Studies on gastrointestinal nematodes of alpacas in Australia

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DEDICATION

This thesis is dedicated to the memory of my father who always dreamt me as a successful agricultural scientist. You are gone but your belief in me has made this journey possible.

SUMMARY

The Australian alpaca industry has grown remarkably since the re-introduced of alpaca into the country in the 1980s. This relatively new and small industry faces various challenges that affect the overall production (fibre, meat etc.) of animals. For example, production losses and deaths associated with gastrointestinal nematodes (GINs) have become one of the major concerns for alpaca producers in Australia and elsewhere as there is a scarcity of reliable information about the epidemiology of GINs in alpacas, worm control practices used by alpaca farmers and the efficacy status of widely used unregistered anthelmintics. The present thesis aimed to (i) assess worm control practices used by Australian alpaca farmers, (ii) determine the epidemiology GINs and (iii) the efficacy of commonly used anthelmintics against GINs of alpacas in Australia. A comprehensive literature review along with seven results chapters and a general discussion chapter are included in this thesis.

In Chapter 2, an online questionnaire survey was conducted to assess worm control practices used by Australian alpaca farmers. Results showed that more than half of the respondents perceived that GINs was an important health problem. Macrocyclic lactones (MLs) were the most commonly used anthelmintics in alpacas. Almost half of the respondents (47%) used anthelmintics at the dose rate recommended for sheep. The majority of alpaca farmers were unaware of pasture spelling and one-third of respondents had shared paddocks with ruminants. This study led to subsequent studies to understand the epidemiology of GINs of Australian alpacas using sensitive diagnostic methods and assess the efficacy of commonly used anthelmintics against GINs of Australian alpacas.

In chapters 3 and 4, sensitive diagnostic methods were developed to determine the faecal eggs counts and identify nematode genus/species in the faeces of alpacas. In chapter 3, a newly developed diagnostic method, the FECPAK^{G2}, was compared to a routinely used method, the McMaster technique, for the counting of GIN eggs in the faeces of alpacas. Data revealed moderate to good agreement between the two methods. This was the first study to assess the agreement of measurements between two methods for estimating nematode eggs in the faeces of alpacas. In Chapter 4, a molecular diagnostic tool based on multiplexed-tandem PCR (MT-PCR) was developed. This tool is faster and is capable of identifying common nematode genera/species of alpacas, including *Camelostrongylus mentulatus* which was not possible using traditional larval culture method.

The epidemiology of GINs of alpacas in Australia was assessed using cross-sectional (Chapter 5) and longitudinal studies (Chapter 6). A range of GINs are prevalent in Australian alpacas with variable worm burdens in different climatic zones and seasons. The results of both studies were comparable. Both studies showed an overall prevalence of GINs in SACs from 61 – 66%. Weaners had the highest prevalence in both studies ranging from 73 - 80%. However, the pattern of prevalence was not same across the climatic zones. In cross-sectional study, the highest prevalence of GINs (77%) were observed in the summer rainfall zone, whereas in longitudinal study the winter rainfall zone had the highest prevalence (68%). In addition, a mixed-effects zero-inflated negative binomial (ZINB) regression model has been used to design parasite control interventions.

To assess worm burden and the spectrum of GINs infecting Australian alpacas, 100 gastrointestinal tracts of alpacas were examined (Chapter 7). Results revealed a mean burden of 1,300 worms, with the highest burden of 29,000 worms. Nineteen different species of GINs were identified from Australian alpacas, including three camelid specific nematodes: *Graphinema auchenia*, *Camelostrongylus mentulatus* and *Trichuris tenuis*. *Haemonchus contortus* was the most prevalent nematode followed by *C. mentulatus* and *Trichostrongylus* spp.

In Chapter 8, the efficacy of commonly used anthelmintics against GINs of alpacas was assessed. The faecal egg count reduction tests were conducted on 20 alpaca farms across the country. The results showed that a commercially available combination of levamisole, closantel, albendazole and abamectin was the most effective dewormer followed by single anthelmintic such as, monepantel, moxidectin, closantel, fenbendazole and ivermectin. *Haemonchus* spp. were the most commonly resistant nematodes. This was the first comprehensive study to investigate the efficacy of commonly used anthelmintics against GINs of alpacas.

This study provides significant information on GINs of Australian alpacas. Results of this study advance our knowledge on the epidemiology and control of GINs and efficacy status of most commonly used anthelmintics which could be used to develop control strategies against GINs of alpacas in Australia.

DECLARATION

I declare that this thesis:

I. Comprises only my original work towards the PhD, except where explicitly stated otherwise in the text or acknowledgements;

II. Due acknowledgement has been made in the text to all other materials used; and

III. The thesis is less than 100,000 words in length, exclusive of tables, figures, bibliographies and appendices

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The publications, conference proceedings, symposiums and seminars resulting from this thesis are listed below:

Peer-reviewed papers published in international scientific journals

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Rashid, M.H., Stevenson M.A., Beveridge I., Vaughan J., Campbell A.J.D., Jabbar A., 2016. A questionnaire survey to assess current worm control practices used by Australian alpaca farmers. International Congress for Tropical Medicine and Malaria, Brisbane, Australia, September 18-22, 2016.

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Rashid, M.H., Stevenson M.A., Beveridge I., Vaughan J., Campbell A.J.D., Jabbar A., 2016. A questionnaire survey to assess current worm control practices used by Australian alpaca farmers. Australian Sheep Veterinarians Conference 2016. Dubbo, NSW, September 9-11, 2016.

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CHAPTER 1. LITERATURE REVIEW

1.1. Introduction

Domesticated South American camelids (SACs) such as alpacas (*Vicugna pacos*) and llamas (*Lama glama*) are becoming increasingly popular as commercial livestock enterprise due to their superior quality fibre, meat and hides as well as their ability to adapt to diverse climatic conditions (Leguía, 1991; Saeed et al., 2018). The Australian alpaca industry has been growing substantially since alpacas were introduced into the country in the 1980s. This relatively new industry faces various challenges directly or indirectly affecting overall production (fibre, meat etc.) of animals. Production losses and deaths due to internal parasites are major challenges for the industry. Problems associated with gastrointestinal nematodes (GINs) have become one of the major concerns for alpaca farmers and veterinarians in recent years (Q-Alpaca 9th Annual Report, April 2013 – March 2014; Newsletter of Australian Alpaca Veterinarians, Number 81, October 2015). In addition, a recent report on anthelmintic resistance (AR) in alpacas (Jabbar et al., 2013) has also raised concerns about this problem in Australia. No systematic study has been undertaken on GINs of alpacas in Australia. Similarly, there is a scarcity of reliable information about the control measures for GINs used by alpaca farmers in Australia. Farmers are using sheep and cattle products in the absence of registered anthelmintic agents for alpacas in Australia. Therefore, the industry needs support based on scientific knowledge to address these challenges and thus ensure sustainable growth in the alpaca industry.

1.2. South American Camelids

Apart from the two domesticated SACs (alpacas and llamas), there are two additional species belonging to this group, the vicugna (*Vicugna vicugna*) and the guanaco (*Lama guanicoe*). These animals are native to South America but are also reared/farmed/kept in zoos in small numbers in other countries, including Australia, Canada, China, France, Germany, New Zealand, Switzerland, the UK and the USA. In their native countries, SACs are most common in the region called ‘Altiplano’, the elevated Andean plain, around 3,750 metres above sea level in Peru, Bolivia, Argentina and Chile.

The main purposes of farming alpacas in South America are for fibre, meat and hides (Tait et al., 2002). Among South American countries, Peru has the largest population of alpacas (~3 million; ACIL Consulting 2001). Peru is also the largest alpaca wool producer and exporter among other alpaca growing countries. Alpacas are also used as a meat source in Peru. In 2012, the annual alpaca meat and fibre produced in Peru was about 11,648 tonnes and 4,917 tonnes, respectively.

Alpaca farming in the countries outside South America was initiated primarily for the luxury fibre - renowned for its softness and warmth. Farmers have endeavoured to improve the genetics responsible for good quality fibre production. More recently, the alpaca meat industry has become commercialised in Australia (Australian Alpaca Association conference, Adelaide 2014). The other purposes of keeping alpacas are as guard animals on sheep farms, or simply for lifestyle (Australian Alpaca Association and ACIL Consulting, 2001; Hertzberg and Kohler, 2006; Ballweber, 2009).

The size of the registered herd in Australia has grown to around 169,000 in 2011–12. However, the current estimated number could be 450,000, including unregistered herds, leading to the largest alpaca population outside South America (Clarke, 2017). Major alpaca populations in the world are shown in the Table 1.1.

Table 1.1. Major alpaca populations in the world.

Country	Population
Peru[1]	2,500,000
Bolivia[1]	500,000
Chile & Argentina[1]	50,000
Australia[2]	450,000
United States[3]	150,000
England[4]	25,000
Canada[5]	20,000
New Zealand[3]	26,000

Sources: [1]ACIL Report 2001; [2]Alpaca Market Assessment 2016; [2]Australian Alpaca Association (AAA); Clarke, 2017; [3]Alpaca Association New Zealand; [4]http://www.purealpacas.co.uk/; [5]Alpaca Canada (http://www.alpacainfo.ca/).

1.3. The Australian alpaca industry

Over the last 25 years, the Australian alpaca industry has been increasing its alpaca herd with emphasis on genetic improvement for a quality fibre. There has been a substantial trade in breeding stock and little meat production to date. A commercial alpaca meat industry has evolved over the last 5-10 years in response to lower prices for genetically inferior animals, combined with excellent meat quality. The Australian Alpaca Association Inc (AAA), which is the main representative body for the industry, has established a genetic database in which parentage is recorded and allows breeders to breed alpacas to improve genetics in a controlled and informed manner. A number of countries have been choosing to import alpacas from Australia because of the genetic database and also because Peru has strict limitations on exportation of their best animals. Australian alpacas are also attractive to other countries for their potential breeding value which opens the door for export to many countries, including Canada, China, Japan, New Zealand and UK and mainland Europe. The Australian alpaca fibre industry has also had substantial growth with an estimated 188 tonnes of alpaca fibres produced in the year 2011–12, with a gross value of $2.6 million. According to the AAA, in 2015, there were approximately 5,500 members in Australia, with more than 2,000 alpaca breeders, of which only 393 members' farms had 50 or more animals. Around 93% of the farms had fewer than 50 animals. However, the remaining 7% of farms contained more than 55% of the total alpaca population in Australia. The two breeds of alpaca are Huacayas and Suri. Huacaya make up 90% of the herd in both Australia and around the world (ACIL Consulting, 2001).

In Australia, most alpaca herds are located in the south-eastern states of New South Wales and Victoria and some are present in Queensland, Western Australia, South Australia and Tasmania. Alpacas are distributed in the four distinct climatic zones in Australia: the Mediterranean-type, non-seasonal rainfall, summer rainfall and winter rainfall zone. The distribution of the alpaca herds in Australia is shown in the map (Fig. 1.1). Alpacas are grazed year-round on pastures with variable provision of supplementary feed. Routine vaccinations are implemented against clostridial diseases (caused by *Clostridium perfringens* type D, *C. tetani*, *C. novyi* type B, *C. septicum* and *C. chauvoei*). Although alpacas may be shorn at different times of the year, spring is the usual season for their single annual shearing. Likewise, female alpacas usually give birth during

autumn, although this practice may vary among farms. Crias are weaned at an average age of five to six months.

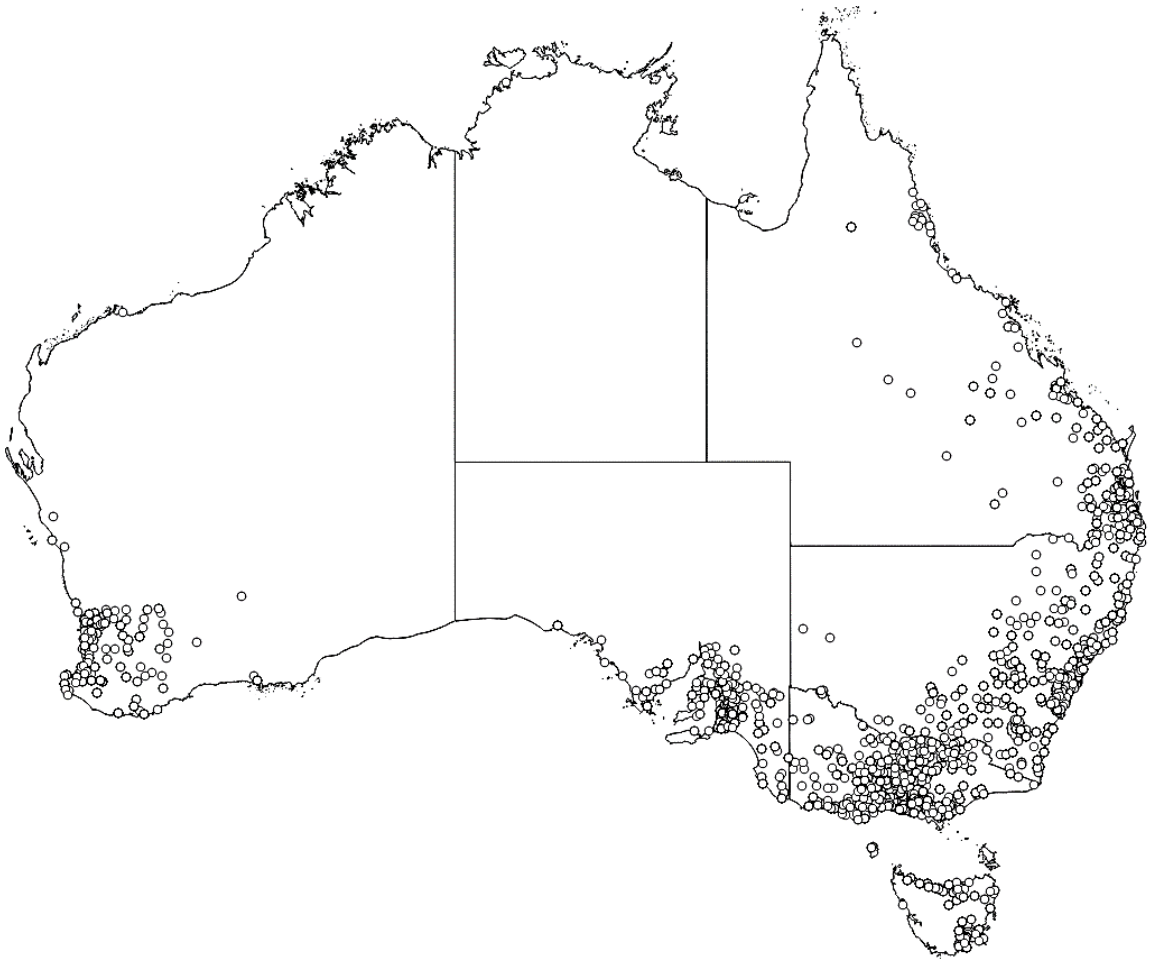


Fig. 1.1. Distribution of alpaca herds in Australia.

1.4. Parasitic challenges to the alpaca industry

The health and productivity of alpacas can be compromised by GINs, resulting in substantial economic losses to the fibre and meat industries (Ballweber, 2009; Jabbar, 2018; Leguía, 1991; Windsor et al., 1992). Alpacas can be infected with shared (i.e. common in sheep, goat and cattle) and camelid-specific GINs (Ballweber, 2009; Hill et al., 1993; Jabbar, 2018). In Australia, according to a recent Q-Alpaca report the most commonly diagnosed (12%) cause of death in alpacas was parasitism and *Haemonchus* spp. were found to be involved in 53% of the total deaths (Q-Alpaca 9th Annual Report, April 2013 – March 2014).

1.4.1. GIN species of alpacas and llamas

A wide range of GINs can infect alpacas across the globe (Table 1.2). Nematode genera such as *Trichostrongylus*, *Ostertagia*, *Teladorsagia*, *Cooperia*, *Haemonchus*, *Nematodirus*, *Oesophagostomum*, *Chabertia*, *Trichuris* and *Capillaria* are cross-species transmissible with domestic ruminants (Ballweber, 2009; Fowler, 2010). However, some SAC-specific GINs have also been reported, including *Graphinema auchenia*, *Lamanema chavezi*, *Nematodirus lamae*, and *Spiculopteragia peruviana* (Cafrune et al., 2001; Guerrero and Chávez, 1967; Mitchell et al., 2016). *Lamanema chavezi* is considered one of the most pathogenic nematodes for alpacas in the South American countries because of the severe liver damage it causes during the enterohepatic migration of larvae (Cafrune, 2001). Outside South America, *L. chavezi* has been identified recently in the USA and New Zealand (Jarvinen et al., 2014; McKenna et al., 2009) but has yet to be found in Australia. *Camelostrongylus mentulatus* is thought to be a SAC-specific GIN, however it has been found in sheep, feral goats and also in camels in Australia (Beveridge et al., 1974, 1987; Beveridge and Ford, 1982; Copland, 1965; Rogers, 1939).

GINs of SACs have been the subject of intermittent studies for the last few decades in many regions of the world, including South American countries (Alcaino et al., 1991; Contreras et al., 2014), Japan (Hyuga and Matsumoto, 2016), New Zealand (Dittmer et al., 2018; Hill et al., 1993), Switzerland (Hertzberg and Kohler, 2006), the UK (Tait et al., 2002; Welchman et al., 2008), and the USA (Cebra and Stang, 2008; Rickard, 1993). Most SAC-specific GINs (except *L. chavezi*) were initially reported in alpacas from South America (Alcaino et al., 1991; Ballweber, 2009; Franz et al., 2015; Guerrero and

Chávez, 1967). However, recent reports have indicated the presence of *N. lamae* in alpacas from New Zealand (Hinkson, 2015) and the UK (Mitchell et al., 2016), indicating that the movement of alpacas outside South America has resulted in the dissemination of parasites to other geographical regions, though quarantine measures are in place in the importing countries. Such reports of GINs in alpacas from outside South America warrant epidemiological studies to understand the spectrum of GINs infecting alpacas and also the pathogenic effects they can have on SACs.

Based on published data, a wide range of GINs occurred in alpacas and llamas (Table 1.2). The most commonly diagnosed shared- genera in Australian alpacas are *Haemonchus*, *Trichostrongylus*, *Ostertagia*/*Teladorsagia*, *Nematodirus*, *Cooperia*, *Chabertia*, *Oesophagostomum* (Jabbar et al., 2013; Carmichael, 1999; RIRDC Publication No 99/140). These nematodes pose a significant threat due to the nature of diseases they can produce in alpacas (Besier, 2009). Endoparasites have also been identified as a major disease issue in a recent survey conducted by the Australian Alpaca Veterinarians (Vaughan, 2015).

Although Australia has the largest alpaca population outside South America, very limited information is available on the GINs in Australian alpacas (Carmichael, 1999, 2014; Presidente, 2007). These studies were either based on the opportunistic collection of alpaca faecal samples (Presidente, 2007) or of limited scope (only South Australia and some parts of Victoria were included). Knowledge from these studies may not be directly applicable to Australian alpacas due to variations in climatic conditions. Therefore, the epidemiology and control of GINs in Australian alpacas remain largely unknown.

Table 1.2. Common gastrointestinal nematodes identified in alpacas and llamas.

Location	*Species of nematodes*	*Selected references*
Third compartment of the stomach (C3)	***Camelostrongylus mentulatus*, *Ostertagia ostertagi* (=*O. lyrata), Teladorsagia circumcincta*, *Trichostrongylus axei*, *Marshallagia marshalli*, *Haemonchus contortus*, *Graphinema auchenia*, *Gongylonema spp.*, *Spiculopteragia peruviana***	**Welchman et al., 2008; Ballweber, 2009; Franz et al., 2015**
Small intestine	***Lamanema chavezi*, *Nematodirus lamae*, *N. battus*, *N. filicollis*, *N. spathiger*, *N. helvetianus*, *Cooperia oncophora* (= *C. surnabada*, *C. mcmasteri*), *Trichostrongylus vitrinus*, *T. colubriformis*, *T.longispicularis*, *T. askivali*, *Capillaria* spp., *Strongyloides* sp.**	**Ballweber, 2009; Franz et al., 2015; Mitchell et al., 2016; Lambacher et al., 2016; McKenna, 2018)**
Large intestine	***Trichuris tenuis*, *T. discolor*, *T. skrjabini*, *Oesophagostumum columbianum*, *O. venulosum***	**Ballweber, 2009; Franz et al., 2015**

1.4.2. Life cycle

The life cycle of GINs is well known in the case of sheep and cattle. In SACs, it is presumed that GINs have a similar life cycle to that of ruminants based on the studies conducted in South America (Ballweber, 2009). Most GINs follow a simple direct lifecycle, which may vary according to the species of nematode. In trichostrongyloid nematodes, such as *Camelostrongylus*, *Ostertagia, Teladorsagia, Trichostrongylus, Haemonchus,* and *Cooperia*, fertilised females produce large numbers of eggs that are passed in the faeces. It usually takes 1-2 days for the first-stage larvae (L1) to hatch from eggs under optimal conditions: moisture (100%) and temperature (22 - 26˚C). Larvae feed on bacteria to develop to the second-stage larva (L2) and subsequently to ensheathed third-stage larvae (L3) in the environment. After being ingested by the hosts, the L3 loses its protective sheath and develops through to the fourth-stage (L4) either in the gastrointestinal lumen or in the mucosa and then to immature adults. Exsheathment sites vary depending on species. For example, *T. circumcincta* larvae exsheath within the rumen whereas, *Cooperia curticei* exsheath within the abomasum (Soulsby, 1982). Generally, somatic migration does not occur except for *L. chavezi*. However, larvae may invade the digestive glands or gastrointestinal wall depending on the type of nematodes. If the environmental conditions are not favourable (e.g. extreme hot or cold, lack of forage), the larvae cease development for few weeks to several months. This phenomenon is known as hypobiosis. Generally, the prepatent period of trichostrongyloid nematodes is 14 - 21 days, unless they undergo hypobiosis (Soulsby, 1982). Evidence of hypobiotic infections in SACs is available but is not abundant (Rickard, 1993; Windsor, 1997). Other nematodes such as *Nematodirus* spp. and *L. chavezi* have a slightly different life cycle. In *Nematodirus* spp. and *L. chavezi* the L3s develops within eggs (Rickard et al., 1989; Guerrero et al., 1981). Unlike other nematodes, the L3 of *L. chavezi* migrates to the liver and develops to the fourth-stage larvae (L4) and then returns to the small intestine as adults through the bile ducts (Guerrero et al., 1981). For the large intestinal worms such as *Oesophagostomum* and *Trichuris*, there are variations in the life cycle. Depending on temperature and humidity, the development of larval stages for *Oesophagostomum* may take 4 – 14 days. After ingestion by the host, the infective larvae penetrate the intestinal wall of the small or large bowel and form numerous nodules. Therefore, *Oesophagostomum* spp. are also known as 'nodule worms' (Beaver et al., 1984). The whole cycle takes approximately six

to seven weeks (Olsen, 1974). *Trichuris* spp. also known as whipworms, have a direct life cycle. The eggs become embryonated by around 10 days and infective larvae (L1s) develop within eggs in about three weeks and then are ingested by definitive host. After ingestion, infective larvae emerge from the eggs and invade the mucosa of the caecum and colon and moult to form adults. The pre-patent period of infections may vary from 8-12 weeks (Mehlhorn, 2001).

1.4.3. Epidemiology

It is important to understand the factors influencing nematode populations in order to develop control program for nematodes. Very little or no information is available on the epidemiology of GINs in SACs. However, due to the similarity of the nematode populations, it is presumed that they will behave similarly in SACs as they do in ruminants (Ballweber, 2009). There are many factors including host factors (e.g. immunity), weather and pasture conditions that affect the population dynamics of nematodes and thus influence the worm burden (Donald et al., 1978). Seasonal factors directly affect parasite populations. In southern Australia, autumn and spring are commonly favourable for the survival and development of larvae of the most GINs on the pasture due to moderate temperatures and evaporation rates and the availability of herbage. Infective larvae on the pasture in autumn may be affected if the winter is dry and frosty. Spring contamination of pasture may be shortened due to high temperatures and the lack of rain in spring (Donald et al., 1978). Therefore, temperature and moisture have major effects on the survival and development of eggs and larvae. For instance, eggs of *Haemonchus* cannot tolerate hot, dry conditions or cold temperatures (Donald et al., 1978). In contrast, the infective stages of *Trichostrongylus* spp. are capable of surviving desiccation and low temperatures (Donald et al., 1978). Similarly, *Ostertagia* spp. can develop at low temperatures (Donald et al., 1978). The population dynamics of GINs differ in the various climatic zones of Australia. The most important GINs prevalent in the Australian summer rainfall zones in domestic ruminants are *H. contortus* and *T. colubriformis*. Other species present are *T. vitrinus*, *T. axei*, *T. rugatus*, *Teladorsagia* and *Nematodirus* but they are not as important as the species mentioned earlier (Cole, 1986). Parasitic gastroenteritis in sheep in the winter rainfall zone is caused primarily by *Te. circumcincta* and *T. axei*, *T. colubriformis* and *T. vitrinus* (Beveridge et

al, 1989; De Chaneet et al., 1988; Anderson, 1972). The most important nematodes in the non-seasonal and uniform rainfall zones in Australia are *Te. circumcincta*, *T. colubriformis*, *T. vitrinus* and *H. contortus* (Donald, 1978). Alpacas in Australia may exhibit a similar pattern of parasitic infections as they commonly share sheep parasites.

Parasitic burdens may increase due to changes in the susceptibility of the stock. For example, ewes suffer a temporary relaxation in immunity during the peri-parturient period which increases their susceptibility to establishment of new infections and/or an increase in egg production of existing parasites (Armour, 1980; Salisbury et al., 1970). Young alpacas on contaminated pasture with poorly developed immunity are more seriously affected by parasitic infections and thus perform poorly (Ballweber, 2009; Fowler, 2010).

1.4.4. Pathology

Generally, alpacas with parasitic infections do not exhibit noticeable changes in terms of body condition and general wellbeing even at high infection levels (Ballweber, 2009; Fowler, 2010). Alpacas with parasitic infections may show non-specific clinical signs such as diarrhoea, anorexia, weight loss, reduced growth, and anaemia. In domestic ruminants, most infections remain asymptomatic, subclinical and chronic (Zajac and Conboy, 2006). However, *H. contortus* infections have been associated with marked anaemia in SACs (Welchman et al., 2008; Jabbar et al., 2013). The severity of the pathological changes caused by parasites depends on the species and the predilection sites in the host. For example, severe pallor of the mucosae, skeletal musculature, liver and kidneys with remarkably watery blood within the heart and major blood vessels were observed in a young alpaca in Australia diagnosed with haemonchosis (Jabbar et al., 2013). *Haemonchus* is a blood-sucking parasite, and it is estimated that each worm can remove 0.05 mL of blood per day and thus causing severe anaemia (Clark et al., 1962). Similarly, nodular lesions are found in the abomasum of sheep when *Te. circumcincta* infect and invade the gastric glands (Armour, 1980). On the other hand, the predilection site for intestinal *Trichostrongylus* spp. is in the epithelium of the duodenum, and as a result villus atrophy may be observed at this stage, leading to poor performance due to malabsorption. Greer et al. (2005) hypothesised that pathological changes due to *T.*

colubriformis infection is the result of host's own immunological response to the parasites, rather than mechanical damage caused by the parasites.

Nematodirus is generally less pathogenic but can be a serious problem in young sheep and calves in cool regions. Infective larvae (L3) of *Nematodirus* disrupt the mucosa of the small intestine, leading to malabsorption, and causing protein loss, profuse watery diarrhoea, dehydration and weight loss. *Nematodirus battus* is a sheep parasite but can infect cattle. It has been found in calves causing severe diarrhoea (Armour et al., 1988; McCoy et al., 2004). Other species of *Nematodirus* such as *N. filicollis* and *N. spathiger* are less pathogenic (Jackson and Coop, 2007).

Trichuris spp. can also infect SACs (Ballweber, 2009; Franz et al., 2015). Infection due to *Trichuris* is usually asymptomatic however, presence of large numbers can cause inflammation in the caecal mucosa and can be fatal in camels. *Trichuris* is less significant in production animals however, evidence of sporadic heavy infections with watery diarrhoea have been recorded in pigs (Urquhart et al., 1996).

1.5. Diagnosis

Worm burdens caused by GINs are usually based on the estimation of the number of eggs excreted in faeces or estimation and identification of adult worms collected from the gastrointestinal tracts at post mortem. The estimation of number of worm eggs in the host's faeces is generally achieved by a laboratory diagnostic method, known as the faecal egg count (FEC) test. The FEC is one of the most popular and routinely practiced methods for assessing nematode worm burden in domestic animals and humans (Denwood et al., 2012).

There are several FEC methods which have been developed over time. However, the most commonly used method is the McMaster method, which was developed by Gordon and Whitlock in Australia (Gordon and Whitlock, 1939). It is commonly used because it is simple, cheap and rapid (Ballweber et al., 2014).

The McMaster method, however, has limitations such as, interference of egg visibility in thick and dark faecal slurries, and the requirement of skills in microscopy and experience to identify and count the different nematode eggs (Ballweber et al., 2014). Other factors such as the type of feed, consistency of faeces, flotation time, flotation solution and interaction among factors can also affect FEC results (Dunn and Keymer,

1986). The FEC results do not necessarily represent the actual worm burdens. Some studies have shown evidence of relationship between FEC and worm burden (Roberts, 1957; Thomas and Boag, 1973; McKenna, 1981), whereas other studies have not shown a correlation (Rubin, 1967; Michel, 1969 a, b; Brunsdon, 1971; Rose and Small, 1980).

The flotation solution is one of the essential components of most FEC tests. There are different types of flotation solutions such as saturated sucrose solution or a saturated salt solution of sodium chloride, zinc chloride or sodium nitrate. However, the most commonly used flotation solutions are saturated salt solution (either sodium chloride or sodium nitrate) or sucrose solution. The specific gravity of the above-mentioned solutions varies from 1.19 – 1.27. Studies have shown that FEC results can be affected by the type and specific gravity of flotation solutions. Saturated sugar solution was found to be superior to salt solutions by Cebra and Stang (2008).

To overcome the limitations of the FEC methods, alternative diagnostic tools such as Kato-Katz, FLOTAC and FECPAC^{G2} have been developed and validated in animals and humans (Bosco et al., 2014; Dias de Castro et al., 2017; Paras et al., 2018; Levecke et al., 2011). Of these, FECPAC^{G2} is one of the most recent techniques and it has been validated in sheep, cattle and horses (Bosco et al., 2014; Godber et al., 2015; Presland, 2005). However, no study has been conducted to assess the performance of FECPAC^{G2} in alpacas. In this technique, a known amount of homogenized faeces is diluted in water and allowed to sediment. Then the supernatant is removed, and a flotation solution is added and transferred to a double-filtered container. An aliquot is taken by a pipette to fill two mini wells in a cassette. The cassette is inserted into a device called MICRO-I which replaces the need for a microscope. MICRO-I captures digital images of the surfaces of the wells in the cassette and uploads these images to an online system where the images are examined for counting of the eggs by a trained technician who is either on-site or at a distant location. Results can be read online or can be sent back to the user by email. The sensitivity of the FECPAC^{G2} for alpacas is 35 eggs per gram of faeces. This new method has the potential to be used in alpacas for estimation of worm burdens.

1.5.1. Larval identification

As stated above, FEC provides an estimation of the parasite burden of the host. Faecal egg counting can be used to make decisions about treatment and control, however, it

cannot be used to identify all types of nematodes involved in the infection. Under the microscope, the eggs of strongylid nematodes are morphologically similar apart from *Nematodirus* and *Trichuris* spp. To determine the genera and/or species of strongylid parasites involved in an infection, larval culture (LC) is used. Larval culture is a widely used technique. However, this technique has limitations. It is time consuming (usually 7 -10 days), and usually does not allow nematodes to be identified to species level. The results of larval culture may vary, depending on temperature and relative humidity of incubation, and thus may lead to a bias in the results (Dobson et al., 1992). To overcome some of these limitations, DNA based techniques have been developed (Roeber et al., 2013; 2015).

1.5.2. Molecular diagnosis

As in domestic ruminants, the diagnosis of GINs in SACs is mainly based on FECs and LC. However, these tests are time-consuming and lack sensitivity and specificity. As an alternative, a molecular diagnostic tool can be used for the accurate identification of GINs of SACs like those used in domestic ruminants (Roeber et al., 2013). For instance, the multiplexed-tandem PCR (MT-PCR) assay is a type of real-time PCR method that uses multiple primer pairs for the detection of several pathogens in one sample (Stanley and Szewczuk, 2005). This assay consists of two amplification steps where the primary amplification involves a 'target enrichment' using a small number of PCR cycles and outer primer sets, whereas the secondary amplification 'quantification step' utilises a diluted product from the primary amplification as a template and target specific, nested or inner primers (Stanley and Szewczuk, 2005). To date, MT-PCR has been applied to the simultaneous detection of a number of parasites of ruminants (Roeber et al., 2017a, b). However, very little is known about the use of molecular tools for diagnosis of GINs in alpacas. More studies are required to validate molecular tools that are being used in other ruminants, for identification of GINs of alpacas.

1.5.3. Total worm count

Total worm count (TWC) is a technique of enumeration of adult worms recovered from the gastrointestinal tract of an animal. Other than using the FEC, LC and MT-PCR

methods, parasitic burdens can be estimated using the TWC technique. As mentioned earlier, the FEC and/or LC may not be able to provide reliable estimation of worm burden. Moreover, there is not always a good correlation between egg counts and worm counts. The total worm count is a better estimation of actual burden of nematodes in the animal. It is a useful diagnostic technique, often used in ruminants. Limited information about TWC in alpacas in Australia is available apart from a study conducted by Jabbar et al. (2013). Results of this study revealed a burden of 460 *H. contortus* in one alpaca. Globally, there is a scarcity of TWC data for camelids. One study in the UK reported the recovery of *Nematodirus* spp., *Capillaria* spp. and *Trichuris* spp. from the gastrointestinal tracts of alpacas (Tait et al., 2002). A comprehensive TWC study could be useful to explore the prevalence and intensity of GINs of alpacas in Australia.

1.6. Control practices

The word "control" in parasitology generally indicates the suppression of parasite burdens in the host at the level below which economic losses occur. The main objectives of worm control practices are to i) prevent heavy exposure in susceptible hosts ii) reduce overall levels of pasture contamination iii) minimise the effects of parasite burdens and iv) enhance the development of immunity in the animals. To achieve these objectives, a comprehensive knowledge of the epidemiological and ecological factors that play an important role in pasture larval populations and host immunity in combating infection is required. Knowledge of GIN control practices are highly relevant to designing a control strategy as demonstrated in the control of GINs of ruminants (Anene et al., 1994; Borgsteede, 1998; Pedreira et al., 2006; Cernanska et al., 2008; Zanzani et al., 2014; Papini et al., 2015). Knowledge on worm control practices in SACs is very limited compared to those available in sheep, goats, cattle and horses.

Control of GIN infections in ruminants is predominantly based on the use of anthelmintic drugs. Other than therapeutic control, there are options of non-therapeutic controls such as grazing management, biological control, nutritional manipulation and use of herbal products amongst other things.

1.6.1. Therapeutic control

For therapeutic control, a wide range of anthelmintics is being used off-label in alpacas for therapeutic control of GINs as there is no registered anthelmintic for alpacas in Australia. The most commonly used anthelmintics are benzimidazoles (e.g. albendazole and fenbendazole; BZs), levamisole (LEV), macrocyclic lactones (abamectin, ivermectin and moxidectin; MLs), closantel and monepantel. A very limited number of studies have been conducted on the efficacy and pharmacokinetics of various anthelmintics globally in alpacas. Lack of knowledge of the pharmacokinetics of anthelmintic agents in SACs can lead to problems with off-label use. Off-label and/or indiscriminate use of anthelmintics may result in treatment failure, possibly due to the development of AR in GINs. In a recent study, *H. contortus* was found to be resistant to macrocyclic lactones (ML) in Australian alpacas (Jabbar et al., 2013). The following section provides a brief account on the commonly used anthelmintics in SACs.

1.6.1.1. Benzimidazoles (BZ)

Benzimidazoles (BZ) are a class of anthelmintics widely used against fungi, protozoa, and helminths. Primarily these drugs interact with the β-tubulin of the organism. The β-tubulin is a cytoskeletal protein and constitutes the main component of microtubules of the organisms along with α-tubulin. Tubulin-binding drugs bind to tubulin proteins in the cytoplasm of the intestinal cells of nematodes, preventing polymerisation of tubulin to form microtubules. BZs have relatively low mammalian toxicity. They have a broad spectrum with high efficacy, are easy to administer and cost effective (Borgers et al., 1975; Lacey, 1990; Jaeger & Carvalho-Costa, 2017). The first case of AR to the BZ class in Australia was reported in 1966. This class of anthelmintics now has resistant populations of *Te. circumcincta*, *T. colubriformis* and *H. contortus* in the majority of the sheep properties across the country (Playford et al., 2014). Resistance has also been reported in small ruminants in many other countries of the world, including South Africa, Malaysia, Netherlands, North America and South America (Sangster et al., 2002). The mechanism of resistance against BZs is now well understood (Conder and Campbell, 1995): the loss of affinity of binding tubulin (Sangster & Gill, 1999; Sangster et al., 2002).

A number of studies have been conducted on the efficacy and pharmacokinetics of fenbendazole in South American camelids. Beier et al. (1999) found the absorption rate of fenbendazole in llamas similar to sheep, goats and cattle. However, the elimination rate was prolonged compared to ruminants. Similar findings have been reported in alpacas by Lakritz et al. (2015). However, the metabolites of fenbendazole produced in alpacas were similar to those found in other species. Another study showed that fenbendazole paste given at 5 mg/kg effectively reduced GINs in naturally infected llamas (Beier et al., 2000), whereas another study found 10mg/kg as the minimum effective dose for alpacas and llamas (Shaw, 2001).

1.6.1.2. Levamisole (LEV)

Levamisole is an imidothiazole anthelmintic. It is a cholinergic agonist and kills GINs by depolarizing nicotinic neuromuscular junctions (Sangster, 1996). It is a widely used anthelmintic but resistance to it has been reported in small ruminants across the globe. Loss of cholinergic receptors in nematodes results in resistance against levamisole (Sangster et al., 2002). The mechanism of resistance is similar in different species of nematodes (Sangster & Gill, 1999).

Very little is known about the efficacy of levamisole in SACs. One study conducted by Gillespie et al. (2010) found levamisole at 8 mg/kg body weight effective in llamas. Levamisole was found to be the most effective drug to control of *L. chavezi* in SACs by Guerrero et al. (1981).

1.6.1.3. Macrocyclic lactones (ML)

This group of anthelmintics comprises the avermectins and milbemycins. The most commonly used drugs of this class are ivermectin, doramectin, moxidectin and eprinomectin. They cause paralysis by binding to glutamate-gated chloride channel receptors in nematode and arthropod nerve cells (Shoop et al., 1995). They are effective against ecto- and endo-parasites and this is one of the reasons why they are widely used in cattle, sheep, goat, horses, pigs, dogs, camelids and human (Fisher, 1997).

Macrocyclic lactones became available for sheep in Australia in 1988, beginning with ivermectin (Playford et al., 2014). Moxidectin is usually more potent than abamectin, i.e.

at the same dose rate, moxidectin kills more worms. In turn, abamectin is more potent than ivermectin. If a short-acting ML is required, abamectin rather than ivermectin should be considered, due to abamectin's greater potency.

Moxidectin has persistent activity against some susceptible worms, meaning that it continues to kill ingested infective larvae for days or weeks – depending on the product – following administration. A first sign of resistance with longer acting products such as moxidectin is a reduction in the period of protection it normally provides.

Resistance of *Trichostrongylus* to the MLs has been reported in sheep in Australia, but confirmed cases are still uncommon (Playford et al., 2014). Many sheep farms in Australia, especially in the uniform and winter rainfall areas, have ML-resistant *Teladorsagia*. Resistance of *H. contortus* to this group occurs on most properties in summer rainfall regions and elsewhere (Playford et al., 2014).

Historically, ivermectin has been the most widely used member of MLs. Studies on the efficacy and pharmacokinetics of ivermectin have been conducted in SACs. Ivermectin injected subcutaneously at a dose rate of 200 µg/kg bodyweight subcutaneously was 100% effective in alpacas in Belgium (Geurden and Van Hemelrijk, 2005). Burkholder et al. (2004) showed that a subcutaneous dose of 200 µg/kg failed to reach therapeutic blood concentrations in llamas. Ivermectin has been used for ectoparasites control with variable levels of efficacy (Geurden, 2003, Twomey et al., 2009).

Moxidectin is also used in SACs to control both endo and ectoparasites with inconsistent results. In llamas, moxidectin drenched at 200 µg/kg bodyweight orally was effective on one farm and ineffective on another farm (Gillespie et al. 2010). Limited information is available on the pharmacokinetics of moxidectin in SACs. In alpacas, bioavailability of the 200 µg/kg bodyweight dose was found to be higher when administered subcutaneously compared to oral administration (Cocquyt et al., 2016). Moxidectin is not recommended for topical use in alpacas due to poor efficacy (Hunter et al., 2004a).

Doramectin is also used in SACs to control external and internal parasites. Slow absorption was reported in alpacas and llamas when used topically at 0.5 mg/kg bodyweight (Hunter et al., 2004b). To control GIN in alpacas, a dose rate of 0.3 mg/kg bodyweight was unsuccessful. Eprinomectin is not a widely used ML. It has been studied for efficacy and pharmacokinetics against the ectoparasites of alpacas and llamas (Pollock et al., 2017; Plant et al., 2007).

1.6.1.4. Closantel

Closantel, nitroxynil and oxyclozanide have activity against blood-sucking parasites, namely *H. contortus* and *Fasciola hepatica* (except for early immatures) and *H. contortus*. These anthelmintics uncouple oxidative phosphorylation in targeted worms, resulting in loss of cellular integrity. Resistance to closantel in *H. contortus* has been reported in Australian farms located mainly in northern New South Wales and southern Queensland of Australia (Besier et al., 2003). Resistance of *Fasciola hepatica* to closantel has been found but is thought to be uncommon (Novobilský et al., 2015).

1.6.1.5. Monepantel

Monepantel is the only commercially available (Zolvix®) amino-acetonitrile derivative (AAD). It is a new anthelmintic, with New Zealand the first country of release in 2009, and available in Australia in 2010. Monepantel acts on a particular type of nicotinic acetylcholine receptor, which is unique in that it is found only in nematodes. Binding of monepantel to the receptor causes irreversible paralysis of nematodes. Resistance to this group has been reported on a few sheep and goat in Australia (Sales and Love, 2016), New Zealand (Scott et al., 2013), Brazil (Martins et al., 2017; Cintra et al., 2016), New Zealand (Scott et al., 2013), Netherlands (Van den Brom et al., 2015) and Uruguay (Mederos et al., 2014). Resistant worms have included *H. contortus*, *Trichostrongylus* spp. and *Te. circumcincta*, but the resistance profile has not been the same on all affected farms in different locations. For example, in the first reported case in Australia (goat farm, central western NSW (Love, 2014), monepantel remained effective against *H. contortus* but had reduced efficacy against *Trichostrongylus* spp. In other cases, monepantel-resistant *H. contortus* has been reported in some regions of Queensland (Constantinoiu and Cat, 2015).

Monepantel, a new anthelmintic has not been studied well in SACs. One recent study conducted by Dadak et al. (2013) found it to be ineffective at the recommended dose rate of 2.5mg/kg bodyweight or two times of that dose used in llamas. Three times the recommended dose achieved 100% efficacy (Dadak et al., 2013).

1.6.2. Non-therapeutic control

Anthelmintics play a vital role in worm control practices. However, due to the widespread problem of AR, alternative approaches such as pasture management have become the focus of research for integrated parasite management in livestock. 'Clean-grazing' systems through pasture management are one of the recommended and effective worm control methods (Rutter et al. 1984). Year-round grazing is a common practice in Australian livestock industries, which increases the exposure to nematode parasites (Barger, 1993). Strategic planning to maintain a pasture with low worm burden can be achieved by alternate grazing with cattle or horses for sheep, grazing crop stubbles, hay etc. (Besier and Love, 2004).

Selective breeding for worm resistance is another alternative approach and has been found effective in reducing worm burdens in sheep. Results from the studies in Australian sheep showed success in consistently reduced worm burdens (Eady et al., 1996, 1998). This approach has a potential for worm control in sheep in Australia (Besier and Love, 2004).

Controlling parasites by immunological means has also been investigated. *Haemonchus* is one of the most significant parasites of small ruminants. Attempts were made to develop vaccines against *Haemonchus* with significant success in sheep (Newton, 1995) and in goats (Meier et al., 2016). Barbervax® (Wormvax Australia Pty Ltd), a commercially manufactured vaccine from the membrane proteins of the nematode's intestines was launched in October 2014 in Australia and registered for use in sheep. Field trials showed significant success in reducing *Haemonchus* burden in sheep (Besier et al., 2015; de Matos et al., 2017; Smith et al., 2013). Barbervax® vaccine has also been studied in alpacas recently with the successful production of antibodies against *Haemonchus* (VanHoy et al., 2018).

Another biological control by the use of predaceous fungi has become a reality recently. BioWorma®, a commercial product containing the chlamydospores of the nematophagous fungus *Duddingtonia flagrans* strain IAH 1297 has been used as a pasture supplement to reduce larval contamination. Studies found this product effective in reducing the worm burden in sheep, goats, cattle and horses (Knox et al., 2013; Healey et al., 2018a, b).

Many of the approaches mentioned above have been successful in sheep, goats and cattle and may show similar results in alpacas. More study is required to assess their performance in SACs.

1.7. Anthelmintics resistance

Drug resistance in any organism is defined by a change in the drug's pharmacokinetics and pharmacodynamics (absorption, distribution, metabolism, excretion, and site of action) (Lacey, 1990) that allows some individuals in a population to tolerate the therapeutic dose of a given compound that would not normally be tolerated.

The use of various classes of anthelmintic agents against GINs is one of the most widely practiced control measures in sheep, cattle and other animals, including alpacas (Franz et al., 2015). For anthelmintic drugs, very little is known about their proper effective dose regime for SACs. As a result, following the dose rates and routes of administration recommended for sheep, cattle or horses may or may not be effective to eliminate GINs of SACs (Dadak et al., 2013). Anthelmintic resistance is a widely recognised problem in GINs of sheep, cattle, horse and other species. There are many risk factors contributing to the development of AR (Edwards et al., 1986). Under-dosing due to lack of scales and underestimation of body weight of animals is the most commonly identified risk factor (Domke et. el., 2011; Maingi et al., 1996). Lack of the use of scales leads to an increased use of visual appraisal for estimating body weight (Maingi et al., 1996) which may lead to under-dosing. A significant number of studies have been conducted in production animals such as sheep and cattle to identify the resistant nematodes and anthelmintic agents as well as the risk factors of developing AR (Domke et al., 2011; Falzon et al., 2013). Very little is known about AR in alpacas. Nevertheless, resistance to albendazole, fenbendazole, ivermectin and doramectin in GINs of alpacas and llamas has been documented across the globe (Galvan et al., 2012; Marino, 2011; Gillespie et al., 2010; Jabbar et al., 2013). Caution must be taken stating that these cases are 'true resistance' as effective dose rates have not been determined for SACs.

Treating all animals at a time when the numbers of infective larvae on pasture are low is considered one of the major factors associated with the rapid and widespread development of AR in Australia (Besier, 2001; Besier and Love, 2004). Maintaining refugia by leaving a proportion of adult sheep untreated is practiced delaying the

development of AR. Studies conducted in sheep found maintaining refugia is likely to reduce building up of resistant worms (Martin et al., 1981; Besier et al., 2012; Leathwick et al., 2008; Waghorn et al., 2008, 2009). Thus, maintaining refugia in conjunction with justified use of anthelmintic could be another tool to be considered as worm control method for alpacas and llamas.

1.8. Aims

Aims of the thesis were:

1. To assess worm control practices used by alpaca farmers in Australia.
2. To establish and validate new diagnostic tools to assess worm burden in alpacas.
3. To determine the epidemiology of GINs of alpacas in Australia.
4. To assess the efficacy of commonly used anthelmintics against GINs of Australian alpacas.

