0.1822
85	0.1203	0.1363	0.1578	0.1381
86	0.1300	0.1449	0.1468	0.1406
88	0.3125	0.1158	0.1441	0.1908
92	0.1380	0.1502	0.1395	0.1426
94	0.1439	0.1479	0.1461	0.1460
122	0.2237	0.1975	0.2481	0.2231
123	0.2257	0.2602	0.2129	0.2329
124	0.2212	0.2424	0.1898	0.2178
126	0.1688	0.1742	0.1704	0.1711
135	0.1015	0.1066	0.1050	0.1044
137	0.1819	0.1945	0.1921	0.1895
139	0.1155	0.1306	0.1258	0.1240
141	0.1503	0.1524	0.1506	0.1511
155	0.1267	0.1250	0.1199	0.1239

156	0.1384	0.1456	0.1425	0.1422
159	0.1215	0.1239	0.1282	0.1245
163	0.1140	0.1196	0.1255	0.1197
166	0.1732	0.1892	0.1943	0.1856
169	0.1019	0.1150	0.1250	0.1140
171	0.1844	0.1960	0.1879	0.1894
172	0.1366	0.1299	0.1227	0.1297
173	0.1218	0.1291	0.1278	0.1262
175	0.1205	0.1191	0.1213	0.1203
176	0.1236	0.1205	0.1187	0.1209
183	0.1082	0.1179	0.1142	0.1134
185	0.1356	0.1312	0.1269	0.1312
186	0.1029	0.0975	0.0973	0.0992
187	0.0915	0.0970	0.0779	0.0888
188	0.1035	0.1189	0.0953	0.1059
189	0.0951	0.1077	0.0881	0.0970
195	0.1026	0.1015	0.0969	0.1003
196	0.1422	0.1448	0.1433	0.1434
219	0.1601	0.2020	0.1751	0.1791
220	0.1067	0.1107	0.1046	0.1073
224	0.1951	0.1932	0.1966	0.1950

Appendix II-d. O.D values for all samples of fox samples.

Serial Code	Triplicate O.D value			Mean O.D value
Fox1	0.1097	0.1019	0.1019	0.1058
Fox2	0.1650	0.1678	0.1686	0.1671
Fox3	0.0942	0.1124	0.1219	0.1095
Fox4	0.0949	0.0998	0.1023	0.0990
Fox5	0.1659	0.1763	0.1692	0.1705
Fox6	0.1817	0.2154	0.2071	0.2014
Fox7	0.1068	0.1104	0.1145	0.1106
Fox8	0.1111	0.1169	0.1109	0.1130
Fox9	0.0858	0.0911	0.1049	0.0939
Fox10	0.1169	0.1168	0.1136	0.1158
Fox11	0.1117	0.1044	0.0988	0.1050
Fox12	0.0894	0.0884	0.0853	0.0877
Fox13	0.0827	0.1194	0.0917	0.0979
Fox14	0.0814	0.0941	0.0886	0.0880
Fox15	0.1099	0.1180	0.1225	0.1168
Fox16	0.1116	0.1208	0.1123	0.1149
Fox17	0.0846	0.0851	0.0819	0.0839
Fox18	0.1085	0.1029	0.1080	0.1065
Fox19	0.1482	0.1346	0.1361	0.1396
Fox20	0.0992	0.0989	0.1005	0.0995
Fox21	0.0977	0.0872	0.0878	0.0909
Fox22	0.0873	0.0925	0.0756	0.0851