1.9. References

Alcaino, H., Gorman, T., & Burgos, M. (1991). Helmintiasis gastrointestinal en llamas (*Lama glama*) de la I Región de Chile. *Parasitolog´ıa al d´ıa*, 15, 93-96.

Anderson, N. (1972). Trichostrongylid infections of sheep in a winter rainfall region. I. Epizootiological studies in the Western District of Victoria, 1966–67. *Australian Journal of Agricultural Research,* 23, 1113-1129.

Anene, B.M., Onyekwodiri, E.O., Chime, A.B., & Anika, S.M. (1994). A survey of gastro-intestinal parasites in cattle of southeastern Nigeria. *Preventive Veterinary Medicine* 20, 297-306.

Armour, J., Bairden, K., Dalgleish, R., Ibarra-Silva, A. M., & Salman, S. K. (1988). Clinical nematodiriasis in calves due to *Nematodirus battus* infection. *The Veterinary Record,* 123, 230-231.

Armour, J. (1980). Epidemiology of Helminth Disease in Farm-Animals. *Veterinary Parasitology*, 6, 7-46.

Australian Alpaca Association (AAA) (2001). Commercial Viability of the Australian Alpaca Industry. *A Report by ACIL Consulting.*

Ballweber, L.R., Beugnet, F., Marchiondo, A.A., & Payne, P.A. (2014). American Association of Veterinary Parasitologists' review of veterinary fecal flotation methods and factors influencing their accuracy and use--is there really one best technique? *Veterinary Parasitology*, 204, 73-80.

Ballweber, L.R. (2009). Ecto- and endoparasites of new world camelids. The Veterinary Clinics of North America. *Food Animal Practice,* 25, 295-310.

Barger, I.A. (1993). Control of gastrointestinal nematodes in Australia in the 21st century. *Veterinary Parasitology*, 46, 23-32.

Beier III, E. (1999). Oral pharmacokinetic and clinical efficacy profiles of fenbendazole in Llamas, South American Camelids (Doctoral dissertation, Oklahoma State University).

Beier, E., Lehenbauer, T.W., & Sangiah, S. (2000). Clinical efficacy of fenbendazole against gastrointestinal parasites in llamas. *Small Ruminant Research*, 36, 17-23.

Beveridge I, Pullman AL, Phillips PH, Martin RR, Barelds A, & Grimson R. (1989). Comparison of the effects of infection with *Trichostrongylus colubriformis*, *T. vitrinus* and *T. rugatus* in Merino lambs. *Veterinary Parasitology,* 1989, 32, 229-245

Beveridge, I., Barker, I., Rickard, M., & Burton, J. (1974). Experimental infection of sheep with *Camelostrongylus mentulatus* and associated gastritis. *Australian Veterinary Journal,* 50, 36-37.

Beveridge, I., & Ford, G.E. (1982). The trichostrongyloid parasites of sheep in South Australia and their regional distribution. *Australian Veterinary Journal,* 59, 177-179.

Beveridge, I., Pullman, A.L., Henzell, R., & Martin, R.R. (1987). Helminth parasites of feral goats in South Australia. *Australian Veterinary Journal,* 64, 111-112.

Besier, B., Kahn, L., Dobson, R., & Smith, D. (2015). Barbervax− a new strategy for haemonchus management. In: Proceedings of the Australian Veterinary Association (AVA) Annual Conferences, Pan Pacific (NZVA and AVA) Veterinary Conference 2015, Combined proceedings, pp. 168-174.

Besier, R. (2012). Refugia-based strategies for sustainable worm control: factors affecting the acceptability to sheep and goat owners. *Veterinary Parasitology*, 186, 2-9.

Besier, B. (2009) Worm control in alpacas in Western Australia. Department of Agriculture and Food, WA, Australia

Besier, R., & Love, S. (2004). Anthelmintic resistance in sheep nematodes in Australia: the need for new approaches. *Animal Production Science*, 43, 1383-1391.

Besier, R.B. (2001). Re-thinking the summer drenching program. *Western Australian Journal of Agriculture,* 42, pp. 6-9.

Beaver, P.C., Jung, R.C., Cupp, E.W. (1984). Oesophagostomum species. In Beaver et al.,

eds., 1984. *Clinical Parasitology.* Lea & Febiger*,* 287-288.

Borgers, M., De, S. N., De, M. B. & Thienpont, D. (1975a). Influence of the anthelmintic mebendazole on microtubules and intracellular organelle movement in nematode intestinal cells. *American Journal of Veterinary Research,* 36, 1153-1166.

Borgsteede, F.H., Sol, J., van Uum, A., de Haan, N., Huyben, R. & Sampimon, O. (1998). Management practices and use of anthelmintics on dairy cattle farms in The Netherlands: results of a questionnaire survey. *Veterinary Parasitology,* 78, pp.23-36.

Bosco, A., Rinaldi, L., Maurelli, M.P., Musella, V., Coles, G.C., & Cringoli, G. (2014). The comparison of FLOTAC, FECPAK and McMaster techniques for nematode egg counts in cattle. *Acta Parasitologica,* 59, 625-628.

Brunsdon, R.V. (1971) Trichostronglye worm infection in cattle: further studies on problems of diagnosis and on seasonal patterns of occurrence. *New Zealand Veterinary Journal,* 19, 203-212.

Cafrune, M.a.M., Aguirre, D.H., & Rickard, L.G. (2001). First report of *Lamanema chavezi* (Nematoda: Trichostrongyloidea) in llamas (*Lama glama*) from Argentina. *Veterinary Parasitology*, 97, 165-168.

Carmichael, I. (1999). Internal parasitism in alpacas in Southern Australia, In: Hack, W., McGregor, B., Ponzoni, R., Judson, G., Carmichael, I., Hubbard, D. (Eds.) Australian Alpaca Fibre: Improving Productivity and Marketing. *Rural Industries Research and Development Corporation, Australia*, pp. 92-130.

Carmichael, I. (2014). Internal parasitism in Australian alpacas. In: *Australian Alpaca Association National Conference Adelaide, Australia,* pp. 13-28.

Cebra, C.K., & Stang, B.V. (2008). Comparison of methods to detect gastrointestinal parasites in llamas and alpacas. *Journal of the American Veterinary Medical Association,* 232, 733-741.

Chaneet, G.C., Dunsmore, J.D. (1988). Climate and the distribution of intestinal *Trichostrongylus* spp. of sheep. *Veterinary Parasitology,* 26, 273-283

Cintra, M.C.R., Teixeira, V.N., Nascimento, L.V., & Sotomaior, C.S. (2016). Lack of efficacy of monepantel against *Trichostrongylus colubriformis* in sheep in Brazil. *Veterinary Parasitology,* 216, 4-6.

Clark, C.H., Kiesel, G.K., & Goby, C.H. (1962). Measurements of blood loss caused by *Haemonchus contortus* infection in sheep. *American Journal of Veterinary Research,* 23, 977-980.

Cernanska, D., Varady, M., Cudekova, P., & Corba, J. (2008). Worm control practices on sheep farms in the Slovak Republic. *Veterinary Parasitology,* 154, 270-276.

Clarke, M. (2017). Alpaca Market Assessment 2016. http://www.agrifutures.com.au/wp-content/uploads/publications/17-010.pdf (retrieved 20 March 2018).

Cocquyt, C.M., Van Amstel, S., Cox, S., Rohrbach, B., & Martin-Jimenez, T. (2016). Pharmacokinetics of moxidectin in alpacas following administration of an oral or subcutaneous formulation. *Research in Veterinary Science,* 105, 160-164.

Cole, V.G. (1986). Helminth Parasites of Sheep and Cattle. Australian Government Publishing Service.

Conder, G.A. & Campbell, W.C. (1995). Chemotherapy of nematode infections of veterinary importance, with special reference to drug resistance. *Advances in Parasitology,* 35, 1-84.

Constantinoiu, C., Cat, S.de. (2015). Lack of efficacy of monepantel against *Haemonchus contortus* and *Trichostrongylus* spp. in small ruminants Proceedings of the 4th AVA/NZVA Pan Pacific Conference, Brisbane, Australia, 24th–29th May (2015).

Contreras, S., Chávez, V., Pinedo, V., Leyva, V., & Suárez, A. (2014). Helminthiasis in alpacas (*Vicugna pacos*) of two peasant communities in Macusani, Puno during the dry season. *Revista de Investigaciones Veterinarias del Perú (RIVEP)*, 25, 268-275.

Copland, J.W. (1965). *Ostertagia mentulata* recorded in New South Wales. *Australian Veterinary Journal,* 41, 27-27.

Dadak, A.M., Asanger, H., Tichy, A., & Franz, S. (2013). Establishing an efficacious dose rate of monepantel for treating gastrointestinal nematodes in llamas under field conditions. *Veterinary Record*, 172, 155-.

Denwood, M.J., Love, S., Innocent, G.T., Matthews, L., McKendrick, I.J., Hillary, N., Smith, A., & Reid, S.W.J. (2012). Quantifying the sources of variability in equine faecal egg counts: Implications for improving the utility of the method. *Veterinary Parasitology,* 188, 120-126.

Dias de Castro, L.L., Abrahão, C.L.H., Buzatti, A., Molento, M.B., Bastianetto, E., Rodrigues, D.S., Lopes, L.B., Silva, M.X., de Freitas, M.G., Conde, M.H., & Borges, F.d.A. (2017). Comparison of McMaster and Mini-FLOTAC fecal egg counting techniques in cattle and horses. *Veterinary Parasitology: Regional Studies and Reports*, 10, 132-135.

Dobson, R.J., Barnes, E.H., Birclijin, S.D., & Gill, J.H. (1992). The survival of *Ostertagia circumcincta* and *Trichostrongylus colubriformis* in faecal culture as a source of bias in apportioning egg counts to worm species. *International Journal for Parasitology,* 22, 1005-1008.

Domke, A.V.M., Chartier, C., Gjerde, B., Leine, N., Vatn, S., Osteras, O., & Stuen, S. (2011). Worm control practice against gastro-intestinal parasites in Norwegian sheep and goat flocks. *Acta Veterinaria Scandinavica,* 53, 29.

Donald, A.D., Southcott, W.H., & Dineen, J.K. (1978) In: The Epidemiology and Control of Gastrointestinal Parasites of Sheep in Australia. *Commonwealth Scientific and Industrial Research Organisation*, Melbourne, Australia.

Dittmer, K., Hinkson, J., Dwyer, C., Adlington, B., van & Andel, M. (2018). Prevalence of Candidatus *Mycoplasma haemolamae*, bovine viral diarrhoea virus, and gastrointestinal parasitism in a sample of adult New Zealand alpaca (*Vicugna pacos*). *New Zealand Veterinary Journal*, 66, 9-15.

Dunn, A., & Keymer, A. (1986). Factors affecting the reliability of the McMaster technique. *Journal of Helminthology,* 60, 260-262.

Dwyer, C., Pomroy, W., Gedye, K., Dittmer, K., Roe W., Aberdein, D., Adlington, B., Dann, H., & Wehrne, A. (2014). *Trichostrongylus askivali* – a new record for alpacas in New Zealand. Proceedings of the New Zealand Society for Parasitology: The 42nd Conference, Wellington, New Zealand.

Eady, S., Woolaston, R., Mortimer, S., Lewer, R., Raadsma, H., Swan, A., & Ponzoni, R. (1996). Resistance to nematode parasites in Merino sheep: sources of genetic variation. *Australian Journal of Agricultural Research*, 47, 895-915.

Eady, S., Woolaston, R., Ponzoni, R., Lewer, R., Raadsma, H., & Swan, A. (1998). Resistance to nematode parasites in Merino sheep: correlation with production traits. *Australian Journal of Agricultural Research*, 49, 1201-1212.

Edwards, J.R., Wroth, R., de Chaneet, G.C., Besier, R.B., Karlsson, J., Morcombe, P.W., Dalton-Morgan, G., & Roberts, D. (1986). Survey of anthelmintic resistance in Western Australian sheep flocks. 2. Relationship with sheep management and parasite control practices. *Australian Veterinary Journal*, 63, 139-144.

Falzon, L.C., Menzies, P.I., Vanleeuwen, J., Jones-Bitton, A., Shakya, K.P., Avula, J., Jansen, J.T., & Peregrine, A.S. (2013). A survey of farm management practices and their associations with anthelmintic resistance in sheep flocks in Ontario, Canada. *Small Ruminant Research,* 114, 41-45.

Fisher, M. H. (1997). Structure-activity relationships of the avermectins and milbemycins. In Phytochemicals for Pest Control, Vol. 658 (eds. Hedin, P. A., Hollingworth, R. M., Masler, E. P., Miyamoto, J., and Thompson, D. G.), pp. 220-238.

Fowler, M.E. (2010). Parasites. In: Medicine and Surgery of Camelids, Third Ed. Wiley-Blackwell, Ames, IA, USA, pp. 231-271.

Franz, S., Wittek, T., Joachim, A., Hinney, B., & Dadak, A.M. (2015). Llamas and alpacas in Europe: endoparasites of the digestive tract and their pharmacotherapeutic control. *The Veterinary Journal,* 204, 255-262.

Galvan, N., Middleton, J.R., Nagy, D.W., Schultz, L.G., & Schaeffer, J.W. (2012). Anthelmintic resistance in a herd of alpacas (*Vicugna pacos*). *Canadian Veterinary Journal,* 53, 1310-1313.

Geurden, T., & Van Hemelrijk, K. (2005). Ivermectin treatment against gastrointestinal nematodes in New World camelids in Belgium. *Small Ruminant Research,* 58, 71-73.

Geurden, T., & Vercruysse J. (2003). Treatment of sarcoptic, psoroptic and chorioptic mange in a Belgian alpaca herd. *Veterinary Record*, 153, 331-332.

Gillespie, R.A., Williamson, L.H., Terrill, T.H., & Kaplan, R.M. (2010). Efficacy of anthelmintics on South American camelid (llama and alpaca) farms in Georgia. *Veterinary Parasitology,* 172, 168-171.

Godber, O.F., Phythian, C.J., Bosco, A., Ianniello, D., Coles, G., Rinaldi, L., & Cringoli, G. (2015). A comparison of the FECPAK and Mini-FLOTAC faecal egg counting techniques. *Veterinary Parasitology,* 207, 342-345.

Gordon, H.M., & Whitlock, H.V. (1939). A new technique for counting nematode eggs in sheep faeces. *Journal of the Council for Scientific and Industrial Research,* 12, 50-52.

Greer, A.W., Stankiewicz, M., Jay, N.P., McAnulty, R.W., & Sykes, A.R. (2005). The effect of concurrent corticosteroid induced immuno-suppression and infection with the intestinal parasite *Trichostrongylus colubriformis* on food intake and utilization in both immunologically naive and competent sheep. *Animal Science,* 80, 89-99.

Guerrero, C.A., Rojas, M. & Alva, J. (1981). *Lamanema chavezi*, an enterohepatic nematode of South American Camelidae and its control using levamisole. *Revista Latinoamericana de Microbiologia,* 23, p.121.

Guerrero, C., & Chávez, C. (1967). Helmintos comunicados por primera vez en alpacas (*Lama pacos*) con una descripción de *Spiculopteragia peruvianus* n. sp. *Boletin Chileno de Parasitologia,* 22, 147-150.

Healey, K., Lawlor, C., Knox, M.R., Chambers, M., & Lamb, J. (2018a). Field evaluation of *Duddingtonia flagrans* IAH 1297 for the reduction of worm burden in grazing animals: tracer studies in sheep. *Veterinary Parasitology*, 253, 48-54.

Healey, K., Lawlor, C., Knox, M.R., Chambers, M., Lamb, J., & Groves, P. (2018b). Field evaluation of *Duddingtonia flagrans* IAH 1297 for the reduction of worm burden in grazing animals: Pasture larval studies in horses, cattle and goats. *Veterinary Parasitology,* 258, 124-132.

Hertzberg, H., & Kohler, L. (2006). Prevalence and significance of gastrointestinal helminths and protozoa in South American camelids in Switzerland. *Berliner und Münchener Tierärztliche Wochenschrift,* 119, 291-294.

HInkson, J. A. (2015). Investigations into common farm management practices and diseases on alpaca farms in New Zealand: a thesis presented in partial fulfilment of the requirements for the degree of Master of Veterinary Studies in Pathobiology at Massey University, Palmerston North, New Zealand (Doctoral dissertation, Massey University).

Hill, F.I., Death, A.F., & Wyeth, T.K. (1993). Nematode burdens of alpacas sharing grazing with sheep in New Zealand. *New Zealand Veterinary Journal,* 41, 205-208.

Hunter, R.P., Isaza, R., Koch, D.E., Dodd, C.C., & Goately, M.A. (2004a). Moxidectin plasma concentrations following topical administration to llamas (*Lama glama*) and alpacas (*Lama pacos*). *Small Ruminant Research*, 52, 275-279.

Hunter, R.P., Isaza, R., Koch, D.E., Dodd, C.C., & Goatley, M.A. (2004b). The pharmacokinetics of topical doramectin in llamas (*Lama glama*) and alpacas (*Lama pacos*). *Journal of Veterinary Pharmacology and Therapeutics*, 27, 187-189.

Hyuga, A., & Matsumoto, J. (2016). A survey of gastrointestinal parasites of alpacas (*Vicugna pacos*) raised in Japan. *Journal of Veterinary Medical Science,* 78, 719-721.

Jabbar, A. 2018. A review of helminth infections in alpacas. In Australian Veterinary Association Annual Conference (Brisbane, Australia).

Jabbar, A., Campbell, A.J.D., Charles, J.A., & Gasser, R.B. (2013). First report of anthelmintic resistance in *Haemonchus contortus* in alpacas in Australia. *Parasites and Vectors,* 6, 243-249.

Jackson, F., & Coop, R. L. (2007). Gastrointestinal helminthosis. In I. Aitken (Ed.), Diseases of Sheep (4th Edition, pp. 185–195). Wiley-Blackwell.

Jarvinen, J.A., Whitley, E.M., Kreuder, A.J., & Schleining, J.A. (2014). Identification of *Lamanema chavezi* Becklund 1963 infection in a llama (*Lama glama*) in the United States. *Journal of Veterinary Diagnostic Investigation*, 26, 178-183.

Jaeger, L. H. & Carvalho-Costa, F. A. (2017). Status of benzimidazole resistance in intestinal nematode populations of livestock in Brazil: a systematic review. *BMC Veterinary Research,* 13, 358.

Knox, M., Healey, K., Lawlor, C., Lamb, J., Chambers, M., & Groves, P. (2013). BioWorma® for control of nematode parasites of livestock. In: *Proceedings World Association for the Advancement of Veterinary Parasitology conference,* Perth, Australia, pp. 25-29.

Lambacher, B., Wittek, T., Joachim, A., Dadak, A., Stanitznig, A., Hinney, B., Tichy, A., Duscher, G., & Franz, S. (2016). From the New World to the Old World: endoparasites of South American camelids in Austria. *Wiener Tierärztliche Monatsschrift,* 103, pp.33-42.

Leathwick, D.M., Miller, C.M., Atkinson, D.S., Haack, N.A., Waghorn, T.S., & Oliver, A.M. (2008). Managing anthelmintic resistance: Untreated adult ewes as a source of unselected parasites, and their role in reducing parasite populations. *New Zealand Veterinary Journal*, 56, 184-195.

Levecke, B., Behnke, J.M., Ajjampur, S.S.R., Albonico, M., Ame, S.M., Charlier, J., Geiger, S.M., Hoa, N.T.V., Kamwa Ngassam, R.I., Kotze, A.C., McCarthy, J.S., Montresor, A., Periago, M.V., Roy, S., Tchuem Tchuenté, L.-A., Thach, D.T.C., & Vercruysse, J. (2011). A comparison of the sensitivity and fecal egg counts of the McMaster Egg counting and Kato-Katz thick smear methods for soil-transmitted helminths. *PLOS Neglected Tropical Diseases*, 5, e1201.

Lacey, E. (1990). Mode of action of benzimidazoles. *Parasitology Today*, 6, 112-115.

Lakritz, J., Linden, D., Anderson, D.E., & Specht, T.A. (2015). Plasma concentrations of fenbendazole (FBZ) and oxfendazole in alpacas (*Lama pacos*) after single intravenous and oral dosing of FBZ. *Veterinary Medicine: Research and Reports,* 71.

Leguía, G. (1991). The epidemiology and economic impact of llama parasites. *Parasitology Today* 7, 54-56.

Love, S. (2014). Monepantel (Zolvix®) resistance confirmed in goats in NSW Australia. *WormMail Newsletter,* 6 June 2014.

Maingi, N., Bjorn, H., Thamsborg, S.M., Dangolla, A., & Kyvsgaard, N.C. (1996). A questionnaire survey of nematode parasite control practices on goat farms in Denmark. *Veterinary Parasitology,* 66, 25-37.

Matos, A.F.I.M., Nobre, C.O.R., Monteiro, J.P., Bevilaqua, C.M.L., Smith, W.D., & Teixeira, M. (2017). Attempt to control *Haemonchus contortus* in dairy goats with Barbervax®, a vaccine derived from the nematode gut membrane glycoproteins. *Small Ruminant Research*, 151, 1-4.

Martin, P.J., Le Jambre, L.F., & Claxton, J.H. (1981). The impact of refugia on the development of thiabendazole resistance in *Haemonchus contortus*. *International Journal for Parasitology,* 11, pp.35-41.

Martins, A.C., Formigoni Bergamasco, P.L., Felippelli, G., Tebaldi, J.H., Duarte Moraes, M.F., Pereira Testi, A.J., Lapera, I.M., & Lux Hoppe, E.G. (2017). *Haemonchus contortus* resistance to monepantel in sheep: fecal egg count reduction tests and randomized controlled trials. *Semina-Ciencias Agrarias,* 38, 231-238.

Marino, T.A.C. (2011). Determinación de resistencia antihelmíntica frente a ivermectina de nematodos gastrointestinales en alpacas (*Vicugna pacos*) Puno-Perú. *Abanico Veterinario* 1, 11-20.

McKenna, P.B. (2018). Additions to the checklists of helminth and protozoan parasites of terrestrial mammals and birds in New Zealand. *New Zealand Journal of Zoology,* 45, pp.1-7.

McKenna, P.B., Morley, C., Koning, M., Tahana, J.S., & Taylor, M.J. (2009). Confirmation of the occurrence of the nematode parasite *Lamanema chavezi* Becklund, 1963 in South American camelids in New Zealand. *New Zealand Veterinary Journal,* 57, 395-396.

McKenna, P.B. (1981) The diagnostic value and interpretation of faecal egg counts in sheep. *New Zealand Veterinary Journal,* 29, 129-132.

McCoy, M. A., Kenny, J., & Hill, J. (2004). Outbreak of *Nematodirus battus* infection in calves. *The Veterinary Record,* 154, 370-371.

Mederos, A.E., Ramos, Z., & Banchero, G.E. (2014). First report of monepantel *Haemonchus contortus* resistance on sheep farms in Uruguay. *Parasites and Vectors,* 7, 598.

Meier, L., Torgerson, P.R., & Hertzberg, H. (2016). Vaccination of goats against *Haemonchus contortus* with the gut membrane proteins H11/H-gal-GP. *Veterinary Parasitology,* 229, 15-21.

Mehlhorn, H. (2001) Trichuriasis. In: Encyclopedic Reference of Parasitology. Springer, Berlin, Heidelberg.

Michel, J.F. (1969a). Observations on the faecal egg count of calves naturally infected with *Ostertagia ostertagi*. *Parasitology,* 59, 829-835

Michel, J.F. (1969b). The regulation of egg output by *Ostertagia ostertagi* in calves infected once only. *Parasitology,* 59, 767-774.

Mitchell, S., Hopkins, B., & Corfield, C. (2016). *Nematodirus lamae* identified in an alpaca in the UK. *Veterinary Record,* 178, 271-272.

Newton, S.E. (1995). Progress on vaccination against *Haemonchus contortus*. *International Journal for Parasitology,* 25, 1281-1289.

Novobilský, A., & Höglund, J. (2015). First report of closantel treatment failure against *Fasciola hepatica* in cattle. *International Journal for Parasitology: Drugs and Drug Resistance,* 5, 172-177.

Olsen, O.W. (1974). *Oesophagostomum columbianum*. In: Animal parasites. Their life cycles and ecology. *Baltimore, University Park Press,* 417-420.

Papini, R.A., De Bernart, F.M., & Sgorbini, M. (2015). A questionnaire survey on intestinal worm control practices in horses in Italy. *Journal of Equine Veterinary Science*, 35, 70-75.

Paras, K.L., George, M.M., Vidyashankar, A.N., & Kaplan, R.M. (2018). Comparison of fecal egg counting methods in four livestock species. *Veterinary Parasitology,* 257, 21-27.

Pedreira, J., Paz-Silva, A., Sanchez-Andrade, R., Suarez, J.L., Arias, M., Lomba, C., Diaz, P., Lopez, C., Diez-Banos, P., & Morrondo, P. (2006). Prevalences of gastrointestinal parasites in sheep and parasite-control practices in NW Spain. *Preventive Veterinary Medicine,* 75, 56-62.

Pollock, J., Bedenice, D., Jennings, S., & Papich, M. (2017). Pharmacokinetics of an extended-release formulation of eprinomectin in healthy adult alpacas and its use in alpacas confirmed with mange. *Journal of Veterinary Pharmacology and Therapeutics,* 40, 192-199.

Plant, J.D., Kutzler, M.A., & Cebra, C.K. (2007). Efficacy of topical eprinomectin in the treatment of *Chorioptes* sp infestation in alpacas and llamas. *Veterinary Dermatology*, 18, 59-62.

Playford, M. C., Smith, A. N., Love, S., Besier, R. B., Kluver, P., & Bailey, J. N. (2014). Prevalence and severity of anthelmintic resistance in ovine gastrointestinal nematodes in Australia (2009-2012). *Australian Veterinary Journal,* 92, 464-471.

Presland, S.L. (2005). Counting nematode eggs in equine faecal samples. *Veterinary Record*, 156, 208.

Presidente, P.J.A. (2007). Alpaca parasites & their control: recent experiences. In: *Australian Alpaca Veterinarians Annual Conference,* Melbourne, Australia, pp. 1-14.

Rickard, L.G. (1993). Parasitic gastritis in a llama (*Lama glama*) associated with inhibited larval *Teladorsagia* spp. (Nematoda: Trichostrongyloidea). *Veterinary Parasitology,* 45, 331-335.

Rickard, L.G., Hoberg, E.P., Bishop, J.K., & Zimmerman, G.L. (1989). Epizootiology of *Nematodirus battus, N. filicollis,* and *N. spathiger* (Nematoda: Trichostrongyloidea) in western Oregon. *Proceedings of the Helminthological Society of Washington,* 56, 104-15

Roberts, F. (1957). Reactions of calves to infestation with the stomach worm, *Haemonchus place*i (Place, 1893) Ransom, 1911. *Australian Journal of Agricultural Research,* 8, 740-767.

Roeber, F., Hassan, E.B., Skuce, P., Morrison, A., Claerebout, E., Casaert, S., Homer, D.R., Firestone, S., Stevenson, M., Smith, L., & Larsen, J. (2017a). An automated, multiplex-tandem PCR platform for the diagnosis of gastrointestinal nematode infections in cattle: An Australian-European validation study. *Veterinary Parasitology,* 239, 62-75.

Roeber, F., Morrison, A., Casaert, S., Smith, L., Claerebout, E., & Skuce, P. (2017b). Multiplexed-tandem PCR for the specific diagnosis of gastrointestinal nematode infections in sheep: an European validation study. *Parasites and Vectors,* 10, 226.

Roeber, F., Jex, A.R., & Gasser, R.B. (2015). A Real-Time PCR Assay for the diagnosis of gastrointestinal nematode infections of small ruminants, In: *Veterinary Infection Biology: Molecular Diagnostics and High-Throughput Strategies*, pp. 145-152.

Roeber, F., Jex, A.R., & Gasser, R.B. (2013). Comparative evaluation of two DNA isolation techniques for PCR-based diagnosis of gastrointestinal nematode infections in sheep. *Molecular and Cellular Probes*, 27, 153-157.

Rogers, W.P. (1939). Nematode parasites of sheep in Western Australia. *Journal of Helminthology,* 17, 151-158.

Rose, J.H., & Small, A.J. (1980). Transmission of *Oesophagostomum* spp among sows at pasture. *Veterinary Record*, 107, 223-225.

Rubin, R. (1967). Some observations on the interpretation of fecal egg counts. *American Journal of Clinical Pathology,* 1, 145-148.

Rutter, W., Black, W.J.M., Fitzsimons, J., & Swift, G. (1984). Clean grazing system for sheep, its development and extension. *Research and Development in Agriculture* 1, 41-46.

Saeed, M.A., Rashid, M.H., Vaughan, J., & Jabbar, A. (2018). Sarcocystosis in South American camelids: the state of play revisited. *Parasites and Vectors,* 11, 146.

Sales, N., & Love, S. (2016). Resistance of *Haemonchus* sp. to monepantel and reduced efficacy of a derquantel/abamectin combination confirmed in sheep in NSW, Australia. *Veterinary Parasitology,* 228, 193-196.

Salisbury, J.R., Arundel, J.H. (1970). Peri-parturient deposition of nematode eggs by ewes and residual pasture contamination as sources of infection for lambs. *Australian Veterinary Journal,* 46, 523-529.

Sangster, N., Batterham, P., Chapman, H. D., Duraisingh, M., Le Jambre, L., Shirley, M., Upcroft, J., & Upcroft, P. (2002). Resistance to antiparasitic drugs: the role of molecular diagnosis. *International Journal for Parasitology,* 32, 637-653.

Sangster, N. (1996). Pharmacology of anthelmintic resistance. *Parasitology,* 113 Suppl, S201-216.

Sangster, N. C., & Gill, J. (1999). Pharmacology of anthelmintic resistance. *Parasitology Today,* 15, 141-146.

Sarre, C., Claerebout, E., Vercruysse, J., Levecke, B., Geldhof, P., Pardon, B., Alvinerie, M., Sutra, J.F., & Geurden, T. (2012). Doramectin resistance in *Haemonchus contortus* on an alpaca farm in Belgium. *Veterinary Parasitology,* 185, 346-351.

Scott, I., Pomroy, W.E., Kenyon, P.R., Smith, G., Adlington, B., Moss, A., 2013. Lack of efficacy of monepantel against *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. *Veterinary Parasitology,* 198, 166-171.

Shoop, W. L., Mrozik, H., & Fisher, M.H. (1995). Structure and activity of avermectins and milbemycins in animal health. *Veterinary Parasitology,* 59, 139-156.

Shaw, G.C. (2001). Most efficient single dose of fenbendazole to control gastrointestinal parasites of llamas and alpacas (Doctoral dissertation, Kalamazoo College).

Smith, W.D., Newlands, G.F., Fitzpatrick, J.L., Besier, R.B. (2013). Barbervax, a potential commercial vaccine for Haemonchus contortus: field efficacy trials with sheep in a high risk region of Australia. Proceedings World Association for the Advancement of Veterinary Parasitology congress; 25-29 August 2013; Perth, Australia.

Soulsby, E.J.L. (1982). Helminths, Arthropods and Protozoa of Domesticated Animals, 7th ed, Baillière Tindall.

Stanley, K.K., & Szewczuk, E. (2005). Multiplexed tandem PCR: gene profiling from small amounts of RNA using SYBR Green detection. *Nucleic Acids Research,* 33, e180.

Tait, S.A., Kirwan, J.A., Fair, C.J., Coles, G.C., & Stafford, K.A. (2002). Parasites and their control in South American camelids in the United Kingdom. *The Veterinary Record,* 150, 637-638.

Thomas, R.J, & Boag, B. (1973). Epidemiological studies on gastro-intestinal nematode parasites of sheep. The control of infection in lambs on contaminated pasture. *Research in Veterinary Science,* 14, 11-20.

Twomey, D.F., Birch, E.S., & Schock, A. (2009). Outbreak of sarcoptic mange in alpacas (*Vicugna pacos*) and control with repeated subcutaneous ivermectin injections. *Veterinary Parasitology,* 159, 186-191.

Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M., Jennings, F.W. (1996). Veterinary Parasitology 2nd edn. Blackwell Science Ltd; Oxford, UK.

Van den Brom, R., Moll, L., Kappert, C., Vellema, P. (2015). *Haemonchus contortus* resistance to monepantel in sheep. *Veterinary Parasitology,* 209, 278-280.

VanHoy, G., Carman, M., Habing, G., Lakritz, J., Hinds, C.A., Niehaus, A., Kaplan, R.M., & Marsh, A.E. (2018). Safety and serologic response to a *Haemonchus contortus* vaccine in alpacas. *Veterinary Parasitology,* 252, 180-186.

Vaughan, J.L. (2015). Survey of livestock veterinarians to identify common medical & surgical conditions seen in Australian alpacas. Newsletter of Australian Alpaca Veterinarians, Number 81, October 2015.

Waghorn, T., Leathwick, D., Miller, C., & Atkinson, D. (2008). Brave or gullible: testing the concept that leaving susceptible parasites in refugia will slow the development of anthelmintic resistance. *New Zealand Veterinary Journal,* 56, 158-163.

Waghorn, T., Miller, C., Oliver, A.-M., & Leathwick, D. (2009). Drench-and-shift is a high-risk practice in the absence of refugia. *New Zealand Veterinary Journal*, 57, 359-363.

Welchman, D.d.B., Parr, J., Wood, R., Mead, A., & Starnes, A. (2008). Alpaca and llama nematodes in Britain. *Veterinary Record,* 162, 832-832.

Windsor, R.S. (1997). Type II ostertagiasis in llamas. *Veterinary Record,* 141, 608.

Windsor, R., Windsor, R., & Teran, M. (1992). Economic benefits of controlling internal and external parasites in South American camelids. *Annals of the New York Academy of Sciences,* 653, 398-405.

Zanzani, S.A., Gazzonis, A.L., Di Cerbo, A., Varady, M., & Manfredi, M.T. (2014). Gastrointestinal nematodes of dairy goats, anthelmintic resistance and practices of parasite control in Northern Italy. *BMC Veterinary Research,* 10, 114.

CHAPTER 2. AN ASSESSMENT OF WORM CONTROL PRACTICES USED BY ALPACA FARMERS IN AUSTRALIA

2.1. Abstract

This study aimed to assess current worm control practices used by Australian alpaca farmers with an online questionnaire survey. The questionnaire contained questions about farm demography and general husbandry practices, farmers' knowledge about gastrointestinal nematodes (GINs) and their importance, the use of worm control strategies and anthelmintics, and grazing management. A link for the questionnaire survey was sent to all (n = 954) registered members of the Australian Alpaca Association in July 2015. The response rate for the questionnaire was 25% (239/954). The majority of respondents were from small (≤ 50 alpacas; 64%, 153/239) followed by medium (50-100 alpacas; 24%, 57/239) and large (>100 alpacas; 12%, 29/239) farms. Findings revealed that the majority of respondents kept Huacaya alpacas to produce high-quality fibre and alpacas were usually kept with other domestic ruminants (e.g. cattle and sheep). Although half of alpaca farmers (114/220) perceived that GINs were an important health problem of alpacas, with *Haemonchus* spp. being the most common nematode, the majority of them (174/220) used anthelmintics for nematode control. Macrocyclic lactones, a commercial combination of four anthelmintics (abamectin, albendazole, closantel and levamisole) and monepantel were the three most commonly used dewormers by Australian alpaca farmers. Although a significant proportion (166/213) of respondents used a quarantine drench for alpacas, very few respondents were aware of strategic deworming and the issue of anthelmintic resistance. Alpaca farmers mostly used anthelmintics at the dose rate recommended for sheep (47%, 79/167) and cattle (9%, 15/167), though some used 1.5 (31%, 51/167) and 2 (13%, 22/167) times the dose rate recommended for sheep. The majority of small herds used anthelmintics at the dose rate recommended for sheep and cattle while medium and large herds used anthelmintics at 1.5 to 2 times the dose rate recommended for sheep. This study provides invaluable insights into the demography of alpaca farms in Australia, husbandry practices used by alpaca farmers and their knowledge about worms and their control, thereby paving the way for developing guidelines for the control of GINs of alpacas.

2.2. Introduction

Alpacas (*Vicugna pacos*) and llamas (*Lama glama*) are native to the high Andean Plateau region in South America. For centuries, alpacas and llamas have been used by Andean communities for their socioeconomic value (Leguia, 1991; Windsor et al., 1992a). Recently, the commercial farming of alpacas has also been exploited outside South America, mainly in Australia, Canada, Europe, New Zealand, the UK and the USA, for their fibre, meat and hides. Additionally, alpacas are kept as guard animal on sheep farms, or simply for lifestyle purposes (Ballweber, 2009; Tait et al., 2002).

As with other grazing livestock species, gastrointestinal nematode (GIN) infections are considered as one of the important challenges alpaca farmers face globally, causing diarrhoea, reduced growth rate, anaemia and mortality (Edwards et al., 2016; Leguia, 1991; Rojas et al., 2016; Thomas and Morgan, 2013; Twomey et al., 2014; Windsor et al., 1992a, b). For instance, a wide range of GINs such as *Haemonchus contortus*, *Camelostrongylus mentulatus*, *Ostertagia* spp., *Trichostrongylus* spp. and *Lamanema chavezi* have been recorded in alpacas from Australia, Europe, New Zealand, the UK and the USA (Ballweber, 2009; Cebra and Stang, 2008; Fowler, 2001; Franz et al., 2015; Hill et al., 1993; Leguia, 1991; Rickard, 1994; Rickard and Bishop, 1991). Although economic losses due to parasitism in alpacas have not been quantified in intensive grazing systems, it is expected that parasitic gastroenteritis in alpacas would result in substantial production losses as reported by Windsor et al. (1992a) in Peruvian alpacas.

Currently, the control of nematode infections in South American camelids (SACs) relies mainly on the use of anthelmintics, although no anthelmintic is registered for use against GINs in these animals in Australia. However, anthelmintic resistance (AR) is now recognised as an important threat to the health, productivity and welfare of alpacas and llamas globally (Galvan et al., 2012; Gillespie et al., 2010; Jabbar et al., 2013; Sarre et al., 2012) as limited information is available on pharmacokinetic properties (Hunter et al., 2004), and appropriate dose rates and routes of administration of anthelmintics used in SACs.

Although Australia has the largest alpaca population (>450,000) outside South America (Clarke, 2016), very little is known about the epidemiology and control of GINs in alpacas (Carmichael 1999, 2014; Presidente 2007). Furthermore, there is no information available on parasite control practices used by Australian alpaca farmers. To date, only two reports are available documenting the understanding of parasites and their

control by alpaca and llama farmers from Switzerland (Hertzberg and Kohler, 2006) and the UK (Tait et al., 2002). However, implementation of an integrated parasite management approach is more likely to work and be sustained when embedded into the solid and cooperative social structures of farming communities (Geels, 2002). Therefore, it is crucial to understand farmers' perceptions about the control of GINs in alpacas as has been demonstrated in the control of GINs in ruminants (Borgsteede et al., 1998; Ploeger et al., 2016; Reinemeyer et al., 1992).

The objective of this study was to document the current worm control practices used by Australian alpaca farmers for the treatment and control of GINs using an online questionnaire survey.

2.3. Materials and methods

2.3.1. Alpaca farms in Australia

In Australia, most alpaca farms are located in the south-eastern states of New South Wales and Victoria, with smaller numbers in Queensland, Western Australia, South Australia and Tasmania. Alpacas are routinely vaccinated against clostridial diseases (caused by *Clostridium perfringens* type D, *C. tetani*, *C. novyi* type B, *C. septicum* and *C. chauvoei*). They are generally shorn once annually in spring, although at variable times throughout the year. Timing and duration of the birthing periods vary among farms but often occur for about two months in autumn.

2.3.2. Questionnaire

A questionnaire was conducted using an online programme, Research Electronic Data Capture (Harris et al., 2009). The questionnaire contained 131 questions about (i) farm demography and general husbandry practices of alpacas, (ii) farmers' knowledge about GINs and their importance, (iii) the use of worm control strategies and anthelmintics, and (iv) grazing management. The majority of the questions were close-ended, with a few semi-open (i.e., a close-ended question with the addition of a category "other'). The questionnaire was first validated using a pilot survey, including 29 alpaca farmers which were then excluded from data analyses of the definitive survey.

The participants of the survey were registered members of the Australian Alpaca Association (AAA) and their participation in the study was entirely voluntary. Following a pilot survey, all active members (n = 954) of the AAA received an invitation through email (20 July 2015) from the head office of the AAA to participate in the survey. The participants were reminded via email three times before it closed on 31 August 2015. In addition, alpaca farmers were encouraged (three times via email) to participate in the survey using regional offices of the AAA as well as social media platforms such as the Facebook page of the AAA. The questionnaire survey was approved by the Human Ethics Committee (Ethics ID 1443529) of the University of Melbourne.

Farmers could submit the questionnaire anonymously but were asked to enter the four digits of their postal code to allow a general assessment of the geographical distribution of respondents.

2.3.3. Data analyses

Analyses were performed using Stata (StataCorp., 2013) and R (R Core Team, 2017). Questionnaire data were downloaded from REDCap as a comma delimited (CSV) file. This database contained 254 questionnaires, and data validation and cleaning were performed manually using Microsoft Excel 2013. Questionnaires submitted with incomplete responses were discarded, leaving a total of 239 questionnaires.

Based on the number of alpacas per farm, herds were divided into three categories: small ≤ 50 alpacas, medium 51-100 alpacas and large >100 alpacas. For each variable, a descriptive analysis was carried out, producing frequencies for categorical variables and means and medians with standard deviations for continuous variables. Subsequently, differences between types of herds for various variables such as worm problems, treatment, and observation on clinical signs were compared using Pearson's Chi-square test. When percentages of responses were calculated, missing values were excluded from the denominator. In all cases, the reported percentages represent the percentage of those who responded to the question.

Association plots were used to visualise cross tabulation (contingency table) for complex data. This kind of plot is used to present a complex relationship from a contingency table in a meaningful way where the area of a box is proportional to the difference in observed and expected frequencies (Lewis, 2013). The rectangles above the

dashed lines in the plot indicate that observed frequencies are greater than expected, showing a positive association. Similarly, rectangles below the dashed lines show observed frequencies are less than expected, demonstrating a negative association. The size and the shape of a rectangle indicate strength of the associations, with a wider rectangle indicating a higher frequency while a taller rectangle a higher proportion.

2.4. Results

2.4.1. General characteristics of alpaca farms

The response rate for the questionnaire was 25% (239/954). Although responses were received from all alpaca farming regions of Australia, the highest response rate (44%) was from the states of New South Wales and Victoria (30%) (Fig. 2.1) where the majority of the national alpaca population exists. The majority of respondents (69%, 165/238) had a tertiary level of education followed by secondary level (25%, 59/238). The average farming experience of respondents was 10.5 years (minimum 0, maximum 28 years). The main purpose of keeping alpacas was fibre production (78%, 186/239) followed by breeding (77%, 183/239), guard animal (42%, 100/239), hobby farming (40%, 95/239) and meat production (16%, 38/239).

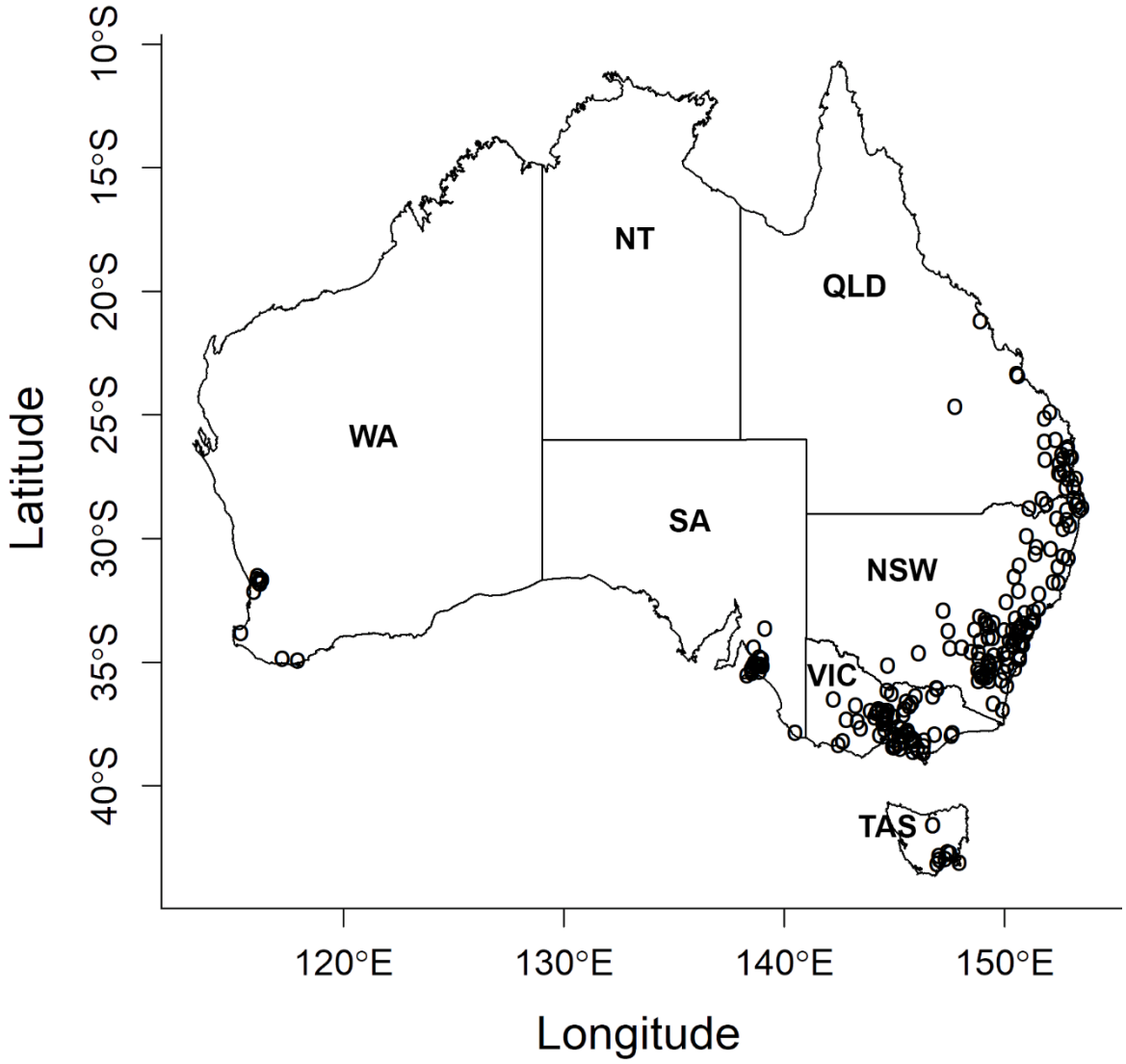


Fig. 2.1. Australian map showing distribution of alpaca farmers (o) who participated in the worm control practices questionnaire survey. States and territories of Australia: NSW, New South Wales; NT, the Northern Territory; QLD, Queensland; SA, South Australia; TAS, Tasmania; VIC, Victoria; WA, Western Australia.

The majority of respondents were from small herds (64%, 153/239) followed by medium (24%, 57/239) and large (12%, 29/239) herds. Of the 238 alpaca farmers, 67% (160/238) kept the Huacaya breed, 20% (47/238) both Huacaya and Suri breeds and only 13% (31/238) the Suri breed (Table 2.1). Overall, the mean herd size was 57 alpacas, with a range of 2-50, 51-100 and 105-1150 animals in small-, medium- and large-sized alpaca herds, respectively (Table 2. 1). Similarly, the mean grazing area was highest for large herds (mean 123 ha, range 14-696 ha) followed by medium (60, 2-931) and small herds (43, 0.04-3,440) (Table 2.1). About 75% (9,730/12,917) of the surveyed alpaca population were female, with 68% (8,800/12,917) being more than 1-year-old females.

Table 2.1. Demographic information of alpaca herds that participated in the survey.

Question	*Herd size*		
	Small	**Medium**	**Large**
No. of respondents (%)	**153 (64)**	**57 (24)**	**29 (12)**
Alpaca breed(s)			
No. of Huacaya (%)	**102 (64)**	**41 (26)**	**17 (10)**
No. of Suri (%)	**24 (77)**	**3 (10)**	**4 (13)**
No. of Huacaya and Suri (%)	**26 (55)**	**13 (28)**	**8 (17)**
No. of alpacas			
Mean (SD)	**24 (13)**	**72 (16)**	**199 (189)**
Median (Q1, Q3)	**22 (13, 34)**	**70 (59, 81)**	**160 (130, 200)**
Min – Max	**2 – 50**	**51 - 100**	**105 - 1150**
Grazing area (hectares) of the farms			
Mean (SD)	**43 (283)**	**60 (153)**	**123 (176)**
Median (Q1, Q3)	**7 (3, 17)**	**14 (8, 40)**	**59 (26, 95)**
Min – Max	**0.04 – 3440**	**2 – 931**	**14 - 696**

SD, standard deviation; Q1, first quartile; Q3, third quartile; Min, minimum; Max, maximum.

2.4.2. Husbandry practices

Herd replacements were homebred on 88% (199/226) of farms across all alpaca herd sizes (Table 2.2), with the most common weaning age ≥ 5-months. Overall, only 15% (35/238) of respondents had agisted non-home-bred alpacas on their farms at least once during the last five years, with a higher percentage (34%, 10/29) in large herds. Raising weaned crias separated from their mothers was a common practice in the large herds. The majority of respondents (92%, 212/231) provided supplementary feed (hay and pelleted feed) to alpacas across all herd sizes, particularly during winter due to insufficient feed (59%, 124/212) or the provision of additional energy to lactating females (58%, 122/212) (Table 2.2).

Almost half (53%, 121/229) of the respondents farmed domestic ruminants along with alpacas across all herd sizes, with sheep (29%, 35/121) and cattle (24%, 29/121) being the most common species, and horses, donkeys, pigs, llamas and Huarizos (a cross between male llama and a female alpaca) being kept by 22% (52/239) of respondents. Alpacas co-grazed with sheep (68%, 23/34), cattle (29%, 10/34), and horses and donkeys (35%, 12/34), and the sharing of paddocks with other livestock species was similar across all herd sizes. A few respondents practised rotational grazing (17%, 20/119) or alternate grazing (7%, 8/119) using horses. Seventy-three percent of respondents (191/262) removed dung, with a frequency from once per week (13%, 33/262) to once in every 2-6 months (15%, 38/262), whereas 27% (71/262) of respondents never removed dung from pastures. The practice of removing dung was more common in small (79%, 131/166) and medium (75%, 47/63) herds rather than large (39%, 13/33) herds (Table 2.2). Harrowing of pastures was practised by 22% (49/227) of respondents.

Table 2.2. Farm husbandry and management practices used by Australian alpaca farmers included in this study.

Husbandry/management practice	*Herd size*						*Total*	
	Small		Medium		Large			
	Proportion	% (95 CI)	Proportion	% (95 CI)	Proportion	% (95 CI)	Proportion	% (95 CI)
On-farm birth of crias	127/146	87 (80 – 92)	47/52	90 (79 – 97)	25/28	89 (72 - 98)	199/226	88 (84 – 92)
Keeping agisted alpacas	12/152	8 (4 – 13)	13/57	22 (13 – 36)	10/29	34 (18 – 54)	35/238	15 (10 – 20)
Supplementary feed	142/150	95 (92 – 98)	47/52	90 (82 – 98)	23/29	79 (64 – 94)	212/231	92 (89 – 96)
Supplementation due to insufficient feed in winter	80/142	56 (48 – 65)	32/47	68 (53 – 81)	12/23	52 (31 – 73)	124/212	59 (52 – 65)
Supplementation to lactating females as extra diet	78/142	55 (45 – 63)	28/47	60 (44 – 74)	16/23	70 (47 – 87)	122/212	58 (51 – 64)
Keeping alpacas with other livestock species	70/148	47 (39 – 56)	28/52	54 (39 – 68)	23/29	79 (60 – 92)	121/229	53 (47 – 59)
Co-grazing of alpacas with other livestock species	46/68	68 (55 – 78)	20/28	71 (51 – 87)	15/23	65 (43 – 84)	81/119	68 (60 – 76)
Removal of alpaca dung from paddocks	131/166	79 (72 -85)	47/63	75 (62- 85)	13/33	39 (23 – 58)	191/262	73 (67 – 78)

CI, confidence interval.

2.4.3. Knowledge of worms

The level of knowledge of worms among respondents from different alpaca herd sizes was similar. Almost one half of respondents (52%, 114/220) perceived that GINs were an important health problem (Table 2.3), and the clinical signs they commonly associated with parasitic gastroenteritis were weight loss, anaemia, scouring and death (Fig. 2.2). Parasitic infections were diagnosed either using laboratory tests such as faecal egg counts (FEC) (86%, 98/114), larval culture (26%, 30/114) or by post-mortem examination conducted by veterinarians (17%, 20/114) (Table 2.3). Usually, FECs were performed in veterinary diagnostic laboratories (59%, 69/116) while a small percentage were performed by alpaca farmers (29%, 34/116). A small proportion of respondents (10%, 12/114) suspected parasitic infections in alpacas by observing low body condition scores or visual observation of worms (mostly tapeworms) in the faeces of their animals (Table 2.3). The majority of respondents (78%, 70/90) believed that the barber's pole worm (*H. contortus*) was the most commonly diagnosed GIN in their animals, particularly during autumn followed by black scour worms (*Trichostrongylus* spp.) and brown stomach worms (*Ostertagia* spp.) (Fig. 2.3; Table 2.3). Almost a quarter of respondents (24%, 22/90) thought that coccidia and tapeworm were also important parasites for their animals (Table 2.3). Females over two years old were the most common group of alpacas (39%, 44/114) affected by GINs.

Table 2.3. Knowledge of parasites known by Australian alpaca farmers included in this study.

Question	*Herd size*						*Total*	
	Small		Medium		Large			
	Proportion	% (95 CI)	Proportion	% (95 CI)	Proportion	% (95 CI)	Proportion	% (95 CI)
Worms are an important health issue of alpacas	**65/142**	**46 (37 – 54)**	**32/51**	**63 (48 – 76)**	**17/27**	**63 (42 – 81)**	**114/220**	**52 (45 – 59)**
Diagnostic method(s) used:								
Faecal egg count (FEC)	**56/65**	**86 (75 – 93)**	**27/32**	**84 (67 – 95)**	**15/17**	**88 (64 – 99)**	**98/114**	**86 (78 – 92)**
Larval culture	**15/65**	**23 (14 – 35)**	**9/32**	**28 (14 – 47)**	**6/17**	**35 (14 – 62)**	**30/114**	**26 (19 – 35)**
Post mortem	**8/65**	**12 (5 – 23)**	**5/32**	**16 (5 – 33)**	**7/17**	**41 (18 – 67)**	**20/114**	**17 (11 – 16)**
Other method[1]	**6/65**	**9 (3 – 19)**	**4/32**	**13 (4 – 29)**	**2/17**	**12 (1 – 36)**	**12/114**	**11 (10 – 18)**
Identified worms on alpaca farms:								
Barber's pole (*Haemonchus* spp.)	**39/53**	**74 (60 – 85)**	**20/24**	**83 (63 – 95)**	**11/13**	**85 (55 – 98)**	**70/90**	**78 (68 – 86)**
Black scour (*Trichostrongylus* spp.)	**15/53**	**28 (17, 42)**	**9/24**	**38 (19 – 59)**	**6/13**	**46 (19 – 75)**	**30/90**	**33 (24 – 44)**
Brown stomach (*Ostertagia* spp.)	**10/53**	**19 (9 – 32)**	**11/24**	**46 (26 – 67)**	**4/13**	**31 (9 – 61)**	**25/90**	**28 (19 – 38)**
Other[2]	**13/53**	**25 (14 – 38)**	**6/24**	**25 (10 – 47)**	**3/13**	**23 (5 – 54)**	**22/90**	**24 (16 – 15)**

[1]Condition scoring, anaemia, diarrhoea, worms in faeces; [2]coccidia, tapeworm; CI, confidence interval.

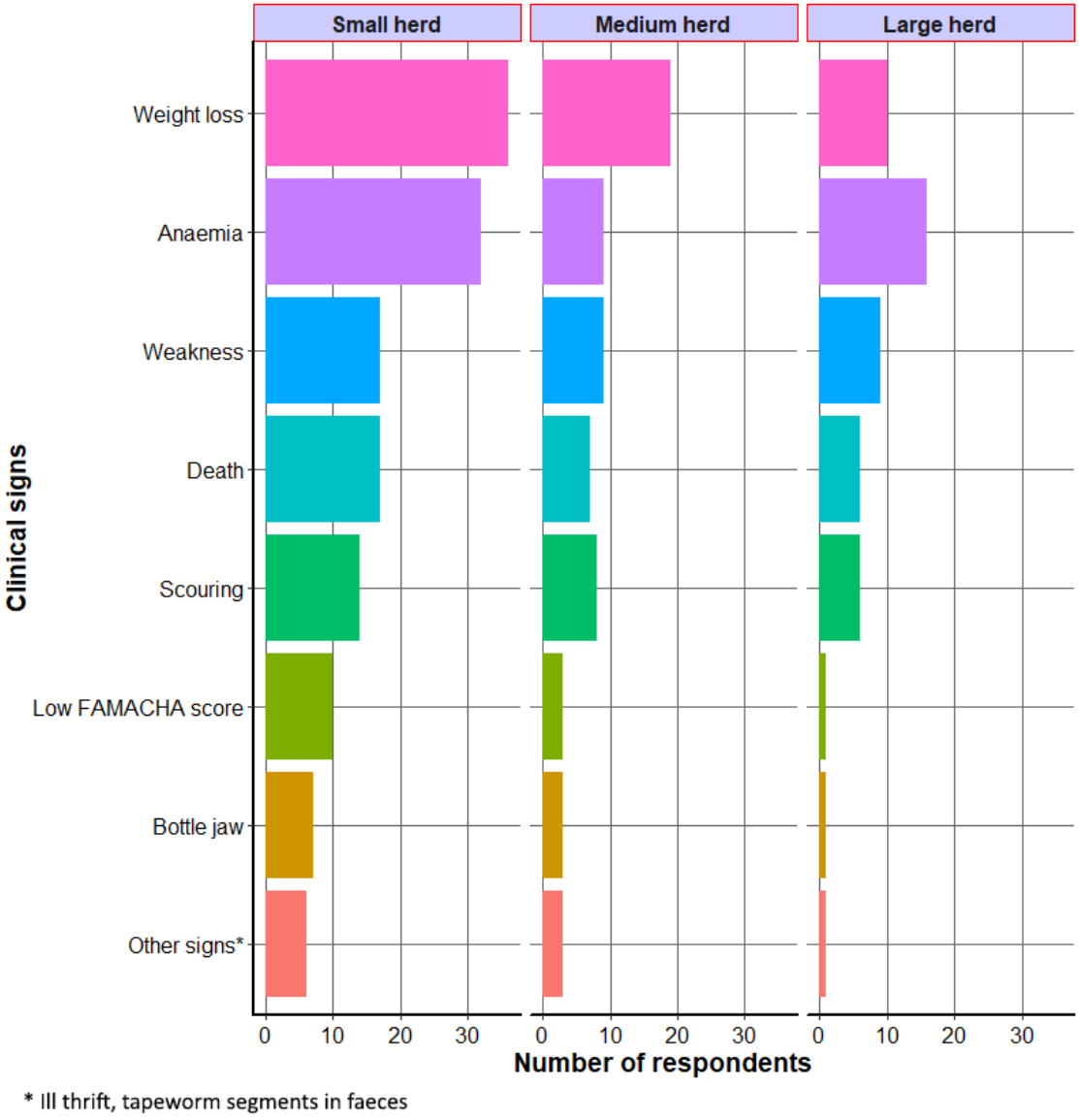


Fig. 2.2. Frequency of clinical signs reported by Australian alpaca farmers to be associated with parasitic gastroenteritis in different seasons from herds of different sizes.

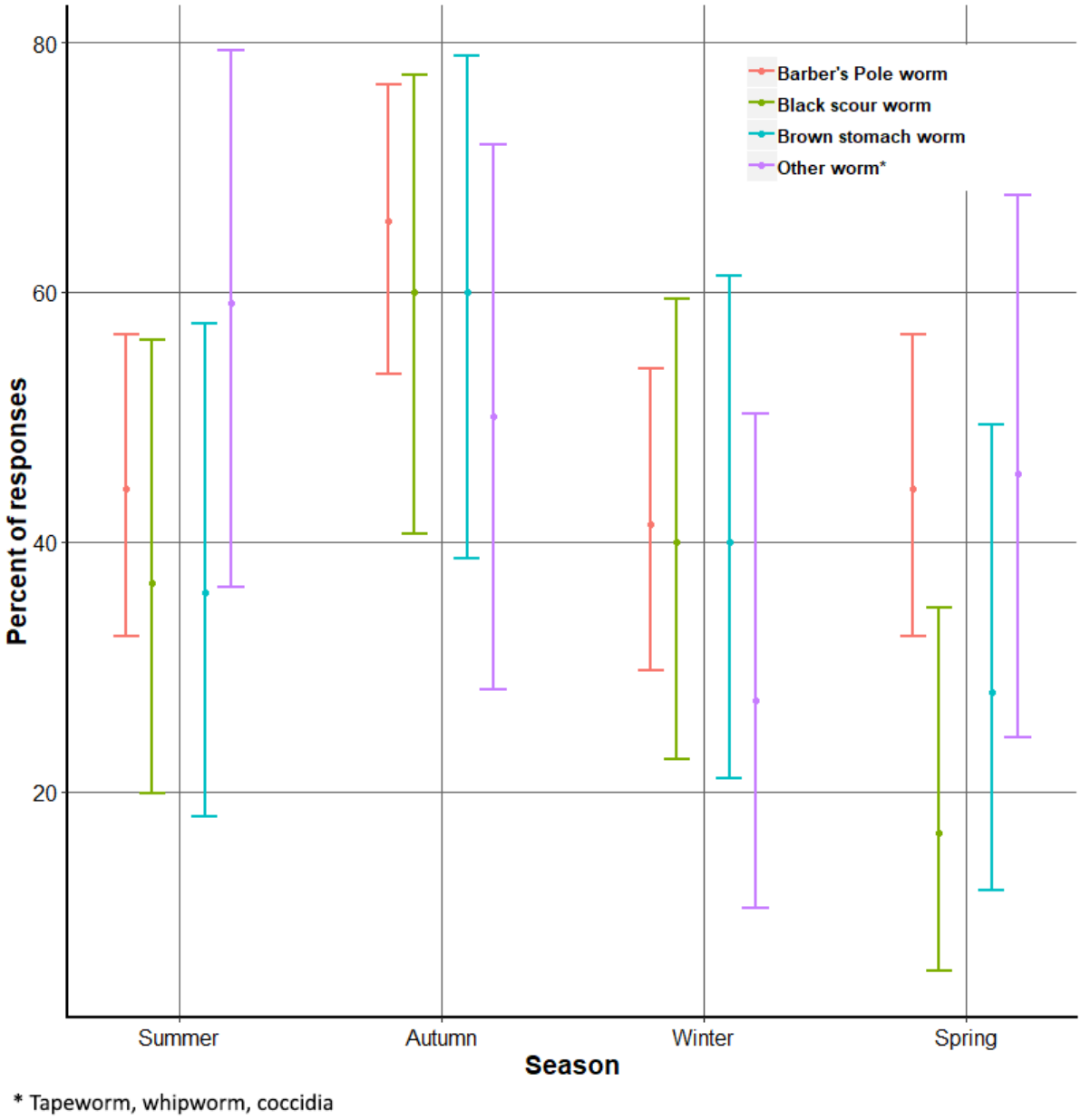


Fig. 2.3. Proportion of surveyed alpaca farmers reporting infections with different gastrointestinal nematodes in different seasons diagnosed by faecal culture (bars indicate 95% confidence interval).

2.4.4. Anthelmintics

The majority of respondents (79%, 174/220) used anthelmintics for the control of GINs of alpacas and this trend of deworming was similar across all herd sizes of alpacas (Table 2.4). Of the respondents having mixed livestock farming, only 34% (38/112) simultaneously dewormed alpacas and other livestock species. Most of the respondents (64%; 112/174) dewormed alpacas based on either the visual appraisal of their poor body condition or the results of recent FECs, and only 33% (57/174) and 8% (14/174) of them followed veterinarians' recommendations or any strategic deworming program respectively.

The commonly used anthelmintics were macrocyclic lactones (MLs) (e.g. ivermectin, moxidectin; 38% [127/331] respondents), monepantel (15%, 50/331), benzimidazoles (BZs; 9%, 31/331), levamisole (LEV; 5%, 15/331), closantel (5%, 15/331), and their combinations, including two (BZ and MLs) and three (BZ, LEV and MLs) (6%, 21/331), and four (closantel, BZ, LEV and MLs, 22% [72/331]) anthelmintics (Table 2.4; Fig. 2.4). No differences were observed among different herd sizes of alpacas for the use of anthelmintic classes. A small proportion of alpaca farmers (11%, 24/216) used diatomaceous earth powder and garlic powder as non-chemical deworming agents. Almost half of the respondents used anthelmintics at the dose rate recommended for sheep (47%, 79/167), though some used 1.5 (31%, 51/167) and 2 (13%, 22/167) times the dose rate recommended for sheep or the dose rate recommended for cattle (9%; 15/167). The majority of small herds used anthelmintics at the dose rate recommended for sheep and cattle while medium and large herds used anthelmintics at 1.5 to 2 times the dose rate recommended for sheep (Fig. 2.5). The majority of alpaca farmers calculated the dose of anthelmintics based on the visual estimation of the weight of alpacas (71%, 123/174) whereas only 20% (34/174) used the actual body weight of animals. Almost half of the respondents (52%, 88/169) did not use a drenching gun and of those who used a drenching gun, only 14% (11/81) of them calibrated the gun.

Forty-three percent of alpaca farmers (75/174) did not follow any deworming schedule (month/season) to deworm their animals, though some indicated preference for the months of March (20%, 35/174) and October (20%; 34/174). The first deworming of alpacas was performed at the time of weaning by 33% (56/170) of respondents whereas another 30% (51/170) did not follow any fixed time for deworming. Sixty-six percent (112/170) of respondents rotated anthelmintics, and 55% (56/102) of them rotated

anthelmintics every year. Furthermore, 47% (80/169) of farmers indicated that they had been using the same anthelmintic for the last two years or more, and the main reason for changing a class of anthelmintic was to prevent AR in GINs (60%, 58/96).

Seventy-eight percent of alpaca farmers (166/213) used quarantine drenching (Table 2.4), with macrocyclic lactones as the most commonly (48%, 79/166) used quarantine dewormer followed by a commercial combination of four anthelmintics (abamectin, albendazole, closantel and levamisole; 37%, 79/166) and monepantel (17%, 29/166). Alpaca farmers used quarantine periods of one (18%, 28/156) and two weeks (26%, 40/156), and only 23% (39/169) of respondents moved alpacas to cleaner pastures after deworming (Table 2.4). The majority of medium (90%, 46/51) and large (84%, 21/25) alpaca herds used a quarantine period for introduced alpacas whereas only 34% (46/137) of small alpaca herds followed this practice (Table 2.4).

The majority of respondents (60%, 143/239) were unaware of AR, and only 12% (21/172) of respondents assessed the status of AR on their farms by assessing pre- and post-treatment FECs. Macrocyclic lactone resistant GINs were reported by only 2% (4/172) of respondents. Alpaca farmers did not appear to have an understanding of the faecal egg count reduction test as 34% (38/113) only performed FECs pre-treatment while 30% (34/113) performed pre- and post-treatment FECs. The majority of respondents received advice on deworming of alpacas from veterinarians (66%, 154/235) followed by word-of-mouth from fellow alpaca farmers (53%, 125/235), various types of journals and magazines (24%, 56/235), the newsletter of the AAA (23%, 55/235) and online resources (such as wormboss.com.au, 18%, 43/235).

Table 2.4. Anthelmintics used by Australian alpaca farmers to control gastrointestinal nematodes of alpacas.

Question	*Herd size*						*Total*	
	Small		Medium		Large			
	Proportion	% (95 CI)	Proportion	% (95 CI)	Proportion	% (95 CI)	Proportion	% (95 CI)
Use of anthelmintics	107/144	74 (66 – 81)	45/51	88 (76 - 96)	22/25	88 (69 - 97)	174/220	79 (73 – 84)
Simultaneous deworming of mixed livestock species	25/66	38 (26 – 51)	9/27	33 (17 – 54)	4/19	21 (6 – 46)	38/114	33 (25 – 43)
Anthelmintics:								
Benzimidazoles (BZ)	15/189	8 (5 – 13)	13/97	13 (7 – 22)	3/45	7 (1 – 18)	31/331	9 (7 – 13)
Levamisole (LEV)	9/189	5 (2 – 8)	5/97	5 (2 – 12)	1/45	2 (0 – 12)	15/331	5 (3 – 7)
Macrocyclic lactones (MLs)	74/189	39 (32 – 47)	35/97	36 (27 – 47)	18/45	40 (26 – 57)	127/331	38 (33 – 44)
Monepantel	27/189	14 (9 – 20)	16/97	17 (10 – 25)	7/45	16 (7 – 30)	50/331	15 (11 – 19)
Closantel	7/189	4 (1 – 8)	6/97	6 (2 – 13)	2/45	4 (1 – 15)	15/331	5 (3 – 7)
Combination of two (BZ and MLs) and three (BZ, LEV and MLs) anthelmintics	10/189	5 (3 – 10)	5/97	5 (2 – 12)	6/45	13 (5 – 27)	21/331	6 (4 – 10)
Combination of four (closantel, BZ, LEV and MLs) anthelmintics	47/189	25 (19 – 32)	17/97	18 (11 – 27)	8/45	18 (8 – 32)	72/331	22 (17 – 27)
Rotation of anthelmintics	62/103	60 (50 – 70)	35/45	78 (63 – 89)	15/22	68 (45 – 86)	112/170	66 (58 – 73)
Quarantine drench	98/138	78 (71 – 83)	44/50	71 (63 – 78)	24/26	92 (75 – 99)	166/214	78 (71 – 83)
Deworming and moving of alpacas to clean pasture	23/103	22 (15 – 32)	10/44	23 (11 – 38)	6/22	27 (11 – 50)	39/174	22 (17 – 29)
Quarantine for introduced alpacas	46/137	34 (26 – 42)	46/51	90 (79 – 97)	21/25	84 (64 – 95)	113/213	53 (46 – 60)
Drench resistance test	7/105	7 (3 – 13)	12/45	27 (15 – 42)	2/22	9 (1 – 29)	21/172	12 (8 – 18)
Source of advice on deworming								
Veterinarian	98/153	64 (51 – 72)	36/53	68 (54 – 80)	20/29	69 (49 – 85)	154/235	66 (59 – 72)
Fellow farmers	91/153	59 (51 – 67)	25/53	47 (33 – 61)	9/29	31 (15 – 51)	125/235	53 (47 – 60)
Journals/Magazines	33/153	12 (7 – 18)	15/53	11 (4 – 23)	8/29	21 (8 – 40)	56/235	24 (19 – 30)
Australian Alpaca Association newsletter	38/153	25 (18 – 32)	13/53	25 (14 – 38)	4/29	14 (4 – 32)	55/235	23 (18 – 29)
Online (e.g. wormboss.com)	25/153	16 (11 – 23)	14/53	26 (15 – 40)	4/29	14 (4 – 32)	43/235	18 (14 – 24)

CI, confidence interval.

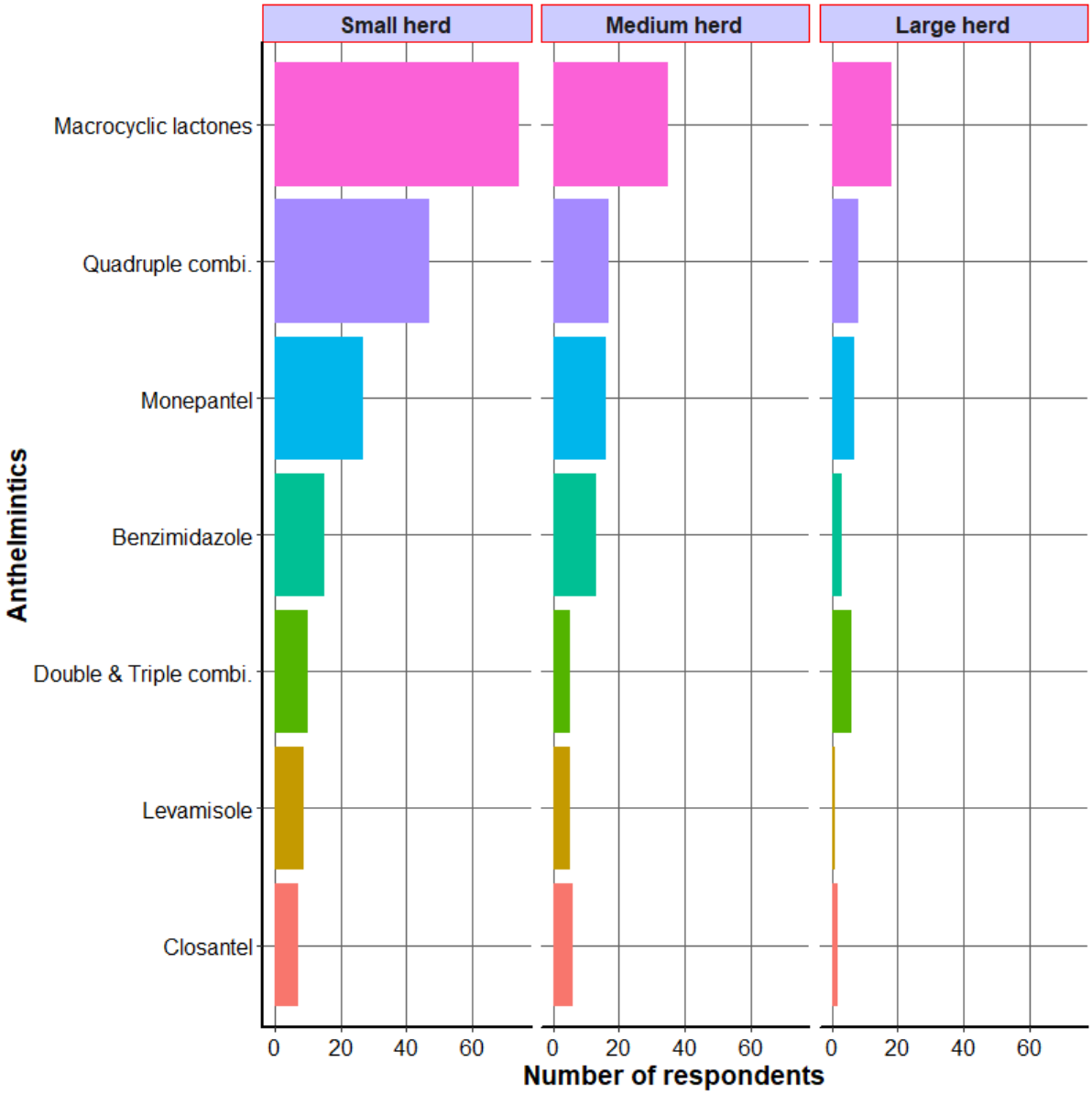


Fig. 2.4. Frequency of use of different anthelmintics on surveyed alpaca farms with herds of different sizes. (Quadruple combi = commercial combination of abamectin, albendazole, closantel and levamisole, Double combi = benzimidazole and macrocyclic lactone, Triple combi = benzimidazole, levamisole and macrocyclic lactone).

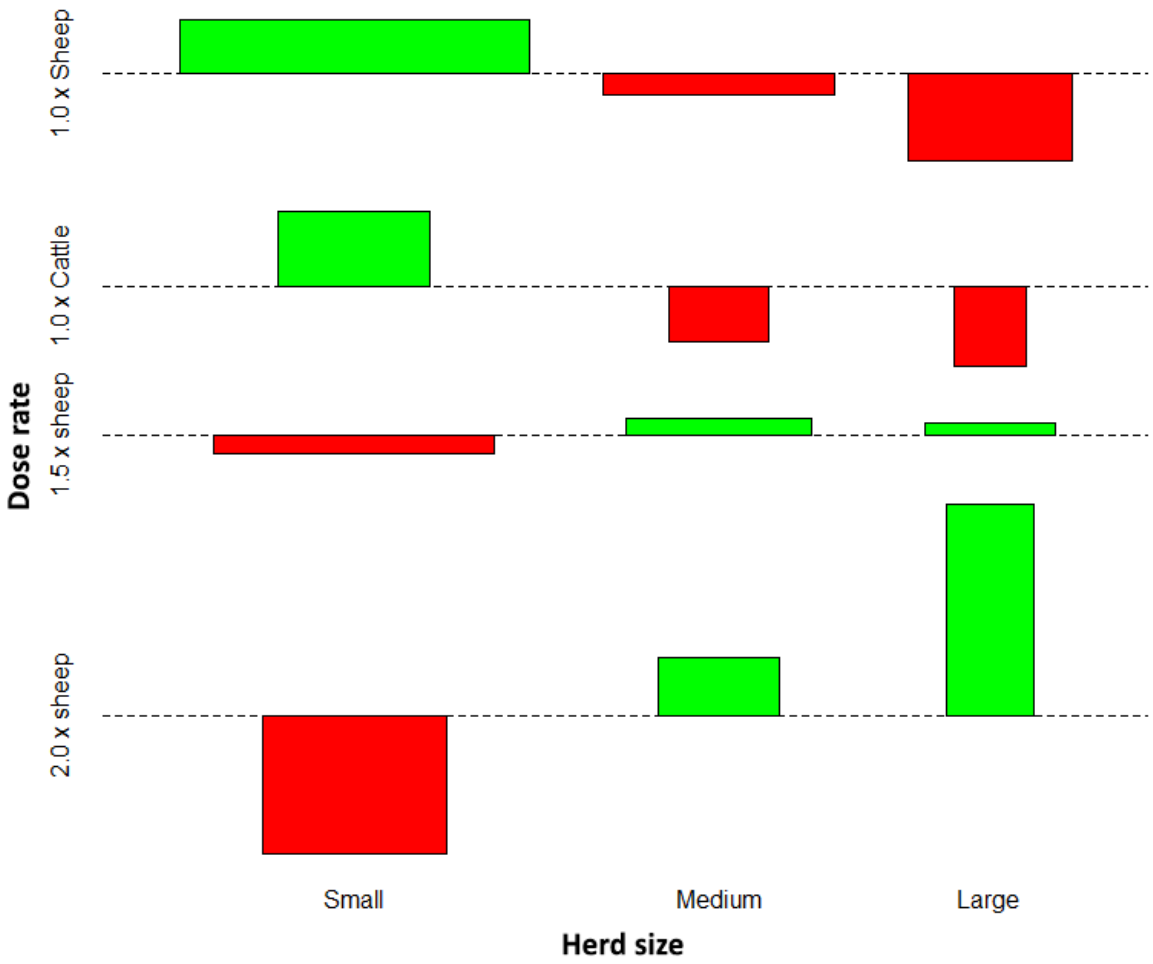


Fig. 2.5. Association between dose rates of anthelmintics used by alpaca farmers and alpaca herd sizes included in this study. The rectangles above the dashed lines indicate that observed frequencies are greater than expected, showing a positive association. Similarly, rectangles below the dashed lines show observed frequencies are less than expected, demonstrating a negative association. The size and the shape of a rectangle indicate strength of the associations, with a wider rectangle indicating a higher frequency while a taller rectangle a higher proportion.

2.5. Discussion

This is the first study to assess the worm control practices used by alpaca farmers in Australia. Australian alpaca farmers mainly keep Huacaya alpacas to produce high-quality fibre and alpacas are usually kept with cattle and sheep. Although only half of alpaca farmers (114/220) perceived that GINs were an important health issue in their alpacas, the majority of them (174/220) used anthelmintics for GIN control. Macrocyclic lactones, a commercial combination of four anthelmintics (abamectin, albendazole, closantel and levamisole) and monepantel were the three most commonly used dewormers by Australian alpaca farmers. Although a significant proportion (166/213) of respondents used a quarantine drench for alpacas, very few respondents were aware of strategic deworming and the issue of AR. An understanding of worm control practices used by livestock farmers is crucial to develop guidelines for effective control of ruminants and such studies have been conducted for both small and large ruminants (Bloemhoff et al., 2014; Brito et al., 2013; Domke et al., 2011; Falzon et al., 2013; Patten et al., 2011; Ploeger et al., 2016; Theodoropoulos et al., 2010; Zanzani et al., 2014). By contrast, very little is known about the worm control practices used by SAC farmers globally, and only two such studies have been conducted on a smaller scale in Switzerland (Hertzberg and Kohler, 2006) and the UK (Tait et al., 2002). This study provides invaluable insights into the demography of alpaca farmers in Australia, husbandry practices used by alpaca farmers and their knowledge about worms and their control which will provide pivotal information for developing guidelines for the control of GINs of alpacas.

This study confirmed that about half of the respondents (121/229) kept alpacas with other domestic ruminants, including cattle, goats and sheep. Furthermore, alpacas on such farms co-grazed with these ruminants. Such husbandry practices can expose alpacas to GINs of domestic ruminants (e.g. *H. contortus*) which they have not evolved with in their natural habitats in South America. Previously, a number of studies from Chile (Alcaino et al., 1991), Ecuador (Robayo, 2015), Japan (Hyuga and Matsumoto, 2016), New Zealand (Dittmer et al., 2018; Hill et al., 1993), Peru (Masson et al., 2016), Switzerland (Hertzberg and Kohler, 2006), the UK (Tait et al., 2002; Welchman et al., 2008), and the USA (Cebra and Stang, 2008; Rickard, 1993) have shown that alpacas commonly harbour GINs of sheep and cattle in importing countries and they can cause significant pathogenic effects in SACs (Ballweber, 2009; Franz et al., 2015).

Furthermore, Hertzberg and Kohler (2006) from Switzerland reported that the mean strongyle egg output was three-fold higher in alpacas and llamas co-grazing with sheep and/or goats. This suggests that alpacas and domestic ruminants share GINs in mixed grazing practices and control programs for GINs of alpacas should be similar to those of GINs of domestic ruminants, particularly where alpacas co-graze with cattle, goats and sheep.

An understanding of the importance of worms in livestock species and the regular monitoring of worm burdens by farmers is crucial for effective and sustainable worm control programs for ruminants. In this study, almost half (114/220) of respondents perceived that GINs were an important health problem of alpacas, and 86% (98/114) of them assessed worm burdens in their animals using faecal egg counts. Although only 26% (30/114) of respondents sent alpaca faecal samples for identification using the larval culture technique, farmers' knowledge about the presence of different species of GINs (i.e. *Haemonchus* spp., *Ostertagia* spp. and *Trichostrongylus* spp.) was similar to the findings of Carmichael (1999, 2014) and Presidente (2007) from Australia. These results indicate that alpaca farmers should regularly monitor the worm burden in alpacas and the genus/species identification of nematodes should also be performed as certain parasites (e.g. *H. contortus*) are known to cause significant morbidity and mortality in alpacas (Ballweber, 2009; Jabbar et al., 2013). Such information may facilitate the treatment/control of GINs in alpacas.

In this study, a large proportion of alpaca farmers (74%, 174/220) used anthelmintics to control GINs of alpacas. However, only half of the respondents (52%, 114/220) perceived that GINs were an important health problem of alpacas. This difference might to be due to farmers' perceptions of using anthelmintics as a part of routine management and health plan for their animals rather than using them to treat alpacas suffering from clinical cases of parasitic gastroenteritis. The results of this study showed that the percentage of respondents (74%) using anthelmintics against GINs was lower than the proportion of sheep farmers controlling GINs (Fraser et al., 2006; Maingi et al., 1996a; Ploeger et al., 2016), goats (Hoste et al., 2000; Maingi et al. 1996b) and cattle (Barton et al., 2006; Schnieder and Ilchmann, 1999) in these studies almost all respondents used dewormers. These differences could be due to lower stocking rates for alpacas as compared to higher flock/herd stocking rates for sheep and goats/cattle, so that alpaca farmers may not have felt the need for a regular deworming program. However, the differences could also be due to farmers' lack of knowledge of parasites and parasite

control in alpacas although a majority of respondents (69%) in this study had a tertiary education. However, this hypothesis requires further testing.

In grazing livestock species, strategic use of anthelmintics is an integral component of parasite control strategies. Although no anthelmintics are registered for use in alpacas in Australia, various classes of anthelmintics are frequently used to control parasitic gastroenteritis in alpacas in Australia and other countries (Jabbar et al. 2013; Tait et al., 2002). This survey has revealed that MLs were the most commonly used anthelmintics in alpacas followed by a commercial combination of four anthelmintics (abamectin, albendazole, closantel and levamisole) and monepantel. MLs have been found to be widely used in sheep and cattle (Barton et al., 2006; Cernanska et al., 2008; Domke et al., 2011; Falzon et al., 2013; Fraser et al., 2006; Patten et al., 2011; Schnieder et al., 1999; Tait et al., 2002). The frequent use of MLs in alpacas could be due to availability of a wide range of sheep and cattle products in oral and injectable forms, endectocidal properties and a wide margin of safety. Additionally, this may be due to the education of farmers by veterinarians and managers/salespersons at agricultural supply stores regarding the widespread resistance of BZs and LEV against GINs of small ruminants in Australia (Playford et al., 2014).

In this study, almost half of the respondents used anthelmintics at the dose rate recommended for sheep (47%, 79/167), although some used 1.5 (31%, 51/167) and 2 (13%, 22/167) times the dose rate recommended for sheep. As no anthelmintics are registered for use in alpacas in Australia, the therapeutic dose of commonly used (off-label) anthelmintics, registered for cattle and sheep, remains unknown. Studies have recommended different anthelmintic dose rates to treat GINs in alpacas but commonly used dose rates in alpacas in Australia are unknown, veterinarians have been recommending 1.5 times the dose rate recommended for sheep, though conflicting reports regarding the efficacy of various anthelmintics against GINs of SACs using various dose rates have been published (Dadak et al., 2013; Geurden and Hemelrijk, 2005; Gillespie et al., 2010; Jabbar et al., 2013). Recently, Dadak et al. (2013) used 1 (2.5 mg/kg), 2 and 3 times the dose rate of monepantel recommended for sheep in llamas in Austria and found that only the three times dose rate recommended for sheep was fully effective in treating SACs naturally infected with GINs. Interestingly, it was found that small alpaca herd farmers used anthelmintics at the dose rate recommended for sheep and cattle whereas medium and large herds farmers used anthelmintics at 1.5 to 2 times the dose rate recommended for sheep. These differences in the dose rate of anthelmintics

among different alpaca herd sizes could be attributed to the variation in farmers' perceptions of the significance of GINs in the productivity of animals as medium and large alpaca herds are primarily kept for commercial gains while small herds can have various purposes such as those of hobby farmers, or a greater awareness by medium and large herd owners of the different pharmacokinetics of anthelmintics in alpacas.

A very important finding in this study was the use of monepantel at a dose rate that is likely incompletely effective in alpacas (Dadak et al., 2013). Monepantel was registered for use in sheep in Australia less than 10 years ago and remains widely effective against all general of GINs (Playford et al., 2014). Its off-label use at likely sub-lethal doses in alpacas is a critical threat to the continued efficacy of monepantel in both alpacas and sheep.

Given that alpacas in Australia share GINs with domestic ruminants (Carmichael, 1999, 2014; Presidente, 2007), Australia is among the world leaders for the high prevalence of multiple AR in GINs of small ruminants (Playford et al., 2014) and no anthelmintics are registered for use in alpacas in this country, Australian alpaca farmers need to use anthelmintics against GINs of alpacas and llamas very carefully to avoid the rapid development of AR. Several factors have been identified that can contribute to the development of anthelmintic resistance in GINs of ruminants (see Jabbar et al., 2006). For example, under-dosing can lead to the development of AR due to the exposure of sub-lethal doses to nematodes (Jabbar et al., 2006; Smith et al., 1999; Waller, 1997; Wolstenholme et al., 2004). In this study, it was found that the majority of respondents (71%, 123/174) calculated the dose of anthelmintics based on visual estimation of the weight of alpacas. In addition, almost half of the respondents (52%, 88/169) did not use a drenching gun and of those who used a drenching gun, only 14% (11/81) calibrated it. In a situation where the therapeutic dose of anthelmintics is unknown for alpacas, the calculation as well as the administration of an accurate dose is important. Previously, the under-dosing of anthelmintics in domestic ruminants by farmers has been found in several studies (Zanzani et al., 2014; Falzon et al., 2013; Domke et al., 2011; Cernanska et al., 2008; Castillo-Alcala et al., 2007; Hoste et al., 2000). Nevertheless, a questionnaire survey conducted by Edwards et al. (1986) in sheep showed that AR was significantly higher on farms where sheep were treated based on the bodyweight of the heaviest sheep (Edwards et al., 1986) which infers sheep were *not* under-dosed. Given that AR in GINs of alpacas has already been reported in different regions of the world (Gillespie et al., 2010; Galvan et al., 2012; Sarre et al., 2012), including Australia (Jabbar et al., 2013),

further research into identifying sustainable strategies to control GINs in alpacas are essential to minimise the development of multiple AR.

Rotation of anthelmintics by changing drug class is one useful strategy used by sheep and goat farmers to reduce the risk of AR development (Coles and Roush, 1992; McKenna, 1990; Waller et al., 1990). This survey showed that 66% (112/170) of respondents rotated anthelmintics to avoid the development of AR. However, the majority of respondents (60%, 143/239) were unaware of AR on their farms as only 12% of them assessed the status of resistant dewormers on their farms. Alpaca farmers did not seem to have a sound understanding of the faecal egg count reduction test as several respondents performed only pre-treatment faecal egg counts and did not see the value of performing post-treatment FECs as well. This shows that alpaca farmers need to be better educated about assessing the efficacy of anthelmintics and its importance in delaying the development of AR.

Designing a worm control program relies on three essential components: (i) an understanding of current worm control practices, (ii) insights into the epidemiology of worms and (iii) the efficacy of commonly used anthelmintics. This study has laid the foundation for designing an effective worm control program for Australian alpacas by assessing the worm control practices currently used by alpaca farmers.

2.6. Conclusions

This is the first study to comprehensively assess the current worm control practices used by alpaca farmers in Australia. The findings of this study indicate that although a low number of alpaca farmers perceive that GINs are an important health problem for their animals, the majority of them use anthelmintics for GIN control. Macrocyclic lactones, a commercial combination of four anthelmintics (abamectin, albendazole, closantel and levamisole) and monepantel are the most commonly used dewormers by Australian alpaca farmers. A number of existing worm control practices may favour the development of AR in GINs of alpacas in Australia.

2.7. References

Alcaino, H. (1991). Helmintiasis gastrointestinal en llamas (*Lama glama*) de la I Región de Chile. *Parasitologia al Dia,* 15, 93-96.

Ballweber, L.R. (2009). Ecto- and endoparasites of New World camelids. *Veterinary Clinics of North America: Food Animal Practice,* 25, 295-310.

Barton, C.H., Dale, E.F., Dixon, C., & Coles, G.C. (2006). Survey of parasite control on beef farms in south-west England. *Veterinary Record,* 159, 682-683.

Bloemhoff, Y., Danaher, M., Forbes, A., Morgan, E., Mulcahy, G., Power, C., & Sayers, R. (2014). Parasite control practices on pasture-based dairy farms in the Republic of Ireland. *Veterinary Parasitology,* 204, 352-363.

Borgsteede, F.H.M., Sol, J., van Uum, A., de Haan, N., Huyben, R., & Sampimon, O. (1998). Management practices and use of anthelmintics on dairy cattle farms in The Netherlands: results of a questionnaire survey. *Veterinary Parasitology,* 78, 23-36.

Brito, D.L., Lima Dallago, B.S., Louvandini, H., Verdolin dos Santos, V.R., Figueiredo de Araujo Torres, S.E., Gomes, E.F., Talamini do Amarante, A.F., de Melo, C.B., & McManus, C.M. (2013). Effect of alternate and simultaneous grazing on endoparasite infection in sheep and cattle. *Revista Brasileira de Parasitologia Veterinária,* 22, 485-494.

Carmichael, I. (1999). Internal parasitism in alpacas in southern Australia. In: Hack, W., McGregor, B., Ponzoni, R., Judson, G., Carmichael, I., Hubbard, D., (eds) Australian Alpaca Fibre Improving Productivity and Marketing. *Rural Industries Research and Development Corporation, Australia,* p 92-130.

Carmichael, I. (2014). Internal parasitism in Australian alpacas. *Australian Alpaca Association National Conference Adelaide, Australia.* p 13-28.

Castillo-Alcala, F., Wilson, P.R., Pomroy, W.E., & Hoskin, S.O. (2007). A survey of anthelmintic use and internal parasite control in farmed deer in New Zealand. *New Zealand Veterinary Journal,* 55, 87-93.

Cebra, C.K., & Stang, B.V. (2008). Comparison of methods to detect gastrointestinal parasites in llamas and alpacas. *Journal of the American Veterinary Medical Association,* 232, 733-741.

Cernanska, D., Varady, M., Cudekova, P., & Corba, J. (2008). Worm control practices on sheep farms in the Slovak Republic. *Veterinary Parasitology,* 154, 270-276.

Clarke, M. (2016). Market Assessment - New and Emerging Animal Industries Tranche 1: Mohair, Alpaca and Camel Milk. NSW, Australia 2016. http://www.agrifutures.com.au/publications/ market-assessment-new-and-emerging-animal-industries-tranche-1-mohair-alpaca-and-camel-milk. Accessed 15 Jan 2018.

Coles, G.C., & Roush, R.T. (1992). Slowing the spread of anthelmintic resistant nematodes of sheep and goats in the United Kingdom. *Veterinary Record*, 130, 505-510.

Dadak, A., Asanger, H., Tichy, A., & Franz, S. (2013). Establishing an efficacious dose rate of monepantel for treating gastrointestinal nematodes in llamas under field conditions. *Veterinary Record,* 172, 155-155.

Dittmer, K., Hinkson, J., Dwyer, C., Adlington, B., & van Andel, M. (2018). Prevalence of Candidatus *Mycoplasma haemolamae*, bovine viral diarrhoea virus, and gastrointestinal parasitism in a sample of adult New Zealand alpaca (*Vicugna pacos*). *New Zealand Veterinary Journal,* 66, 9-15.

Domke, A.V., Chartier, C., Gjerde, B., Leine, N., Vatn, S., Østerås, O., & Stuen, S. (2011). Worm control practice against gastro-intestinal parasites in Norwegian sheep and goat flocks. *Acta Veterinaria Scandinavica,* 53, 29.

Edwards, J.R., Wroth, R., de Chaneet, G.C., Besier, R.B., Karlsson, J., Morcombe, P.W., Dalton-Morgan, G., & Roberts, D. (1986). Survey of anthelmintic resistance in Western Australian sheep flocks. 2. Relationship with sheep management and parasite control practices. *Australian Veterinary Journal,* 63, 139-144.

Edwards, E.E., Garner, B.C., Williamson, L.H., Storey, B.E., & Sakamoto, K. (2016). Pathology of *Haemonchus contortus* in New World camelids in the southeastern United States: a retrospective review. *Journal of Veterinary Diagnostic Investigation,* 28, 105-109.

Falzon, L.C., Menzies, P.I., Vanleeuwen, J., Jones-Bitton, A., Shakya, K.P., Avula, J., Jansen, J.T., & Peregrine, A.S. (2013). A survey of farm management practices and their associations with anthelmintic resistance in sheep flocks in Ontario, Canada. *Small Ruminant Research,* 114, 41-45.

Fowler, M.E. (2001). Selected diseases of South American camelids. *Journal of Camel Practice and Research,* 8, 99-112.

Franz, S., Wittek, T., Joachim, A., Hinney, B., & Dadak, A.M. (2015). Llamas and alpacas in Europe: Endoparasites of the digestive tract and their pharmacotherapeutic control. *Veterinary Journal,* 204, 255-262.

Fraser, D.E., Hunt, P.J., Skinner, R.J., & Coles, G.C. (2006). Survey of parasite control on sheep farms in south-west England. *Veterinary Record,* 158, 55-57.

Galvan, N., Middleton, J.R., Nagy, D.W., Schultz, L.G., & Schaeffer, J.W. (2012). Anthelmintic resistance in a herd of alpacas (*Vicugna pacos*). *Canadian Veterinary Journal,* 53, 1310-1313.

Geels, F.W. (2002). Technological transitions as evolutionary reconfiguration processes: a multi-level perspective and a case-study. *Research Policy,* 31, 1257-1274.