Appendix II-e. Species of animal feeding on cull sites in the orders in which they arrived at the location as well as the number of visits.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Head of the Bay							
Sp-1- Crested Caracara	7	10	20	8	9	10	10
Sp-2- Turkey Vulture	10	62	42	21	55	45	32
Sp-3- Striated Caracara	5	13	34	15	27	19	14
Sp-4- Feral Cat	1	4	3	5	6	7	5
Sp-5- Giant Petrel	0	26	37	102	83	64	11
Sp-6- Hawk (Unknown)	0	1	0	0	0	0	0
Sp-7- Sea gull	0	0	5	3	7	1	1
Sp-8- Sheep	0	0	0	0	0	0	8
Peaks							
Sp-1- Red Backed Hawk	3	3	1	2	2	5	2
Sp-2- Turkey Vulture	1	0	0	0	0	0	0
Sp-3- Feral Cat	3	6	5	4	4	7	5
Sp-4- Giant Petrel	0	54	0	0	0	85	0
Sp-5- Crested Caracara	0	0	0	0	0	3	0
Sheffield							
Sp-1- Turkey Vulture	1	0	1	2	N/A	N/A	N/A
Sp-2- Feral Cat	0	2	1	0	N/A	N/A	N/A
Sp-3- Striated Caracara	0	1	4	1	N/A	N/A	N/A
Sp-4- Domestic Dog	0	0	0	2	N/A	N/A	N/A
Saladero							
Sp-1- Striated Caracara	1	0	6	1	5	2	1
Sp-2- Turkey Vulture	1	36	35	51	36	12	46
Sp-3- Giant Petrel	0	8	34	42	13	6	19
Sp-4- Crested Caracara	0	0	3		2		2
Sp-5- Red Backed hawk	0	0	1	0	0	0	0
Sp-6- Hawk Unknown	0	0	0	1	0	0	0
Sp-7- Feral Cat	0	0	0	0	0	0	1

III. Appendix III. Chapter five material

Appendix III-a. O.D values for soil samples

Sample Serial code	Triplicate O.D value			Mean O.D 620
S1	0.0554	0.0600	0.0623	0.0592
S2	0.0480	0.0544	0.0542	0.0522
S3	0.0819	0.0784	0.0832	0.0812
S4	0.0572	0.0565	0.0636	0.0591
S5	0.0595	0.0572	0.0505	0.0557
S6	0.0577	0.0563	0.0499	0.0546
S7	0.0671	0.0674	0.0693	0.0679
S8	0.1026	0.1018	0.1047	0.1030
S9	0.0478	0.0543	0.0560	0.0527
S10	0.1830	0.1807	0.1816	0.1818
S11	0.2615	0.0715	0.0603	0.1311
S12	0.0719	0.0556	0.0515	0.0597
S13	0.0617	0.0710	0.0638	0.0655
S14	0.0582	0.0571	0.0610	0.0588
S15	0.0603	0.0637	0.0640	0.0627
S16	0.3319	0.3838	0.3112	0.3423
S17	0.0671	0.0767	0.0675	0.0704
S18	0.0480	0.0545	0.0470	0.0498
S19	0.0599	0.0632	0.0760	0.0664
S20	0.0902	0.0773	0.0869	0.0848
S21	0.0520	0.0538	0.0574	0.0544
S22	0.0432	0.0411	0.0477	0.0440
S23	0.1123	0.0976	0.1007	0.1035
S24	0.1199	0.1741	0.1179	0.1373
S25	0.0713	0.0493	0.0542	0.0583
S26	0.0562	0.0513	0.0541	0.0539
S27	0.0628	0.0600	0.0617	0.0615
S28	0.4230	0.3594	0.4292	0.4039
S29	0.0587	0.0647	0.0643	0.0626
S30	0.0738	0.0739	0.2026	0.1168
S31	0.0926	0.0990	0.1034	0.0983
S32	0.0743	0.0715	0.0713	0.0724
S33	0.0645	0.0636	0.0629	0.0637
S34	0.0513	0.0514	0.0557	0.0528
S35	0.0613	0.0697	0.0659	0.0656
S36	0.0608	0.0664	0.0615	0.0629
S37	0.0818	0.1063	0.0610	0.0830
S38	0.0842	0.0730	0.0809	0.0794
S39	0.2579	0.2287	0.0808	0.1891
S40	1.0401	0.7002	0.5555	0.7653
S41	0.0943	0.0872	0.0860	0.0892
S42	0.1738	0.1439	0.1353	0.1510
S43	0.1462	0.1407	0.1497	0.1455