Geurden, T., & Van Hemelrijk, K. (2005). Ivermectin treatment against gastrointestinal nematodes in New World camelids in Belgium. *Small Ruminant Research,* 58, 71-73.

Gillespie, R.A.M., Williamson, L.H., Terrill, T.H., & Kaplan, R.M. (2010). Efficacy of anthelmintics on South American camelid (*llama and alpaca*) farms in Georgia. *Veterinary Parasitology,* 172, 168-171.

Harris, P.A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N., & Conde, J.G. (2009). Research electronic data capture (REDCap) - A metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of Biomedical Informatics,* 42, 377-381.

Hertzberg, H., & Kohler, L. (2006). Prevalence and significance of gastrointestinal helminths and protozoa in South American camelids in Switzerland. *Berliner und Münchener tierärztliche Wochenschrift,* 119, 291-294.

Hill, F.I., Death, A.F., & Wyeth, T.K. (1993). Nematode burdens of alpacas sharing grazing with sheep in New Zealand. *New Zealand Veterinary Journal,* 41, 205-208.

Hoste, H., Chartier, C., Etter, E., Goudeau, C., Soubirac, F., Lefrileux, Y. (2000). A questionnaire survey on the practices adopted to control gastrointestinal nematode parasitism in dairy goat farms in France. *Veterinary Research Communications,* 24, 459-469.

Hunter, R.P., Isaza, R., Koch, D.E., Dodd, C.C., & Goately, M.A. (2004). Moxidectin plasma concentrations following topical administration to llamas (*Lama glama*) and alpacas (*Lama pacos). Small Ruminant Research,* 52, 275-279.

Hyuga, A., Matsumoto, J. (2016). A survey of gastrointestinal parasites of alpacas (*Vicugna pacos*) raised in Japan. *The Journal of Veterinary Medical Science,* 78, 719-721.

Jabbar, A., Iqbal, Z., Kerboeuf, D., Muhammad, G., Khan, M.N., & Afaq, M. (2006). Anthelmintic resistance: the state of play revisited. *Life Sciences,* 79, 2413-2431.

Jabbar, A., Campbell, A.J.D., Charles, J.A., & Gasser, R.B. (2013). First report of anthelmintic resistance in *Haemonchus contortus* in alpacas in Australia. *Parasites and Vectors,* 6, 243.

Leguía, G. (1991). The epidemiology and economic impact of llama parasites. *Parasitology Today,* 7, 54-56.

Lewis, N. D. (2013). Visualizing Complex Data Using R. *CreateSpace Independent Publishing Platform*, pp. 13-16.

Maingi, N., Bjørn, H., Thamsborg, S.M., Dangolla, A., & Kyvsgaard, N.C. (1996a). Worm control practices on sheep farms in Denmark and implications for the development of anthelmintic resistance. *Veterinary Parasitology,* 66, 39-52.

Maingi, N., Bjørn, H., Thamsborg, S.M., Bøgh, H.O., & Nansen, P. (1996b). A survey of anthelmintic resistance in nematode parasites of goats in Denmark. *Veterinary Parasitology,* 66, 53-66.

Masson, M., Gutiérrez, G., Puicón. V., & Zárate, D. (2016). Helmintiasis y eimeriosis gastrointestinal en alpacas criadas al pastoreo en dos granjas comunales de la región Pasco, Perú, y su relación con el peso y condición corporal. *La Revista de Investigaciones Veterinarias del Perú es*, 27, 805-812.

McKenna, P.B. (1990). The use of benzimidazole-levamisole mixtures for the control and prevention of anthelmintic resistance in sheep nematodes: an assessment of their likely effects. *New Zealand Veterinary Journal,* 38, 45-49.

Patten, T., Good, B., Hanrahan, J.P., Mulcahy, G., & de Waal, T. (2011). Gastrointestinal nematode control practices on lowland sheep farms in Ireland with reference to selection for anthelmintic resistance. *Irish Veterinary Journal,* 64, 4.

Playford, M.C., Smith, A.N., Love, S., Besier, R.B., Kluver, P., & Bailey, J.N. (2014). Prevalence and severity of anthelmintic resistance in ovine gastrointestinal nematodes in Australia (2009-2012). *Australian Veterinary Journal,* 92, 464-471.

Presidente, P.J.A. (2007). Alpaca parasites and their control: recent experiences. *Australian Alpaca Veterinarians Annual Conference,* Melbourne, Australia. p 1-14.

R Core Team. (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ploeger, H.W., Antonis, A.F.G., Verkaik, J.C., Vellema, P., & Bokma-Bakker, M.H. (2016). Perceptions and actions of Dutch sheep farmers concerning worm infections. *Veterinary Parasitology,* 229, 150-158.

Reinemeyer, C.R., Rohrbach, B.W., Grant, V.M., & Radde, G.L. (1992). A survey of ovine parasite control practices in Tennessee. *Veterinary Parasitology,* 42, 111-122.

Rickard, L.G. (1994). Update on llama medicine. Parasites. *Veterinary Clinics of North America: Food Animal Practice,* 10, 239-247.

Rickard, L.G., & Bishop, J.K. (1991). Helminth parasites of llamas (*Lama glama*) in the Pacific Northwest. *Journal of the Helminthological Society of Washington,* 58, 110-115.

Rojas, M., Manchego, A., Rocha, C.B., Fornells, L.A., Silva, R.C., Mendes, G.S., Dias, H.G., Sandoval, N., Pezo, D., & Santos, N. (2016). Outbreak of diarrhea among preweaning alpacas (*Vicugna pacos*) in the southern Peruvian highland. *Journal of Infection in Developing Countries,* 10, 269-274.

Robayo, C.I.S. (2015). Prevalencia de parásitos gastrointestinales en alpacas del Inga Alto, Pichincha. Thesis, Universidad San Francisco de Quito, Ecuador.

Sarre, C., Claerebout, E., Vercruysse, J., Levecke, B., Geldhof, P., Pardon, B., Alvinerie, M., Sutra, J.F., & Geurden, T. (2012). Doramectin resistance in *Haemonchus contortus* on an alpaca farm in Belgium. *Veterinary Parasitology,* 185, 346-351.

Schnieder, T., Epe, C., & Ilchmann, G. (1999). Survey on parasite control in dairy cattle in northern Germany. *Veterinary Record,* 145, 704-706.

Smith, G., Grenfell, BT., Isham, V., & Cornell, S. (1999). Anthelmintic resistance revisited: under-dosing, chemoprophylactic strategies, and mating probabilities. *International Journal for Parasitology,* 29, 77-91.

StataCorp. (2013). Stata Statistical Software: Release 13. College Station, TX: StataCorp LP.

Tait, S.A., Kirwan, J.A., Fair, C.J., Coles, G.C., & Stafford, K.A. (2002). Parasites and their control in South American camelids in the United Kingdom. *Veterinary Record,* 150, 637-638.

Theodoropoulos, G., Peristeropoulou, P., Kouam, M.K., Kantzoura, V., & Theodoropoulou, H. (2010). Survey of gastrointestinal parasitic infections of beef cattle in regions under Mediterranean weather in Greece. *Parasitology International,* 59, 556-559.

Thomas, S.M., & Morgan, E.R. (2013). Effect on performance of weanling alpacas following treatments against gastro-intestinal parasites. *Veterinary Parasitology,* 198, 244-249.

Twomey, D.F., Wu, G., Nicholson, R., Watson, E.N., & Foster, A.P. (2014). Review of laboratory submissions from New World camelids in England and Wales (2000–2011). *Veterinary Journal,* 200, 51-59.

Waller, P.J., Dobson, R.J., & Haughey, K.G. (1990). The effect of combinations of anthelmintics on parasite populations in sheep. *Australian Veterinary Journal,* 67, 138-140.

Waller, P.J. (1997). Anthelmintic resistance. *Veterinary Parasitology,* 72, 391-405; Discussion 405-12.

Welchman, D.B., Parr, J., Wood, R., Mead, A., & Starnes, A. (2008). Alpaca and llama nematodes in Britain. *Veterinary Record,* 162, 832-832.

Windsor, R.S., Windsor, R.H.S., & Teran, M. (1992a). Economic benefits of controlling internal and external parasites in South American camelids. *Annals of the New York Academy of Sciences,* 653, 398-405.

Windsor, R.H.S., Teran, M., & Windsor, R.S. (1992b). Effects of parasitic infestation on the productivity of alpacas (*Lama pacos*). *Tropical Animal Health and Production,* 24, 57-62.

Wolstenholme, A.J., Fairweather, I., Prichard, R., von Samson-Himmelstjerna, G., & Sangster, N.C. (2004). Drug resistance in veterinary helminths. *Trends in Parasitology,* 20, 469-476.

Zanzani, S.A., Gazzonis, A.L., Di Cerbo, A., Varady, M., & Manfredi, M.T. (2014). Gastrointestinal nematodes of dairy goats, anthelmintic resistance and practices of parasite control in Northern Italy. *BMC Veterinary Research,* 10, 114.

CHAPTER 3. COMPARISON OF MCMASTER AND FECPAK^{G2} METHODS FOR COUNTING NEMATODE EGGS IN THE FAECES OF ALPACAS

3.1. Abstract

This study aimed to compare the FECPAK^{G2} and the McMaster techniques for counting of gastrointestinal nematode eggs in the faeces of alpacas using two flotation solutions (saturated sodium chloride and sucrose solutions). Faecal eggs counts from both techniques were compared using the Lin's concordance correlation coefficient and Bland and Altman statistics. Results showed moderate to good agreement between the two methods, with better agreement achieved when saturated sugar is used as a flotation fluid, particularly when faecal egg counts are less than 1,000 eggs per gram of faeces. This is the first study conducted to assess agreement of measurements between McMaster and FECPAK^{G2} methods for estimating faecal eggs in South American camelids.

3.2. Introduction

Parasitic gastroenteritis caused by gastrointestinal nematodes (GINs) is responsible for significant clinical and subclinical problems in domesticated South American camelids (SACs), alpacas and llamas, resulting in economic losses arising from lowered production of fibre, meat and/or leather (Leguia, 1991; Windsor, et al., 1992). Although no drugs are registered for use against GINs of SACs in Australia, anthelmintics are routinely used to deworm alpacas and llamas, mostly without assessment of worm burdens prior to treatment (Jabbar et al., 2013).

In SACs, the burden of GINs can be assessed using various coprological methods originally developed for domestic ruminants (Gordon and Whitlock, 1939), with estimation of the number of GIN eggs per gram of faeces (faecal egg count, FEC). The most commonly used coprological method is the McMaster technique (Cebra and Stang, 2008; Ballweber et al., 2014). The choice of flotation solution (saturated sugar or salt) is important for this technique because the type and specific gravity of flotation solutions can affect FEC results (Ballweber et al., 2014). Previously, saturated sugar was found to be superior to salt as a flotation solution for the detection of some GINs in SACs as the rate of water loss and distortion of GIN eggs was slower using sugar (Cringoli et al.,

2004; Cebra and Stang, 2008. Although the McMaster technique has stood the test of time because it is a relatively simple and cheap procedure to carry out, it has a number of disadvantages, including difficulties identifying eggs when faeces are thick and dark, and high levels of technical skill and experience are required to identify and count different species of nematode eggs (Ballweber et al., 2014). To overcome these limitations, other FEC estimation methods such as Kato-Katz®, FLOTAC® and FECPAK have been developed and validated in both humans and animals (Levecke et al., 2011; Ballweber et al., 2014; Bosco et al., 2014. Recently, smartphone applications have been found to be provide a more efficient approach for counting parasite eggs in the faeces of animals compared with the McMaster technique (Saeed and Jabbar, 2018).

The FECPAK method is based on a modified McMaster technique with a minimum detection limit of 30-35 eggs per gram (EPG) of faeces (Presland et al., 2005). The original FECPAK method was developed in New Zealand to provide a simple on-farm method for FEC estimation. The updated FECPAK^{G2} method uses a flotation-dilution approach similar to the McMaster technique, but involves capturing digital images of samples without the use of a microscope. Digital images of samples are stored, ready for assessment by trained technicians for identification and counting nematode eggs (Sowerby et al., 2016). Each digital image remains available for reference and auditing purposes. Setting up the FECPAK^{G2} test does not require specialised laboratory equipment or technical skills, and preparation can be done easily in the field by a lay operator. As a result, large numbers of samples can be processed at one time and images analysed later.

The aim of this study was to compare FEC estimates of GINs in alpacas using FECPAK^{G2} and the McMaster technique using two flotation solutions (saturated sodium chloride and sucrose solutions).

3.3. Materials and methods

Defining $\alpha = 0.05$, a study power of 0.80 and agreement limits of ±2100 EPG, it was estimated that a sample size of 94 faecal samples was required to assess agreement between the two methods of measurement (Lu et al., 2016). Briefly, fresh faecal samples (n = 94) were collected directly from the rectum of 3-month to 16-year old Huacaya alpacas, from commercial farms (n = 10) in New South Wales, Queensland and Victoria, Australia. Samples were stored at 4°C for up to seven days until the time of testing.

Each faecal sample was tested using modified McMaster technique [4,6]. Briefly, four grams of faeces were soaked for 5-30 minutes in 11 mL of water in 60 mL plastic containers. The faecal slurry was then mixed with either 45 mL of saturated sodium chloride (specific gravity [SG] 1.20, Merck, Germany) or white sugar (SG 1.27, www.csrsugar.com.au) solution and homogenised using a metal spatula. After 30-45 minutes, a sample was drawn from the suspension using a sieve-top pipette (sieve aperture size 12 meshes per cm). Following agitation, the sample was introduced into two chambers of a Whitlock egg counting slide (www.whitlock.com.au) which was then placed on the stage of a compound light microscope. After five minutes, eggs were counted. The minimum detection limit using this method was 15 EPG.

All of the faecal samples that were processed using the McMaster technique were then processed using the FECPAK^{G2} (Techion Group Ltd., New Zealand; www.techiongroup.com) method using salt and sugar solution as per the manufacturer's protocol. Four grams of faeces were selected for each sample.

3.4. Results and discussion

The arithmetic means of EPG were calculated for faecal egg counts obtained using the two methods and differences in arithmetic means were tested using the Wilcoxon signed rank test. A P-value < 0.05 was considered as statistically significant. Differences in EPG estimates assessed using the McMaster and FECPAK^{G2} methods using sugar and salt solutions were statistically significant (P-values 0.003 and 0.001 for salt and sugar solutions, respectively). Due to the highly skewed distribution of the FEC data, individual FECs were log transformed ($\log_{10}$[EPG + 15]) (Nodtvedt et al., 2002), and agreement of the FEC estimates using the McMaster and FECPAK^{G2} methods assessed using the Lin's concordance correlation coefficient (Lin et al., 2002) and Bland-Altman plot (Bland and Altman, 2010). To provide interpretable Bland and Altman plots, limits of agreement for the log-transformed data were calculated and transformed back to the original scale using the approach described by Euser et al. (2008). These limits of agreement were plotted on the original scale using conventional Bland and Altman plots. Statistical analyses were carried out using the epiR package (Stevenson et al., 2018) implemented in R (R Core Team., 2017).

A total of 64-89% faecal samples was test-positive using the McMaster and FECPAK^{G2} methods (Table 3.1). However, when saturated salt solution was used, more

samples had at least more than one EPG using the McMaster technique (89%; 81/91) compared with FECPAK^{G2} (77%; 70/91). Using sugar solution, more samples had at least more than one EPG using the McMaster technique (73%; 69/94) compared with FECPAK^{G2} (64%; 60/94; Table 3.1). There was a significant difference ($P = 0.03$) in the proportions of positive samples tested by both methods when salt solution was used (Table 3.1).

Table 3.1. Descriptive statistics of the untransformed faecal egg counts of alpaca gastrointestinal nematodes using McMaster and FECPAK^{G2} methods.

Flotation solution	*Method*	*% Test-positive samples (proportion)*	*P-value*	*Arithmetic mean EPG ± SE*	*95% CI*	*Range of EPG*
Salt	**McMaster**	**89 (81/91)**	**0.03**	**335 ± 62**	**(211, 458)**	**(0, 3435)**
	FECPAK^{G2}	**77 (70/91)**		**438 ± 83**	**(273, 603)**	**(0, 5180)**
Sugar	**McMaster**	**73 (69/94)**	**0.16**	**448 ± 138**	**(174, 723)**	**(0, 10515)**
	FECPAK^{G2}	**64 (60/94)**		**280 ± 86**	**(109, 450)**	**(0, 6930)**

EPG = eggs per gram of faeces; SE = standard error of mean; CI = confidence interval.

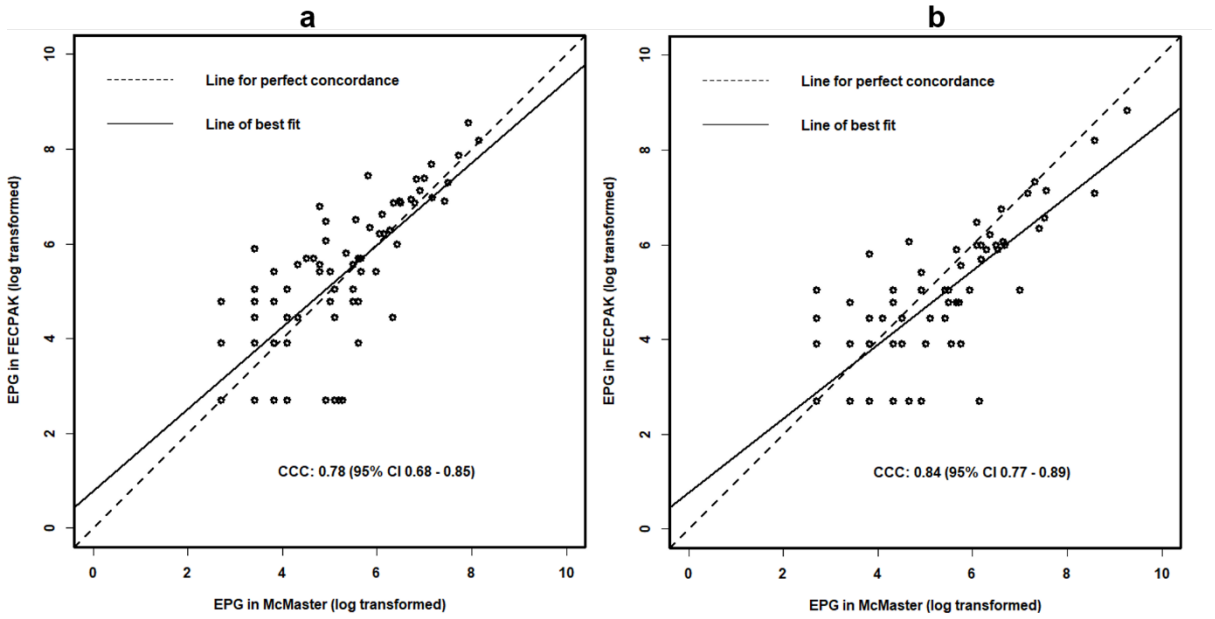


Fig. 3.1. Concordance correlation coefficient (CCC) plots showing line of perfect concordance (dotted line) and estimated concordance (solid line) between McMaster and FECPAKG2 methods using salt solution (**a**) and sugar solution (**b**).

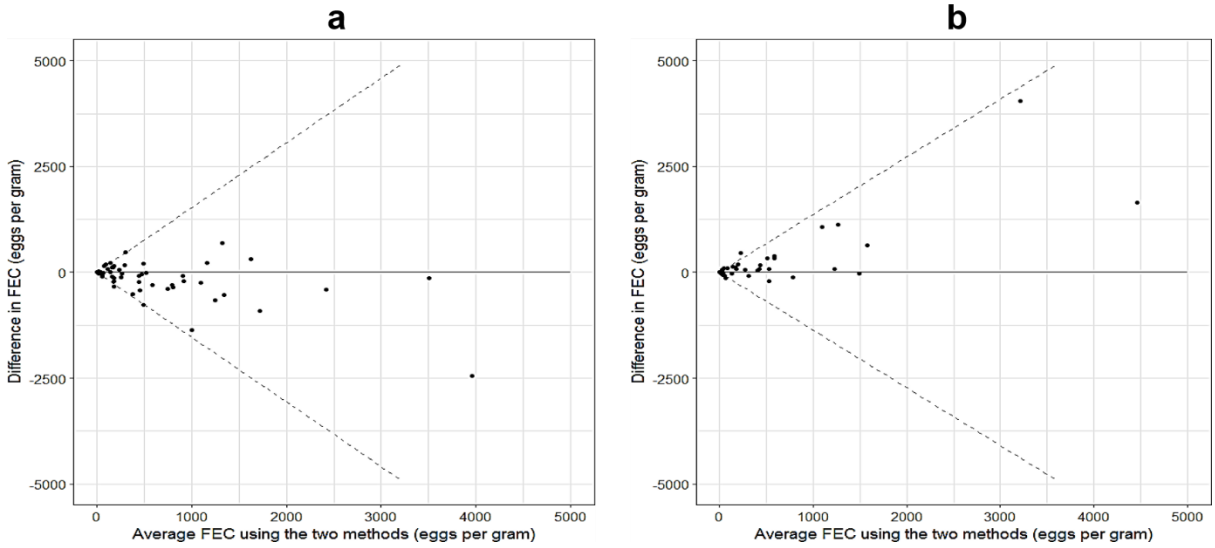


Fig. 3.2. Bland-Altman agreement plots for McMaster and FECPAKG2 methods. The dashed lines on each plot show the back-transformed limits of agreement using salt solution (**a**) and sugar solution (**b**).

Lin's concordance correlation coefficient was greater when sugar solution was used as a flotation fluid compared with salt (0.84, 95% CI 0.77 to 0.89 and 0.78, 95% CI 0.68 to 0.85, respectively) (see Fig. 3.1). These metrics are supported by the Bland-Altman plots shown in Fig. 3.2. where FEC differences for sugar are more tightly clustered around zero (Fig. 3.2b) compared with salt (Fig. 3.2a), particularly when mean EPGs were less than 1,000. For the relatively small numbers of samples where mean EPGs were greater than 2,500, differences in the two methods were greater for sugar (Fig. 3.2b).

3.5. Conclusions

This is the first study conducted to assess agreement between SAC FECs estimated using the McMaster and FECPAK^{G2} methods. The present results show moderate to good agreement between the two methods, with better agreement achieved when saturated sugar is used as a flotation fluid, particularly when FECs are less than 1,000 EPG. The advantages of the FECPAK^{G2} method are that it does not require specialised laboratory equipment or highly trained staff on farm, and images are stored online for perpetuity.

3.6. References

Ballweber, L.R., Beugnet, F., Marchiondo, A.A., & Payne, P.A. (2014). American Association of Veterinary Parasitologists' review of veterinary fecal flotation methods and factors influencing their accuracy and use - is there really one best technique? *Veterinary Parasitology,* 204, 73-80.

Bland, J.M., & Altman, D.G. (2010). Statistical methods for assessing agreement between two methods of clinical measurement. *International Journal of Nursing Studies,* 47, 931-936.

Bosco, A., Rinaldi, L., Maurelli, M.P., Musella, V., Coles, G.C., & Cringoli, G. (2014). The comparison of FLOTAC, FECPAK and McMaster techniques for nematode egg counts in cattle. *Acta Parasitologica,* 59, 625-628.

Cebra, C.K., & Stang, B.V. (2008). Comparison of methods to detect gastrointestinal parasites in llamas and alpacas. *Journal of the American Veterinary Medical Association,* 232, 733-741.

Cringoli, G., Rinaldi, L., Veneziano, V., Capelli, G., & Scala, A. (2004). The influence of flotation solution, sample dilution and the choice of McMaster slide area (volume) on the reliability of the McMaster technique in estimating the faecal egg counts of gastrointestinal strongyles and *Dicrocoelium dendriticum* in sheep. *Veterinary Parasitology,* 123, 121-131.

Euser, A., Dekker, F., & le Cessie, S. (2008). A practical approach to Bland-Altman plots and variation coefficients for log transformed variables. Journal of Clinical Epidemiology, 61, 978-982.

Gordon, H.M., & Whitlock, H. (1939). A new technique for counting nematode eggs in sheep faeces. *Council for Scientific and Industrial Research,* 12, 50-52.

Jabbar, A., Campbell, A.J.D., Charles, J.A., & Gasser, R.B. (2013). First report of anthelmintic resistance in *Haemonchus contortus* in alpacas in Australia. *Parasites and Vectors,* 6, 243.

Leguia, G. (1991). The epidemiology and economic impact of llama parasites. *Parasitology Today,* 7, 54-55.

Levecke, B., Behnke, J.M., Ajjampur, S.S.R., Albonico, M., Ame, S.M., Charlier, J., Geiger, S.M., Hoa, N.T.V., Kamwa, Ngassam, R.I., Kotze, A.C., McCarthy, J.S., Montresor, A., Periago, M.V., Roy, S., Tchuem Tchuenté, L-A., Thach, D.T.C., & Vercruysse, J. (2011). A comparison of the sensitivity and fecal egg counts of

the McMaster egg counting and Kato-Katz thick smear methods for soil-transmitted helminths. *PLOS Neglected Tropical Diseases,* 5, e1201.

Lin, L., Hedayat, A.S., Sinha, B., & Yang, M. (2002). Statistical methods in assessing agreement: models, issues, and tools. *Journal of the American Statistical Association,* 97, 257-270.

Lu, M.J., Zhong, W.H., Liu, Y.X., Miao, H.Z., Li, Y.C., & Ji, M.H. (2016). Sample size for assessing agreement between two methods of measurement by Bland-Altman method. *The International Journal of Biostatistics,* 12, 20150039-46.

Nodtvedt, A., Dohoo, I., Sanchez, J., Conboy, G., DesCoteaux, L., Keefe, G., & Leslie, K., Campbell, J. (2002). The use of negative binomial modelling in a longitudinal study of gastrointestinal parasite burdens in Canadian dairy cows. *Canadian Journal of Veterinary Research,* 66, 249-257.

Presland, S.L., Morgan, E.R., & Coles, G.C. (2005). Counting nematode eggs in equine faecal samples. *Veterinary Record,* 156, 208-210.

R Core Team. (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria.

Saeed, M.A., Jabbar, A. (2018). Smart diagnosis of parasitic diseases by use of smartphones. *Journal of Clinical Microbiology*, 56, e01469-17.

Sowerby, S.J., Crump, J.A., Johnstone, M.C., Krause, K.L., & Hill, P.C. (2016). Smartphone microscopy of parasite eggs accumulated into a single field of view. *The American Journal of Tropical Medicine and Hygiene*, 94, 227-230.

Stevenson, M., Nunes, T., Heuer, C., Marshall, J., Sanchez, J., Thornton, R., Reiczigel, J., Robison-Cox, J., Sebastiani, P., Solymos, P., Yoshida, K., Jones, G., Pirikahu, S., Firestone, S., Kyle, R., Popp, J., & Mathew, J.(2018). epiR: Tools for the Analysis of Epidemiological Data. Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Melbourne, Australia; 2018.

Windsor, R.H.S., Teran, M., & Windsor, R.S. (1992). Effects of parasitic infestation on the productivity of alpacas (Lama pacos). *Tropical Animal Health and Production*, 24, 57-62.

CHAPTER 4. MULTIPLEXED-TANDEM PCR (MT-PCR) ASSAY TO DETECT AND DIFFERENTIATE GASTROINTESTINAL NEMATODES OF ALPACAS

4.1. Abstract

Gastrointestinal nematodes (GINs) frequently infect South American camelids (alpacas and llamas) and cause economic losses due to reduced production of fiber, meat and/or leather. The knowledge about the epidemiology and diagnosis of GINs in llamas and alpacas is limited, and reliable keys for the identification of the third-stage larvae (L3s) of some common nematodes (such as *Camelostrogylus mentulatus*) that infect alpacas and llamas remain undescribed. In this study, two existing semi-quantitative multiplexed-tandem (MT)-PCR assays, originally developed for the GINs of sheep and cattle have been modified, to reliably detect and differentiate the common genera/species of GINs in the faeces of alpacas. Following the establishment of the MT-PCR assay using positive and negative control samples, alpaca faecal samples were tested to validate the assay. Selective sequencing of the purified MT-PCR products demonstrated specific (100%) amplification of the target nematode genera/species. Additionally, a comparison of results of the MT-PCR assay and the morphological identification of adult female and male worms collected from the same 35 alpacas revealed that there was a good agreement between the two methods in identifying nematode genera/species, including *C. mentulatus*, *Cooperia* spp. *Haemonchus* spp., *Oesophagostomum* spp., *O. ostertagi, T. circumcincta* and *Trichostrongylus* spp. However, some discrepancies were observed between the results of the MT-PCR assay and the morphological identification of adult worms. The MT-PCR platform is an accurate, sensitive and rapid method for the species/genus-specific diagnosis of GIN infections in alpacas, and it can be used as a substitute to larval culture to identify common GINs in the faeces of alpacas and llamas.

4.2. Introduction

In South American camelids (SACs) such as alpacas (*Vicugna pacos*) and llamas (*Lama glama*), parasitic gastroenteritis is caused by a variety of gastrointestinal nematodes (GINs). Some of them are host-specific (e.g. *Graphinema auchenia* and *Lamanema chavezi*) and occur in native habitats (i.e. South America) of alpacas and

llamas, while others are shared between SACs and domestic ruminants (e.g., *Cooperia* spp., *Haemonchus contortus*, *Nematodirus* spp., *Oesophagostomum* spp., *Ostertagia ostertagi*, *Teladorsagia circumcincta*, *Trichostrongylus* spp.) (Rickard, 1994; Ballweber, 2009; Franz et al., 2015). These nematodes can lead to considerable morbidity and even death in SACs, leading to significant economic losses (Leguia, 1991; Windsor et al., 1992). Farmers frequently use various classes of anthelmintics to control parasitic gastroenteritis in alpacas and llamas (Ballweber, 2009; Franz et al., 2015), although no anthelmintic is registered against GINs in SACs. The under-dosing of anthelmintics could potentially lead to the development of resistance in GINs of alpacas and llamas (Dadak et al., 2013; Jabbar et al., 2013; Franz et al., 2015) as it has been reported in those of small ruminants (Jabbar et al., 2006).

Although GINs of SACs have been the subject of intermittent studies over the past 25 years, very limited information is available on the epidemiology and control GINs in SACs (Franz et al., 2015). For instance, like domestic ruminants, the diagnosis of GINs in SACs is based on faecal egg counts (FEC) and larval culture (LC). However, these tests are time-consuming and lack sensitivity and specificity. In addition, the LC requires experienced personnel for accurate identification of the third-stage larvae (L3s) as many nematode species are difficult to distinguish morphologically (Roeber and Kahn, 2015). Furthermore, keys for the identification of L3s of some GINs that commonly infect SACs (e.g., *C. mentulatus*) remain undescribed. To overcome these challenges, molecular diagnostic tools can be used to accurately identify GINs of SACs like those of domestic ruminants (Roeber et al., 2013). For instance, the multiplexed-tandem PCR (MT-PCR) assay is a type of real-time PCR method that uses multiple primer pairs for the detection of several pathogens in one sample (Stanley and Szewczuk, 2005). This assay consists of two amplification steps where the primary amplification involves a 'target enrichment' using a small number of PCR cycles and outer primer sets, whereas the secondary amplification 'quantification step' utilises a diluted product from the primary amplification as a template and target specific, nested or inner primers (Stanley and Szewczuk, 2005). To date, MT-PCR has been applied to the sensitive and simultaneous detection of a number of pathogens of veterinary and medical significance, including fungi (Lau et al., 2008), enteric pathogens of humans (Jex et al., 2012), GINs of sheep (Roeber et al., 2012a, 2017a) and cattle (Roeber et al., 2017b), and toxigenic cyanobacteria (Baker et al., 2018).

A recent study in Australia showed that alpacas can be infected with a variety of GINs which also infect sheep and cattle. Furthermore, a high prevalence of a stomach worm, *C. mentulatus*, was found in alpacas. Given that very little is known about the epidemiology and diagnosis of GINs of SACs and reliable keys for the identification of L3s of some nematodes (such as *C. mentulatus*) that infect alpacas and llamas remain undescribed, two existing semi-quantitative MT-PCR assays were modified for the GINs of sheep (Roeber et al., 2017a, b) and cattle (Roeber et al., 2017b) to accurately detect and differentiate the common GINs, including *C. mentulatus*, *Cooperia* spp. *Haemonchus* spp., *Oesophagostomum* spp., *O. ostertagi, T. circumcincta* and *Trichostrongylus* spp. in the faeces of alpacas.

4.3. Materials and methods

4.3.1. Faecal samples and DNA extraction

A total of 35 alpaca faecal samples were available from a previous study. Following the processing of faecal samples for the faecal egg counts (FECs) of GINs in alpacas by employing the McMaster technique, 5 mL of the suspension containing the saturated sugar solution form each sample was drawn and transferred to a 50 mL Falcon tube to extract eggs of GINs as previously described (Roeber et al., 2013). The washed eggs in each sample were transferred into a microcentrifuge tube and stored at -20 °C until further use. Following thawing, a 250 µL of the concentrated eggs was used to extract and isolate DNA using Powersoil® DNA purification kit (MoBio, USA) as per manufacturer's recommended protocol.

4.3.2. Multiplexed-tandem PCR

In this study, MT-PCR assay (AusDiagnostics Pty. Ltd., Australia) has been validated to detect and differentiate seven GINs of alpacas (*C. mentulatus*, *Cooperia* spp. *Haemonchus* spp., *Oesophagostomum* spp., *O. ostertagi, T. circumcincta* and *Trichostrongylus* spp.) based on two existing MT-PCR assays for cattle (Roeber et al., 2017b), and sheep (Roeber et al., 2012a, 2017a).

The assay was conducted in the 24 wells variant of the Easy-Plex platform, including the 96-well Easy-Plex Analyzer and PC with Easy-Plex software (Cat. No. 9350, AusDiagnostics). The primary amplification ('target enrichment') was conducted using nematode specific primer pairs designed to the sequences of the internal transcribed spacer 2 (ITS-2) (Step 1 tubes for nematodes [8-well], Cat. No. 78150S, AusDiagnostics). The secondary amplification for semi-quantification employed nested primer pairs to internal regions of the ITS-2 (Alpaca Nematodes MP96 8-well, Cat. No.78150E, AusDiagnostics) specific to *C. mentulatus*, *Cooperia* spp. *Haemonchus* spp., *Oesophagostomum* spp., *O. ostertagi, T. circumcincta* and *Trichostrongylus* spp. and these internal primer pairs amplify a region of ~ 90 to 110 bp from the ITS-2 region. Furthermore, another primer pair was included in each run as a reference for quantitation and to assess the efficiency of amplification from 10,000 copies of a synthetic oligonucleotide template (internal 'spike control').

For primary amplification (15 cycles of 10 s at 95 °C, 20 s at 60 °C, and 20 s at 72 °C), 5 µl of genomic DNA representing each DNA sample or 5 µl of water (negative control) were dispensed into 0.2 ml PCR strips, and placed into a 24-well thermocycling block in the Gene-Plex robotic platform (AusDiagnostics). Following the dispensing of each sample and the initiation of the assay, the following set-up process and analysis were executed by the program Easy-Plex Assay Setup (AusDiagnostics), with the secondary amplification in MT-PCR and the melting curve analysis being semi-automated. Each sample was recorded as test-positive using the auto-call function of the Easy-Plex software (AusDiagnostics) if the amplicon produced a single melting curve which was within 1.5°C of the expected melting temperature, the height of the peak was higher than 0.2 dF/dT (where dF/dT is the derivative of fluorescence over temperature), and the peak width was ≤ 3.5°C, otherwise the sample was considered as negative (AusDiagnostics). Additionally, cycle threshold (Ct) values for each nematode per sample were determined by comparing with the data obtained from the internal spike control which had a known 10,000 DNA copy numbers. Based on the peak high-resolution melting (HRM) temperature analyses, nematode genera/species were assigned according to their mean HRM temperatures. Randomly selected amplicons representing each nematode genus/species were subjected to sequencing to verify the target nematodes.

The analytical sensitivity (using a 10-fold serial dilution) was determined using known positive (assessed by amplifying the ITS-2 region from each individual worm

using a conventional PCR) samples (*Cooperia oncophora*, *Haemonchus contortus*, *Oesophagostomum venulosum, O. ostertagi, T. circumcincta,* and *Trichostrongylus colubriformis*), and the analytical specificity of the assay was determined by testing known positive samples (assessed by amplifying the ITS-2 region from each individual worm using a conventional PCR) for each of the targeted nematode species (*Chabertia ovina, Cooperia curticei*, *C. oncophora* (*C. surnabada)*, *H. contortus*, *H. placei*, *Oesophagostomum radiatum, O. columbianum, O. venulosum, Nematodirus spathiger, N. filicollis, O. ostertagi, T. circumcincta, Trichostrongylus vitrinus, T. colubriformis, T. rugatus* and *T. axei*). Repeatability of the assay for the detection of the expected nematode genera/species and among different runs were also assessed, and the coefficient of variation (CV) was estimated using the program Microsoft Excel (2016) to determine the repeatability.

4.3.3. Comparison of the MT-PCR data and morphological examination of adult worms

Conventionally, data on the detection of GINs using molecular methods is compared with those of LC (Roeber et al., 2012b, 2017b). However, this study used the morphological identification of adult female and male worms from the same animals because such data are more accurate and specific, and were incidentally available to us from a previous study. The nematode genera/species found using MT-PCR in the faeces of the 35 alpacas were compared with the results of morphological examination of adult worms collected from the third compartment of the stomach and the small intestine during the total worm counts from the same animals to assess the concordance of identification of nematode genera/species.

4.3.4. Statistical analysis

The morphological identification of adult worms and MT-PCR datasets were imported into R statistical package for agreement calculations. Adult worms as well as MT-PCR data were converted into binary data based on the presence or absence of nematode genera/species. Frequency table (2 x 2) was constructed for genera/species of GINs and the level of agreement using Kappa values was calculated using ‘epiR’ package (Stevenson et al., 2018). Kappa measures the proportion of agreement and its values are

used to compare results of different tests for one set of samples. Kappa has a range from -1 to 1. A benchmark can be used arbitrarily to interpret Kappa values as 0.00: poor agreement; 0.00–0.20: slight agreement; 0.21–0.40: fair agreement; 0.41–0.60: moderate agreement; 0.61–0.80: substantial agreement; ≥0.81: almost perfect agreement (Conraths and Schares, 2006).

4.4. Results and discussion

Using the MT-PCR assay, each of the seven primer sets designed to the ITS-2 region amplified products exclusively from the expected nematode genera/species of alpacas (Fig. 4.1). The identity of individual products was confirmed by sequencing, and no products were amplified from other nematodes tested. Repeatability of the MT-PCR assay revealed that the seven GINs of alpacas were always correctly assigned (CV of 0%). Based on the peak HRM temperature analyses, each GIN of alpacas was produced as a single and distinct melt curve, and nematodes *C. mentulatus*, *Cooperia* spp. *Haemonchus* spp., *Oesophagostomum* spp., *O. ostertagi, T. circumcincta* and *Trichostrongylus* spp. had mean HRM temperatures of 77.0 ± 1.5 °C, 78.4 ± 1.5 °C, 80.5 ± 1.5 °C, 78.9 ± 1.5 °C, 82.3 ± 1.5 °C, 79.4 ± 1.5 °C and 80.3 ± 1.5 °C, respectively (see Fig. 4.1).

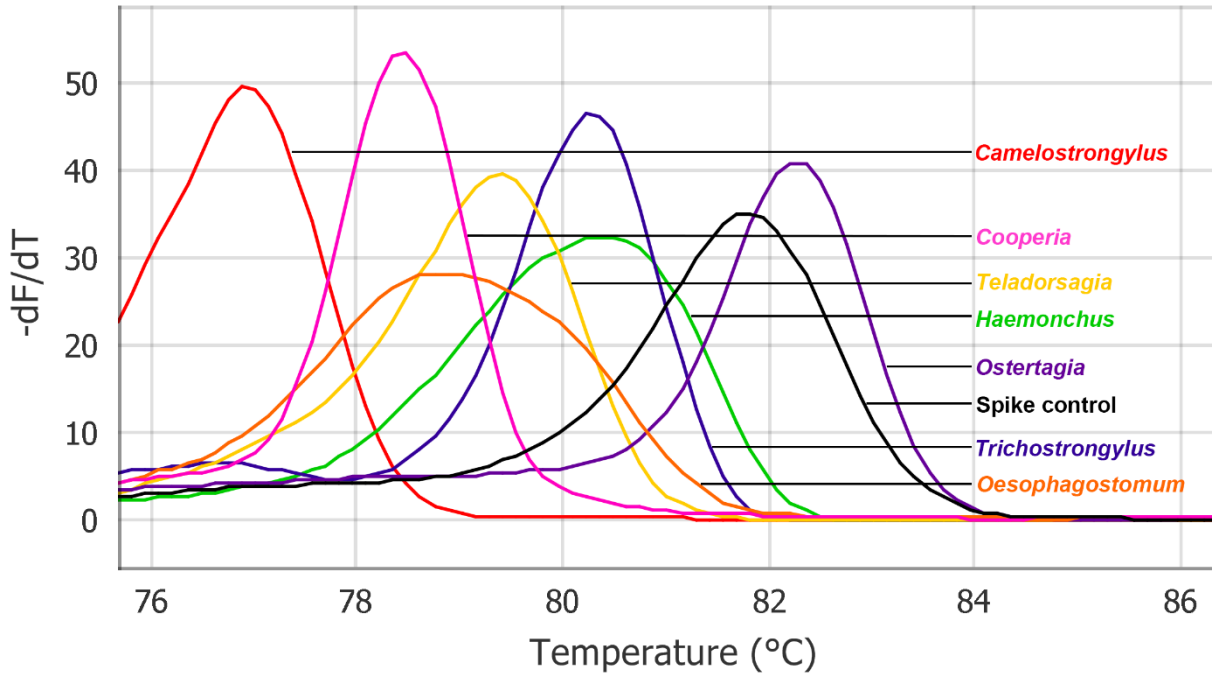


Fig. 4.1. High-resolution melting (HRM) curve analysis of the gastrointestinal nematodes of alpacas and spike control using the multiplexed-tandem polymerase chain reaction (MT-PCR) assay.

The MT-PCR assay was then validated using the alpaca faecal samples (n = 35) known to be positive for nematode eggs (determined by FECs). All 35 samples were test-positive using MT-PCR for at least one nematode genus/species (Table 4.1). Furthermore, all the seven nematode genera/species (i.e. *C. mentulatus*, *Cooperia* spp., *Haemonchus* spp., *Oesophagostomum* spp., *O. ostertagi, T. circumcincta* and *Trichostrongylus* spp.) were detected. *Haemonchus* spp. was detected in most of samples (94.29%; 33/35) followed by *Trichostrongylus* spp. (68.57%; 24/35), *C. mentulatus* (57.14%; 20/35), *O. ostertagi* (31.43%; 11/35)*, Cooperia* spp. (28.57%; 10/35), *Oesophagostomum* spp. (8.57%; 3/35) and *T. circumcincta* (2.86%; 1/35) (Table 4.1). Mixed infections with two or more nematode genera/species were more common (82.86%; 29/35) than single infections (17.14%; 6/35).

Highest agreement between morphological identification of adult worms and MT-PCR results was observed for *T. circumcincta* (94.23%) followed by *Haemonchus* spp. (85.71%) and *C. mentulatus* (65.71%) (Table 4.1). Based on the Kappa statistic, a fair agreement was found between results of the MT-PCR assay and the morphological identification of worms such as *Haemonchus* spp. and *C. mentulatus*. However, two GINs, *Oesophagostomum* and *Ostertagia* were only detected by the MT-PCR assay (Table 4.1). Thus, the MT-PCR assay was able to detect more genera/species of alpaca GINs than morphological examination of adult worms.

Table 4.1. Agreement (%) between the identification of gastrointestinal nematodes of alpacas using the MT-PCR assay and morphological identification of adult worms.

Genera /Species	*No. of sample*	*No. of samples (%) identified by morphological examination*	*No. of samples identified by MT-PCR*	*Agreement (%)*	*Kappa value*
Camelostrongylus mentulatus	**35**	**22 (62.85)**	**20 (57.14)**	**65.71**	**0.29**
***Haemonchus* spp.**	**35**	**30 (85.71)**	**33 (94.29)**	**85.71**	**0.22**
***Cooperia* spp.**	**35**	**18 (51.43)**	**10 (28.57)**	**37.14**	**-0.24**
Teladorsagia circumcincta	**35**	**1(2.86)**	**1 (2.86)**	**94.23**	**-0.03**
***Trichostrongylus* spp.**	**35**	**15 (42.86)**	**24 (68.57)**	**38.29**	**-0.25**
***Oesophagostomum* spp.**	**35**	**-**	**3 (8.57)**	**-**	**-**
***Ostertagia* spp.**	**35**	**-**	**11 (31.43)**	**-**	**-**

This is the first study to identify and differentiate GINs using molecular tools in the faeces of SACs. In spite of the development of molecular tools for identifying common GINs in faeces of domestic ruminants in the last two decades (Roeber et al., 2013a), LC remains to be the most commonly used method for identifying nematodes at genus/species level in faeces of domestic ruminants, including alpacas and llamas (Cebra and Stang, 2008; Ballweber, 2009). However, this technique is not only time and labour-intensive but it also lacks sensitivity and specificity (Roeber et al., 2013b). Furthermore, the taxonomic keys for the identification of L3s of uncommon species of GINs (such as *C. mentulatus*) in domestic ruminants are not described which lead to 'false' diagnosis of GINs. Contrarily, molecular techniques that rely on the amplification of nucleic acids, particularly those coupled to the polymerase chain reaction are effective for the specific identification of GINs, and aid the diagnosis of infections from minute amounts of target template utilizing specific markers (Gasser et al., 1993). Such methods offer accurate, reliable, specific and sensitive tools to traditional approaches such as LC that can not only offer better diagnostic tools but also provide alternate fundamental research into epidemiology and control of GINs (Gasser et al., 2006). A range of molecular methods have been developed to detect GINs using DNA isolated from embryonated nematodes eggs or larvae from faeces of ruminants (Bott et al., 2009; Sweeny et al., 2011; Roeber et al., 2012a, b, 2017a, b). However, MT-PCR offers advantages over most of commonly used molecular tools as it is semi-automated, more sensitive (using nested-PCR) in detecting low infection levels and utilizes genus/species specific primers, thereby allowing simultaneous detection of multiple pathogens in one sample (Stanley and Szewczuk, 2005).

In this study, the results of MT-PCR have been compared with those of morphological identification of adult female and male worms that were available from the same animals whose faecal samples were tested using MT-PCR. Morphological examination of adult worms is considered to be a 'gold standard' technique in the identification of GINs as this involves the examination of adult male and female worms which provides an accurate and rapid diagnosis of worms. However, LC is time-consuming to perform (takes one week) and it could be non-specific as it relies on the morphological features of L3s of nematodes developed *in vitro* which can vary depending on culture conditions (temperature and relative humidity), leading to a bias when assigning larvae to nematode genera/species (Dobson et al., 1992). Furthermore, significant differences in the protocols of LC often limit direct comparisons of results between or among laboratories

(Dobson et al., 1992; Roeber et al., 2011) whereas the examination of adult worms does not have such issues. Such differences between the identification of GINs based on the examination of adult worms and L3s should be interpreted carefully as adult worms are collected after necropsy while LC is performed based on the collection of faeces of animals using a non-invasive method. However, the use of a 'gold standard' test such as the identification of adult worms is critical in the validation of new tests such as the MT-PCR assay developed in this study.

Although a good agreement between morphological identification of adult worms and MT-PCR results were found for *T. circumcincta*, *Haemonchus* spp. and *C. mentulatus* (see Table 4.1). Furthermore, this study also found discrepancies between the results of the examination of adult worms and MT-PCR for *Oesophagostomum* spp. and *O. ostertagi* as these were only detected by the MT-PCR assay. This difference between two methods could be attributed to the inherent drawback with the routine total worm count technique which does not involve a meticulous collection of worms (such as *Oesophagostomum* spp.) that occur in the large intestine. Contrarily, molecular tools involving PCRs such as MT-PCR can detect the DNA from even low number of nematode eggs passed in faces of domestic ruminants (Gasser et al., 1993). Therefore, MT-PCR assay could detect more nematode genera/species of alpacas than morphological examination of adult worms. This difference of sensitivity between the two methods could also be due to the variation in the relative abundance of adult worms collected from gastrointestinal tracts of alpacas as aliquots (rather than complete contents) of stomach and intestinal contents were examined to collect and identify worms during total worm counts. Previously, Roeber et al. (2017a) also found that the MT-PCR was more sensitive in the detection and differentiation of GINs of sheep. However, these authors compared the results of MT-PCR with those of LC as opposed to identification of adult worms herein.

4.5. Conclusions

The MT-PCR assay established and validated herein, is a rapid, sensitive and effective molecular diagnostic tool to detect and differentiate seven common nematode genera/species of alpacas and llamas.

4.6. References

Baker, L., Sendall, B.C., Gasser, R.B., Menjivar, T., Neilan, B.A., & Jex, A.R. (2018). Rapid, multiplex-tandem PCR assay for automated detection and differentiation of toxigenic cyanobacterial blooms. *Molecular and Cellular Probes,* 27, 208-214.

Ballweber, L.R. (2009). Ecto- and endoparasites of New World camelids. *Veterinary Clinics of North America: Food Animal Practice,* 25, 295-310.

Bott, N.J., Campbell, B.E., Beveridge, I., Chilton, N.B., Rees, D., Hunt, P.W., & Gasser, R.B. (2009). A combined microscopic-molecular method for the diagnosis of strongylid infections in sheep. *International Journal for Parasitology,* 39, 1277-1287.

Cebra, C.K., & Stang, B.V. (2008). Comparison of methods to detect gastrointestinal parasites in llamas and alpacas. *Journal of the American Veterinary Medical Association,* 232, 733-741.

Conraths, F., & Schares, G. (2006). Validation of molecular-diagnostic techniques in the parasitological laboratory. *Veterinary Parasitology,*136, 91-98.

Dadak, A.M., Asanger, H., Tichy, A., & Franz, S. (2013). Establishing an efficacious dose rate of monepantel for treating gastrointestinal nematodes in llamas under field conditions. *Veterinary Record,* 172, 155.

Dobson, R.J., Barnes, E.H., Birclijin, S.D., & Gill, J.H. (1992). The survival of *Ostertagia circumcincta* and *Trichostrongylus colubriformis* in faecal culture as a source of bias in apportioning egg counts to worm species. *International Journal for Parasitology,* 22, 1005-1008.

Franz, S., Wittek, T., Joachim, A., Hinney, B., & Dadak, A.M. (2015). Llamas and alpacas in Europe: endoparasites of the digestive tract and their pharmacotherapeutic control. *Veterinary Journal,* 204, 255-262.

Gasser, R.B. (2006). Molecular tools-advances, opportunities and prospects. *Veterinary Parasitology,*136, 69-89.

Gasser, R.B., Chilton, N.B., Hoste, H., & Beveridge, I. (1993). Rapid sequencing of rDNA from single worms and eggs of parasitic helminths. *Nucleic Acids Research,* 21, 2525-2526.

Jabbar, A., Campbell, A.J.D., Charles, J.A., & Gasser, R.B.(2013). First report of anthelmintic resistance in *Haemonchus contortus* in alpacas in Australia. *Parasites and Vectors,* 6, 243.

Jabbar, A., Iqbal, Z., Kerboeuf, D., Muhammad, G., Khan, M.N., & Afaq, M. (2006). Anthelmintic resistance: the state of play revisited. *Life Science,* 79, 2413-2431.

Jex, A.R., Stanley, K.K,, Lo, W., Littman, R., Verweij, J.J., Campbell, B.E., Nolan, M.J., Pangasa, A., Stevens, M.A., Haydon, S., & Gasser, R.B. (2012). Detection of diarrhoeal pathogens in human faeces using an automated, robotic platform. *Molecular and Cellular Probes,* 26, 11-15.

Lau, A., Sorrell, T.C., Chen, S., Stanley, K., Iredell, J., & Halliday, C. (2008). Multiplex tandem PCR: a novel platform for rapid detection and identification of fungal pathogens from blood culture specimens. *Journal of Clinical Microbiology,* 46, 3021-3027.

Leguia, G. (1991). The epidemiology and economic impact of llama parasites. *Parasitology Today,* 7, 54-55.

Rickard, L.G. (1994). Parasites. *Veterinary Clinics of North America: Food Animal Practice,* 10, 239-47.

Roeber, F., & Kahn, L. (2015). The specific diagnosis of gastrointestinal nematode infections in livestock: larval culture technique, its limitations and alternative DNA-based approaches. *Veterinary Parasitology,* 205, 619-628.

Roeber, F., Hassan, E.B., Skuce, P., Morrison, A., Claerebout, E., Casaert, S, Homer, D.R., Firestone, S., Stevenson, M., Smith, L., & Larsen, J. (2017b). An automated, multiplex-tandem PCR platform for the diagnosis of gastrointestinal nematode infections in cattle: an Australian-European validation study. *Veterinary Parasitology,* 239, 62-75.

Roeber, F., Jex, A.R., & Gasser, R.B. (2013a). Comparative evaluation of two DNA isolation techniques for PCR-based diagnosis of gastrointestinal nematode infections in sheep. *Molecular and Cellular Probes,* 27, 153-157.

Roeber, F., Jex, A.R., & Gasser, R.B. (2013b). Next-generation molecular-diagnostic tools for gastrointestinal nematodes of livestock, with an emphasis on small ruminants: a turning point? *Advances in Parasitology,* 83, 267-333.

Roeber, F., Jex, A.R., Campbell, A.J., Campbell, B.E., Anderson, G.A., & Gasser, R.B. (2011). Evaluation and application of a molecular method to assess the composition of strongylid nematode populations in sheep with naturally acquired infections. *Infection, Genetics and Evolution,* 11, 849-854.

Roeber, F., Jex, A.R., Campbell, A.J., Nielsen, R., Anderson, G.A., Stanley, K.K., & Gasser, R.B. (2012a). Establishment of a robotic, high-throughput platform for

the specific diagnosis of gastrointestinal nematode infections in sheep. *International Journal for Parasitology,* 42, 1151-1158.

Roeber, F., Larsen, J.W., Anderson, N., Campbell, A.J,, Anderson, G.A., Gasser, R.B., & Jex, A.R. (2012b). A molecular diagnostic tool to replace larval culture in conventional faecal egg count reduction testing in sheep. *PLoS One*, 7, e37327.

Roeber, F., Morrison, A., Casaert, S., Smith, L., Claerebout, E., & Skuce, P. (2017a). Multiplexed-tandem PCR for the specific diagnosis of gastrointestinal nematode infections in sheep: an European validation study. *Parasites and Vectors,*10, 226.

Stanley, K.K., & Szewczuk, E. (2005). Multiplexed tandem PCR: gene profiling from small amounts of RNA using SYBR Green detection. *Nucleic Acids Research,* 33, e180.

Stevenson, M., Nunes, T., Heuer, C., Marshall, J., Sanchez, J., Thornton, R., Reiczigel, J., Robison-Cox, J., Sebastiani, P., Solymos, P. & Yoshida, K. (2018). epiR: Tools for the analysis of epidemiological data. R package version 0.9–79:[Available from: https://cran. r-project. org/package= epiR.

Sweeny, J.P., Robertson, I.D., Ryan, U.M., Jacobson, C., & Woodgate, R.G. (2011). Comparison of molecular and McMaster microscopy techniques to confirm the presence of naturally acquired strongylid nematode infections in sheep. *Molecular and Biochemical Parasitology,* 180, 62-67.

Windsor, R.H.S., Teran, M., & Windsor, R.S. (1992). Effects of parasitic infestation on the productivity of alpacas (*Lama pacos*). *Tropical Animal Health and Production,* 24, 57-62.

CHAPTER 5. EPIDEMIOLOGY OF GASTROINTESTINAL NEMATODES OF ALPACAS IN AUSTRALIA: I. A CROSS-SECTIONAL STUDY

5.1. Abstract

This study involved a national cross-sectional survey of gastrointestinal nematodes (GINs) of alpacas in Australia. A total of 1,545 fresh faecal samples were collected from both sexes of alpacas and processed for faecal egg counts (FEC) and molecular identification of nematodes using the multiplexed-tandem PCR assay. Based on egg morphology, the overall prevalence of GINs was 66% while that for strongyles was 59%. The overall mean FEC was 276 eggs per gram (EPG) of faeces, with the highest count of 17,415 EPG. Male alpacas had a higher prevalence (68%, 334/490) as well as mean FEC (328±60 EPG) of GINs than females (63%, 602/954; 227±26, respectively). Weaners had the highest prevalence (80%) whereas tuis had the highest FEC (402 EPG) of nematodes. The highest prevalence (77%, 293/383) and FEC (630 EPG) of GINs were observed in the summer rainfall zone followed by the Mediterranean-type rainfall, non-seasonal rainfall, and winter rainfall zones. The characterization of nematode DNA isolated from faeces revealed the occurrence of seven different GINs, including *Camelostrongylus mentulatus, Cooperia* spp., *Haemonchus* spp., *Oesophagostomum* spp., *Ostertagia ostertagi*, *Teladorsagia circumcincta* and *Trichostrongylus* spp. Besides, *Nematodirus* spp. and *Trichuris* spp. were also found during FECs. The prevalence of *Haemonchus* spp. was highest in the summer rainfall zone while that of *C. mentulatus* was highest in the Mediterranean-type rainfall, non-seasonal rainfall and winter rainfall zones. The findings of this study revealed that alpacas harbour many of the same nematodes as sheep and cattle.

5.2. Introduction

Domesticated South American camelids (SACs), namely alpacas (*Vicugna pacos*) and llamas (*Lama glama*), are increasingly popular as a commercial livestock species due to their fine fibre, lean meat and hides as well as their ability to adapt to diverse climatic conditions around the world (Leguía, 1991; Saeed et al., 2018). The health and productivity of alpacas and llamas can be compromised by gastrointestinal nematodes

(GINs), resulting in substantial economic losses (Ballweber, 2009; Leguía, 1991; Windsor et al., 1992). Alpacas and llamas are usually infected with GINs of sheep and cattle (Ballweber, 2009; Hill et al., 1993). However, some SAC-specific GINs such as *Graphinema auchenia*, *Lamanema chavezi*, *Nematodirus lamae* and *Mazamastrongylus peruvianus*, have also been reported (Cafrune et al. 2001; Guerrero and Chávez, 1967; Mitchell et al., 2016).

Gastrointestinal nematodes of SACs have been the subject of intermittent studies over the last few decades in many regions of the world, including Chile (Alcaino et al., 1991), Ecuador (Robayo, 2015), Japan (Hyuga and Matsumoto, 2016), New Zealand (Dittmer et al., 2018; Hill et al., 1993), Peru (Masson et al., 2016), Switzerland (Hertzberg and Kohler, 2006), the UK (Tait et al., 2002; Welchman et al., 2008), and the USA (Cebra and Stang, 2008; Rickard, 1993). Most SAC-specific GINs were initially reported in alpacas and llamas from South America (Alcaino et al., 1991; Ballweber, 2009; Franz et al., 2015; Guerrero and Chávez, 1967). However, recent reports have indicated the presence of *N. lamae* in alpacas from New Zealand (Hinkson, 2015) and the UK (Mitchell et al., 2016). Such reports of GINs in alpacas from outside South America warrant epidemiological studies to understand better the spectrum of GINs infecting alpacas.

Although Australia has the largest alpaca population outside South America, minimal information is available on the GINs of Australian alpacas (Carmichael, 1999, 2014; Presidente, 2007). The latter studies were either based on the opportunistic collection of alpaca faecal samples (Presidente, 2007) or were of limited scope (from South Australia and some parts of Victoria) (Carmichael, 1999). Therefore, the epidemiology of GINs in Australian alpacas remain mostly unknown.

This study aimed to conduct a national cross-sectional survey of GINs of alpacas to establish baseline data on the epidemiology of GINs of alpacas in Australia. Also, an online questionnaire survey was conducted to understand the important husbandry practices that might play a role in the prevalence of GINs of alpacas in Australia.

5.3. Materials and methods

5.3.1. Study population

In Australia, climatic zones can be categorized as follows: (i) Western Australian winter rainfall, (ii) South Australian winter rainfall, (iii) Victorian winter rainfall, (iv) Tasmania, (v) New South Wales (NSW) non-seasonal rainfall, (vi) Queensland (Qld)/NSW summer rainfall/slopes and plains, (vii) Qld/NSW summer rainfall /tablelands and slopes and (viii) Pastoral (www.wormboss.com.au). Based on the distribution and density of alpaca populations in different climatic zones of Australia, the climatic zones were designated as Mediterranean-type (by combining sheep zones 1 and 2), non-seasonal (5), summer (6-8) plus the north Queensland coast and winter (3 and 4) rainfall zones in this study (Fig. 5.1). The majority of alpaca farms contain 50 or fewer animals, which graze year-round on pastures with a variable provision of supplementary feed. According to the age, there are four main categories of alpacas such as cria (< 6 months), weaner (6-12 months), tuis (1-2 years) and adult (>2 years). Alpacas are routinely vaccinated against clostridial diseases. Although alpacas are shorn at variable times throughout the year, spring is the usual season for annual shearing. Likewise, female alpacas usually give birth during two months in autumn, although this practice varies among farms. Crias are weaned at an average age of five to six months.

5.3.2. Questionnaire

An online survey was conducted using the software “Research Electronic Data Capture” (REDCap; Harris et al., 2009). The online questionnaire contained 30 questions focused on (i) farm demography and general husbandry practices, (ii) grazing management practices and (iii) the use of anthelmintics. The majority of the questions were closed with a few semi-open (a closed question with the addition of a category ‘other’) questions.

5.3.3. Sample size calculation for faecal sampling

During a previous study on worm control practices used by Australian alpaca farmers (Rashid et al. 2019), a total of 172 (out of 954) alpaca farms provided their consent to participate in epidemiological studies on GINs of alpacas. Based on the herd size, farms were divided into three groups, i.e. (i) small (having 10 to 50 alpacas), (ii) medium (>50 to ≤100), and (iii) large (>100). The required sample size was calculated by employing Generalized Random Tessellation Stratified (GRTS) sampling technique (Rosanowski et al. 2012) in the software R (R Core Team, 2017). This sampling technique allows random sampling by taking into account the spatial distribution of samples. To calculate the sample size, a 60% (based on Rashid et al. unpublished data) expected prevalence of GIN infections at the farm level and the four climatic zones of Australia was used as a prior. Based on these requirements, a total of 92 alpaca farms across various climatic zones of Australia was selected for this study (Fig. 5.1).

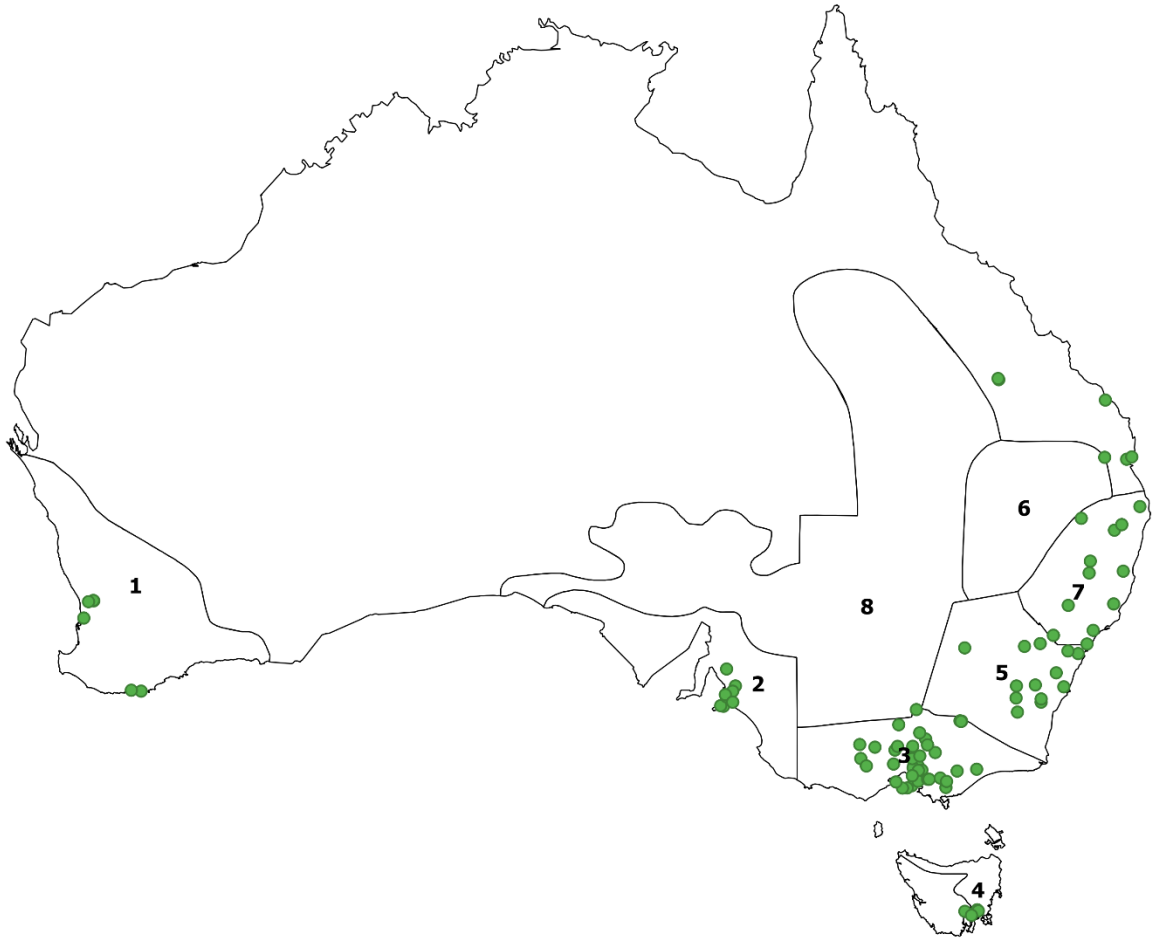


Fig. 5.1. Map of Australia showing the locations of alpaca farms included in the cross-sectional epidemiological study. Each green circle represents one alpaca farm. The climatic zones are drawn based on climatic zones described by www.wormboss.com.au. 1. Western Australian winter rainfall; 2. South Australian winter rainfall; 3. Victorian winter rainfall; 4. Tasmania; 5. NSW non-seasonal rainfall; 6. Qld/NSW Summer rainfall/slopes and plains; 7. Qld/NSW Summer rainfall /tablelands and slopes; 8. Pastoral.

5.3.4. Collection of faecal samples

The alpaca faecal samples were collected from 92 farms from February 2016 to October 2017, and these samples were collected only from those animals which had not been dewormed in the last four to six weeks. Each alpaca farm manager was sent the sample collection kits. Participating herd managers were asked to collect rectal faecal samples from 15 to 20 mixed age and sex alpacas. Faeces from individual alpacas were collected into zip-lock plastic bags appropriately labelled with the unique identifier of the alpaca. At the time of each sampling event, herd managers provided details of the animals that were sampled, including their herd identifier (name or number), age, sex, bodyweight (if recorded) and body condition score (expressed on a 1 to 5 scale). Individual faecal samples were collected directly from the rectum into zip-lock plastic bags, sent unrefrigerated in a container with an ice-pack by overnight post and then stored at 4°C until further processing.

5.3.5. Faecal egg count

Faecal egg counts (FEC) were undertaken using the modified McMaster technique with a saturated sugar solution (specific gravity 1.27), as described previously (see Chapter 3). The minimum detectable limit of the McMaster technique was 15 eggs per gram (EPG) of faeces.

5.3.6. Identification of nematodes

Following the processing of fresh faecal samples (n = 15-20) for the FECs from each farm, as described above, a 1-2 mL of the suspension from each specimen was transferred to a 50 mL Falcon tube to extract eggs as previously described (Roeber et al., 2013a). The washed eggs in each pooled sample were transferred into a microcentrifuge (Eppendorf) tube and stored at -20 °C until further use.

To accurately detect and differentiate the seven common GINs of alpacas, a newly established molecular diagnostic test has been used, the multiplexed-tandem polymerase chain reaction (MT-PCR) (see Chapter 4).

5.3.7. Statistical analyses

Data validation and cleaning were performed by using Microsoft Excel 2013. Descriptive statistical analysis was performed using the statistical package '*epiR*' in R (R Core Team 2017; Stevenson et al. 2018). To compare the difference in EPG between sexes, different age groups and in various climatic zones, Kruskal-Wallis ('*kruskal.test*') and Wilcoxon rank sum tests ('*pairwise.wilcox.test'*) were employed in the R software (R Core Team, 2017). To compare the prevalence of nematode genera/species (determined by the MT-PCR assay) among different climatic zones, Pearson's Chi-squared test with continuity correction and pairwise comparison of proportions test with adjusted *P*-value ('bonferroni' method) were employed using '*chisq.test*' and '*pairwise.prop.test*' functions in the R software. A *P*-value <0.05 was considered as statistically significant.

5.4. Results

The response rate for the questionnaire was 94% (91/97). The mean herd size was 73 animals. Thirty-four per cent (31/91) of herds had 50-100 alpacas, 29% had 21-50, alpacas and 37% of farms had >100 alpacas. About two-thirds (67%) of alpaca farms had the Huacaya breed of alpacas whereas 11% only kept the Suri breed. The area of alpaca farms ranged from 2-565 hectares, with a mean size of 66 hectares; however, the mean grazing area for alpacas was only 59 hectares. Adult females constituted the major proportion of alpaca herds, with a mean of 43 and a maximum number of 300 animals. Approximately 50% of alpaca farms had other livestock species (e.g. cattle, sheep and/or goats) and more than half of them co-grazed with alpacas. The survey results related to worm control practices have been published elsewhere (see Chapter 8).

Of 1,545 alpaca faecal samples examined, 66% (1012/1545) had at least one type of GIN egg. Overall, the mean FEC (± standard error of the mean) of GINs was 291±29 EPG (Table 5.1). Male alpacas had a higher prevalence (68%, 334/490) as well as mean FEC (328±60 EPG) of GINs than females (63%, 602/954 and 227±26 EPG, respectively), and this difference in FECs was statistically ($P < 0.05$) different. Based on egg morphology, strongyle nematodes were the most prevalent (59%, 916/1545), followed by *Nematodirus* spp. (17%, 261/1545) and *Trichuris* spp. (6%, 103/1545) (Table 5.1). Strongyle eggs had the highest mean FEC (276±28 EPG), and it was

statistically different ($P < 0.05$) from those of *Nematodirus* spp. (12±1 EPG) and *Trichuris* spp. (3±1 EPG).

Table 5.1. Prevalence and faecal egg counts of gastrointestinal nematodes in Australian alpacas.

Sex of alpacas	% Prevalence (proportion)	EPG (mean ± SE)	95% CI		Range
Female	63 (602/954)	227±26[a]	176	277	0 – 10,800
Male	68 (334/490)	328±60[b]	210	447	0 – 17,415
Type of nematode egg					
Strongyle	59 (916/1545)	276±28[a]	220	332	0 – 17,400
Nematodirus spp.	17 (261/1545)	12±1[b]	10	14	0 – 600
Trichuris spp.	6 (103/1545)	3±1[b]	2	4	0 – 420
Overall	66 (1012/1545)	291 ±29	235	347	0 – 17,415

Lowercase letters in a column indicate significant differences in EPG
EPG eggs per gram of faeces, SE standard error of mean, CI confidence interval

Based on the age of alpacas, the highest prevalence of GINs was observed in weaners (80%, 165/206) followed by tuis (74%, 157/211), crias (66%, 56/85) and adults (58%, 547/936). However, the mean FEC was highest in tuis (402±63 EPG) followed by weaners (331±58 EPG), adults (214±31 EPG) and crias (159±26 EPG) (Table 5.2). Furthermore, the prevalence of GINs was significantly different between weaners and crias ($P < 0.008$), and adults and tuis ($P < 0.05$) and weaners ($P < 0.05$).

Table 5.2. Prevalence and faecal egg count of gastrointestinal nematodes in different age groups of Australian alpacas.

Age group	% Prevalence (proportion)	EPG (mean ± SE)	95% CI		Range
Crias	66 (56/85)	159±26[a,d]	107	211	(0 – 2,490)
Weaners	80 (165/206)	331±58[b,c]	218	445	(0 – 12,390)
Tuis	74 (157/211)	402±63[a,b]	278	526	(0 – 9,490)
Adults	58 (547/936)	214±31[d]	152	275	(0 – 1,7425)

Lowercase letters in a column indicate significant differences in EPG among age groups EPG eggs per gram of faeces, SE standard error of mean, CI confidence interval

Overall, the highest prevalence (77%, 293/383) and mean FEC (630±89 EPG) were observed in the summer rainfall zone followed by the Mediterranean-type, non-seasonal and winter rainfall zones (Table 5.3). The prevalences of nematodes were significantly different ($P < 0.05$) among various climatic zones except between winter rainfall and non-seasonal zones. Both female and male alpacas had the highest prevalences (76%, 183/242; 78%, 110/141, respectively) as well as mean FECs (577±92 EPG and 720±185 EPG, respectively) of GINs in the summer rainfall zone (Table 5.4). The mean FEC of GINs in female alpacas was statistically different ($P < 0.05$) between the summer rainfall and the other three climatic zones but that for males was different among various climatic zones (Table 5.4). All age groups of alpacas had the highest prevalence (except cria) as well as mean FECs in the summer rainfall zone (Table 5.5).

Based on alpaca herd size, the highest prevalence (70%, 248/352) of GINs was observed in large herds followed by medium (65%, 319/488) and small (63%, 445/705) herds, whereas the highest FEC (352±49 EPG) was recorded in small herds followed by large (328±65 EPG) and medium (177±30 EPG) herds (Table 5.6).

Table 5.3. Prevalence and faecal egg counts of gastrointestinal nematodes in alpacas in different climatic zones of Australia.

Climatic zones	% Prevalence (proportion)	EPG (mean ± SE)	95% CI		Range
Mediterranean type	70 (157/223)	433±101[a]	235	631	(0 – 14,355)
Winter rainfall	58 (411/703)	104±11[b]	83	125	(0 – 3,015)
Non-seasonal rainfall	64 (151/236)	165±50[b]	65	264	(0 – 10,980)
Summer rainfall	77 (293/383)	630±89[c]	454	806	(0 – 17,415)

Lowercase letters in a column indicate significant differences in EPG among climatic zones EPG eggs per gram of faeces, SE standard error of mean, CI confidence interval

Table 5.4. Prevalence and faecal egg counts of gastrointestinal nematodes in female and male alpacas in different climatic zones of Australia.

Sex	Climatic zones	% Prevalence (proportion)	EPG (mean ± SE)	95% CI		Range
Female	Mediterranean type	62 (79/127)	138±30[a]	78	198	(0 – 1,980)
	Winter rainfall	58 (249/433)	98±13[a]	72	124	(0 – 3,015)
	Non-seasonal rainfall	60 (91/152)	109±25[a]	59	159	(0 – 3,090)
	Summer rainfall	76 (183/242)	577±92[b]	396	759	(0 – 10,800)
Male	Mediterranean type	75 (40/53)	293 ±105[a,d]	83	503	(0 – 4,575)
	Winter rainfall	58 (124/212)	102 ±17[b,c]	67	136	(0 – 2,730)
	Non-seasonal rainfall	71 (60/84)	266±134[d]	0	532	(0 – 10,980)
	Summer rainfall	78 (110/141)	720 ±185[a,b]	355	1,085	(0 – 17,415)

Lowercase letters in a column for each sex indicate significant differences in EPG among different climatic zones

EPG eggs per gram of faeces, SE standard error of mean, CI confidence interval

Table 5.5. Prevalence and eggs per gram (EPG) of faeces of gastrointestinal nematodes in different age groups of alpacas in various climatic zones of Australia.

Age group	Climatic zone	% Prevalence (proportion)	EPG (mean ± SE)	95% CI		Range
Cria	Mediterranean type	64 (9/14)	191±130 [b]	-89	470	(0 – 1,845)
	Winter rainfall	61 (33/54)	92±21 [a]	50	134	(0 – 705)
	Non-seasonal rainfall	89 (8/9)	238±99 [a]	15	461	(0 – 885)
	Summer rainfall	75 (6/8)	469±300 [b]	-222	1161	(0 – 2,490)
Weaner	Mediterranean type	81 (26/32)	262±66 [c]	128	395	(0 – 1,395)
	Winter rainfall	77 (60/78)	144 ±25 [b,c]	95	193	(0 – 1,125)
	Non-seasonal rainfall	72 (33/46)	155 ±42 [b,c]	70	239	(0 – 1,260)
	Summer rainfall	93 (46/50)	833 ±275 [a,c]	280	1385	(0 – 12,390)
Tuis	Mediterranean type	65 (20/31)	401±180 [a]	35	768	(0 – 4,575)
	Winter rainfall	66 (65/98)	130±35 [a]	60	199	(0 – 2,265)
	Non-seasonal rainfall	86 (25/29)	76±17 [a]	4	110	(0 – 360)
	Summer rainfall	89 (47/53)	1,083±261 [b]	559	1607	(0 – 9,480)
Adult	Mediterranean type	62 (64/103)	93±24 [a]	45	140	(0 – 1,980)
	Winter rainfall	50 (204/409)	61±7 [a]	47	75	(0 – 1,650)
	Non-seasonal rainfall	56 (85/152)	180±77 [a]	28	332	(0 – 10,980)
	Summer rainfall	71 (194/272)	509±102 [b]	308	711	(0 – 17,415)

Lowercase letters in a column for each age group indicate significant differences in EPG among different climatic zones

EPG eggs per gram of faeces, SE standard error of mean, CI confidence interval

Table 5.6. Prevalence and faecal egg counts of gastrointestinal nematodes in different herd sizes of Australian alpacas.

Herd size	% Prevalence (proportion)	EPG (mean ± SE)	95% CI		Range
Small	63 (445/705)	352±49[a]	255	448	(0 – 17,415)
Medium	65 (319/488)	177±30[a]	119	235	(0 – 10,980)
Large	70 (248/352)	328±65[a]	201	455	(0 – 14,355)

Lowercase letters in a column indicates that no significant difference was shown among different herd sizes EPG eggs per gram of faeces, SE standard error of mean, CI confidence interval

The identification of nematode genera/species using MT-PCR assay revealed that overall farm-level prevalence of *Trichostrongylus* spp. was the highest (75%, 67/89) in alpacas followed by *C. mentulatus* (69%), *Haemonchus* spp. (67%), *O. ostertagi* (64%), *Cooperia* spp. (34%), *Oesophagostomum* spp. (10%), and *T. circumcincta* (3%) (Fig. 5.2). However, the prevalence of individual nematode species/genera varied according to the climatic zone. For example, *C. mentulatus* was the most common nematode in Mediterranean-type (92%, 11/12) and winter rainfall (79%, 33/42) zones, whereas *Trichostrongylus* spp. and *Haemonchus* spp. were the most common nematodes in non-seasonal (93%, 13/14) and summer rainfall (86%, 18/21) zones, respectively (Table 5.7). The prevalence of *C. mentulatus* was significantly different between summer rainfall and Mediterranean-type ($P < 0.05$) and winter rainfall ($P < 0.05$) zones. Similarly, the prevalence of *O. ostertagi* was significantly different between summer rainfall and Mediterranean-type zones ($P < 0.05$) (Table 5.7).

Table 5.7. Prevalence of gastrointestinal nematodes (detected by the MT-PCR assay) in alpacas in four climatic zones of Australia.

Climatic zone	Nematodes	% Prevalence (proportion)	95% Confidence Interval	
Mediterranean type	*Haemonchus* spp.	67 (8/12)	35	90
	Camelostrongylus mentulatus	92 (11/12) [a]	62	100
	Cooperia spp.	33 (4/12)	10	65
	Oesophagostomum spp.	8 (1/12)	0	38
	Ostertagia ostertagi	92 (11/12) [c]	62	100
	Teladorsagia circumcincta	0 (0/12)	0	0
	Trichostrongylus spp.	83 (10/12)	52	98
Winter rainfall	*Haemonchus* spp.	55 (23/42)	39	70
	C. mentulatus	79 (33/42) [a]	63	90
	Cooperia spp.	24 (10/42)	12	39
	Oesophagostomum spp.	14 (6/42)	5	29
	O. ostertagi	69 (29/42) [c]	53	82
	T. circumcincta	7 (3/42)	2	19
	Trichostrongylus spp.	69 (29/42)	53	82
Non-seasonal rainfall	*Haemonchus* spp.	79 (11/14)	49	95
	C. mentulatus	79 (11/14) [a]	49	95
	Cooperia spp.	43 (6/14)	18	71
	Oesophagostomum spp.	7 (1/14)	0	34
	O. ostertagi	71 (10/14) [c]	42	92
	T. circumcincta	0 (0/14)	0	0
	Trichostrongylus spp.	93 (13/14)	66	100
Summer rainfall	*Haemonchus* spp.	86 (18/21)	64	97
	C. mentulatus	29 (6/21) [b]	11	52
	Cooperia spp.	48 (10/21)	26	70
	Oesophagostomum spp.	5 (1/21)	0	24
	O. ostertagi	33 (7/21) [d]	15	57
	T. circumcincta	0 (0/21)	0	0
	Trichostrongylus spp.	71 (15/21)	48	89

Proportions of seven nematode genus/species were compared among different climatic zones. Letters *a* and *b* (for *C. mentulatus*), and *c* and *d* (for *O. ostertagi*) indicate significant difference in the prevalences of nematodes among different climatic zones.

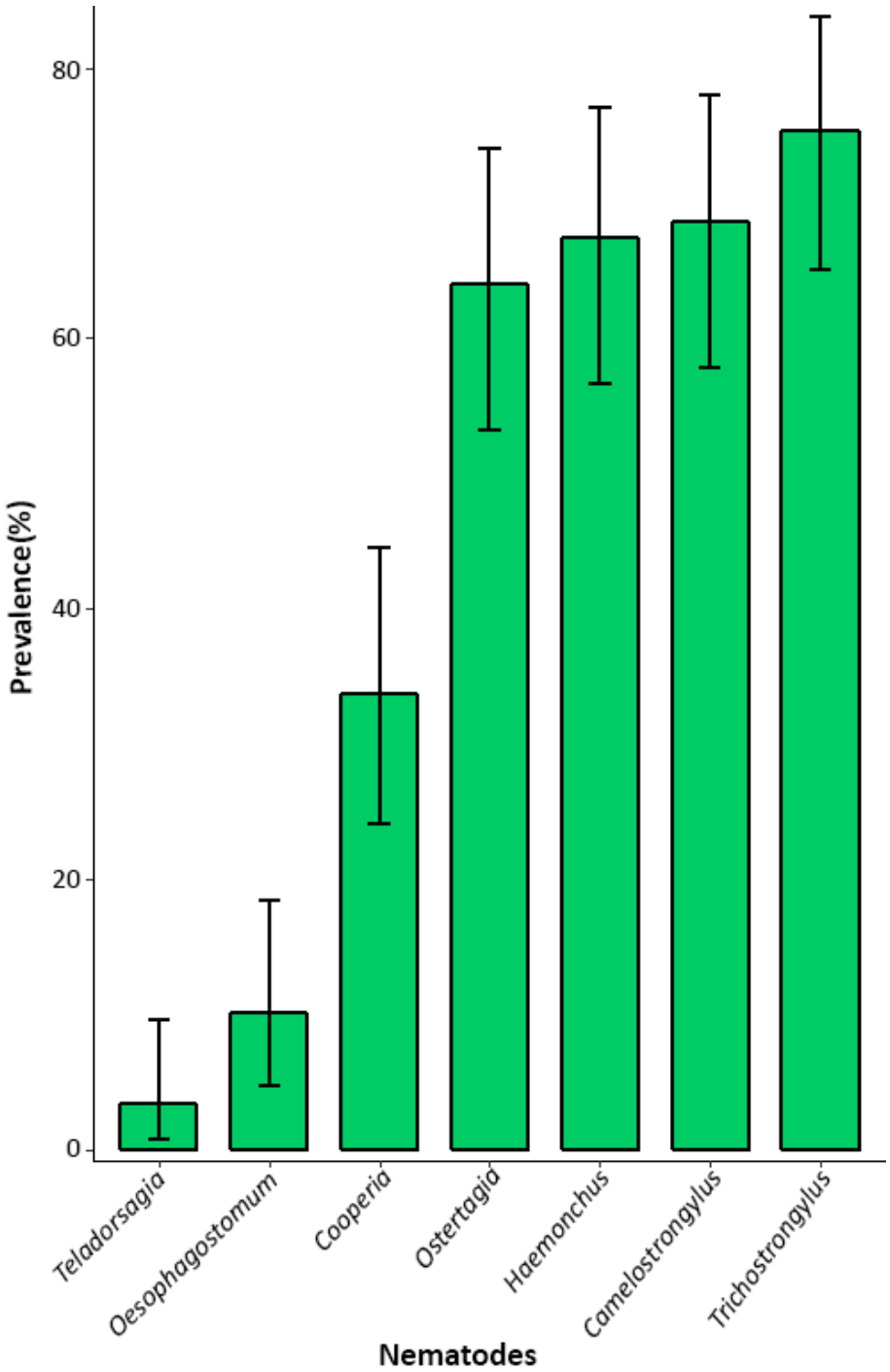


Fig. 5.2. Overall prevalence of gastrointestinal nematodes of alpacas in Australia. Each green bar shows the percentage of the farms found positive for a nematode genus/species using the multiplexed-tandem PCR assay, and each vertical line shows upper and lower 95% confidence intervals.

5.5. Discussion

This is the first study to investigate the prevalence and worm burden (based on FECs) of GINs in domesticated alpacas at a national level in Australia. Australian alpacas were infected with GINs which also infect domestic ruminants (*C. mentulatus*, *Haemonchus* spp. and *Trichostrongylus* spp.). Weaners were the most susceptible age group while tuis had the highest mean EPG. Alpacas in the summer rainfall zone had the highest prevalence as well as EPG.

In this study, the overall prevalence of GINs in alpacas was 66% (Table 5.1) which was higher than that (48%) reported previously by Carmichael (1999) and Presidente (2007) from southern Australia. Similarly, the prevalence of GINs in different age groups of alpacas, including crias, weaners, tuis and adults was higher than those reported from the same age groups previously (51%, 61%, 52% and 43%, respectively) from southern Australia (Carmichael, 1999). The differences found among different studies might be due to geographical variation, and the total number of alpaca faecal samples analysed, as 1,545 faecal samples were collected from 92 alpaca farms across six states compared with 1,030 faecal samples from South Australia and Victoria only. Furthermore, a country-wide increase in the population of alpacas, mixed grazing with domestic ruminants and more intensive commercial farming in Australia might have also contributed to an increased prevalence of GINs in different age groups of alpacas.

The prevalence of GINs (66%) in alpacas in this study was comparable to those (64% and 62%) previously reported from Peru (Contreras et al., 2014) and New Zealand (Dittmer et al., 2018), respectively but lower compared to those (90-91%) reported from Japan (Hyuga and Matsumoto, 2016) and Ecuador (Bermudez, 2015) although the prevalences reported from the latter studies included other gastrointestinal parasites such as *Eimeria* spp. By contrast, a lower prevalence (52%) of GINs in alpacas has been reported from Ecuador (Robayo, 2015). Individual parasite data indicated that the prevalence of *Nematodirus* spp. (17%) herein was comparable to that observed in alpacas (13%) from Japan (Hyuga and Matsumoto, 2016) but lower than those reported (28-53%) from Peru (Contreras, 2013; Masson et al., 2016). Similarly, in this study a prevalence of 6% for *Trichuris* spp. was found, which was lower than those reported from Brazil (14%; Sprenger et al., 2018) and Japan (11%; Hyuga and Matsumoto, 2016) but higher than that reported from Peru (2%; Lucas et al., 2016). These differences in the prevalences of GINs of alpacas could be due to (i) variation in climatic conditions in

various geographical locations, (ii) time/season of sampling, (iii) mixed grazing practices with other animals such as sheep and cattle (Hill et al. 1993), and (iv) differences in husbandry and management practices (e.g. herd density and hygienic conditions on alpaca farms).

The present study found an overall mean FEC of 291 EPG, with the highest count of 17,415 EPG (Table 5.1). These values appear much higher than those previously reported in alpacas from other countries. For instance, the FECs of 50 EPG and 200 EPG, with the highest counts of 4,700 EPG and 450 EPG were reported from New Zealand (Dittmer et al., 2018) and the UK (Tait et al., 2002), respectively. Likewise, the mean FEC for strongyle eggs in this study was higher (276 EPG) than those reported from Chile (100 EPG; Valenzuela et al., 1998) and Switzerland (53 EPG; Hertzberg and Kohler, 2006). All these studies used the McMaster method (like this study) for the quantification of GIN eggs. Therefore, minimal methodological variations could be expected (and hence neglected) while making comparisons of FECs. The McMaster method used in this study has the potential for false negatives, particularly for those nematodes whose EPG is <15 EPG (i.e. lower than the minimal detectable limit). However, an overall mean FEC of 291 EPG in this study is much higher than the lowest detection limit of the McMaster (i.e. 15 EPG). Therefore, as an assumption this detection limit has minimal impact on the overall prevalence of GINs in this study.

Currently, no guidelines are available for the threshold of FECs of GINs at which alpacas should be treated with appropriate anthelmintic(s). However, based on anecdotal evidence, veterinarians and/or veterinary parasitologists have proposed treating adult and tuis alpacas (>1 year of age) if the FECs are more than 125 EPG, 50 EPG and 75 EPG for strongyles, *Nematodirus* spp., and strongyles plus *Nematodirus* spp., respectively (Love 2017). Similarly, for young animals (<1 year of age), the values proposed are >350 EPG (for strongyles), >100 EPG (*Nematodirus* spp.) and >250 EPG (strongyle plus *Nematodirus* spp.) (Carmichael, 2014; Love, 2017). These values were recommended for 'scour worms' including *Trichostrongylus* spp., *Teladorsagia*/*Ostertagia* spp., and *Nematodirus* spp. in non-seasonal and/or winter rainfall areas of South Australia and Victoria. However, findings in this study revealed that an overall worm burden in adult animals was higher (overall: 214 EPG; strongyle: 207 EPG; *Nematodirus* spp. 5 EPG) while those in crias (overall: 159 EPG; strongyle: 125 EPG; *Nematodirus* spp. 28 EPG) and weaners (overall: 331 EPG; strongyle: 291 EPG; *Nematodirus* spp. 27 EPG) were lower when compared to previously suggested thresholds (Carmichael, 2014; Love,

2017). Notably, alpacas sampled in this study did not have any clinical signs of parasitic gastroenteritis at the time of sample collection, thereby indicating that the precise threshold of FECs of GINs at which to treat alpacas remains unknown due to discrepant results among published reports and anecdotal evidence. Further studies are recommended to determine the threshold of FECs for various GINs in alpacas using experimental infections with monospecific infections.

In this study, tuis had the highest FEC (402 EPG) followed by weaners (331 EPG), adults (214 EPG), and crias (159 EPG) (Table 5.2). Overall, these values of FECs were higher than those previously reported for various age groups of alpacas from Australia (Carmichael, 1999, 2014; Presidente, 2007) and New Zealand (Hill et al., 1993). For example, Carmichael (2014) and Hill et al. (1993) observed the highest FECs in crias followed by weaners, tuis and adult alpacas. It is important to consider that the previous studies were either based on the opportunistic collection of alpaca faecal samples (Presidente, 2007) or were of limited scope (only South Australia and some parts of Victoria were included) (Carmichael, 1999) as opposed to this study that examined higher number of alpaca faecal samples across six states of Australia. Other potential reason(s) of a higher prevalence in this study could be due to (i) differences in the geographical locations of alpacas (i.e. only South Australia and a few parts of Victoria in the previous studies); (ii) differences in climatic conditions across various sampling locations; (iii) high infection pressures of GINs due to increased (intensive) commercial alpaca farming in Australia (Ballweber 2009; Leguía 1991; Saeed et al. 2018); (iv) sampling time/season of the year; and (v) increased anthelmintic resistance in GINs infecting alpacas from Australia (see Chapter 8).

In this study, seven GINs have been found, including *C. mentulatus*, *Cooperia* spp., *Haemonchus* spp., *Oesophagostomum* spp., *O. ostertagi*, *T. circumcincta* and *Trichostrongylus* spp. (see Fig. 5.2). Some of these GINs (such as *Cooperia* spp., *Haemonchus* spp., *Nematodirus* spp., *Ostertagia* spp. and *Trichostrongylus* spp.) have previously been reported from alpacas in Australia (Carmichael, 2014; Jabbar et al., 2013). It is worth noting that *Haemonchus* spp., *Ostertagia*/*Teladorsagia* spp., *Trichostrongylus* spp. and *Nematodirus* spp., have been found to be associated with clinical infections in alpacas in Australia (Carmichael, 1999; Love, 2017; Love and Hutchinson, 2003). Similarly, *C. mentulatus, H. contortus, T. circumcincta* and *Trichostrongylus* spp. have been found to be linked with parasitic gastroenteritis in both alpacas and llamas in other parts of the world (Edwards et al., 2016; Rickard, 1993). All

of the GINs found in alpacas herein have already been described in sheep and cattle from Australia (Anderson, 1972, 1973; Beveridge and Ford, 1982; Roeber et al., 2011, 2013b) which suggests that alpacas might have acquired these nematodes while co-grazing with domestic ruminants after their importation from South America. This is further supported by the fact that about half of the alpaca farms in this study had other livestock (e.g. cattle, sheep and/or goats) besides alpacas and they used mixed livestock grazing practices

In the current study, the highest prevalence (77%) and FEC (630 EPG) in alpacas were observed in the summer rainfall zone. Furthermore, *Haemonchus* spp. were the most common parasite of alpacas living in this climatic zone (see Table 5.7). This finding is supported by previous reports that *Haemonchus* was the most common nematode in alpacas dying of anaemia, poor condition and oedema (Jabbar et al., 2013; Q-Alpaca, 2014). Similarly, high worm burdens have been reported in alpacas and llamas from regions with high rainfall and low altitude (Erp, 2012; Wolf, 2010), which favour larval development on pastures. For instance, depending on the temperature and rainfall, a variable strongyle larval load (10-780/Kg of herbage) was recorded at different times of the year on a pasture grazed by alpacas and sheep in New Zealand (Hill et al., 1993). In fact, each GIN species may require varying levels of temperature and humidity for development and differences in such requirements may influence the nature/type of parasite species in a particular geographic location (Craig, 2018; Hill et al., 1993).

5.6. Conclusion

A national cross-sectional survey was conducted to understand the epidemiology of GINs of Australian alpacas. The findings of this study revealed that alpacas harbour many of the same nematodes as sheep and cattle and attention should be paid to the transmission of these parasites where different hosts co-graze. The prevalence and burden of GINs of alpacas vary according to age, sex, climatic zone and the type of alpaca herds in Australia. Further studies are required to determine the prevalence of different GINs of alpacas in different seasons across various climatic zones of Australia.

5.7. References

Alcaino, H., Gorman, T., & Burgos, M. (1991). Helmintiasis gastrointestinal en llamas (*Lama glama*) de la I Región de Chile. *Parasitología al día,* 15, 93-96.

Anderson, N. (1972). Trichostrongylid infections of sheep in a winter rainfall region. 1. Epizootiological studies in the Western District of Victoria, 1966-67. *Australian Journal of Agricultural Research,* 23, 1113-1129.

Anderson, N. (1973). Trichostrongylid infections of sheep in a winter rainfall region. 2. Epizootiological studies in the Western District of Victoria, 1967-68. *Australian Journal of Agricultural Research,* 24, 599-611.

Ballweber, L.R. (2009). Ecto- and endoparasites of new world camelids. *Veterinary Clinics of North America: Food Animal Practice,* 25, 295-310.

Bermudez, L.F. (2015). Parasitosis gastrointestinal en especies zootécnicas, diagnosticadas en el laboratorio de biotecnología y microbiología animal (ESPOCH-Riobamba). Thesis, Universidad de Guayaquil, Ecuador.

Beveridge, I., & Ford, G.E. (1982). The trichostrongyloid parasites of sheep in South Australia and their regional distribution. *Australian Veterinary Journal*, 59, 177-179.

Cafrune, M.M., Aguirre, D.H., & Rickard, L.G. (2001). First report of *Lamanema chavezi* (Nematoda: Trichostrongyloidea) in llamas (*Lama glama*) from Argentina. *Veterinary Parasitology*, 97,165-168.