S44	0.1244	0.1422	0.1404	0.1357
S45	0.1002	0.0989	0.1013	0.1001
S46	0.1439	0.1458	0.1413	0.1437
S47	0.1121	0.1242	0.1144	0.1169
S48	0.2175	0.2210	0.2161	0.2182
S49	0.1455	0.1469	0.1527	0.1484
S50	0.1528	0.1605	0.1634	0.1589
S51	0.1098	0.1059	0.1087	0.1081
S52	0.1810	0.1820	0.1759	0.1796
S53	0.1291	0.1184	0.1086	0.1187
S54	0.0994	0.0910	0.0951	0.0952
S55	0.0841	0.0886	0.1002	0.0910
S56	0.1260	0.0980	0.1088	0.1109
S57	0.1293	0.1152	0.1205	0.1217
S58	0.1145	0.0960	0.0973	0.1026
S59	0.0921	0.0933	0.0967	0.0940
S60	0.1229	0.1282	0.1113	0.1208
S61	0.1642	0.1566	0.1535	0.1581
S62	0.1224	0.1225	0.1351	0.1267
S63	0.0982	0.0834	0.0883	0.0900
S64	0.0932	0.1066	0.1194	0.1064
S65	0.1215	0.1173	0.2674	0.1687
S66	0.1113	0.1140	0.1870	0.1374
S67	0.1455	0.1446	0.2356	0.1752
S68	0.1353	0.1315	0.1786	0.1485
S69	0.0964	0.1067	0.1855	0.1295
S70	0.0886	0.0948	0.0888	0.0907
S71	0.0905	0.0991	0.0928	0.0941
S72	0.1722	0.1823	0.1772	0.1772
S73	0.1159	0.1113	0.1150	0.1141
S74	0.1371	0.1403	0.1381	0.1385
S75	0.1054	0.1095	0.1127	0.1092
S76	0.1076	0.1072	0.1476	0.1208
S77	0.1016	0.0986	0.0998	0.1000
S78	0.1104	0.1291	0.1053	0.1149
S79	0.1405	0.1257	0.1247	0.1303
S80	0.1014	0.0967	0.1019	0.1000
S81	0.1099	0.1127	0.1137	0.1121
S82	0.1318	0.1451	0.1221	0.1330
S83	0.1593	0.1443	0.1504	0.1513

IIII. Appendix IIII. Chapter six material

Appendix IIII-a. Sheep grazing and dog defecation questionnaire.

Sheep Grazing and Dog defecation Questionnaire

The aim of this questionnaire is to establish the extent at which sheep and dogs share the same area. I am interested in sheep grazing habits and dog defecation habits because these behaviours could result in transmission of *Echinococcus granulosus*. I will use the results of this questionnaire to assess the risk of transmission between dogs and sheep.

Farming Statistics:

How many Hectares is your farm? [ ]

How Many Sheep do you have (pre-lambing)? [ ]

How many dogs do you have? [ ]

During Lambing Season:

a. What proportion (%) of your farm is grazed by sheep during this time of the year (including fertile areas, re-seeds etc, excluding barren or poor-quality land)?

b. Over the course of a whole year, how often do your dogs defecate on the land sheep occupy during the Lambing season?

i. Once or more a day? [ ]
ii. Once or more a week? [ ]
iii. Once or more a month? [ ]
iv. Less than once a month? [ ]
if so, how many times per year? [ ]
v. Never? [ ]

During Shearing Season:

a. What proportion (%) of your farm is grazed by sheep during this time of the year (including fertile areas, re-seeds etc, excluding barren or poor-quality land)?

b. Over the course of a whole year, how often do your dogs defecate on the land sheep occupy during the shearing season?

i. Once or more a day? [ ]
ii. Once or more a week? [ ]
iii. Once or more a month? [ ]
iv. Less than once a month? [ ]
if so, how many times per year? [ ]
v. Never? [ ]

During Winter:

a. What proportion (%) of your farm is grazed by sheep during this time of the year (including fertile areas, re-seeds etc, excluding barren or poor-quality land)?

b. Over the course of a whole year, how often do your dogs defecate on the land sheep occupy during the winter?

i. Once or more a day? [ ]
ii. Once or more a week? [ ]
iii. Once or more a month? [ ]
iv. Less than once a month? [ ]
if so, how many times per year? [ ]
v. Never? [ ]

Appendix IIII. b. R_0 estimation model code

R_0 estimation function

```
R0EstFunc <- function(
  eggProdRate, # mean daily egg production by adult worm
  lifespanAdult, # lifespan of adult worm (years)
  lifespanDefHost, # lifespan of definitive host (years)
  lengthShearingDays, # length of shearing period in days
  lengthLambingDays, # length of lambing period in days
  grazedContamShearing, # probability of dogs defaecating on grazed pasture during shearing
  grazedContamLambing, # probability of dogs defaecating on grazed pasture during lambing
  grazedContamOther, # probability of dogs defaecating on grazed pasture during rest of year
  ingestionProb, # probability of ingestion of egg if contaminated pasture grazed
  eggPersistenceYears, # duration of peristence of eggs on pasture in years
  propIntHostIngest, # probability that egg ingested by intermediate host in question (must sum to 1 over all types)
  propEggsEncyst, # probability that ingested egg encysts in intermediate host
  protGrowth, # total protoscolices per protoscolex before host death (accounts for life expectancy of host but also age at first infection)
  probOffalIngestDef, # probability definitive host ingests offal
  propProtIngestDef, # proportion of all protoscolices ingested if definitive host ingests offal
  propProtEstablish, # proportion of ingested protoscolices which establish in definitive host
  propEstProtAdult # proportion of established protoscolices which reach adulthood
){
  # Total eggs produced by adult worm in definitive host:
  eggProdTot <- eggProdRate*365*(1/((1/lifespanAdult)+(1/lifespanDefHost)))

  # Representing lengths of seasons as proportions
  shearingProp <- (lengthShearingDays/365)
  lambingProp <- (lengthLambingDays/365)
  otherProp <- 1 - (shearingProp+lambingProp)
```

```
# Total eggs on grazed pasture in different seasons
eggGrazedPastureShearing <- eggProdTot*grazedContamShearing*shearingProp
eggGrazedPastureLambing <- eggProdTot*grazedContamLambing*lambingProp
eggGrazedPastureOther <- eggProdTot*grazedContamOther*otherProp

# Assume that grazed area is completely grazed over course of year (within each season)
# Prob of egg ingestion over duration of egg persistence in different seasons
probEggIng <- 1 - (1 - ingestionProb)^eggPersistenceYears

# Number of eggs ingested in different seasons
eggIngShearing <- probEggIng*eggGrazedPastureShearing
eggIngLambing <- probEggIng*eggGrazedPastureLambing
eggIngOther <- probEggIng*eggGrazedPastureOther

# Total eggs ingested by intermediate hosts:
eggIngestIntHost <- eggIngShearing+eggIngLambing+eggIngOther

# Total eggs ingested by hosts in specific intermediate host category:
eggIngestGroup <- eggIngestIntHost*propIntHostIngest

# Total eggs which form protoscolices in intermediate hosts:
protEst <- eggIngestGroup*propEggsEncyst #proportion of eggs ingested by adults at the abattoir that encyst

# Total number of protoscolices in intermediate hosts at slaughter:
protTotSlaught <- protEst*protGrowth

# Number of protoscolices ingested by definitive host
totProtIngDefHost <- protTotSlaught*probOffalIngestDef*propProtIngestDef

# Number of protoscolices establishing in definitive hosts
```

```
  protEstDefTot <- totProtIngDefHost*propProtEstablish

  # Number of protoscolices establishing in definitive hosts
  adultDefTot <- protEstDefTot*propEstProtAdult

  ##### Estimating R0
  R0Est<- adultDefTot
  #R0Est <- eggProdTot*eggPasture*eggIngestIntHost*eggIngestGroup*protEst*protTotSlaught*totProtIngDefHost*protEstDefTot*adultDefTot

  return(R0Est) # return R0 estimate
}
```

Protoscoleces per cyst age function

```
protPerCystFunc <- function(ageInf, ageDeath){
  # This function fits a logistic curve to the Torgerson data to predict numbers of protoscolices for different cyst ages

  # Estimates from Torgerson 2009. Assuming age describes time since infection (i.e. cyst age)
  ageVar <- data.frame(age=c(1,2,3,4,5,6),
                       protPerCyst=c(215, 870, 2095, 3061, 8192, 12603))

  protPreds <- nls(protPerCyst ~ SSlogis(age, Asym, xmid, scal), ageVar)

  if(ageInf<ageDeath){
    protPerCystPred <- predict(protPreds, list(age=(ageDeath-ageInf)))[[1]]
  } else {
    protPerCystPred <- 0
  }
  return(protPerCystPred)
}
```