Carmichael, I. (1999). Internal parasitism in alpacas in Southern Australia. In: Hack W, McGregor B, Ponzoni R, Judson G, Carmichael I, Hubbard D (eds) Australian Alpaca Fibre: Improving Productivity and Marketing. *Rural Industries Research and Development Corporation, Australia,* p 92-130.

Carmichael, I. (2014). Internal parasitism in Australian alpacas. *Australian Alpaca Association National Conference Adelaide, Australia,* p 13-28.

Cebra, C.K., & Stang, B.V. (2008). Comparison of methods to detect gastrointestinal parasites in llamas and alpacas. *Journal of the American Veterinary Medical Association,* 232, 733-741.

Contreras, S., Chávez, V., Pinedo, V., Leyva, V., & Suárez, A. (2014). Helminthiasis in alpacas (*Vicugna pacos*) of two peasant communities in Macusani, Puno during the dry season. *La Revista de Investigaciones Veterinarias del Perú es,* 25, 268-275.

Craig, T.M. (2018). Gastrointestinal nematodes, diagnosis and control. *Veterinary Clinics of North America: Food Animal Practice,* 34, 185-199.

Dittmer, K., Hinkson, J., Dwyer, C., Adlington, B., & van Andel, M. (2018). Prevalence of *Candidatus Mycoplasma haemolamae*, bovine viral diarrhoea virus, and gastrointestinal parasitism in a sample of adult New Zealand alpaca (*Vicugna pacos*). *The New Zealand Veterinary Journal,* 66, 9-15.

Edwards, E.E., Garner, B.C., Williamson, L.H., Storey, B.E., Sakamoto, K. (2016). Pathology of *Haemonchus contortus* in New World camelids in the southeastern United States: a retrospective review. *Journal of Veterinary Diagnostic Investigation,* 28, 105-109.

Erp, MLvan. (2012). The diagnostic of gastrointestinal nematodes and coccidiosis in llamas from intensive and extensive agriculture systems in different areas of Argentina. Thesis, Utrecht University, Netherlands.

Franz, S., Wittek, T., Joachim, A., Hinney, B., & Dadak, A.M. (2015). Llamas and alpacas in Europe: endoparasites of the digestive tract and their pharmacotherapeutic control. *Veterinary Journal,* 204, 255-262.

Guerrero, C., & Chávez, C. (1967). Helmintos comunicados por primera vez en alpacas (*Lama pacos*) con una descripción de *Spiculopteragia peruvianus* n. sp. *Boletin Chileno de Parasitologia,* 22,147-150.

Harris, P.A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N., & Conde, J.G. (2009). Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of Biomedical Informatics*, 42, 377-381.

Hertzberg, H., & Kohler, L. (2006). Prevalence and significance of gastrointestinal helminths and protozoa in South American camelids in Switzerland. *Berliner und Münchener tierärztliche Wochenschrift,* 119, 291-294.

Hill, F., Death, A., & Wyeth, T. (1993). Nematode burdens of alpacas sharing grazing with sheep in New Zealand. *The New Zealand Veterinary Journal,* 41, 205-208.

Hinkson, J.A. (2015). Investigations into common farm management practices and diseases on alpaca farms in New Zealand. Thesis, Massey University, New Zealand.

Hyuga, A., Matsumoto, J. (2016). A survey of gastrointestinal parasites of alpacas (*Vicugna pacos*) raised in Japan. *The Journal of Veterinary Medical Science,* 78,719-721.

Jabbar, A., Campbell, A.J.D., Charles, J.A., & Gasser, R.B. (2013). First report of anthelmintic resistance in *Haemonchus contortus* in alpacas in Australia. *Parasites and Vectors,* 6, 243.

Leguía G (1991) The epidemiology and economic impact of llama parasites. Parasitology Today, 7, 54-56.

Love, S.C. (2017). Alpaca worms - an overview. Department of Primary Industries, NSW Government,Australia. https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0004/318217/Alpaca-worms-an-overview.pdf. Accessed 05 May 2018.

Love, S.C., & Hutchinson, G.W. (2003). Pathology and diagnosis of internal parasites in ruminants. In: Gross Pathology of Ruminants, Proceedings 350, Post Graduate Foundation in Veterinary Science, University of Sydney, p 309-338.

Lucas, Juan R., Siever Morales, Manuel Barrios, José Rodríguez, María Vásquez, Boris Lira, Bernardo Torres, Eva Casas, & Juan Espinoza. (2016). Patógenos involucrados en casos fatales de diarrea en crías de alpaca de la Sierra Central del Perú. *Revista de Investigaciones Veterinarias del Perú es* 27, 169-175.

Masson, M., Gutiérrez, G., Puicón, V., & Zárate, D. (2016). Helmintiasis y eimeriosis gastrointestinal en alpacas criadas al pastoreo en dos granjas comunales de la región Pasco, Perú, y su relación con el peso y condición corporal. *Revista de Investigaciones Veterinarias del Perú es,* 27, 805-812.

Mitchell, S., Hopkins, B., & Corfield, C. (2016). *Nematodirus lamae* identified in an alpaca in the UK. *Veterinary Record,* 178, 271-272.

Presidente, P.J.A. (2007). Alpaca parasites & their control: recent experiences. *Australian Alpaca Veterinarians Annual Conference,* Melbourne, Australia. p 1-14

Q-Alpaca, (2014) Q-Alpaca 9th annual report. Australian Alpaca Association. https://www.alpaca.asn.au. Accessed 5 July 2018.

R Core Team, (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rickard, L.G. (1993). Parasitic gastritis in a llama (*Lama glama*) associated with inhibited larval *Teladorsagia* spp. (Nematoda: Trichostrongyloidea). *Veterinary Parasitology,* 45, 331-335.

Robayo, C.I.S. (2015). Prevalencia de parásitos gastrointestinales en alpacas del Inga Alto, Pichincha. Thesis, Universidad San Francisco de Quito, Ecuador.

Roeber, F., Jex, A.R., Campbell, A.J., Campbell, B.E., Anderson, G.A., & Gasser, R.B. (2011). Evaluation and application of a molecular method to assess the composition of strongylid nematode populations in sheep with naturally acquired infections. *Infection, Genetics and Evolution,* 11, 849-854.

Roeber, F., Jex, A.R., & Gasser, R.B. (2013a). Comparative evaluation of two DNA isolation techniques for PCR-based diagnosis of gastrointestinal nematode infections in sheep. *Molecular and Cellular Probes,* 27, 153-157.

Roeber, F., Jex, A.R., & Gasser, R.B. (2013b). Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance-an Australian perspective. *Parasites & Vectors,* 6, 153.

Rosanowski, S., Cogger, N., Rogers, C., Benschop, J., & Stevenson, M. (2012). A description of the demographic characteristics of the New Zealand non-commercial horse population with data collected using a generalised random-tessellation stratified sampling design. *Preventive Veterinary Medicine,* 107, 242-252.

Saeed, M.A., Rashid, M.H., Vaughan, J., & Jabbar, A. (2018). Sarcocystosis in South American camelids: the state of play revisited. *Parasites and Vectors,* 11, 146.

Sprenger, L.K., Yoshitani, U.Y., Buzatti, A., & Molento, M.B. (2018). Occurrence of gastrointestinal parasites in wild animals in State of Paraná, Brazil. *Annals of the Brazilian Academy of Sciences,* 90, 231-238.

Stevenson, M., Nunes, T., Heuer, C., Marshall, J., Sanchez, J., Thornton, R., Reiczigel, J., Robison-Cox, J., Sebastiani, P., Solymos, P., Yoshida, K., Jones, G., Pirikahu, S., Firestone, S., Kyle, R., Popp, J., & Mathew, J.(2018). epiR: Tools for the Analysis of Epidemiological Data. Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Melbourne, Australia; 2018.

Tait, S., Kirwan, J., Fair, C., Coles, G., & Stafford, K. (2002). Parasites and their control in South American camelids in the United Kingdom. *Veterinary Record,* 150, 637-638.

Valenzuela, G., Leiva, M., & Quintana, I. (1998). Epidemiological studies on infective larvae of gastrointestinal nematodes on pasture grazed by alpacas (*Lama pacos*) in Valdivia, Southern Chile. *Archivos de Medicina Veterinaria*, 30, 79-90.

Welchman, D.d.B., Parr, J., Wood, R., Mead, A., & Starnes, A. (2008). Alpaca and llama nematodes in Britain. *Veterinary Record,* 162, 832-832.

Windsor, R.S., Windsor, R.H., & Teran, M. (1992). Economic benefits of controlling internal and external parasites in South American camelids. *Annals of the New York Academy of Sciences,* 653, 398-405.

Wolf, D. (2010). Untersuchungen zur seroprävalenz von zystenbildenden Kokzidien und gastrointestinalparasitosen bei neuweltkameliden in Peru. Thesis, Universität Giessen, Germany.

CHAPTER 6. EPIDEMIOLOGY OF GASTROINTESTINAL NEMATODES OF ALPACAS IN AUSTRALIA: II. A LONGITUDINAL STUDY

6.1. Abstract

A longitudinal survey was conducted on 13 alpaca farms in four climatic zones of Australia to understand the epidemiology of gastrointestinal nematodes (GINs) of alpacas. A total of 1,688 fresh faecal samples were collected from both sexes of alpacas from May 2015 to April 2016 and processed for faecal egg counts (FEC) and molecular identification of eggs using the multiplexed-tandem PCR assay. Based on egg morphology, the overall prevalence of GINs was 61% while that for strongyles was 53%. The overall mean FEC was 168 eggs per gram (EPG) of faeces, with the highest count of 15,540 EPG. Weaners had the highest prevalence (73%) and mean FEC (295 EPG) of GINs followed by tuis, crias and adults. Alpacas in the winter rainfall zone had the highest prevalence (68%) as well as FEC (266 EPG) followed by Mediterranean-type, non-seasonal and summer rainfall zones. *Trichostrongylus* spp. (83%, 89/107), *Haemonchus* spp. (71%, 76/107) and *C. mentulatus* (63%, 67/107) were the three most common GINs of alpacas across all climatic zones. The mixed-effects zero-inflated negative binomial regression model used in this study showed that it could help to design parasite control interventions targeted at both the herd level and the individual alpaca level. The findings of this study showed that the epidemiology of GINs of alpacas is very similar to those of cattle and sheep, and careful attention should be paid when designing control strategies for domestic ruminants co-grazing with alpacas.

6.2. Introduction

Gastrointestinal nematode infections are one of the most severe disease problems in domesticated South American camelids (SACs), alpacas (*Vicugna pacos*) and llamas (*Lama glama*), leading to substantial economic losses due to lowered production of fibre and meat, and death (Ballweber, 2009; Leguía, 1991; Windsor et al., 1992a, b). In regions where SACs are imported outside South America, animals are kept under intensive to semi-intensive conditions (in contrast to extensive management in South America),

with/without other domestic livestock, thereby exposing them to high infection pressures of gastrointestinal nematodes (GINs) (Ballweber, 2009; Leguía, 1991). Although SACs have their specific GINs (such as *Graphinema auchenia*, *Lamanema chavezi*), they are often infected with GINs of domestic ruminants (Ballweber, 2009; Franz et al., 2015; Hill et al., 1993).

In the last three decades, the knowledge on the epidemiology and control of GINs in SACs has gradually been increasing due to a number of reports from Argentina, Australia, Bolivia, Chile, Ecuador, England, Japan, Libya, New Zealand, Peru, Switzerland and the USA (Abdouslam et al., 2003; Alcaino et al., 1991; Beltrán-Saavedra, 2015; Cebra and Stang, 2008; Dittmer et al., 2018; Hertzberg and Kohler, 2006; Hill et al., 1993; Hyuga and Matsumoto, 2016; Jabbar et al., 2013; Masson et al., 2016; Mitchell et al., 2016; Moreno et al., 2013; Robayo, 2015; Tait et al., 2002; Welchman et al., 2008; Rickard, 1993). These studies mostly used convenience sampling methods such as the collection of faecal samples and focused only on one or a few regions in a country rather than conducting a national study to understand the epidemiology of GINs.

Recently, the first national cross-sectional survey of GINs of alpacas was conducted (see Chapter 5) to establish baseline data of the epidemiology of GINs of alpacas in Australia. This study showed that Australian alpacas were commonly infected with cattle and sheep nematodes (e.g. *Cooperia* spp., *Haemonchus* spp., *Nematodirus* spp., *Oesophagostomum* spp., *Ostertagia ostertagi*, *Teladorsagia circumcincta, Trichostrongylus* spp. and *Trichuris* spp.), and weaners had the highest prevalence whereas tuis had the highest FECs. It was also found that alpacas in the summer rainfall zone had the highest prevalence as well as FECs. Given that Australia has the largest alpaca population outside South America (Clarke, 2016) and alpacas are reared in various climatic zones, it is crucial to understand better the effect of climatic conditions, seasons, gender, age, deworming history and other factors related to the epidemiology of GINs of alpacas. Therefore, in this study, the cross-sectional survey conducted in Australian alpacas (see Chapter 5) was extended to a longitudinal survey of GINs of alpacas to understand the epidemiology of GINs of alpacas in Australia. In addition, a mixed-effects zero-inflated negative binomial regression model has been used to design parasite control interventions.

6.3. Materials and methods

6.3.1. Selection of farms

This was a longitudinal study of monthly faecal egg counts (FECs) in Australian alpacas for the period May 2015 to April 2016. The study included all alpaca herds registered with the Australian Alpaca Association (AAA; www.alpaca.asn.au) on 1 January 2015. The herd had ≥ 50 alpacas on 1 January 2015. Alpaca farmers were contacted via the AAA as well as veterinarians to participate in this study during another study on worm control practices of Australian alpacas (see Chapter 2), and the herd managers of 13 alpaca farms consented to take part in the study in March/April 2015 (Fig. 6.1). Based on the distribution and density of alpaca populations in different climatic zones of Australia, the selected 13 alpaca farms were distributed in Mediterranean-type, non-seasonal, summer and winter rainfall zones in this study (Fig. 6.1). The details of these zones have already been described (see Chapter 5). The annual rainfalls for the farms located in these climatic zones from 2013 to 2017 are presented in Fig. 6.2. The average annual minimum and maximum temperatures for the farms located in four climatic zones from 2013 to 2017 are presented in Fig. 6.3.

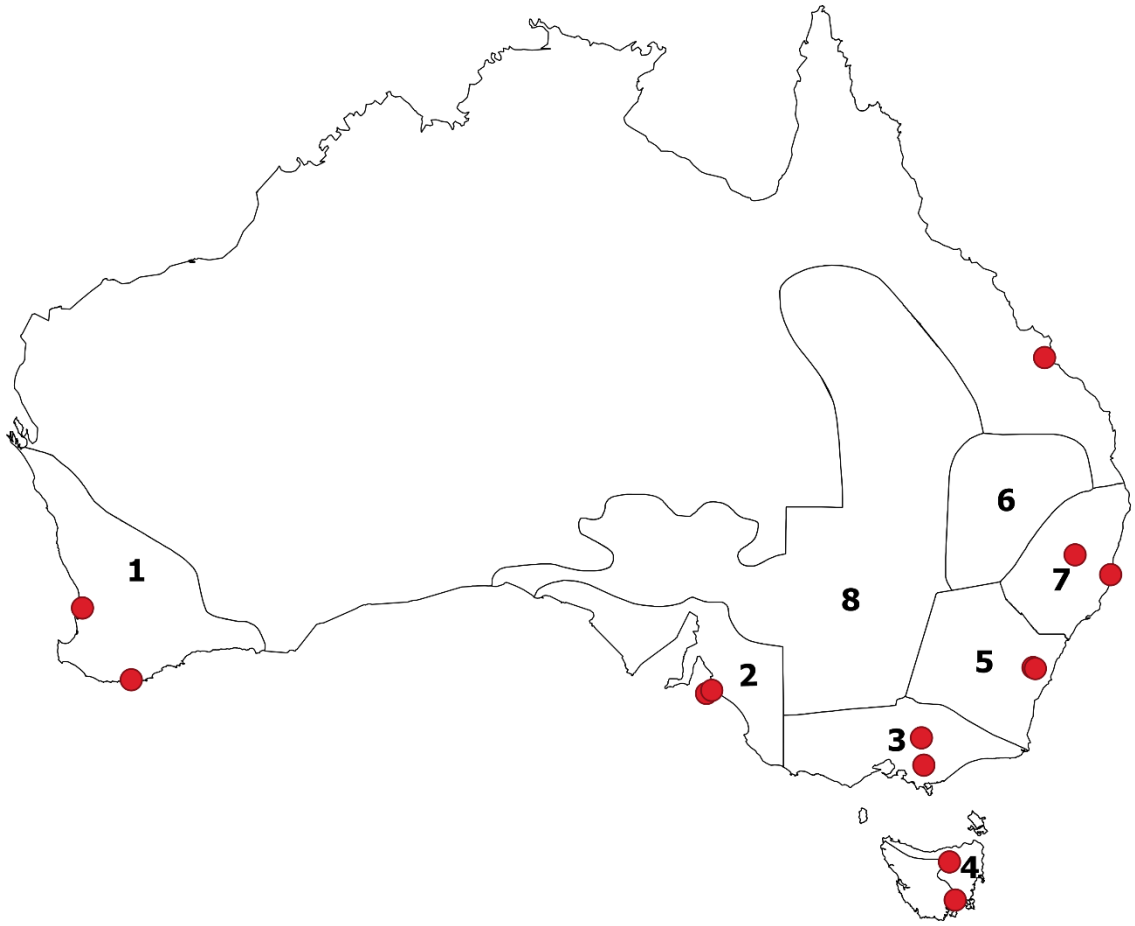


Fig. 6.1. Map of Australia showing the locations of alpaca farms enrolled in longitudinal epidemiological study. Each red circle represents one alpaca farm. The boundaries of different zones are drawn based on climatic zones described by www.wormboss. com.au. 1, Western Australian winter rainfall; 2, South Australian winter rainfall; 3, Victorian winter rainfall; 4, Tasmania; 5, NSW non-seasonal rainfall; 6, Qld/NSW Summer rainfall/slopes and plains; 7, Qld/ NSW Summer rainfall/tablelands and slopes; 8, Pastoral

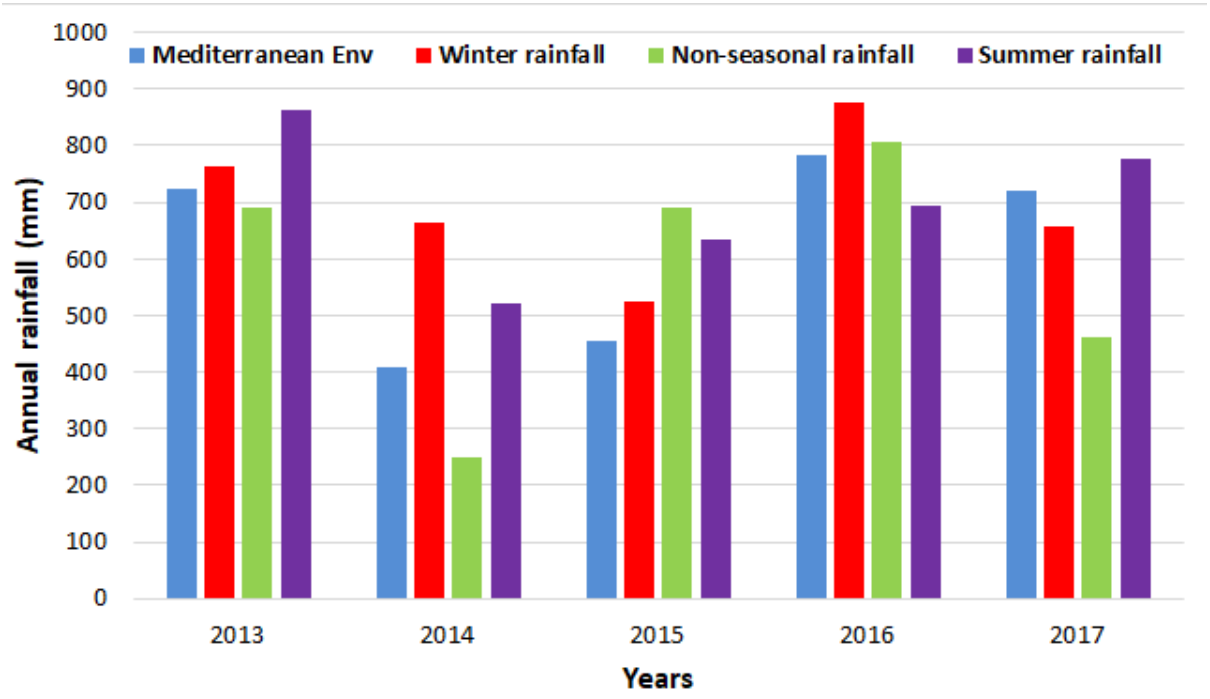


Fig. 6.2. Annual rainfall (mm) of the farms located in four selected climatic zones of Australia from 2013 to 2017 (collated from the Bureau of Meteorology (www.bom.gov.au) stations nearest to the farms).

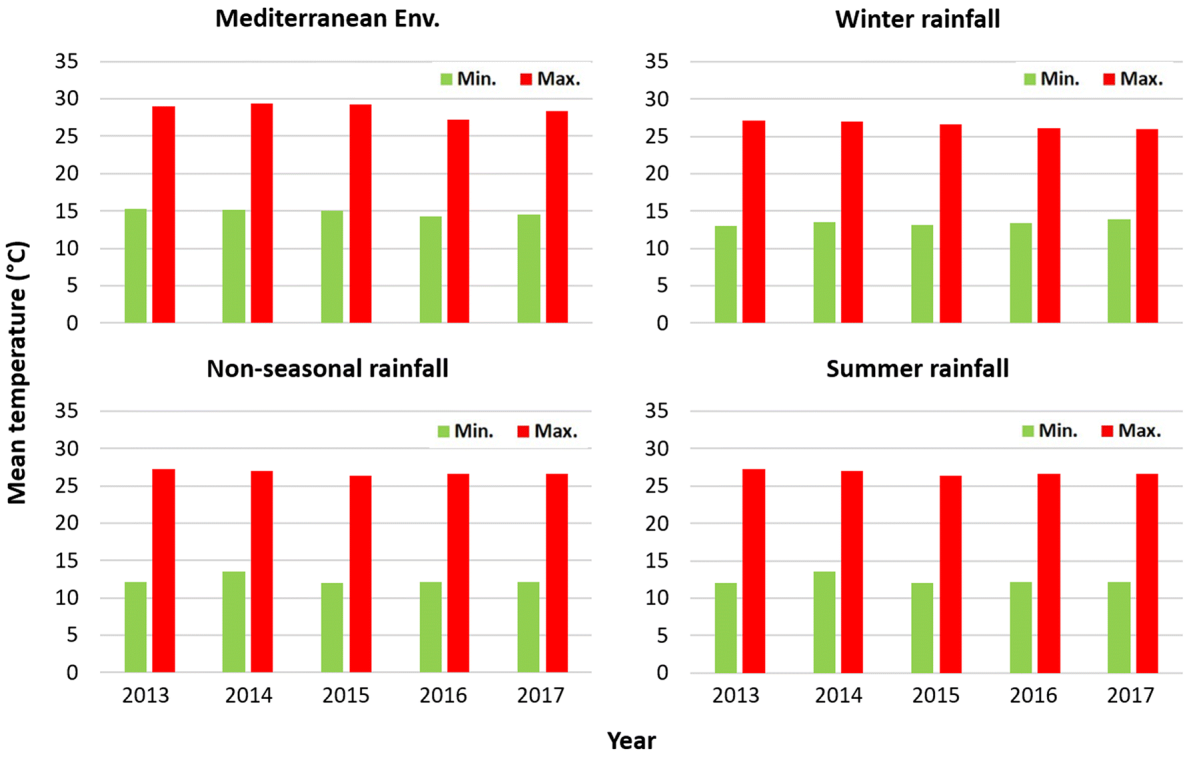


Fig. 6.3. Average minimum and maximum annual temperatures of the farms located in four selected climatic zones of Australia from 2013 to 2017 (collated from the Bureau of Meteorology (www.bom.gov.au) stations nearest to the farms).

6.3.2. Collection of faecal samples

Each alpaca farm manager was sent the sample collection kits containing submission forms, faecal collection bags, gloves, ice-packs for shipment and overnight courier envelopes. The instructions for the collection and shipping of alpaca faecal samples have previously described (see Chapter 2).

6.3.3. Faecal egg count

Following receipt of the overnight courier envelopes by the research team, faecal samples were refrigerated at 4°C until processing which occurred within seven days of the date of faeces collection. Each faecal sample was tested using a modified McMaster technique with a saturated sugar solution (specific gravity 1.27), as described previously (see Chapter 5). The estimated limit of detection of this modified McMaster method was 15 EPG.

6.3.4. Molecular identification of nematodes

Following the processing of fresh faecal samples (n = 10-20) for the FECs from each farm per month, as described above, a 1-2 mL of the suspension containing a saturated sugar solution from each sample was transferred to a 50 mL Falcon tube to extract eggs as previously described (Roeber et al., 2013a). The washed eggs in each pooled sample were transferred into a microcentrifuge tube and stored at -20 °C until DNA isolation. Eggs in faecal samples were identified using a newly established molecular diagnostic kit for the identification of common GINs of alpacas (see Chapter 2).

6.3.5. Statistical analyses

The prevalence of parasitism in a herd for a given sampling event was calculated as the number of alpacas with nematodes of a particular type divided by the number of individual alpacas sampled expressed as a percentage (Margolis et al., 1982). The Kruskal-Wallis rank sum and Wilcoxon rank sum tests were used to statistically test for

differences in FEC for different age groups and different climatic zones. A *P-value* <0.05 was used to declare statistically significant differences in FECs.

A mixed-effects zero-inflated negative binomial (ZINB) regression model (Nodtvedt et al., 2002; Denwood, 2008) has been used to quantify the influence of herd-level and individual alpaca-level characteristics on FEC at the time of sampling. A zero-inflated negative binomial model addressed over-dispersion in the data arising from the relatively large number of zero FECs while at the same time allowing us to quantify the effect of factors influencing FEC when nematodes were actually present. A multilevel approach was used because of the study design. Faecal samples were collected from the same alpacas within individual herds over time (giving rise to repeated measures) and individual alpacas were clustered within herds.

In a zero-inflated negative binomial model the expected FEC for alpaca j from herd i at time k, Z_{ijk} was modelled conditional on the observed FEC for alpaca j from herd i at time k, O_{ijk}:

$$\boldsymbol{Z_{ijk} \sim \begin{cases} Bernoulli\ (p)\ with\ probability\ p_{ijk}\ if\ O_{ijk} = 0, \\ Negative\ binomial\ (\mu_{ijk}, s) \end{cases}} \qquad \textbf{Equation 1}$$

In Equation 1, O_{ijk} was an independent Bernoulli random variable with a mean of p_{ijk} if

$O_{ijk} = 0$, otherwise O_{ijk} was assumed to follow a negative binomial distribution defined by a mean μ_{ijk} and a shape parameter s. Thus:

$$\boldsymbol{logit(p_{ijk}) = \alpha_0 + \alpha_1 x_{1ijk} + zH_i + zA_{jk}} \qquad \textbf{Equation 2}$$

and

$$\boldsymbol{log(\mu_{ijk}) = \beta_0 + \beta_1 x_{1ijk} + \cdots + \beta_m x_{mijk} + nbA_{jk}.} \qquad \textbf{Equation 3}$$

For the N herds included in the study, the herd-level random effect term for the zero-count component of the model, zH_i, was given by:

$$\boldsymbol{zH_i = (zH_1, \ldots zH_N) \sim N(0, \sigma_{zH})} \qquad \textbf{Equation 4}$$

and for the M individual alpacas included in the study, the individual alpaca-level random effect term for the zero-count component of the model, zA_{jk}, was given by:

$$zA_{jk} = (zA_{1k}, \ldots zA_{Mk}) \sim N(0, \sigma_{zA}) \quad \text{Equation 5}$$

Similarly, the individual alpaca-level random effect term for the negative binomial component of the model (nbA_{jk}) was parameterised to follow a normal distribution with zero mean and standard deviation σ_{nbA}. A random effect term for herd was not included in the negative binomial component of the model because the effect of a herd on FEC was accounted-for by the inclusion of a herd-day of the sampling interaction term, as described below.

The zero-count component of the model was parameterised using a single binary variable representing the presence or absence of herd anthelmintic treatment at any time during the month before sampling. The negative binomial component of the model was parameterised as a function of four fixed effects: (1) the WormBoss control region in which the farm was located (a categorical variable comprised of four levels); (2) sex of the alpaca (a categorical variable comprised of two levels); (3) age of the alpaca (in years) at the time of sampling; and (4) a binary variable representing the presence or absence of herd anthelmintic treatment at any time during the month prior to sampling. A term was included in the model to account for the interaction between farm and the date of sampling.

Estimation of model parameters was carried out using the glmmTMB package (Brooks et al., 2017) in R version 3.5.0 (R Core Team, 2017). Model fit was assessed by comparing time series plots of FECs for individual alpacas within individual herds with expected FECs, computed from the fixed- and random-effects included in the model.

6.4. Results

Based on the alpaca population in different climatic zones, the highest number of faecal samples was collected from the summer rainfall zone (475 individual samples from 3 farms) followed by Mediterranean-type environment (417 samples from 4 farms), winter rainfall zone (330 faecal samples from 4 alpaca farms) and non-seasonal rainfall zone (299 samples from 2 farms) during the study period. Of 1,688 alpaca faecal samples tested using the McMaster technique, 61% had at least one type of GIN egg. Overall, the mean FEC (i.e. EPG ± standard error of the mean) was 168±14 EPG (Table 6.1). Based on egg morphology, strongyle nematodes were the most prevalent (53%, 876/1,666)

followed by *Nematodirus* spp. (18%) and *Trichuris* spp. (11%) and this difference was statistically significant ($P < 0.05$) (Table 6.1).

Table 6.1. Prevalence and faecal egg counts gastrointestinal nematodes in Australian alpacas.

Type of nematode egg	% Prevalence (proportion)	EPG (mean ± SE)	95% CI		Range
Strongyle	53 (876/1,666)	151±14[b]	123	179	(0 – 15,540)
Nematodirus spp.	18 (305/1,666)	12±1[a]	10	14	(0 – 615)
Trichuris spp.	11 (180/1,665)	6±1[a]	4	8	(0 – 1,275)
Overall	61 (1,037/1,688)	168±14	140	196	(0 – 15,630)

CI = confidence interval; EPG = eggs per gram of faeces; SE = standard error of mean.

Similar letters in a column indicate that no significant difference was shown among different nematode eggs.

Based on the age of alpacas, the highest prevalence and mean FEC were recorded for weaners (73%, 233/321) followed by tuis (69%, 240/346), crias (59%, 58/98) and adults (55%, 502/919). Similarly, the mean FEC was highest in weaners (295±60 EPG) followed by tuis (187±23 EPG), adults (126±13 EPG) and crias (68±13 EPG) (Table 6.2). Furthermore, the prevalence of GINs was significantly different between weaners and crias ($P < 0.05$) and adults ($P < 0.05$), and tuis and crias ($P < 0.05$) and adults ($P < 0.05$).

The highest prevalence (68%, 233/330) and mean FEC (266±59 EPG) were recorded in the winter rainfall zone followed by the Mediterranean-type (65%, 270/417; 149±19 EPG). However, the prevalence of GINs in the non-seasonal rainfall zone was higher (60%, 179/299) than that in the summer rainfall (54%, 258/475) but the mean FEC was higher (167±21 EPG) in the summer rainfall zone than the non-seasonal rainfall zone (101±17) (Table 6.3). The prevalences of GINs were significantly different between Mediterranean-type and winter rainfall ($P < 0.05$) and summer rainfall ($P < 0.05$) zones, and winter rainfall and summer rainfall ($P < 0.05$) and non-seasonal rainfall ($P < 0.05$) zones.

Table 6.2. Prevalence and faecal egg counts of gastrointestinal nematodes in different age groups of Australian alpacas.

Age group	% Prevalence (proportion)	EPG (mean±SE)	95% CI		Range
Crias	59 (58/98)	68±13[a]	42	94	(0 – 870)
Weaners	73 (233/321)	295±60[b]	178	413	(0 – 15,630)
Tuis	69 (240/346)	187±23[b]	142	231	(0 – 4,635)
Adults	55 (502/919)	126±13[a]	101	151	(0 – 4,770)

CI = confidence interval; EPG = eggs per gram of faeces; SE = standard error of mean.

Similar letters in a column indicate that no significant difference was shown among age groups.

Table 6.3. Prevalence and faecal egg counts of gastrointestinal nematodes in alpacas in different climatic zones of Australia.

Climatic zones	% Prevalence (proportion)	EPG (mean ± SE*)	95% CI		Range
Mediterranean-type	65 (270/417)	149±19[a]	111	188	(0 – 4,635)
Winter rainfall	68 (223/330)	266±59[b]	151	382	(0 – 15,630)
Non-seasonal rainfall	60 (179/299)	101±17[a,c]	68	134	(0 – 3,690)
Summer rainfall	54 (258/475)	167±21[c]	126	207	(0 – 4,695)

CI = confidence interval; EPG = eggs per gram of faeces; SE = standard error of mean.
Similar letters in a column indicate that no significant difference was shown among climatic zones.

The effect of climatic conditions (rainfall and temperature) on nematodes of alpacas in the four climatic zones was studied over the study period (i.e. May 2015 to April 2016). In the winter rainfall zone, though the highest mean FEC (364±119 EPG) was observed from late winter to early spring (i.e. August-September), a second peak (319±111 EPG) was seen unexpectedly in summer (i.e. December-February) (Fig. 6.4). Within the winter rainfall zone, there was a statistical difference in the prevalence of GINs between winter and spring ($P < 0.05$), summer ($P < 0.05$) and autumn ($P < 0.05$) seasons. In the Mediterranean-type environment, the highest mean FEC (194±36 EPG) was noted in winter (June-August) followed by a second peak (190±74 EPG) in summer, and there was no difference in the prevalence of GINs across different seasons in this zone. Conversely, the highest mean FEC was recorded in late summer to early autumn (January-March) in both non-seasonal (157±45 EPG) and summer rainfall (255±52 EPG) zones (Fig. 6.4). Furthermore, there was a statistical difference in the prevalence of GINs between summer and winter seasons in non-seasonal ($P < 0.009$) and summer rainfall ($P < 0.004$) zones.

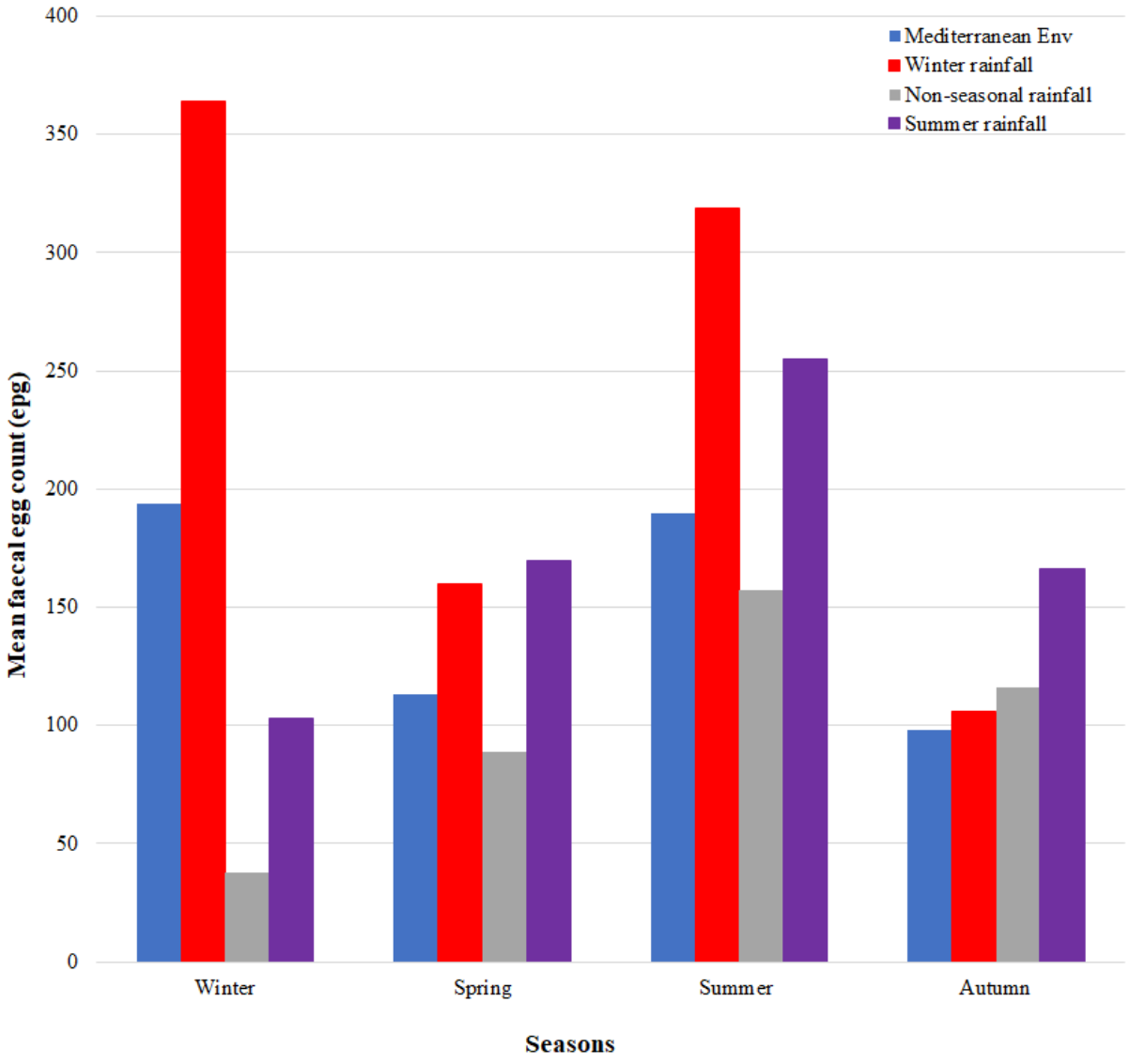


Fig. 6.4. Seasonal variation in the mean faecal egg counts (i.e. eggs per gram [epg] of faeces) of gastrointestinal nematodes in alpacas from the four climatic zones of Australia during the study period (May 2015 to April 2016).

The identification of nematode genera/species using MT-PCR assay revealed that overall farm-level prevalence of *Trichostrongylus* spp. was the highest (83%, 89/107) followed by *Haemonchus* spp. (71%), *C. mentulatus* (63%), *O. ostertagi* (53%), *Cooperia* spp. (11%), *T. circumcincta* (8%) and *Oesophagostomum* spp. (6%) (Fig. 6.5). *Trichostrongylus* spp. were the most common nematodes in both the Mediterranean-type environment (89%, 25/28) and the summer rainfall zone (83%, 30/36) followed by *Haemonchus* spp. (57% and 69%, respectively) and *C. mentulatus* (50% and 39%, respectively) (Fig. 6.6; Table 6.4). *Camelostrongylus mentulatus* was the most common nematode in the winter rainfall (81%, 17/21) and non-seasonal rainfall (100%, 22/22) zones followed by *Trichostrongylus* spp. (76% and 72%, respectively) and *Haemonchus* spp. (71% and 91%, respectively). *Ostertagia ostertagi* was the fourth most common nematode among all climatic zones (Fig. 6.6; Table 6.4). There was a statistical difference in the prevalence of *C. mentulatus* between the non-seasonal rainfall zone and the summer rainfall zone ($P < 0.05$) and the Mediterranean-type environment ($P < 0.05$), and between the winter rainfall and summer rainfall ($P < 0.05$) zones. Similarly, there was a statistical difference in the prevalence of *O. ostertagi* between the non-seasonal rainfall zone and the summer rainfall zone ($P < 0.05$) and the Mediterranean-type environment ($P < 0.05$), and between the winter rainfall and summer rainfall ($P < 0.05$) zones.

Table 6.4. Prevalence of gastrointestinal nematodes in alpacas from the four climatic zones of Australia.

	Mediterranean Env.		Winter rainfall		Non-seasonal rainfall		Summer rainfall	
Nematode	% Prevalence (proportion)	95% Conf. Interval	% Prevalence (proportion)	95% Conf. Interval	% Prevalence (proportion)	95% Conf. Interval	% Prevalence (proportion)	95% Conf. Interval
Camelostrongylus mantulatus	50 (14/28)	(31 - 69)	81 (17/21)	(58 - 95)	100 (22/22)	(85 - 100)	39 (14/36)	(23 - 57)
Cooperia spp.	7 (2/28)	(1 - 24)	14 (3/21)	(3 - 36)	0 (0/22)	-	19 (7/36)	(8 - 36)
Haemonchus spp.	57 (16/28)	(37 - 76)	71 (15/21)	(48 - 89)	91 (20/22)	(71 - 98)	69 (25/36)	(52 - 84)
Oesophagostomum spp.	0 (0/28)	-	24 (5/21)	(8 - 47)	0 (0/22)	-	3 (1/36)	(0 - 15)
Ostertagia ostertagi	46 (13/28)	(30 - 64)	52 (11/21)	(30 - 74)	91 (20/22)	(71 - 99)	36 (13/36)	(22 - 53)
Teladorsagia circumcincta	11 (3/28)	(2 - 28)	24 (5/21)	(8 - 47)	5 (1/22)	(0 - 23)	0 (0/36)	-
Trichostrongylus spp.	89 (25/28)	(72 - 98)	76 (16/21)	(53 - 92)	82 (18/22)	(60 - 95)	83 (30/36)	(67 - 94)

Different letters in a row indicate that significant difference was shown among climatic zone

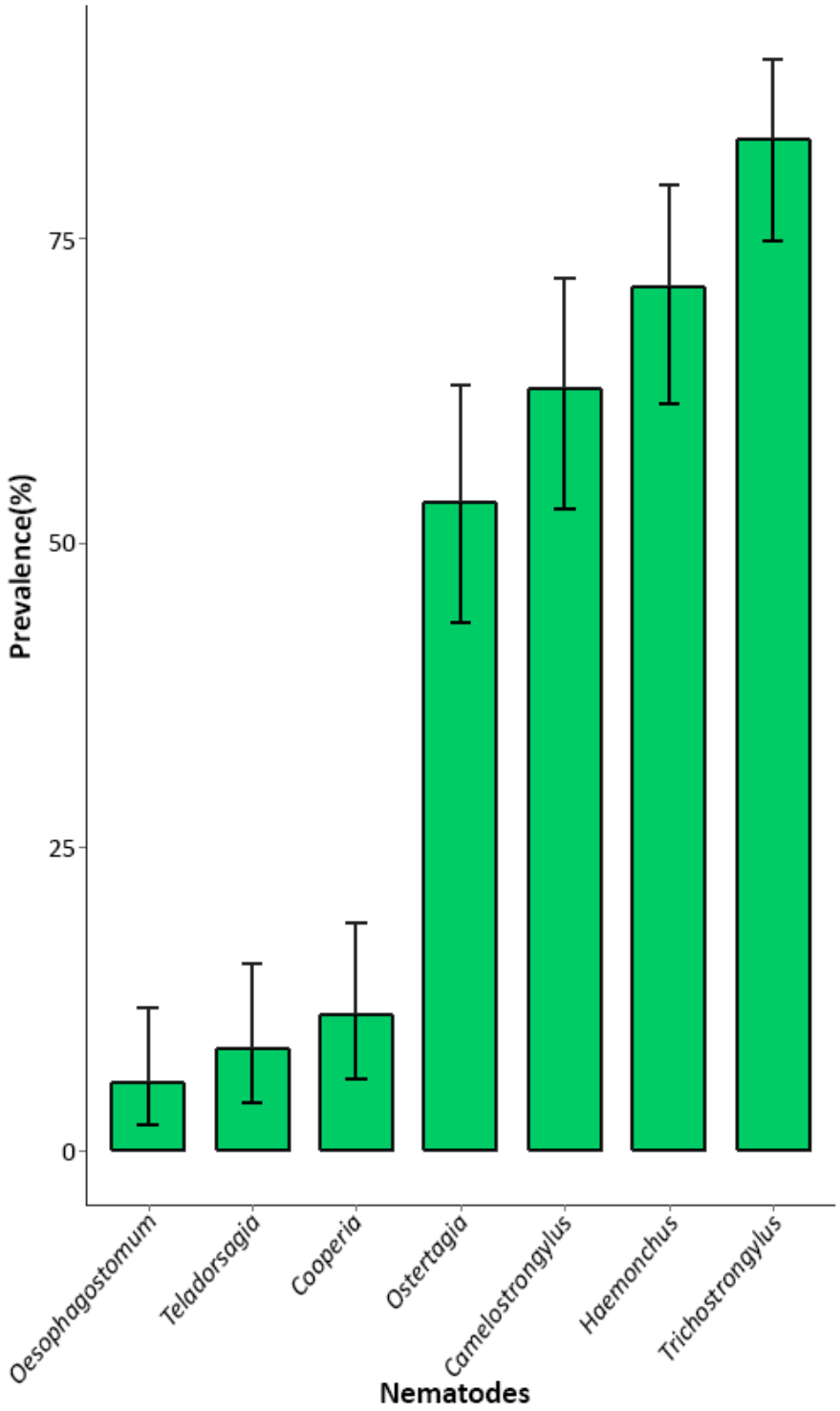


Fig. 6.5. Overall prevalence (%) of gastrointestinal nematodes of alpacas in Australia. Each green bar shows percentage of the farms found positive for a nematode genus/species using the multiplexed-tandem PCR assay, and each vertical line shows upper and lower 95% confidence intervals.

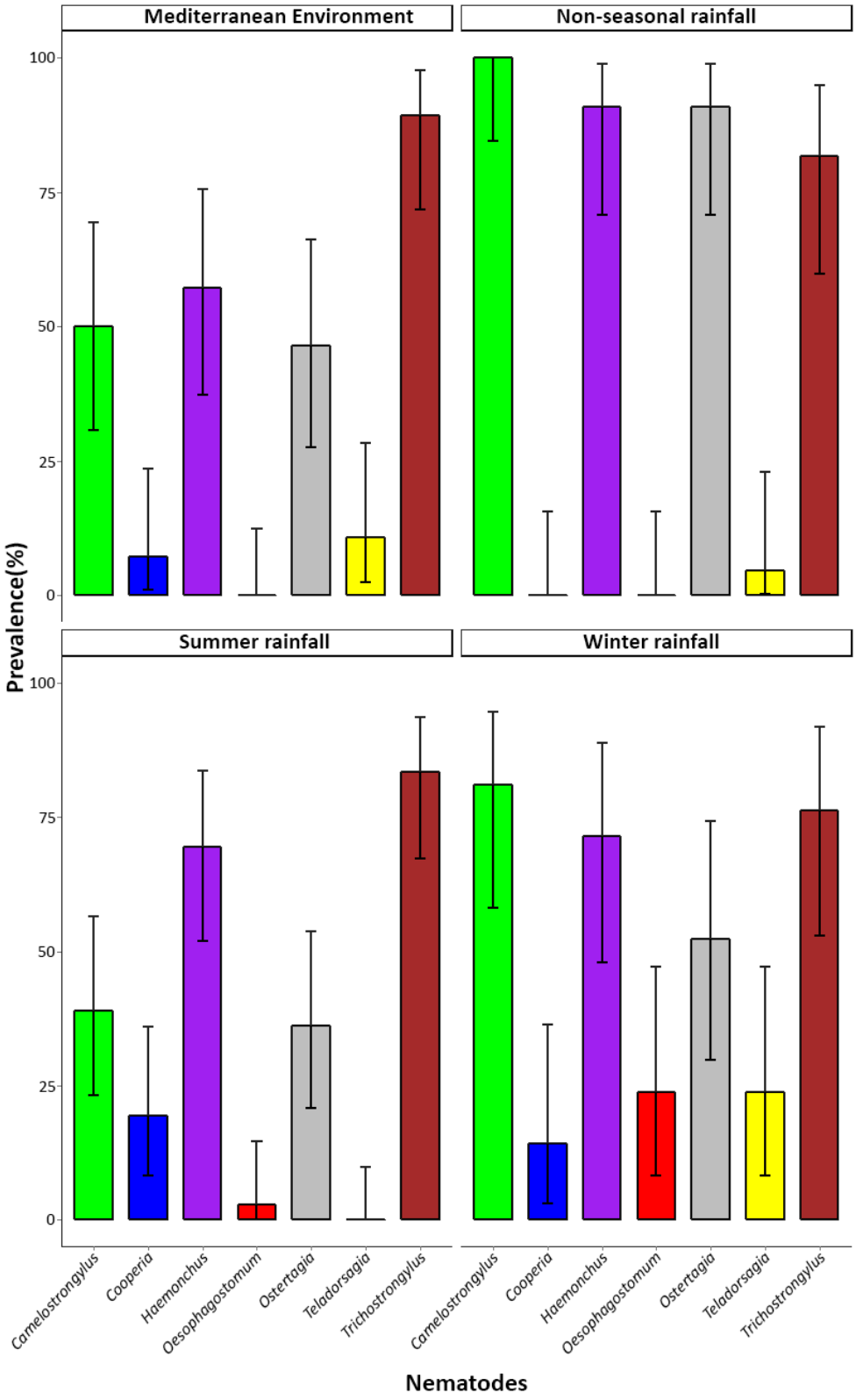


Fig. 6.6. Prevalence (%) of seven common gastrointestinal nematodes of alpacas in the four climatic zones of Australia. Nematodes were identified in pooled DNA samples using the multiplexed-tandem PCR assay. Each vertical line shows upper and lower 95% confidence intervals.