R0 Estimation parameter code

```
source("R0EstFunc.R")
source("protPerCystFunc.R")
#install.packages("tidyverse")

# This is how to find all combinations of different sensitivity analysis parameters (as well as a column to store R0 estimates for each)
allSensCombs <- expand.grid(ingestionProb=c(1e-05,1e-04,1e-03,1e-02,1e-01,1e-00),
                            ageInf=seq(0,10,2),
                            maxProbIngOffal=c(1e-05,1e-04,1e-03,1e-02,1e-01,1e-00),
                            dosingProb=c(0, 0.886, 0.996),
                            r0_Est=NA)
r0EstList <- list()
for(i in 1:nrow(allSensCombs)){
  # Sensitivity analysis parameters:
  ingestionProb <- allSensCombs$ingestionProb[i] # probability of ingestion of egg if contaminated pasture grazed
  ageInf <- allSensCombs$ageInf[i] # age at first infection (would expect to be the same for all animals, unless different types are managed substantially differently)
  maxProbIngOffal <- allSensCombs$maxProbIngOffal[i] # Probability definitive host ingests offal
  dosingProb <- allSensCombs$dosingProb[i] # Probability of correct PZQ dosing

  totSheepFI <- 476767 # total sheep in the FIs
  totDogsFI <- 620 # total dogs in the FIs

  eggProdRate <- 82.3 # mean daily egg production by an adult worm (lambda; from literature)
  lifespanAdult <- 0.75 # mean duration of adult worm lifespan (ignoring host lifespan) (1/mu_A; from literature)
  lifespanDefHost <- 12.5 # mean duration of dog lifespan (from Wang et al)
  lengthShearingDays <- 122 # length of shearing period in days
  lengthLambingDays <- 42 # length of lambing period in days
```

```
grazedContamShearing <- 0.08292727 # probability of dogs defecating on grazed pasture during shearing

grazedContamLambing <- 0.058690909 # probability of dogs defecating on grazed pasture during lambing

grazedContamOther <- 0.058690909 # probability of dogs defecating on grazed pasture during rest of year


eggPersistenceYears <- 3.4167  # mean duration of time an egg remains on pasture (ignoring ingestion by intermediate hosts) (1/mu_E; from literature)


# NOTE THAT ESTIMATE BELOW MUST SUM TO 1 OVER ALL HOST TYPES- estimations of probability that each age category ingests eggs from pasture

AbattoirLambIngest <- 0.07 #less than 1.5 years of age, slaughtered at abattoir.Low probability of ingesting eggs

AbattoirAdultIngest <- 0.15 #5.6 years of age, average taken from abattoir data and age estimates from SP/ZF. Higher risk of ingesting eggs (most common catagorie of infected sheep is cull ewes sent to abattoir)

NatDeathLambIngest <- 0.03 #YL lambs that die naturally over winter

NatDeathAdultIngest <- 0.4 #Adult sheep that die naturally over winter as well as cull ewes. Highest risk

HomeConsumpIngest <- 0.15 #adults 2-4 yoa

DogMeatIngest <- 0.2 #Adult sheep similar to culls


sumHostTypes <- AbattoirLambIngest+AbattoirAdultIngest+NatDeathLambIngest+NatDeathAdultIngest+HomeConsumpIngest+DogMeatIngest

try(if(sumHostTypes != 1) stop("Host probabilities must sum to 1!"))


propEggsEncyst <- 0.0033 # proportion of ingested eggs which form cysts in intermediate hosts (tau; from literature)


# Note: note sure about the estimates below. Double check that they are correct!!!

ageDeathAbattoirLamb <- 1 # age at death for lambs at abattoir

ageDeathAbattoirAdult <- 5.6 # age at death for adults at abattoir
```

```
ageDeathHomeConsump <- 3 # age at death for animals killed for home consumption
ageDeathDogMeat <- 6 # age at death for animals used for dog meat
ageDeathNatDeathLamb <- 1 # age at death for lambs which die on pasture
ageDeathNatDeathAdult <- 10 # age at death for adults which die on pasture

maxAgeDeath <- max(ageDeathAbattoirLamb,ageDeathAbattoirAdult,ageDeathHomeConsump,ageDeathDogMeat,ageDeathNatDeathLamb,ageDeathNatDeathAdult)

totProtAbattoirLamb <- protPerCystFunc(ageInf,ageDeathAbattoirLamb)
totProtAbattoirAdult <- protPerCystFunc(ageInf,ageDeathAbattoirAdult)
totprotHomeConsump <- protPerCystFunc(ageInf,ageDeathHomeConsump)
totProtDogMeat <- protPerCystFunc(ageInf,ageDeathDogMeat)
totprotNatDeathLamb <- protPerCystFunc(ageInf,ageDeathNatDeathLamb)
totProtNatDeathAdult <- protPerCystFunc(ageInf,ageDeathNatDeathAdult)

# Relative probability definitive host ingests offal
# New parameter to reflect the fact that we know something about the relative likelihoods of different intermediate hosts types being ingested by dogs. I've pulled these estimates out of nowhere, so you will probably want to sanity check them with Steve et al.

relProbDogOffalAbattoirLamb <- 0
relProbDogOffalAbattoirAdult <- 0
relProbDogOffalHomeConsump <- 0.2
relProbDogOffalDogMeat <- 0.2
relProbDogOffalNatDeathLamb <- 1
relProbDogOffalNatDeathAdult <- 1 # these are not controlled by disposal or inspection of offal (include cull ewes).

propProtIngestAbattoirLamb <- relProbDogOffalAbattoirLamb*maxProbIngOffal #proportion of protoscolices in abattoir sheep which are likely ingested by dogs (psi_S: Sensitivity analysis needed. Lower proportion to be scavenge at abattoir than home slaughter)
```

```
propProtIngestAbattoirAdult <- relProbDogOffalAbattoirAdult*maxProbIngOffal

propProtIngestNatDeathLamb <- relProbDogOffalNatDeathLamb*maxProbIngOffal

propProtIngestNatDeathAdult <- relProbDogOffalNatDeathAdult*maxProbIngOffal #baseline. Proportion of protoscolices in sheep which die naturally which are likely ingested by dogs

propProtIngestHomeConsump <- relProbDogOffalHomeConsump*maxProbIngOffal

propProtIngestDogMeat <- relProbDogOffalDogMeat*maxProbIngOffal


propProtEst <- 0.047 # Proportion of ingested protoscolices which establish in dogs (epsilon; from literature)

pzqEfficacy <- 0.996 # efficacy of PZQ against Eg

propEstProtAdult <- (1-pzqEfficacy)*(1-dosingProb) # proportion of established protoscolices in dogs which ultimately form an adult worm (sigma_p; analysed to identify the impact of dosing)


numIntHosts <- totSheepFI/11297.23 # number of sheep in area of interest (note: need to define area of interest - assumuing 1km square here)

numDefHosts <- totDogsFI/12173 # number of dogs in area of interest (note: need to define area of interest!) 12,200 published Mcadams


parameters<- data.frame(

group=c("abattoirLamb","abattoirAdult","natDeathLamb","natDeathAdult","homeConsump","dogMeat"),

  eggProdRate=eggProdRate,

  lifespanAdult=lifespanAdult,

  lifespanDefHost=lifespanDefHost,

  lengthShearingDays=lengthShearingDays,

  lengthLambingDays=lengthLambingDays,

  grazedContamShearing=grazedContamShearing,

  grazedContamLambing=grazedContamLambing,

  grazedContamOther=grazedContamOther,

  ingestionProb=ingestionProb,
```

```
   eggPersistenceYears=eggPersistenceYears,

propIntHostIngest=c(AbattoirLambIngest,AbattoirAdultIngest,NatDeathLambIngest,NatDeathAdultIngest,HomeConsumpIngest,DogMeatIngest),
   propEggsEncyst=propEggsEncyst,

protGrowth=c(totProtAbattoirLamb,totProtAbattoirAdult,totprotNatDeathLamb,totProtNatDeathAdult,totprotHomeConsump,totProtDogMeat),

probOffalIngestDef=c(propProtIngestAbattoirLamb,propProtIngestAbattoirAdult,propProtIngestNatDeathLamb,propProtIngestNatDeathAdult,propProtIngestHomeConsump,propProtIngestDogMeat),
   propProtIngestDef=1,
   propProtEstablish = propProtEst,
   propEstProtAdult = propEstProtAdult)



 ageCategories <- as.character(unique(parameters$group))
 r0EstimatesDF <- data.frame(ageCat=ageCategories,
                             r0Est=NA)

 for(ageCat in 1:length(ageCategories)) {
  selectedGroup=ageCategories[ageCat] # name of group
  groupIndex <- which(parameters$group==selectedGroup) # index of group