The prevalence of individual genera/species of nematodes also varied depending upon the season. For instance, the highest farm-level prevalence of *C. mentulatus* (68%, 19/28), *Cooperia* spp., (18%, 5/28), *Haemonchus* spp. (75%, 21/28) and *Trichostrongylus* spp. (86%, 24/28) were recorded in autumn while those of *Oesophagostomum* spp., *O. ostertagi* and *T. circumcincta* were observed in summer. Although the prevalence of individual nematodes varied in different seasons, this variation was not statistically significant.

Estimated regression coefficients and their standard errors for the mixed-effects ZINB model are presented in Table 6.5. Alpacas on farms in the winter rainfall zone, while holding all other variables in the model constant, had FECs 4.93 (95% CI 0.87 to 28.1) times greater than alpacas on farms in the Mediterranean-type environment. Male alpacas, while holding all other variables in the model constant, had FECs 1.38 (95% CI 1.01 to 1.87) times greater than female alpacas. For alpacas in herds where deworming was carried out in the month prior to sampling, the odds of having a zero faecal egg count was increased by a factor of 6.6 (95% CI 4.2 to 10) compared with alpacas in herds where de-worming was not carried out in the month prior to sampling (Table 6.5). The variance of the herd- and alpaca-level random effect terms were similar for both the negative binomial component (herd 0.4938; alpaca 0.4961) and the zero-count component (herd 0.6378; alpaca 0.7790) of the model (Table 6.5). This means that after controlling for each of the fixed effects included in the model, the variation in the residual (unexplained) component of FEC was similar at both the herd and individual alpaca level.

Table 6.5. Coefficients and standard errors from a mixed-effects zero-inflated negative binomial model of faecal egg counts.

Negative binomial component:				
Variable	Coefficient (SE)	*z* value	Pr (> |z|)	IRR 95% CI
Intercept	5.024 (0.4708)	10.67	< 0.01	
Climatic zone:				
Mediterranean-type	Reference	-	-	
Winter rainfall	1.596 (0.8877)	1.80	0.07	4.93 (0.87, 28.1)
Non-seasonal rainfall	-0.7269 (0.7187)	-1.01	0.31	0.48 (0.12, 1.98)
Summer rainfall	-0.6577 (0.6719)	-0.98	0.33	0.52 (0.14, 1.93)
Gender:				
Female	Reference	-	-	
Male	0.3204 (0.1571)	2.04	0.04	1.38 (1.01, 1.87) [a]
Age (years):	-0.0179 (0.0194)	-0.92	0.35	0.98 (0.95, 1.02)
Deworming:				
No	Reference	-	-	
Yes	-0.8277 (0.1912)	-4.33	<0.01	0.44 (0.30, 0.64)
Sample month × herd interaction:				
Month × Herd 1	Reference			
Month × Herd 2	-0.0048 (0.0013)	-3.63	<0.01	-
Month × Herd 3	-0.0057 (0.0013)	-4.34	<0.01	-
Month × Herd 4	0.0009 (0.0012)	0.73	0.46	-
Month × Herd 5	0.0016 (0.0008)	2.02	0.04	-
Month × Herd 6	0.0068 (0.0019)	3.52	<0.01	-
Month × Herd 7	0.0026 (0.0011)	2.36	0.02	-
Month × Herd 8	-0.0001 (0.0018)	-0.05	0.96	-
Month × Herd 9	0.0005 (0.0008)	0.65	0.52	-
Month × Herd 10	-0.0107 (0.0052)	-2.07	0.04	-
Random effects:	Variance			
Herd	0.4938			
Alpaca	0.4961			

Zero-count component:				
Variable	Coefficient (SE)	*z* value	Pr (> |z|)	OR (95% CI)
Intercept	-0.8412 (0.2750)	-3.06	<0.01	-
Deworming:				
No	Reference	-	-	
Yes	1.8907 (0.2338)	8.08	<0.01	6.6 (4.2 to 10) [b]
Random effects:	Variance			
Herd	0.6378			
Alpaca	0.7790			

[a] Interpretation: Male alpacas, while holding all other variables in the model constant, were expected to have FECs 1.38 (95% CI 1.01 to 1.87) times greater than female alpacas.

[b] Interpretation: The odds of a zero faecal egg count for herds where de-worming was carried out in the month prior to sampling was increased by a factor of 6.6 (95% CI 4.2 to 10) compared with herds where de-worming was not carried out in the month prior to sampling.

SE: standard error.

IRR: incidence rate ratio.

OR: odds ratio.

6.5. Discussion

This is the first national longitudinal survey to determine the prevalence and worm burden of GINs in alpacas. *Camelostrongylus mentulatus*, *Haemonchus* spp. and *Trichostrongylus* spp. were the three most common GINs. Weaners were found to be the most susceptible age group as they harboured the highest prevalence as well as FECs. Alpacas in the winter rainfall zone had the highest prevalence and FECs. Overall, the epidemiology of GINs of Australian alpacas determined in this study is very similar to that was observed in a recent cross-sectional survey of GINs of alpacas (see Chapter 5).

The prevalence of GINs (61%) found in this study was comparable to that (66%) recently reported in a cross-sectional survey of GINs in Australian alpacas (see Chapter 5) but higher than those (48% and 20%) reported by Carmichael (1999) and Presidente (2007), respectively, previously from Australia. However, the latter studies were either based on the opportunistic collection of alpaca faecal samples (Presidente, 2007) or were of limited scope (only South Australia and some parts of Victoria were included) (Carmichael, 1999) as opposed to this study being a longitudinal survey across six states of Australia, involving a higher number of alpaca faecal samples.

In this study, weaners had the highest prevalence (73%) of GINs followed by tuis, crias and adults which are concordant with previous findings (see Chapter 5). Similarly, the highest FECs were recorded in weaners whereas that was observed in tuis (402 EPG) in the cross-sectional study. The prevalences of strongyles (53%), *Nematodirus* spp. (18%) and *Trichuris* spp. (11%) were also very similar to those found in a cross-sectional study (see Chapter 5). These differences in GIN prevalence between both studies might be due to geographical variations and the total number of faecal samples analyzed, i.e. higher number (n = 1,688) of faecal samples from 13 alpaca farms across six states (New South Wales, Queensland, South Australia, Tasmania, Victoria and Western Australia) were analyzed in this study. Other important factors underlying substantial differences in GIN prevalence in alpacas could be mixed grazing (with domestic ruminants) practices, intensive commercial farming and the year in which the sampling occurred (Chapters 2 and 5; Saeed et al., 2018).

The overall mean FEC (168 EPG) recorded in this study appears lower than those (291 and 200 EPG) observed in Australia (see Chapter 5) and the UK (Tait et al., 2002), respectively. The main distinguishing feature of this study is that it revealed the temporal distribution of FECs. The acquisition and frequency of GIN infections in a camelid herd

could vary at different times of the year due to variable temperatures and humidity levels throughout the year which ultimately affect larval growth on the pasture (Hill et al., 1993). Thus, the temporal distribution of GIN burdens in alpacas would be more informative in interpreting the epidemiological data. Importantly, the mean FEC for strongyle eggs (151 EPG) in this study was comparable to the overall mean FEC (168 EPG) in alpacas which indicates that alpacas were commonly infected with strongyle nematodes as are other domestic livestock in Australia (Roeber et al. 2011, 2013b) and elsewhere (Ballweber, 2009; Franz et al., 2015).

It is worth considering that some of the GINs (e.g. *T. circumcincta*) reported herein and in the cross-sectional study (see Chapter 5) have not been described in alpacas/llamas from South America. Furthermore, all these GIN parasites are known to exist in sheep and cattle from Australia (Anderson, 1972, 1973; Beveridge and Ford, 1982; Roeber et al., 2011, 2013b). This indicates that the movement of alpacas to new geographical locations outside South America have likely exposed them to novel parasites of ruminants during their co-grazing with sheep and cattle; hence, they may serve as a potential source of GIN infections for sheep and cattle upon their movement between properties.

As with infections in other domestic ruminant species, the distribution of GINs in alpacas is considerably impacted by climatic conditions such as rainfall and temperature which ultimately affects the prevalence and burden of these parasites in camelids and domestic ruminants across different climatic zones worldwide. Therefore, this study assessed the impact of climatic conditions on the prevalence and FECs in alpacas from different climatic zones of Australia throughout one-year. The highest prevalence (68%) and FECs (266 EPG) in alpacas were observed in the winter rainfall zone which encompasses the south-eastern part of the continent. For instance, in the winter rainfall zone, the peak prevalence found in winter, which decreased in spring and increased again in the summer months. The usual trend in this zone is peak prevalence in winter followed by a decrease in spring to the lowest in summer (Roeber et al., 2013b). However, the second peak in the prevalence of GINs in the winter rainfall zone (Fig. 6.3) was most likely associated with higher rainfall (876 mm) during the summer season in 2016 (see Fig. 6.2) as the average annual rainfall in this zone prior to (2013: 764 mm; 2014: 664 mm; 2015: 523mm) and after (2017: 658 mm) 2016 was lower. Overall, this study revealed that the temporal distribution of different GINs of alpacas in various climatic

zones follows the patterns of those previously described for sheep and cattle GINs in Australia (Roeber et al., 2013b).

In this study, a high prevalence of *H. contortus* observed in spring and autumn which is consistent previous studies in sheep from Australia (Roeber et al., 2013b). *Camelostrongylus mentulatus* was the most frequently (81%) observed GIN followed by *Trichostrongylus* spp. (76%) in winter rainfall zone. *Camelostrongylus mentulatus* has been found in sheep, feral goats and also in camels in Australia (Beveridge et al., 1974, 1987; Beveridge and Ford, 1982; Copland, 1965; Rogers, 1939). Hilton et al. (1978) suggested *C. mentulatus* be a potential pathogen as it behaves like *Teladorsagia* spp. in sheep. Outside South American countries, *C. mentulatus* has also been found in alpacas, llamas and zoo animals in New Zealand, the UK and the USA (Averbeck et al., 1981; Dwyer et al., 2014; Flach, 2008; Hinkson, 2015; Rickard, 1993; Welchman et al., 2008). A recent study (see Chapter 7) found four species of *Trichostrongylus* (*T. axei*, *T. colubriformis*, *T. rugatus* and *T. vitrinus*) in alpacas which are known to commonly infect sheep in the winter rainfall zone of Australia, exhibiting high pathogenicity and production losses in the south-eastern states of the country (Beveridge and Ford, 1982; Roeber et al., 2013b). Given that alpacas share GINs with sheep and *C. mentulatus* is known to infect sheep, it is proposed that the presence of this parasite in sheep should be evaluated in Australia, particularly in those regions where mixed grazing is a common practice.

A recent cross-sectional study (see Chapter 5) reported the highest prevalence (77%) and FECs (630 EPG) in alpacas from the summer rainfall zone in Australia, however this study found that these (68%; FEC 266 EPG) were modestly higher in the winter rainfall zone. Similarly, other differences in the prevalence of *C. mentulatus*, *Cooperia* spp., *O. ostertagi* and *T. circumcincta* were recorded in the Mediterranean zone between this study and the cross-sectional study (see Chapter 5). These differences in the prevalence and FECs in alpacas across different climatic zones could be attributed to different timing of the two studies (longitudinal 2015-2016 *versus* cross-sectional 2016-2017), variations in rainfall (see Fig. 6.2) and management practices of alpaca herds sampled. Further longitudinal epidemiological studies, spanning for 3-5 years, are required to further investigate the prevalence of different GINs of alpacas in different seasons across various climatic zones of Australia. Also, studies should focus on examining the gastrointestinal tracts of alpacas to precisely determine the type of GINs as FEC and the MT-PCR assay were only used to identify GIN genera/species in alpacas.

A recent cross-sectional study (see Chapter 5) as well as this longitudinal epidemiological survey found that male alpacas had higher prevalence and burden (i.e. FECs) of GINs than females. Sex could be an important factor in the susceptibility of hosts for infection with GINs, though variable results have been reported previously. For example, male alpacas were found to have higher burden of *H. contortous* than females (Dittmer et al., 2018). Contrarily, in another study by Edwards et al. (2016), no association was found between the occurrence of *H. contortous* and sex of camelids. Further studies are required to investigate the precise effect of the sex on the susceptibility of alpacas and llamas for GINs.

A mixed-effects, zero-inflated negative binomial regression model has been used to derive quantitative estimates of the influence of herd- and individual alpaca-level characteristics on the likelihood of an alpaca having a FEC of zero at the time of sampling and the magnitude of FECs for those alpacas with non-zero counts. The analytical approach used in this study was novel in that a zero-inflated negative binomial model was used to address the issue of over-dispersion in the data arising from the relatively large number of zero FECs while at the same time allowing us to quantify the effect of factors influencing FECs when nematodes were present. A multilevel modelling approach was used to adjust the estimated regression coefficients to account for lack of independence in the data arising from faecal samples collected from the same alpacas within herds over time and because individual alpacas were clustered within herds. At least four advantages arose from the use of this analytical method. Firstly, the zero-inflated regression approach meant that it was not necessary for us to transform the data prior to analysis which meant that the regression coefficients from the regression model were more readily interpretable. Secondly, the extension of the model to account for lack of independence allowed us to quantify the effect of risk factors acting at both the herd (e.g. climatic zone in which the herd was located) and individual alpaca (e.g. gender and age) level. Thirdly, accounting for lack of independence in the data provided more conservative estimates of the uncertainty around each of the regression coefficients for each risk factor making it less likely to make Type I errors (rejecting the null hypothesis when it is actually true). Finally, comparisons of the variance of the herd-level and individual alpaca level random effect terms for both the negative binomial component and a zero-count component of the model provided useful information to inform parasite control strategies. In this analysis, the variation in the residual (unexplained) component of FEC was similar at both the herd and individual alpaca level which means that parasite

control interventions should be targeted at both the herd level (e.g. pasture management) and the individual alpaca level (e.g. anthelmintic treatment) with equal emphasis.

6.6. Conclusions

This is the first comprehensive longitudinal study to investigate the prevalence and worm burden in alpacas. The overall prevalence and burden of GINs appear to have considerably risen in alpacas from Australia over the last two decades. Alpacas harbour the majority of the same GINs as occurred in sheep and cattle and thus the potential transmission of these parasites between domestic ruminant species, including alpacas, should be taken into account for designing effective management and control strategies. The mixed-effects zero-inflated negative binomial regression model can be used to quantify the influence of herd-level and individual alpaca-level characteristics on FEC at the time of sampling which can the help to design parasite control interventions targeted at both the herd level and the individual alpaca level. This study has generated novel information on the epidemiology of GINs in alpacas from Australia with potential implications not only within the country but also in alpacas from similar agroclimatic zones globally.

6.7. References

Abdouslam, O., Al-Bassam, L., Al-Izzi, S., & Azwai, S. (2003). Prevalence of external and internal parasites in llamas (*Lama glama*) at Surman Park in Libya. *Journal of Camel Practice and Research,* 10, 61-65.

Alcaino, H., Gorman, T., & Burgos, M. (1991). Helmintiasis gastrointestinal en llamas (*Lama glama*) de la I Región de Chile. *Parasitología al día,* 15, 93-96.

Anderson, N. (1972). Trichostrongylid infections of sheep in a winter rainfall region. 1. Epizootiological studies in the Western District of Victoria, 1966-67. *Australian Journal of Agricultural Research,* 23, 1113-1129.

Anderson, N. (1973). Trichostrongylid infections of sheep in a winter rainfall region. 2. Epizootiological studies in the Western District of Victoria, 1967-68. *Australian Journal of Agricultural Research,* 24, 599-611.

Averbeck, G.A., Scholtthauer, J.C., & Hinueber, J.G. (1981). *Camelostrongylus mentulatus* infection in a camel (*Camelus dromedarius*): a case report. *Journal of Zoo Animal Medicine*, 12, 24-26.

Ballweber, L.R. (2009). Ecto- and endoparasites of new world camelids. *Veterinary Clinics of North America: Food Animal Practice,* 25, 295-310.

Beltrán-Saavedra, L.F. (2015). Health assessment of free-ranging vicuna of the National Integrated Management Natural Area Apolobamba, Bolivia. *Journal of the Selva Andina Animal Science,* 46, 1-17.

Beveridge, I., Barker, I., Rickard, M., & Burton, J. (1974). Experimental infection of sheep with *Camelostrongylus mentulatus* and associated gastritis. *Australian Veterinary Journal,* 50, 36-37

Beveridge, I., & Ford, G.E. (1982). The trichostrongyloid parasites of sheep in South Australia and their regional distribution. *Australian Veterinary Journal,* 59, 177-179.

Beveridge, I., Pullman, A.L., Henzell, R., & Martin, R.R. (1987). Helminth parasites of feral goats in South Australia. *Australian Veterinary Journal,* 64, 111-112.

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Maechler, M., & Bolker, B.M. (2017). Modeling zero-inflated count data with glmmTMB. bioRxiv 1, 132753.

Carmichael, I. (1999). Internal parasitism in alpacas in southern Australia. In: Hack W, McGregor B, Ponzoni R, Judson G, Carmichael I, Hubbard D (eds) Australian

Alpaca Fibre Improving Productivity and Marketing. *Rural Industries Research and Development Corporation, Australia,* p 92–130.

Cebra, C.K., & Stang, B.V. (2008). Comparison of methods to detect gastrointestinal parasites in llamas and alpacas. *Journal of the American Veterinary Medical Association,* 232, 733-741.

Clarke, M. (2016). Market Assessment - New and Emerging Animal Industries Tranche 1: Mohair, Alpaca and Camel Milk. NSW, Australia 2016. http://www.agrifutures.com.au/publications/ market-assessment-new-and-emerging-animal-industries-tranche-1-mohair-alpaca-and-camel-milk. Accessed 15 Jan 2018.

Copland, J.W. (1965). *Ostertagia mentulata* recorded in New South Wales. *Australian Veterinary Journal,* 41, 27-27.

Denwood, M.J., Stear, M.J., Matthews, L., Reid, S.W., Toft, N., & Innocent, G.T. (2005). The distribution of the pathogenic nematode *Nematodirus battus* in lambs is zero-inflated. *Parasitology,* 135, 1225-1235.

Dwyer, C., W. Pomroy, K. Gedye, K. Dittmer, K. Roe, D. Aberdein, B. Adlington, H. Dann, & A. Wehrne. (2014). *Trichostrongylus askivali* – a new record for alpacas in New Zealand. Proceedings of the New Zealand Society for Parasitology: 42nd Conference, Wellington, New Zealand.

Dittmer, K., Hinkson, J., Dwyer, C., Adlington, B., & van Andel, M. (2018). Prevalence of Candidatus *Mycoplasma haemolamae*, bovine viral diarrhoea virus, and gastrointestinal parasitism in a sample of adult New Zealand alpaca (*Vicugna pacos*). *New Zealand Veterinary Journal,* 66, 9-15.

Edwards, E.E., Garner, B.C., Williamson, L.H., Storey, B.E., & Sakamoto, K. (2016). Pathology of *Haemonchus contortus* in New World camelids in the southeastern United States: a retrospective review. *Journal of Veterinary Diagnostic Investigation,* 28, 105-109.

Flach, E. (2008). Alpaca and llama nematodes in Britain. *Veterinary Record,* 163, 128-128.

Franz, S., Wittek, T., Joachim, A., Hinney, B., & Dadak, A.M. (2015). Llamas and alpacas in Europe: Endoparasites of the digestive tract and their pharmacotherapeutic control. *Veterinary Journal,* 204, 255-262.

Hertzberg, H., & Kohler, L. (2006). Prevalence and significance of gastrointestinal helminths and protozoa in South American camelids in Switzerland. *Berliner und Münchener Tierärztliche Wochenschrift,* 119, 291-294.

Hill, F., Death, A., & Wyeth, T. (1993). Nematode burdens of alpacas sharing grazing with sheep in New Zealand. *New Zealand Veterinary Journal,* 41, 205-208.

Hilton, R.J., Barker, I.K., Rickard, M.D. (1978). Distribution and pathogenicity during development of *Camelostrongylus mentulatus* in the abomasum of sheep. *Veterinary Parasitology,* l4, 231-242.

Hinkson, J.A. (2015). Investigations into common farm management practices and diseases on alpaca farms in New Zealand. Dissertation, Massey University, Palmerston North, New Zealand.

Hyuga, A., & Matsumoto, J. (2016). A survey of gastrointestinal parasites of alpacas (*Vicugna pacos*) raised in Japan. *The Journal of Veterinary Medical Science,* 78, 719-721.

Jabbar, A., Campbell, A.J.D., Charles, J.A., & Gasser, R.B. (2013). First report of anthelmintic resistance in *Haemonchus contortus* in alpacas in Australia. *Parasites and Vectors,* 6, 243.

Leguía, G. (1991). The epidemiology and economic impact of llama parasites. *Parasitology Today,* 7, 54-56.

Margolis, L., Esch, G.W., Holmes, J.C., Kuris, A.M., & Schad, G. (1982). The use of ecological terms in parasitology (report of an *ad hoc* committee of the American Society of Parasitologists). *Journal of Parasitology,* 68, 131-133.

Masson, M., Gutiérrez, G., Puicón, V., & Zárate, D. (2016). Helmintiasis y eimeriosis gastrointestinal en alpacas criadas al pastoreo en dos granjas comunales de la región Pasco, Perú, y su relación con el peso y condición corporal. *La Revista de Investigaciones Veterinarias del Perú es,* 27, 805-812.

Moreno, P.G., Eberhardt, M.A.T., Lamattina, D., Previtali, M.A., & Beldomenico, P.M. (2013). Intra-phylum and inter-phyla associations among gastrointestinal parasites in two wild mammal species. *Parasitology Research,* 112, 3295-3304.

Mitchell, S., Hopkins, B., & Corfield, C. (2016). *Nematodirus lamae* identified in an alpaca in the UK. *Veterinary Record,* 178, 271-272.

Nodtvedt, A., Dohoo, I., Sanchez, J., Conboy, G., DesCoteaux, L., Keefe, G., Leslie, K., & Campbell, J. (2002). The use of negative binomial modelling in a longitudinal

study of gastrointestinal parasite burdens in Canadian dairy cows. *Canadian Journal of Veterinary Research,* 66, 249-257.

Presidente, P.J.A. (2007). Alpaca parasites and their control: recent experiences. *Australian Alpaca Veterinarians Annual Conference, Australia*, p 1-14.

R Core Team, (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rickard, L.G. (1993). Parasitic gastritis in a llama (*Lama glama*) associated with inhibited larval *Teladorsagia* spp.(Nematoda: Trichostrongyloidea). *Veterinary Parasitology,* 45, 331-335.

Robayo, C.I.S. (2015). Prevalencia de parásitos gastrointestinales en alpacas del Inga Alto, Pichincha. Universidad San Francisco de Quito, Ecuador.

Roeber, F., Jex, A.R., Campbell, A.J., Campbell, B.E., Anderson, G.A., & Gasser, R.B. (2011). Evaluation and application of a molecular method to assess the composition of strongylid nematode populations in sheep with naturally acquired infections. *Infection, Genetics and Evolution,* 11, 849-854.

Roeber, F., Jex, A.R., & Gasser, R.B. (2013a). Comparative evaluation of two DNA isolation techniques for PCR-based diagnosis of gastrointestinal nematode infections in sheep. *Molecular and Cellular Probes,* 27, 153-157.

Roeber, F., Jex, A.R., & Gasser, R.B. (2013b). Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance-an Australian perspective. *Parasites and Vectors,* 6, 153.

Rogers, W.P. (1939). Nematode parasites of sheep in Western Australia. *Journal of Helminthology,* 17, 151-158.

Saeed, M.A., Rashid, M.H., Vaughan, J., & Jabbar, A. (2018). Sarcocystosis in South American camelids: The state of play revisited. *Parasites and Vectors,* 11, 146.

Tait, S., Kirwan, J., Fair, C., Coles, G., & Stafford, K. (2002). Parasites and their control in South American camelids in the United Kingdom. *Veterinary Record,* 150, 637-638.

Welchman, D.d.B., Parr, J., Wood, R., Mead, A., & Starnes, A. (2008). Alpaca and llama nematodes in Britain. *Veterinary Record,* 162:832-832.

Windsor, R.S., Teran, M., & Windsor, R.H. (1992a). Effects of parasitic infestation on the productivity of alpacas (*Lama pacos*). *Tropical Animal Health and Production,* 24, 57-62.

Windsor, R.S., Windsor, R.H., & Teran, M. (1992b). Economic benefits of controlling internal and external parasites in South American camelids. *Annals of the New York Academy of Sciences*, 653, 398-405

CHAPTER 7. WORM BURDENS AND ASSOCIATED HISTOPATHOLOGICAL CHANGES CAUSED BY GASTROINTESTINAL NEMATODES IN ALPACAS FROM AUSTRALIA

7.1. Abstract

In this study, one hundred gastrointestinal tracts of Australian alpacas were examined to assess the worm burden and to identify the species of nematode present. Faecal samples were collected from 97 alpacas and processed for faecal egg counts (FECs). For identification of the species, both molecular (multiplexed-tandem polymerase chain reaction [MT-PCR]) and morphological techniques were used. Total worm counts (TWCs) revealed a mean burden of 1,300 worms, with the highest burden of 29,000 worms. The average egg count was 501 eggs per gram of faeces (EPG), with the highest count of 3,500 EPG. Nineteen different species of gastrointestinal nematodes (GINs) were identified, and *Graphinema auchenia*, *Camelostrongylus mentulatus* and *Trichuris tenuis* were recovered from Australian alpacas for the first time. *Haemonchus contortus* was the most prevalent nematode (81%) followed by *C. mentulatus* (60%). The majority of the nematodes found are shared with sheep, goats and cattle. Findings of this study provide useful insights into the spectrum of GINs and their burden in Australian alpacas.

7.2. Introduction

A wide range of gastrointestinal nematodes (GINs) can infect alpacas and llamas, many of which also affect sheep, goats and cattle (Ballweber, 2009; Hill et al., 1993; Schmäschke, 2015). However, the parasitological status of alpacas and llamas in non-native countries such as Australia is unknown.

To date, few total worm count (TWC) studies have been conducted in Australia to assess the nematode burdens of alpacas, and there is a lack of information about the spectrum of GINs occurring in alpacas. *Haemonchus* spp. and other strongylid nematodes (*Cooperia, Chabertia*, *Oesophagostomum*, *Nematodirus*, *Ostertagia/Teladorsagia* and *Trichostrongylus*) have been diagnosed by larval culture from Australian alpacas (Carmichael, 1999; Presidente, 2007) and using molecular

methods (Jabbar et al., 2013). Globally, there is also a scarcity of TWC data from South American camelids (SACs). One study in the UK reported the recovery of *Capillaria* spp., *Nematodirus* spp. and *Trichuris* spp. from three alpacas (Tait et al., 2002); however, a worm count was not performed on these animals. In addition, the molecular method developed so far, to detect and differentiate GINs in the faeces of alpacas (see Chapter 4), can only detect seven common nematodes, thereby they are also unable to provide the full spectrum of GINs infecting SACs.

Hence, the objective of this study was to determine the worm burden in Australian alpacas using TWC. In addition, morphological and molecular diagnostic techniques were compared to identify the nematode species which occur in Australian alpacas.

7.3. Materials and methods

7.3.1. Collection and processing of gastrointestinal tracts of alpacas

Alpacas used in this study were either killed in abattoirs for meat or were culled on a farm. Hence no institutional animal ethics approval was required. The alpacas were of mixed sex and were mature before slaughter. More than 100 alpaca gastrointestinal tracts were collected. The majority of the samples (86) were collected from New South Wales followed by Victoria (11) and South Australia (6). Fresh alpaca gastrointestinal tracts were processed immediately after killing. Total worm counts were performed following Hutchinson (2009) with minor modifications. After killing, the third compartment (C3) of the stomach was isolated by ligating the two ends using cotton thread, separated from the second compartment and the small intestine, and collected into a plastic bag. Similarly, small and large intestine were also collected into other plastic bags and stored on ice. About 20 grams of faeces were also collected from the rectum of each animal and stored on ice for faecal egg counts and the molecular identification of nematodes. All samples were transported to the University of Melbourne and stored at 4° C until processing.

The C3 was placed onto a tray, opened and its contents washed into a graduated bucket using water. The volume of the washed contents was made up to 1 to 2 litres based on the amount of content. The content mixed, then 5-10% aliquots were drawn into a 100

mL sieve-top (38 μm mesh) glass jar. The contents were washed with water and were fixed by adding 70% ethanol.

Six meters of the duodenum were separated from their mesenteric attachments and about 20 mL of water passed through the intestine three times. The content was made up to one litre and an aliquot of 100 mL was transferred into a sieve-top jar for washing. The content was preserved as described earlier. The entire caecum and colon were opened and examined for the presence of nematodes which were preserved using 70% ethanol.

Material collected from the C3 and small intestine was examined under a dissecting microscope and nematodes were counted. Male nematodes were removed, cleared in lactophenol or glycerine and examined under an Olympus BH-2/BHS Systems microscope (Tokyo, Japan) for species identification (Averbeck et al., 1981; Becklund and Walker, 1967; Beldomenico et al., 2003; Chandler, 1930; Gibbons and Khalil, 1982; Rickard and Bishop, 1991). Photographs of spicules and other key features were captured using an attached digital Olympus DP21camera (Tokyo, Japan). Voucher morphological specimens representing each of the nematodes found have been deposited in the South Australian Museum (SAM), Adelaide (SAM 48463-48485).

Tissues from the C3 with gross pathological lesions were preserved in 10% buffered formalin and prepared for histopathological examination. Formalin-fixed tissues were trimmed longitudinally, embedded in paraffin wax, sectioned at a thickness of 5 μm and stained with haematoxylin and eosin. The histopathological slides were examined under an Olympus BX41 microscope (Tokyo, Japan) and photographs of characteristic findings were captured using an attached digital Olympus DP71 camera.

7.3.2. Faecal egg count

Faecal samples were examined using the modified McMaster technique (Gordon and Whitlock, 1939). The minimum detection limit using this method was 15 EPG.

7.3.3. Molecular identification of common nematodes

DNA was extracted from the nematode eggs present in the faeces of alpacas using a method described previously with few modifications (Roeber et al., 2013). A

multiplexed-tandem PCR (MT-PCR) assay was used as per the protocol described (see Chapter 4). The assay was capable of identifying *Camelostrongylus mentulatus*, *Cooperia* spp., *Haemonchus* spp., *Ostertagia ostertagi*, *Oesophagostomum* spp., *Teladorsagia circumcincta* and *Trichostrongylus* spp. by targeting the second internal transcribed spacer of these nematodes. Randomly selected amplicons representing each nematode genus/species were subjected to sequencing to verify the target nematodes.

7.3.4. Statistical analysis

Descriptive analysis of the data was carried out in the R software using *epiR* package (R Core Team, 2017; Stevenson et al., 2018). Kappa values were calculated to compare the agreement between morphological and molecular (MT-PCR) techniques for the identification of nematode species. A benchmark can be used arbitrarily to interpret Kappa values as 0: poor agreement; 0–0.20: slight agreement; 0.21–0.40: fair agreement; 0.41–0.60: moderate agreement; 0.61–0.80: substantial agreement; ≥ 0.81: almost perfect agreement (Altman, 1991).

7.4. Results and Discussion

Ninety-three percent (93/100) of the gastrointestinal tracts examined were infected with nematodes. The mean worm burden was 1,280, with a maximum of 28,640 worms. The mean worm counts in the C3 and the small intestine were 617 and 689, with maxima of 4,400 and 28,530, respectively (Table 7.1). Similar studies of naturally infected alpacas are lacking globally. Previously, Carmichael (1999) reported TWCs of 14,500 and 23,500 in two healthy alpacas from Victoria. In Australia, a total of 460 *H. contortus* was recovered from the C3 of a one-year-old alpaca that was recently treated with abamectin. No other nematodes were found in the C3 and small intestine (Jabbar et al., 2013). A necropsy of a llama found a total of 6,510 adult nematodes in the C3 (Rickard, 1993).

Table 7.1. Worm burden caused by gastrointestinal nematodes in Australian alpacas.

Method	No. of samples	Mean ± SD	95% Conf. Interval	Median	Range
Faecal egg count (EPG)	97	501 ± 609	381 – 622	255	0 – 3,495
Total worm counts					
Third compartment of the stomach	98	617 ± 867	445 – 789	313	0 – 4,400
Small intestine	98	689 ± 2,974	99 – 1,279	70	0 – 28,530
Overall	100	1,280 ± 3,020	681 – 1,879	660	0 – 28,640

SD, standard deviation; EPG, eggs per gram of faeces.

Morphological examination revealed 19 different nematodes species (Table 7.2; Fig. 7.1). *Camelostrongylus mentulatus*, *G. auchenia* and *Tr. tenuis* are primarily camelid nematodes while the remaining species are shared with cattle and sheep. The shared species have been reported previously in sheep, goats and cattle in Australia (Anderson, 1972, 1973; Barger, 1993). Moreover, this study reports the occurrence of significant nematodes of alpacas such as *C. mentulatus, C. pectinata, G. auchenia*, *Oe. venulosum* and *Tr. tenuis* for the first time in Australia.

Morphological examination of the nematodes in the C3 showed a high prevalence of *H. contortus* (81%; 78/96) followed by *C. mentulatus* (60%; 58/96). A similar trend for these two species was also found in the MT-PCR assay. In the small intestine, a low to moderate prevalence was observed (Table 7.3). Furthermore, kappa values showed fair agreement between two methods of identification for *H. contortus, C. mentulatus*, *Cooperia* spp. and slight agreement for *Trichostrongylus* spp., *O. ostertagi* and *Oe. venulosum*. No agreement was found in case of *T. circumcincta*. (Table 7.3). This disagreement between two diagnostic methods for *T. circumcincta* could be due to its low prevalence detected by both morphological (1%, 1/97) and molecular (3%, 3/86) methods.

Table 7.2. Species of gastrointestinal nematodes found in Australian alpacas based on morphological examination of adult male worms.

Organ	Nematode species
Third compartment of the stomach	*Camelostrongylus mentulatus*
	Graphinema auchenia
	Haemonchus contortus
	Ostertagia ostertagi
	Teladorsagia circumcincta
	Trichostrongylus axei
Small intestine	*Capillaria* spp.*
	Cooperia oncophora, *C. punctata*, *C. pectinata*
	Nematodirus spathiger, *N. filicollis*, *N. helvetianus*, *N. abnormalis*
	Trichostrongylus rugatus, *T. colubriformis*, *T. vitrinus*
Large intestine	*Oesophagostomum venulosum*
	Trichuris tenuis

*Detected by faecal examination only.

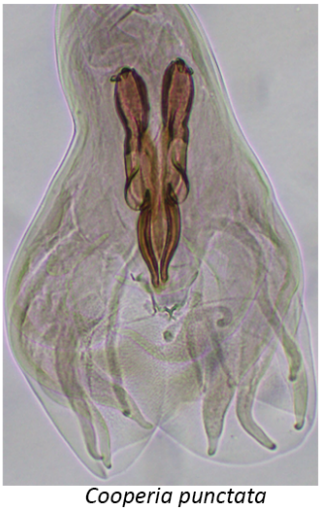
Table 7.3. Prevalence of gastrointestinal nematodes of alpacas in Australia using morphological examination of adult worms and the identification of eggs in the alpaca faeces using MT-PCR. Agreement (%) and kappa values of both techniques for the identification of gastrointestinal nematodes of alpacas are also presented.

Genera/Species	Morphological identification		Molecular identification		Agreement (%)	Kappa	95% Conf. Interval
	% Prevalence (proportion)	95% Conf. Interval	% Prevalence (proportion)	95% Conf. Interval			
Haemonchus contortus	81 (78/96)	72 – 89	78 (67/86)	68 – 86	80	0.33	0.05 – 0.61
Camelostrongylus mentulatus	60 (58/96)	50 – 70	48 (41/86)	37 – 59	64	0.29	0.09 – 0.49
Trichostrongylus spp.	47 (46/97)	37 – 58	56 (48/86)	45 – 67	58	0.16	-0.05 – 0.38
Ostertagia ostertagi	3 (3/97)	1 – 9	31 (27/86)	22 – 42	69	0.01	-0.31 – 0.32
Teladorsagia circumcincta	1 (1/97)	0 – 6	3 (3/86)	1 – 10	96	-0.02	-1.15 – 1.11
Oesophagostomum venulosum	8 (8/97)	4 – 16	12 (10/86)	6 – 20	85	0.15	-0.28 – 0.57
Cooperia spp.	38 (37/97)	29 - 49	17 (15/86)	10 – 27	64	0.20	-0.03 – 0.43
Nematodirus spp.	33 (32/97)	24 – 43	-	-	-	-	-
Trichuris tenuis	6 (6/97)	2 – 13	-	-	-	-	-
Graphinema auchenia	3 (3/97)	1 – 9	-	-	-	-	-

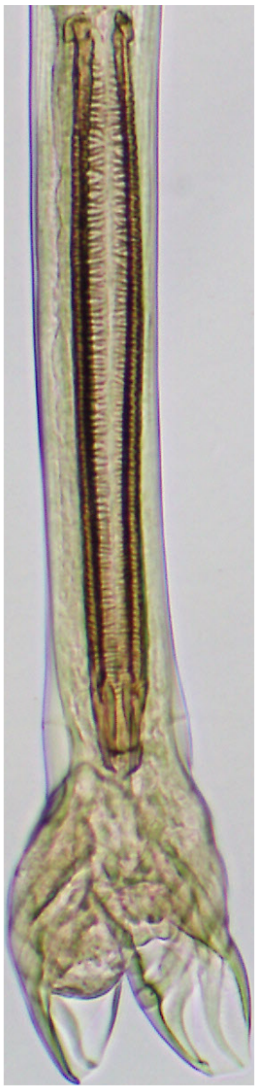
Haemonchus contortus

Cooperia oncophora

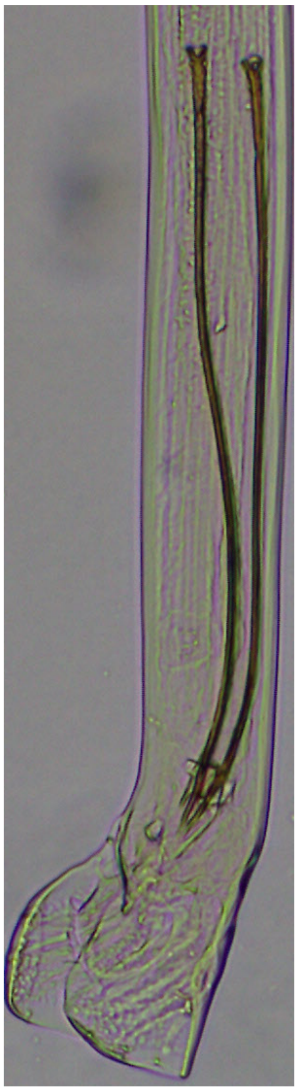
Cooperia pectinata



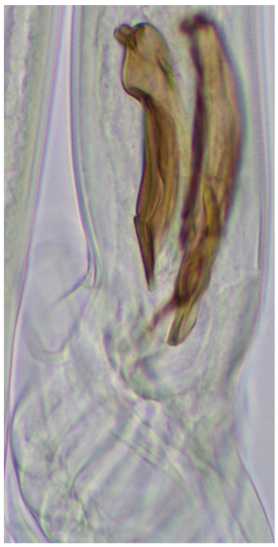
Cooperia punctata



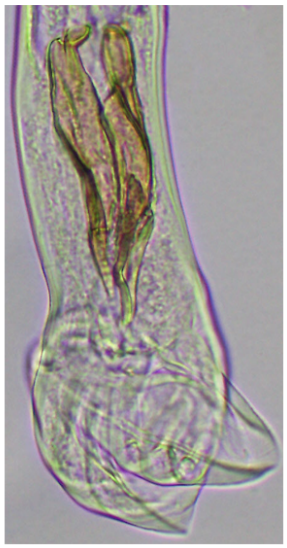
Camelostrongylus mentulatus



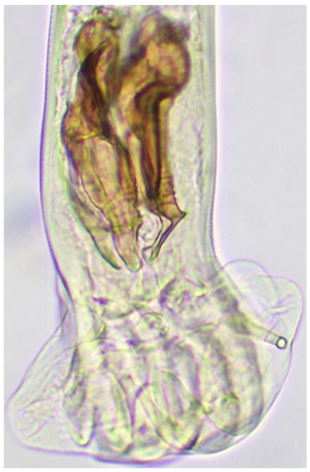
Graphinema auchenia



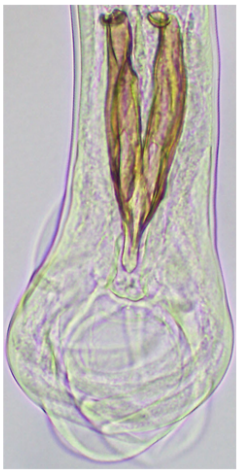
Trichostrongylus axei



Trichostrongylus colubriformis



Trichostrongylus rugatus



Trichostrongylus vitrinus

Fig 7.1 (continued)

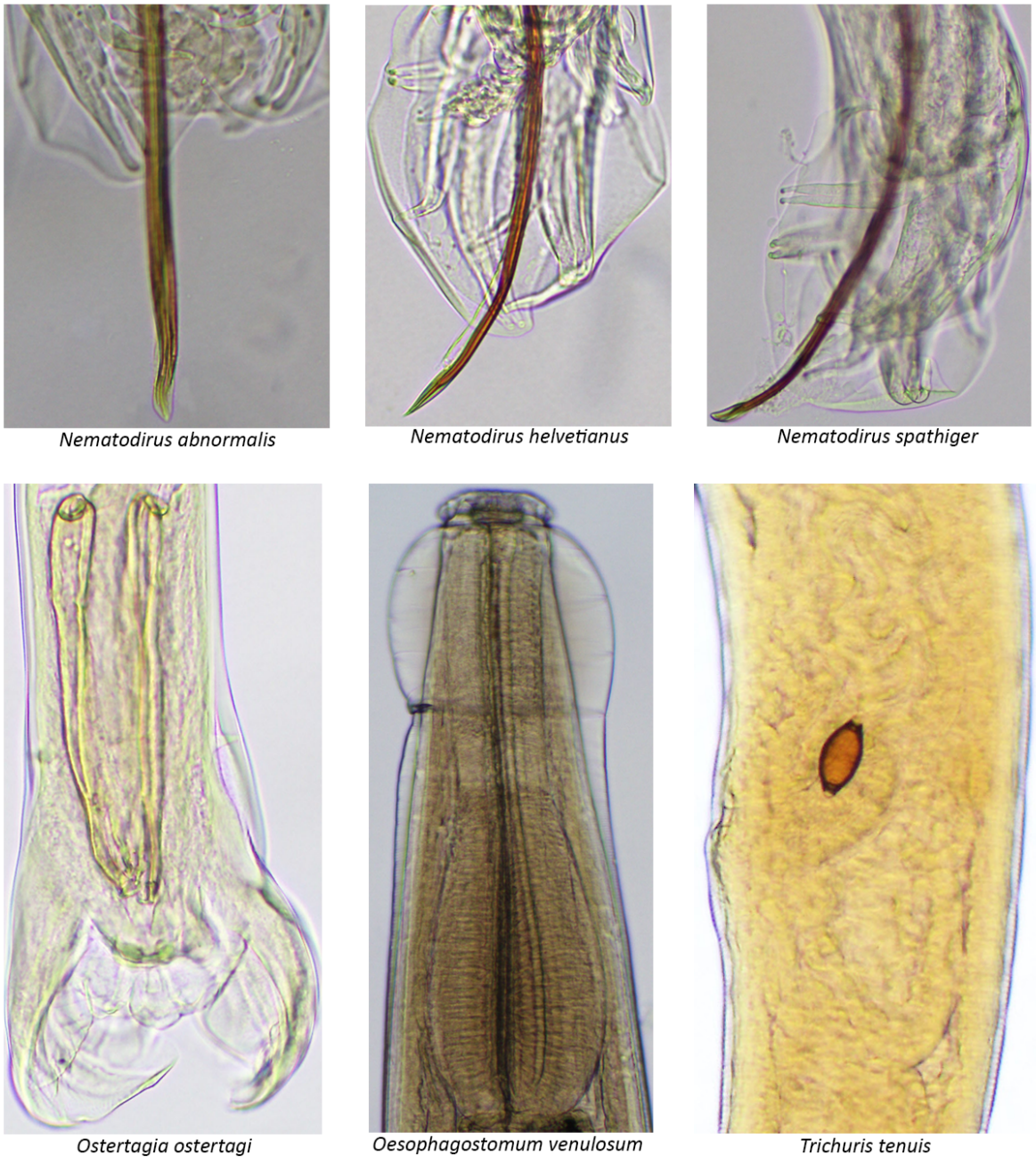


Fig 7.1

Fig. 7.1. Gastrointestinal nematodes found in Australian alpacas based on morphological examination of spicules of male worms.

The nematode species identified from the C3 herein have been previously documented in alpacas and llamas apart from *G. auchenia* which has not been reported outside South American countries (Ballweber, 2009). *Haemonchus contortus* was found to be the cause of death in alpacas in Australia (Jabbar et al., 2013). The prevalence of *C. mentulatus* was 60% and 48% using morphological and molecular techniques, respectively. Similar prevalences of *C. mentulatus* were observed in cross-sectional (69%) and longitudinal (68%) surveys of GINs in Australian alpacas (see Chapters 3 and 4). *Camelostrongylus mentulatus* has been found in sheep, feral goats and also in camels in Australia (Beveridge et al., 1974, 1987; Beveridge and Ford, 1982; Copland, 1965; Rogers, 1939). In this study, the prevalence of *C. mentulatus* in alpacas is being reported for the first time in Australia.

In this study, the prevalence of *O. ostertagi* by morphology (3%, 3/97) was lower compared with MT-PCR (31%, 27/86). There was only a slight agreement between two methods which may be due to very low abundance of this species. The prevalence of *Te. circumcincta* (1-3%) found herein is similar to that (3%) was found in a recent cross-sectional study (see Chapter 5) but lower than that (8%) reported in the longitudinal epidemiological studies on GINs of alpacas in Australia (see Chapter 6).

The morphology of the mucosa of the C3 was examined during the recovery of adult worms. The mucosa of the C3 was studded with pea-sized nodules with no worms protruding from the nodules (Fig. 7.2A). Histopathological examination of the lesions revealed multifocal areas of moderate inflammation in the lamina propria, consisting of eosinophils, lymphocytes and a smaller number of neutrophils (Fig. 7.2B, 7.2C). Sections of nematodes were present in the lumen of the C3 (Fig. 7.2B), and histopathological lesions were consistent with the high abundance of *C. mentulatus* in the same animals. Previously, in Britain, the presence of higher numbers of *C. mentulatus* in an alpaca was associated with comparable lesions in the C3 (Welchman et al., 2008). Similar lesions were also observed in a llama with a relatively low number of *C. mentulatus* (Rickard, 1993). The available data suggest that *C. mentulatus* caused these lesions in the C3 of alpacas. Hilton et al., (1978) studied the pathogenicity of *C. mentulatus* in sheep and found various histopathological changes such as 2-3 mm sized white nodules, scattered over the surface of the abomasum 3-4 days after infection. Light to moderate infiltration of mononuclear and polymorphonuclear cells in the lamina propria was also observed. Findings in sheep and in alpacas and llamas suggest that *C. mentulatus* may be responsible for producing the histopathological changes found in this study.