  r0Estimate <- R0EstFunc(
    eggProdRate = parameters$eggProdRate[groupIndex], # mean daily egg production by adult worm
    lifespanAdult = parameters$lifespanAdult[groupIndex], # lifespan of adult worm (years)
    lifespanDefHost = parameters$lifespanDefHost[groupIndex], # lifespan of definitive host (years)
    lengthShearingDays = parameters$lengthShearingDays[groupIndex], # length of shearing period in days
```

```
    lengthLambingDays = parameters$lengthLambingDays[groupIndex], # length of lambing period in days

    grazedContamShearing = parameters$grazedContamShearing[groupIndex], # probability of dogs defaecating on grazed pasture during shearing

    grazedContamLambing = parameters$grazedContamLambing[groupIndex], # probability of dogs defaecating on grazed pasture during lambing

    grazedContamOther = parameters$grazedContamOther[groupIndex], # probability of dogs defaecating on grazed pasture during rest of year

    ingestionProb = parameters$ingestionProb[groupIndex], # probability of ingestion of egg if contaminated pasture grazed

    eggPersistenceYears = parameters$eggPersistenceYears[groupIndex], # duration of peristence of eggs on pasture in years

    propIntHostIngest = parameters$propIntHostIngest[groupIndex], # probability that egg ingested by intermediate host in question (must sum to 1 over all types)

    propEggsEncyst = parameters$propEggsEncyst[groupIndex], # probability that ingested egg encysts in intermediate host

    protGrowth = parameters$protGrowth[groupIndex], # total protoscolices per protoscolex before host death (accounts for life expectancy of host but also age at first infection)

    probOffalIngestDef = parameters$probOffalIngestDef[groupIndex], # probability definitive host ingests offal

    propProtIngestDef = parameters$propProtIngestDef[groupIndex], # proportion of all protoscolices ingested if definitive host ingests offal

    propProtEstablish = parameters$propProtEstablish[groupIndex], # proportion of ingested protoscolices which establish in definitive host

    propEstProtAdult = parameters$propEstProtAdult[groupIndex] # proportion of established protoscolices which reach adulthood

   )

   r0EstimatesDF[ageCat,2] <- r0Estimate

   #assign(paste0("R0_",selectedGroup),r0Estimate) # assign r0Estimate to suitable name

   rm(list="r0Estimate") # remove r0Estimate variable

  }

  r0EstList[[i]] <- r0EstimatesDF

  allSensCombs$r0_Est[i] <- sum(r0EstimatesDF$r0Est)

}
```

```
library(tidyverse)

allSensCombs <- filter(allSensCombs, ageInf<maxAgeDeath)
allSensCombs$ageInfText <- paste0(allSensCombs$ageInf,"y at first infection")
allSensCombs$dosingProbText <- factor(paste0(100*(1-allSensCombs$dosingProb),"% dosing failure"),
                                      levels=paste0(100*(1-unique(allSensCombs$dosingProb)),"% dosing failure"))

ggplot(data=allSensCombs, aes(x=ingestionProb, y=maxProbIngOffal, fill=log(r0_Est))) +
  geom_tile() +
  facet_grid(dosingProbText ~ ageInfText) +
  scale_x_continuous(trans='log10') + scale_y_continuous(trans='log10') +
  scale_fill_gradient2(name="R0",
                       low="dark green", mid="white", high="red", #colors in the scale
                       midpoint=0,
                       breaks=c(min(log(allSensCombs$r0_Est)), mean(log(allSensCombs$r0_Est)), 0, max(log(allSensCombs$r0_Est))),
                       labels=format(c(min(allSensCombs$r0_Est),mean(allSensCombs$r0_Est), 1, max(allSensCombs$r0_Est)), scientific = TRUE, digits = 3)) +
  labs(x="Probability of sheep infection if grazes eggs on pasture", y="Probability of dog ingesting offal")

write_csv(allSensCombs, path = "E:/PhD/Modelling/R0 estimation model/r0EstimationNew/r0EstimationNew/R0estimates.csv")
```