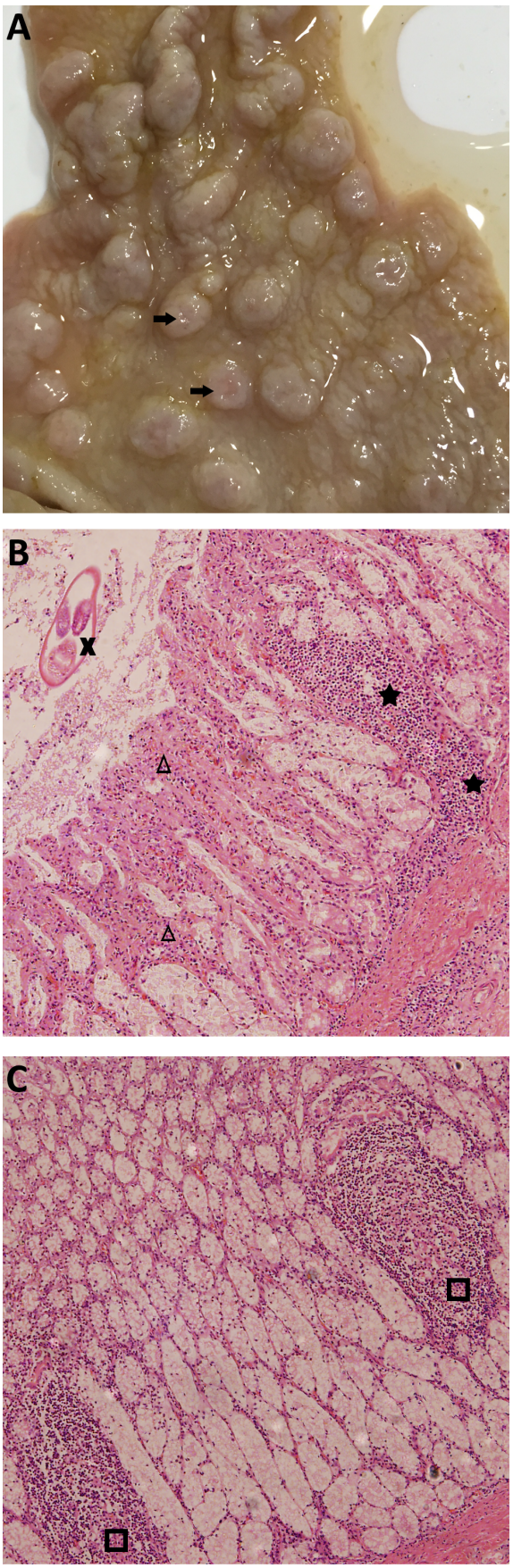


Fig. 7.2. Gross and histopathological changes in the third compartment of the stomach (C3) of alpacas caused by gastrointestinal nematodes. (A) Multiple raised nodular lesions ("Morocco leather" appearance) on the mucosal surface of the C3 (black arrow) caused by high numbers of *Camelostrongylus mentulatus*. (B) Mucosal surface of the C3 showing a transverse section of a nematode (cross), mucosal and crypt necrosis (triangle) and mononuclear cellular infiltrations (star). (C) Histological section of tumour like lesion of the third compartment showing aggregation of mononuclear inflammatory cells (rectangle).

In the small intestine, three species of *Trichostrongylus* spp., four species of *Nematodirus* spp. and three species of *Cooperia* spp. were identified (Table 7.2). These species, except *C. pectinata,* have previously been identified in SACs from Australia (Carmichael, 1999) and elsewhere (Ballweber, 2009). *Trichostrongylus rugatus, T. colubriformis* and *T. vitrinus* occur in sheep in Australia (Beveridge and Ford, 1982; Beveridge et al., 1989), and in this study, they were also encountered in the small intestine of alpacas. Few reports of occurrence of the *Nematodirus* spp. in alpacas are available. The prevalence of *Nematodirus* spp. (33%) found herein was lower than that reported from the UK (93%) in a necropsy of an alpaca (Tait et al., 2002). Three different species of *Cooperia* (*C. oncophora*, *C. punctata* and *C. pectinata*) found in this study, have been reported previously in alpacas from Peru (Contreras et al., 2014) and New Zealand (Hill et al., 1993). This study also found *Capillaria* type eggs in the faecal samples of alpacas for the first time, but no adults of this genus were encountered. *Capillaria* has previously been reported in SACs in the UK (Tait et al., 2002) and Japan (Hyuga and Matsumoto, 2016).

In this study, *Tr. tenuis* and *Oe. venulosum* were recovered from the large intestine. Previous studies have reported the occurrence of these species in SACs (Ballweber, 2009). The prevalence of *Trichuris* in this study was 6%. *Trichuris* has been found in SACs in the USA, UK and Argentina (Rickard and Bishop, 1991; Cafrune et al., 1999; Tait et al., 2002). *Oesophagostomum venulosum* has been recovered from alpacas in New Zealand (Hill et al., 1993).

A total of 97 individual faecal samples was processed for FECs. The mean FEC for 97 samples was 501 EPG, with the highest count of 3,495 eggs in one alpaca (Table 7.1) which is higher than recently found in cross-sectional (291 EPG) and longitudinal (168 EPG) studies of GINs in Australian alpacas (see Chapters 5 and 6). It was also higher than levels reported from other countries (Tait et al., 200; Dittmer et al., 2018; Hertzberg and Kohler, 2006). There was a low correlation (r = 0.23) between faecal egg counts and total worm count. Earlier studies have shown a moderate to high relationship between FEC and worm burden (Bryan and Kerr, 1989; McKenna, 1981; Roberts, 1957; Thomas and Boag, 1973) (for fecund genera of nematodes such as *Haemonchus* spp. and *Cooperia* spp.) whereas other studies found poor correlation between FEC and worm burden (Brunsdon, 1971; Michel, 1969a, b; Rose and Small, 1980; Rubin, 1967) (for less fecund genera such as *Nematodirus* spp., *Oesophagostomum* spp., *O. ostertagia* and *Trichostrongylus axei*).

7.5. Conclusions

This study documented 19 different species of nematodes in Australian alpacas, some for the first time. Overall, the burden of GINs in Australian alpacas was moderate, with the highest burden of 28,640 worms in one alpaca. This study also showed that molecular techniques (MT-PCR) can be used to identify the most important GINs.

7.6. References

Altman, D.G. (1991). Practical Statistics for Medical Research. London: Chapman and Hall.

Anderson, N. (1972). Trichostrongylid infections of sheep in a winter rainfall region. 1. Epizootiological studies in the Western District of Victoria, 1966-67. *Australian Journal of Agricultural Research,* 23, 1113-1129.

Anderson, N. (1973). Trichostrongylid infections of sheep in a winter rainfall region. 2. Epizootiological studies in the Western District of Victoria, 1967-68. *Australian Journal of Agricultural Research,* 24, 599-611.

Averbeck, G.A., Scholtthauer, J.C., & Hinueber, J.G. (1981). *Camelostrongylus mentulatus* infection in a camel (*Camelus dromedarius*): a case report. *Journal of Zoo Animal Medicine,* 12, 24-26.

Ballweber, L.R. (2009). Ecto- and endoparasites of new world camelids. *Veterinary Clinics of North America: Food Animal Practice,* 25, 295-310.

Barger, I.A. (1993). Control of gastrointestinal nematodes in Australia in the 21st century. *Veterinary Parasitology,* 46, 23-32.

Becklund, W.W., & Walker, M.L. (1967). *Nematodirus* of domestic sheep, *Ovis aries*, in the United States with a key to the species. *Journal of Parasitology,* 53, 777-781.

Beldomenico, P.M., Uhart, M., Bono, M.F., Marull, C., Baldi, R., & Peralta, J.L. (2003). Internal parasites of free-ranging guanacos from Patagonia. *Veterinary Parasitology,* 118:71–77

Beveridge, I., Barker, I., Rickard, M., & Burton, J. (1974). Experimental infection of sheep with *Camelostrongylus mentulatus* and associated gastritis. *Australian Veterinary Journal,* 50, 36-37.

Beveridge, I., & Ford, G.E. (1982). The trichostrongyloid parasites of sheep in South Australia and their regional distribution. *Australian Veterinary Journal,* 59, 177-179.

Beveridge, I., Pullman, A.L., Henzell, R., & Martin, R.R. (1987). Helminth parasites of feral goats in South Australia. *Australian Veterinary Journal,* 64, 111-112.

Beveridge, I., Pullman, A.L., Phillips, P.H., Martin, R.R., Barelds, A., & Grimson, R. (1989). Comparison of the effects of infection with *Trichostrongylus*

colubriformis, *T. vitrinus* and *T. rugatus* in Merino lambs. *Veterinary Parasitology*, 32, 229-245.

Brunsdon, R.V. (1971). Trichostronglye worm infection in cattle: further studies on problems of diagnosis and on seasonal patterns of occurrence. *New Zealand Veterinary Journal,* 19, 203-212.

Bryan, R., & Kerr, J. (1989). The relation between the natural worm burden of steers and the faecal egg count differentiated to species. *Veterinary Parasitology,* 30, 327-334.

Cafrune, M.M., Aguirre, D.H., & Rickard, L.G. (1999). Recovery of *Trichuris tenuis* Chandler, 1930, from camelids (*Lama glama* and *Vicugna vicugna*) in Argentina. *Journal of Parasitology,* 85, 961-962.

Carmichael, I.H. (1999). Internal parasitism in alpacas in southern Australia. In: Australian Alpaca Fibre – Improving Productivity and Marketing. *Rural Industries Research and Development Corporation, Canberra,* pp. 92-130.

Chandler, A.C. (1930). Specific characters in the genus Trichuris, with a description of a new species, *Trichuris tenuis*, from a camel. *Journal of Parasitology,* 16, 198-206.

Contreras, S., Chávez, V., Pinedo, V., Leyva, V., & Suárez, A. (2014). Helminthiasis in alpacas (*Vicugna pacos*) of two peasant communities in Macusani, Puno during the dry season. *La Revista de Investigaciones Veterinarias del Perú es,* 25, 268-275.

Copland, J.W. (1965). *Ostertagia mentulata* recorded in New South Wales. *Australian Veterinary Journal,* 41, 27-27.

Dittmer, K.E., Hinkson, J.A., Dwyer, C., Adlington, B., & van Andel, M. (2018). Prevalence of Candidatus *Mycoplasma haemolamae*, bovine viral diarrhoea virus, and gastrointestinal parasitism in a sample of adult New Zealand alpaca (*Vicugna pacos*). *New Zealand Veterinary Journal,* 66, 9-15.

Gibbons, L.M., & Khalil, L.F. (1982). A key for the identification of genera of the nematode family Trichostrongylidae Leiper, 1912. *Journal of Helminthology,* 56, 185-233.

Gordon, H.M., & Whitlock, H. (1939). A new technique for counting nematode eggs in sheep faeces. *Journal of Council of Scientific and Industrial Research,* 12, 50-52.

Hertzberg, H., & Kohler, L. (2006). Prevalence and significance of gastrointestinal helminths and protozoa in South American camelids in Switzerland. *Berliner und Münchener Tierärztliche Wochenschrift,* 119, 291-294.

Hill, F., Death, A., & Wyeth, T. (1993). Nematode burdens of alpacas sharing grazing with sheep in New Zealand. *New Zealand Veterinary Journal,* 41, 205-208.

Hilton, R.J., Barker, I.K., & Rickard, M.D. (1978). Distribution and pathogenicity during development of *Camelostrongylus mentulatus* in the abomasum of sheep. *Veterinary Parasitology,* 4, 231-242.

Hutchinson, G. (2009). Nematode parasites of small ruminants, camelids and cattle: diagnosis with emphasis on anthelmintic efficacy and resistance testing. Aquatic and Terrestrial Australian and New Zealand Standard Diagnostic Procedures (ANZSDPs).
http://www.agriculture.gov.au/animal/health/laboratories/procedures/anzsdp

Hyuga, A., & Matsumoto, J. (2016). A survey of gastrointestinal parasites of alpacas (*Vicugna pacos*) raised in Japan. *The Journal of Veterinary Medical Science,* 78, 719-721.

Jabbar, A., Campbell, A.J.D., Charles, J.A., & Gasser, R.B. (2013). First report of anthelmintic resistance in *Haemonchus contortus* in alpacas in Australia. *Parasites and Vectors,* 6, 243.

McKenna, P.B. (1981). The diagnostic value and interpretation of faecal egg counts in sheep. *New Zealand Veterinary Journal,* 29, 129-132.

Michel, J.F. (1969a). Observations on the faecal egg count of calves naturally infected with *Ostertagia ostertagi*. *Parasitology,* 59, 829-835.

Michel, J.F. (1969b). The regulation of egg output by *Ostertagia ostertagi* in calves infected once only. *Parasitology,* 59, 767-774.

Presidente, P.J.A. (2007). Alpaca parasites & their control: recent experiences. *Australian Alpaca Veterinarians Annual Conference, Australia*, p 1-14.

R Core Team, (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria. https://www.R-project.org/

Rickard, L.G., Bishop, J.K. (1991). Redescription of *Trichuris tenuis* Chandler, 1930, from llamas (*Lama glama*) in Oregon with a key to the species of *Trichuris* present in North American ruminants. *Journal of Parasitology,* 70-75.

Rickard, L.G. (1993). Parasitic gastritis in a llama (*Lama glama*) associated with inhibited larval *Teladorsagia* spp. (Nematoda: Trichostrongyloidea). *Veterinary Parasitology,* 45, 331-335.

Roberts, F. (1957). Reactions of calves to infestation with the stomach worm, *Haemonchus placei* (Place, 1893) Ransom, 1911. Australian Journal of Agricultural Research, 8, 740-767.

Roeber, F.H.S, Jex, A.R., & Gasser, R.B. (2013). Comparative evaluation of two DNA isolation techniques for PCR-based diagnosis of gastrointestinal nematode infections in sheep. *Molecular and Cellular Probes*, 27, 153-157.

Rogers, W.P. (1939). Nematode parasites of sheep in Western Australia. *Journal of Helminthology,* 17, 151-158.

Rose, J.H., & Small, A.J. (1980). Transmission of *Oesophagostomum* spp. among sows at pasture. *Veterinary Record,* 107, 223-225.

Rubin, R. (1967). Some observations on the interpretation of fecal egg counts. *American Journal of Veterinary Clinical Pathology,* 1, 145-148.

Schmäschke, R. (2015). Endo-and ectoparasites of South American camelids and their control. *Tierärztliche Praxis. Ausgabe G, Grosstiere/ Nutztiere ,* 43, 169-179.

Stevenson, M., Nunes, T., Heuer, C., Marshall, J., Sanchez, J., Thornton, R., Reiczigel, J., Robison-Cox, J., Sebastiani, P., Solymos, P., Yoshida, K., Jones, G., Pirikahu, S., Firestone, S., Kyle, R., Popp, J., & Mathew, J. (2018). epiR: Tools for the Analysis of Epidemiological Data. Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Melbourne, Australia.

Tait, S.A., Kirwan, J.A., Fair, C.J., Coles, G.C., & Stafford, K.A. (2002). Parasites and their control in South American camelids in the United Kingdom. *Veterinary Record,* 150, 637-638.

Thomas, R.J., & Boag, B. (1973). Epidemiological studies on gastro-intestinal nematode parasites of sheep. The control of infection in lambs on contaminated pasture. *Research in Veterinary Science,* 14, 11-20.

Welchman, D.d.B., Parr, J., Wood, R., Mead, A., & Starnes, A. (2008). Alpaca and llama nematodes in Britain. *Veterinary Record,* 162, 832-832.

CHAPTER 8 ANTHELMINTIC RESISTANCE IN GASTROINTESTINAL NEMATODES OF ALPACAS (*Vicugna pacos*) IN AUSTRALIA

8.1. Abstract

Gastrointestinal nematodes (GINs) can cause significant economic losses in alpacas due to lowered production of fibre and meat. Although no anthelmintics are registered for use in alpacas, various classes of anthelmintics are frequently used to control parasitic gastroenteritis in alpacas in Australia and other countries. Very little is known about the current worm control practices as well as the efficacy of anthelmintics used against common GINs of alpacas. This study aimed to assess the existing worm control practices used by Australian alpaca farmers and to quantify the efficacy of commonly used anthelmintics against GINs of alpacas. An online questionnaire survey was conducted to assess current worm control practices on 97 Australian alpaca farms, with an emphasis on the use of anthelmintics. Of this group of 97 alpaca farms, 20 were selected to assess the efficacy of eight anthelmintics and/or their combinations (closantel, fenbendazole ivermectin, monepantel, moxidectin and a combination of levamisole, closantel, albendazole, abamectin) using the faecal egg count reduction test (FECRT). A multiplexed-tandem PCR (MT-PCR) was used to identify the prevalent nematode genera/species. The response rate for the questionnaire was 94% (91/97). Almost half of the respondents kept alpacas with sheep and cattle, and 26% of respondents allowed alpacas to co-graze with these ruminants. Although only 63% respondents perceived worms to be an important health concern for alpacas, the majority of respondents (89%) used anthelmintics to control GINs of alpacas. The commonly used anthelmintics were macrocyclic lactones, monepantel, benzimidazoles, levamisole, closantel and their combinations, and they were typically administered at the dose rate recommended for sheep. The FECRT results showed that a combination of levamisole, closantel, albendazole and abamectin was the most effective dewormer followed by single drugs, including monepantel, moxidectin, closantel, fenbendazole and ivermectin. *Haemonchus* spp. were the most commonly resistant nematodes followed by *Trichostrongylus* spp., *Camelostrongylus mentulatus*, *Ostertagia ostertagi* and *Cooperia* spp. This is the first study aimed at assessing worm control practices and efficacy of commonly used

anthelmintics in alpacas in Australia. The findings of the current study document the extent of anthelmintics resistance on Australian alpaca farms and identify those anthelmintics that are still effective against GINs of alpacas.

8.2. Introduction

In the last three decades, the farming of domesticated South American Camelids (SACs), alpacas (*Vicugna pacos*) and llamas (*Lama glama*) has increased in Australia, Europe, New Zealand, the UK and the USA, due to their high-quality fibre and adaptability to many climatic conditions (Gerken and Renieri, 2004; Jabbar et al., 2013). In an intensive farming system, alpacas and llamas can be infected with both shared (those common in domestic ruminants; e.g., *Haemonchus contortus*, *Ostertagia ostertagi*, *Trichostrongylus* spp., and *Nematodirus* spp.) as well as host-specific (e.g., *Lamanema chavezi*) gastrointestinal nematodes (GINs) (Rickard, 1994; Ballweber, 2009; Franz et al., 2015) that can cause significant clinical and subclinical problems, resulting in economic losses from lowered production of fibre, meat and/or leather (Leguia, 1991; Windsor et al., 1992; Rickard, 1993; Ballweber, 2009; Franz et al., 2015). Outside South America, knowledge on the parasites of SACs is limited.

Traditionally, the use of chemotherapeutic agents has been the most commonly used method to treat and control GINs of domestic ruminants. Similarly, farmers regularly use various classes of anthelmintics to control GINs in alpacas and llamas (Ballweber, 2009; Franz et al., 2015), although no anthelmintic is registered for use against GINs in SACs in Australia. Given that very little is known about the pharmacokinetics of drugs in SACs (Hunter et al., 2004), the off-label use of anthelmintics in alpacas registered for domestic ruminants at different dose rates recommended for goats, sheep and cattle is commonplace. However, the dose rate(s) and route(s) of administration recommended for sheep might not be effective against GINs in SACs (Dadak et al., 2013) as found previously in goats (Hennessy et al., 1993). Thus, under-dosing of anthelmintics may promote the development of anthelmintic resistance (AR) in GINs in SACs as under-dosing is known to be one of the risk factors for the development of AR in GINs of sheep (Falzon et al., 2014). Case reports of AR in GINs of SACs have been reported from Australia (Jabbar et al., 2013), Belgium (Sarre et al., 2012), Canada (Galvan et al., 2012) and the USA (Gillespie et al., 2010) in various GINs against two commonly used classes of anthelmintics, benzimidazoles and macrocyclic lactones.

Australia has the largest alpaca population (>450,000) outside South America (Clarke et al., 2018) and the Australian alpaca industry is an important emerging livestock industry. However, very little is known about the epidemiology and control of GINs in alpacas in Australia. Recently, the first case of ivermectin resistance in the Barber's pole worm (*Haemonchus contortus*) was reported (Jabbar et al., 2013). A survey of the worm control practices used by Australian alpaca farmers revealed that the dose of anthelmintics used for alpacas (e.g., one to three times of dose recommended for sheep) and the existence of other potential risk factors for the development of AR known for GINs of sheep, goats and cattle, could lead to the development of AR in GINs of alpacas.

The aims of this study were (i) to undertake a questionnaire survey to obtain insights into farm-level characteristics that might be associated with the development of AR in GINs of alpacas and (ii) to quantify the efficacy of commonly used anthelmintics against GINs of alpacas in Australia.

8.3. Materials and methods

8.3.1. Study population

Australia has various climatic zones, and alpaca farming in Australia mainly occurs in four zones, the Mediterranean, non-seasonal rainfall, summer rainfall and winter rainfall. Most alpaca herds are located in the south-eastern states of New South Wales and Victoria, with fewer in Queensland, Western Australia, South Australia and Tasmania. The majority of alpaca farms contain ≤ 50 animals, which graze year-round on pastures, with variable provision of supplementary feed. Alpacas are routinely vaccinated against clostridial diseases (caused by *Clostridium perfringens* type D, *C. tetani*, *C. novyi* type B, *C. septicum* and *C. chauvoei*). They are generally shorn once annually in spring, although at variable times throughout the year. Timing and duration of the birthing periods vary between farms but often occur for about two months in spring. Crias are weaned at an average age of three months.

8.3.2. Questionnaire survey

The survey aimed to assess current worm control practices of Australian alpaca farmers, with an emphasis on the use of anthelmintics. A questionnaire was conducted

using an online programme, Research Electronic Data Capture (Harris et al., 2009). The questionnaire contained 30 questions about (i) farm demography and general husbandry practices, (ii) the use of anthelmintics, and (iii) grazing management. The majority of questions were close-ended, with a few semi-open (i.e., a close-ended question with the addition of a category "other'). An online questionnaire survey was sent to 97 alpaca farmers who had responded to a larger survey on more general aspects of alpaca husbandry, worm problems and parasite management in Australia.

8.3.3. Selection of farms

Out of 91 alpaca farms that responded to the survey, 20 farms were selected to take part in faecal egg count reduction testing (FECRT) based on herd size and the geographical location of their herd (Fig. 8.1). The following selection criteria were used: (i) the herd was comprised of between 40 and 60 alpacas of different ages and sexes; (ii) deworming had not been carried out within the 8 weeks prior to the scheduled herd visit; (iii) confirmation that average faecal egg counts (FEC) were greater than or equal to 150 eggs per gram (EPG) of faeces; and (iv) there was a history of anthelmintic usage on the farm in the last five years. When a farmer agreed to participate and met the first two criteria, faecal samples were collected from fifteen randomly selected alpacas and tested for FEC. Over 50 farms were tested to obtain 20 suitable farms for the FECRT trial.

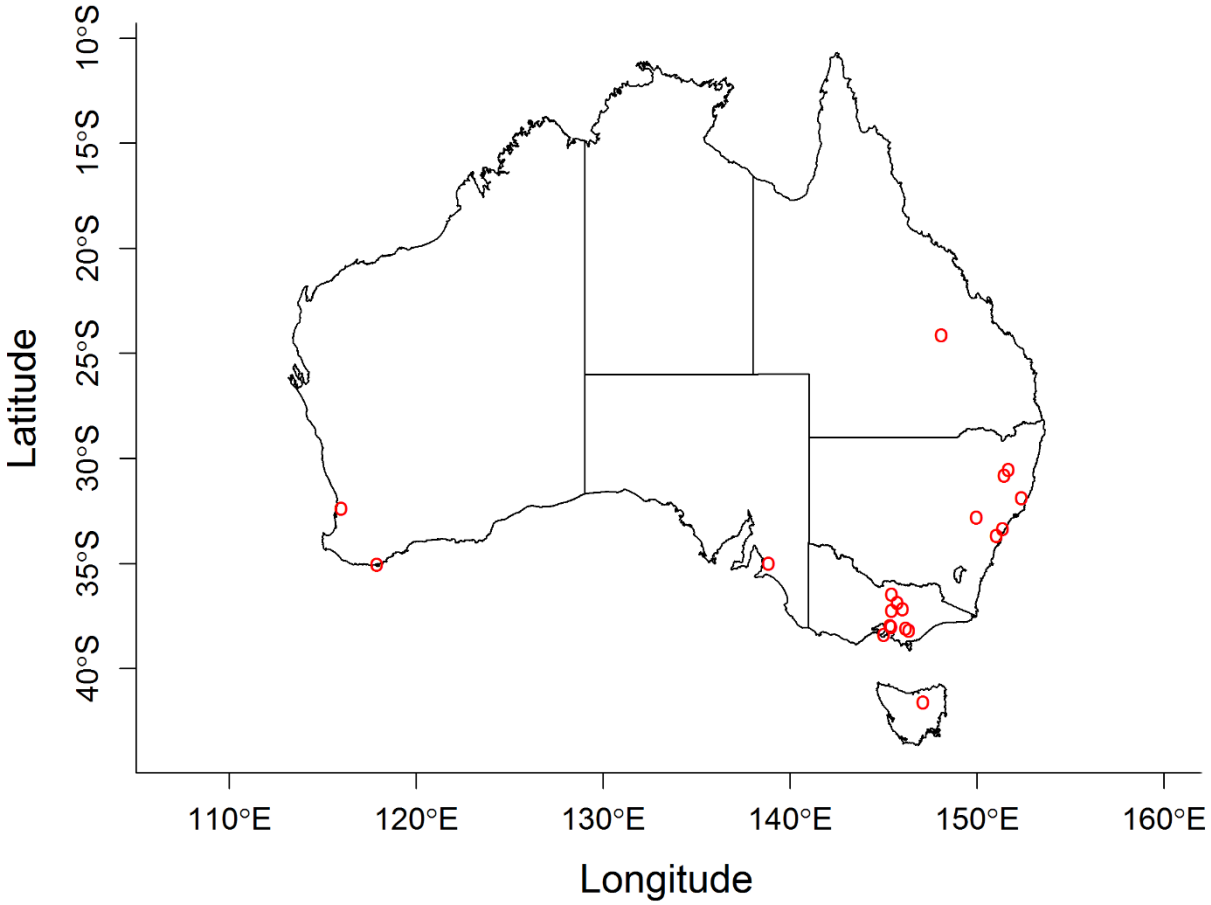


Fig. 8.1. Map of Australia showing the locations of alpaca farms enrolled in the faecal egg count reduction trials in this study. Each circle represents one alpaca farm.

8.3.4. Faecal egg count reduction test (FECRT)

The FECRT was performed on each farm according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for the evaluation of anthelmintic efficacy in ruminants (Coles et al., 1992; Wood et al., 1995). Both female and male - Huacaya and Suri alpacas, aged 3-months to 16-years were randomly selected on each farm and allocated to five or six groups (anthelmintic treatment groups and an untreated control group) comprising 5-15 animals. Six anthelmintics were evaluated in this study: (i) monepantel (Zolvix®, Elanco Pty. Ltd., Australia), (ii) a combination of levamisole, closantel, albendazole and abamectin (Q-drench®, Jurox Pty. Ltd., Australia), (iii) closantel with sodium selenate (Closicare Plus Selenium®, Virbac Pty. Ltd., Australia), (iv) ivermectin (Ivomec®, Boehringer Ingelheim Pty. Ltd., Australia), (v) moxidectin (Cydectin® Injection for cattle, Virbac) and (vi) fenbendazole (Panacur 25®, Intervet Pty. Ltd., Australia). Resistance to ivermectin was found up to the 9th FECR trial. Hence, it was decided to replace ivermectin with a potent macrocyclic lactone in subsequent FECR trials. All anthelmintics were administered orally apart from moxidectin (subcutaneously) at 1.5 times the dose rate recommended for sheep. Animals were dosed individually based on body weight using scales where available

Individual faecal samples were collected from the rectum pre- (day 0) and post-treatment (day 11-14) into zip-lock plastic bags, and were kept at 4°C until processed for FEC using a modified McMaster method (see Chapter 3) within seven days of collection. Briefly, four grams of faeces were mixed with 11 mL of water added into a 60 mL container and soaked for 5-30 minutes before making a homogenized faecal slurry. Saturated sugar (specific gravity 1.27) solution (45 mL) was added and 30-45 minutes later, the sample was agitated, and a sample drawn immediately from the suspension using a sieve-top pipette (sieve aperture size 12 meshes per cm), to fill two chambers of a Whitlock egg counting slide (www.whitlock.com.au). After five minutes, the slide was placed on the stage of a compound light microscope and eggs were counted. The sensitivity of the McMaster technique was 15 EPG.

8.3.5. Identification of nematodes

A newly established molecular diagnostic kit (Easy-Plex, AusDiagnostics Pty. Ltd., Beaconsfield, Australia) has been used for the identification of common GINs of alpacas (see Chapter 4). Faecal DNA was extracted using a method described previously with few modifications (Roeber et al., 2013). Pre- and post-treatment faecal samples for each group were pooled in order to obtain one DNA sample representing 5-15 individual faecal samples per group. This was achieved during processing of individual faecal samples for FEC by withdrawing 1 mL of the faeces suspension in the saturated sugar solution to a 50 mL Falcon tube. This process was repeated for each sample per treatment group. Finally, the Falcon tube was filled by adding more flotation solution to make 50 mL. Following centrifugation (2500 rpm, 10 min), the supernatant (~5 mL) was collected and transferred to another 50 mL Falcon tube. To wash the GIN eggs collected in the Falcon tube, tap water was added to 50 mL and centrifuged (2500 rpm, 10 min). The supernatant was discarded, and the pellet was washed twice more, using the same steps as above. The washed pooled eggs with remaining faecal materials were transferred into a microcentrifuge tube and stored at -20°C until further use. Following thawing, a 250 µL sample of the concentrated faecal material was used to extract and isolate DNA using Powersoil® DNA purification kit (MoBio, USA) as per manufacturer's protocol.

The assay was conducted in the *High*-Plex 24 system with the MT-Assay Setup Software for the first round of PCR and the 96-well MT-Analyzer and the MT Analysis Software (Cat. No. 9150, AusDiagnostics) for the second round of PCR. The primary amplification ('target enrichment') was conducted using nematode-specific primer pairs designed for the sequences of the Internal Transcribed Spacer-2 (ITS-2) (Step 1 tubes for nematodes [8-well], Cat. No. 78150S, AusDiagnostics). The secondary amplification for semi-quantification employed nested primer pairs to the internal regions of the ITS-2 (Alpaca Nematodes MP96 8-well, Cat. No. 78150E, AusDiagnostics) specific to *Camelostrongylus mentulatus*, *Cooperia* spp., *Haemonchus* spp., *Ostertagia ostertagi*, *Oesophagostomum* spp., *Teladorsagia circumcincta* and *Trichostrongylus* spp. These internal primer pairs amplify a region of ~90 to 110 bp from the ITS-2 region. Furthermore, another primer pair was included in each run as a reference to assess the efficiency of amplification from 10,000 copies of a synthetic oligonucleotide template (internal 'spike control').

For primary amplification (15 cycles of 10 s at 95 °C, 20 s at 60 °C, and 20 s at 72 °C), 5 µL of genomic DNA representing each DNA sample or 5 µL of water (negative control) were dispensed into 0.2 ml PCR strips and placed into a 24-well thermocycling block in the *High*-Plex 24 system (AusDiagnostics). Subsequently, the analysis was executed by the program MT-Assay Setup Software (AusDiagnostics). Following the first round of PCR, the secondary amplification and the melting curve analysis were performed in 96-well MT-Analyzer using the MT Analysis Software (AusDiagnostics). Each sample was recorded as test-positive using the auto-call function of the Easy-Plex software (AusDiagnostics) if the amplicon produced a single melting curve which was within 1.5°C of the expected melting temperature, the height of the peak was higher than 0.2 dF/dT (where dF/dT is the derivative of fluorescence over temperature), and the peak width was ≤ 3.5°C, otherwise the sample was considered as negative (AusDiagnostics). Additionally, cycle threshold (Ct) values for each nematode per sample were determined by comparing with the data obtained from the internal spike control which had a known 10,000 DNA copy numbers. Based on the peak high-resolution melting (HRM) temperature analyses, nematode genera/species were assigned according to their mean HRM temperatures. Randomly selected amplicons representing each nematode genus/species were subjected to sequencing to verify the target nematodes.

8.3.6. Statistical analyses

Questionnaire data were downloaded from REDCap as a comma-separated values (CSV) file. Data validation and cleaning were performed by using Microsoft Excel 2013. Calculation of faecal egg count reduction (FECR) was performed using the contributed R package “eggsCount” (Torgerson et al., 2014) and following the WAAVP guidelines (Coles et al., 1992; Falzon et al., 2014). FECR (%) was calculated between treatment and control group at post-treatment collection for each anthelmintic. Faecal egg count data is generally over-dispersed and inherit Poisson errors, which was incorporated in the R package “eggsCount” (Falzon et al., 2014). Thus, it has advantage over the Excel spreadsheet to calculate FECR more precisely by taking the inherent errors of faecal egg count data into account.

8.3.7. Interpretation of the FECRT results

Anthelmintic resistance status was interpreted as recommended by the WAAVP guidelines on AR based on the percentage of faecal egg count reduction (%FECR) and the upper (UCL) and lower (LCL) 95% confidence limits (Wood et al., 1995). Hence, each anthelmintic was declared as (i) effective when the %FECR and UCL were both ≥ 95% and the LCL was ≥ 90%, (ii) suspected resistant when %FECR was < 95% or LCL was < 90%, and (iii) ineffective/resistant when both %FECR was < 95% and LCL was < 90%. Furthermore, multiple AR was declared when parasite populations of GINs were identified, using the above criteria, to be resistant to anthelmintics of different chemical classes (James et al., 2009).

8.4. Results

8.4.1. Questionnaire survey

The response rate for the questionnaire was 94% (91/97). Huacaya was the more popular alpaca breed, with an average herd size of 77 (minimum 9; maximum 600) alpacas. About half of the respondents (51%, 46/91) kept alpacas with other livestock species such as sheep and cattle, and 26% (24/91) of alpaca farmers allowed their animals to co-graze with other domestic ruminants (Table 8.1). Twenty five percent (23/91) of respondents had agisted non- home-bred alpacas on their farms at least once during the last five years. Although 63% (57/91) of respondents reported that worms were an important health issue for their alpacas, the majority of respondents (89%, 81/91) used anthelmintics for the control of GINs in their animals (Table 8.1). The commonly used anthelmintics were macrocyclic lactones (e.g. ivermectin, moxidectin; 65% [53/81] respondents), monepantel (31%, 25/81), benzimidazoles (BZs) (20%, 16/81), levamisole (LEV) (15%, 12/81), closantel (10%, 8/81), and their combinations, including two (BZ and MLs, 7% [6/81]), three (BZ, LEV and MLs, 9% [7/81]) or four (closantel, BZ, LEV and MLs, 37% [29/81]) anthelmintics (Table 8.1). The majority of respondents (53%, 43/81) used these anthelmintics at the dose rate recommended for sheep, though some used 1.5 times the dose rate recommended for sheep (23%, 19/81) or the dose rate recommended for cattle (10%, 8/81). The use of anthelmintics seemed to be need-based (62%, 50/81) rather than a part of any strategic deworming program twice (25%, 20/81)

or once per year (12%, 10/81). Alpacas were dewormed either before winter (43%, 35/81), at shearing (36%, 29/81) or at weaning (32%, 26/81) (Table 8.1). The majority of respondents (60%, 49/81) were using one class of anthelmintics for more than a year, and most of them (72%, 58/81) said that they changed ('rotated') drench classes in the last five years. A relatively small proportion of respondents (12%, 11/91) reported that they tested the efficacy of anthelmintics in use on their farms using FECRT (Table 8.1).

Table 8.1. Worm control practices used by Australian alpaca farms included in this study.

Worm control factor	No. of responses	Responses (%)
Keeping alpacas with other livestock species	46/91	51
Co-grazing of alpacas with other livestock species	24/91	26
Keeping agisted alpacas	23/91	25
Worms is an important health issue of alpacas	57/91	63
Anthelmintic usage	81/91	89
Types of anthelmintics used		
Macrocyclic lactones (MLs)	53/81	65
Monepantel	25/81	31
Benzimidazole (BZ)	16/81	20
Levamisole (LEV)	12/81	15
Closantel	8/81	10
Double combinations (BZ and LEV)	6/81	7
Triple combinations (BZ, LEV and ML)	7/81	9
Q-Drench (a combination of levamisole, closantel, albendazole, abamectin)	29/81	37
Deworming time		
Before winter	35/81	43
At shearing	29/81	36
At weaning	26/81	32
Dose rate		
Sheep dose	43/81	53
1.5 times sheep dose	19/81	23
Cattle dose	8/81	10
Testing of anthelmintics for efficacy (e.g. FECRT)*	11/91	12
Rotation of anthelmintics	58/81	72

*FECRT, faecal egg count reduction test.

8.4.2. Faecal egg count reduction tests

The FECRT results revealed that a combination of levamisole, closantel, albendazole and abamectin was the most effective dewormer (78%, 14/18 susceptible farms), followed by monepantel (75%, 15/20), moxidectin (27%, 3/11), closantel (5%, 1/20), fenbendazole (0%, 0/19) and ivermectin (0%, 0/10) (Figs. 8.2 and 8.3; Table 8.2; see Table 8.3). The molecular identification of GINs from all herds of alpacas showed the presence of mixed GIN infections containing *C. mentulatus*, *Cooperia* spp., *Haemonchus* spp., *O. ostertagi*, *Oesophagostomum* spp. *T. circumcincta* and *Trichostrongylus* spp. *Haemonchus* spp. (49%) were the most commonly resistant nematodes followed by *Trichostrongylus* spp. (37%), *C. mentulatus* (28%), *O. ostertagi* (25%) and *Cooperia* spp. (13%) whereas *Oesophagostomum* spp. (100%) and *T. circumcincta* (99%) were susceptible to anthelmintics tested in this study (Figs. 8.4 and 8.5; see Table 8.4).

Ivermectin and moxidectin, the two compounds tested as single ML anthelmintics, were infrequently effective at the dose rates used in this study (Fig. 8.3; Table 8.2). On the 10 farms where ivermectin was tested, the FECR on six farms was 2-87% and there was no reduction in FEC on the remaining four farms (i.e. FECR -329 to -7%) (Fig 8.3; Table 8.2). Conversely, moxidectin was fully effective on three farms but was not effective on the remaining eight farms, with three farms having suspected resistance and the other five resistant GINs (Fig. 8.3; Table 8.2). BZ used as a single anthelmintic in this study was ineffective against GINs of alpacas on all farms, with FECR ranging from 8-94% on 13 farms, with an increase in EPG (FECR -522 to -18%) on six farms. Similarly, GINs were found to be susceptible to closantel on only one farm, while resistance and suspected resistance to this drug occurred on 15 (FECR of 29-93%) and four (FECR -194 to -13%) alpaca farms, respectively. Monepantel and a combination of anthelmintics were found to be the most effective drenches on alpaca farms in this study, with the majority of prevalent GINs ~100% susceptible to both anthelmintics (Fig. 8.3; Table 8.2). Multiple AR (when two or more anthelmintics from different classes are ineffective) was detected on all alpaca farms included in the study (Table 8.2)

Table 8.2. Faecal egg count reduction percentages (%FECR) and 95% confidence intervals (CI) calculated by the faecal egg count reduction test (FECRT) 10-14 days after anthelmintic treatments in alpacas naturally infected with gastrointestinal nematodes on 20 alpaca farms in Australia.

Farm no.	%FECR (95% CI) of anthelmintics					
	Monepantel	Q-drench*	Closantel	Fenbendazole	Ivermectin	Moxidectin
1	100	NT**	98 (93 – 100)	64 (-55 – 95)	42 (-270 – 91)	NT
2	98 (94 – 100)	NT	54 (-3 – 80)	-60 (-286 – 33)	-329 (-1157 – -46)	NT
3	99 (90 – 100)	100 (94 – 100)	86 (17 – 98)	NT	40 (-224 – 87)	NT
4	99 (87 – 100)	99 (96 – 100)	93 (66 – 98)	87 (34 – 97)	45 (-217 – 91)	NT
5	100	100	92 (72 - 97)	56 (-44 - 87)	-58 (-589 - 63)	NT
6	95 (75 – 99)	100	25 (-168 – 79)	44 (-250 – 91)	-31 (-293 – 56)	NT
7	98 (78 – 100)	100	80 (-1 – 96)	8 (-254 – 76)	21 (-269 – 83)	NT
8	100	100	34 (-32 – 67)	58 (11 – 80)	87 (54 – 97)	NT
9	100	100	-65 (-1291 – 80)	-522 (-4368 – 14)	-7 (-618 – 84)	NT
10	97 (75 –100)	99 (92 - 100)	29 (-44 - 65)	55 (-7 - 81)	2 (-280 - 74)	39 (-355 - 92)
11	100	96 (67 – 100)	66 (10 - 87)	15 (-201 - 76)	NT	93 (65 - 98)
12	100	100	97 (81 - 99)	75 (-59 - 99)	NT	98 (84 - 100)
13	100	99 (92 – 100)	90 (47 – 98)	94 (82 – 98)	NT	99 (85 – 100)
14	100	99 (86 – 100)	95 (69 – 99)	-203 (-1396 – 39)	NT	98 (84 – 100)
15	100	100	49 (-104 - 87)	70 (13 - 90)	NT	100
16	100	93 (42 - 99)	96 (79 - 99)	50 (-174 - 91)	NT	1 (-566 - 85)
17	100	100	-194 (-1262 - 37)	9 (-118 - 62)	NT	89 (-2 - 99)
18	100	100	96 (48 - 100)	78 (-33 - 96)	NT	90 (23 - 99)
19	99 (85 –100)	99 (85 - 100)	-13 (-327 - 70)	-147 (-848 - 36)	NT	100
20	100	100	-96 (-3257 - 89)	-18 (-1560 - 92)	NT	100

*Q-drench contains levamisole, closantel, albendazole, abamectin; **NT, not tested.

Table 8.3. Arithmetic means, minimum faecal egg counts (FEC; eggs per gram of faeces, EPG) and maximum FEC counts before and after treatment with different anthelmintics on 20 Australian alpaca farms.

Farm no.	State	No. of alpacas per group	Anthelmintic	Pre-treatment FEC		Post-treatment FEC	
				EPG ± SD	Min-Max	EPG ± SD	Min-Max
1	VIC	7	MON	624 ± 750	45-2220	0	0
		7	CLO	799 ± 402	255-1275	9 ± 12	0-30
		7	FBZ	208 ± 244	0-600	182 ± 269	0-675
		7	IVM	311 ± 656	0-1785	298 ± 598	0-1650
		7	CON	703 ± 947	15-2730	512 ± 517	0-1515
2	NSW	11	MON	164 ± 135	0-345	5 ± 10	0-30
		11	CLO	217 ± 173	0-570	147 ± 146	0-495
		11	FBZ	374 ± 485	0-1605	513 ± 572	0-1605
		11	IVM	559 ± 983	60-3525	1372 ± 2100	165-7470
		10	CON	426 ± 426	15-1290	320 ± 213	15-600
3	NSW	8	MON	653 ± 1027	0-2745	6 ± 11	0-30
		8	QDR	1174 ± 2575	0-7410	2 ± 5	0-15
		8	CLO	298 ± 462	0-1425	56 ± 96	0-285
		8	IVM	108 ± 97	0-240	236 ± 377	0-1155
		8	CON	204 ± 217	0-675	393 ± 560	0-1590
4	WA	14	MON	931 ± 3093	0-11670	8 ± 28	0-105
		13	QDR	1068 ± 2052	0-7065	3 ± 9	0-30
		14	CLO	205 ± 200	0-735	46 ± 84	0-300
		14	FBZ	378 ± 575	0-1935	100 ± 1	0-615
		14	IVM	470 ± 841	0-3210	338 ± 832	0-3135
		15	CON	1619 ± 3989	0-735	618 ± 1312	0-300
5	NSW	10	MON	743 ± 931	15-2430	0	0
		10	QDR	1349 ± 1539	120-5160	0	0
		10	CLO	335 ± 443	30-1515	63 ± 86	0-225
		10	FBZ	726 ± 1193	0-3315	327 ± 462	0-1335
		10	IVM	1079 ± 1480	0-4575	1185 ± 2126	0-6570
		10	CON	618 ± 547	0-1275	749 ± 824	0-2220
6	VIC	10	MON	366 ± 456	0-1425	11 ± 20	0-60
		11	QDR	345 ± 516	0-1635	0	0
		10	CLO	408 ± 578	0-1725	152 ± 205	0-540
		10	FBZ	300 ± 793	0-2550	113 ± 257	0-795
		10	IVM	327 ± 268	0-915	266 ± 249	30-870
		10	CON	179 ± 286	0-855	203 ± 276	0-870
7	VIC	12	MON	146 ± 144	3 ± 9	0-495	0-30
		10	QDR	341 ± 484	0	30-1650	0
		10	CLO	102 ± 127	26 ± 39	15-345	0-105
		10	FBZ	162 ± 208	117 ± 87	0-630	0-255
		10	IVM	496 ± 937	101 ± 136	0-3075	0-390
		10	CON	81 ± 110	128 ± 241	0-330	0-780

8	VIC	12	MON	236 ± 145	0-570	0	0
		10	QDR	351 ± 577	0-1935	0	0
		10	CLO	228 ± 145	0-465	186 ± 143	0-480
		10	FBZ	260 ± 174	0-615	120 ± 102	0-330
		10	IVM	239 ± 223	0-690	36 ± 65	0-210
		10	CON	279 ± 274	0-885	284 ± 203	0-720
9	NSW	10	MON	327 ± 348	0-1050	0	0
		10	QDR	498 ± 938	0-3075	0	0
		10	CLO	504 ± 777	0-1995	294 ± 645	0-1890
		10	FBZ	1126 ± 1400	0-4500	1108 ± 2026	0-5970
		10	IVM	364 ± 493	0-1665	190 ± 316	0-1050
		9	CON	262 ± 508	0-1545	178 ± 393	0-1215
10	SA	10	MON	1988 ± 4927	30-15915	14 ± 43	0-135
		10	QDR	554 ± 695	90-2235	4 ± 14	0-45
		10	CLO	1194 ± 1857	120-6375	342 ± 168	120-600
		10	FBZ	268 ± 186	45-540	219 ± 196	0-585
		10	IVM	848 ± 1075	40-3360	476 ± 855	15-2850
		10	CYD	1506 ± 3531	0-11520	297 ± 850	0-2715
		10	CON	548 ± 596	90-1710	483 ± 456	60-1155
11	VIC	10	MON	738 ± 1634	30-5265	0	0
		10	QDR	200 ± 351	0-1170	10 ± 30	0-90
		10	CLO	407 ± 378	30-1155	92 ± 107	0-285
		9	FBZ	518 ± 686	15-1680	231 ± 340	0-975
		10	CYD	254 ± 174	45-510	20 ± 43	0-140
		9	CON	384 ± 406	45-1065	270 ± 227	30-720
12	VIC	10	MON	256 ± 359	0-1050	0	0
		10	QDR	296 ± 333	0-1080	0	0
		10	CLO	1274 ± 2393	75-7890	32 ± 37	0-120
		10	FBZ	369 ± 682	30-2265	228 ± 362	0-1170
		10	CYD	465 ± 955	0-3120	20 ± 36	0-105
		10	CON	620 ± 1263	0-3945	914 ± 1843	60-5445
13	WA	8	MON	731 ± 967	0-2415	0	0
		8	QDR	662 ± 1047	0-2880	4 ± 11	0-30
		8	CLO	182 ± 169	30-495	45 ± 80	0-195
		8	FBZ	159 ± 186	60-615	24 ± 28	0-75
		7	CYD	118 ± 230	0-630	6 ± 17	0-45
		8	CON	246 ± 334	0-990	433 ± 422	0-1095
14	QLD	10	MON	240 ± 432	0-1440	0	0
		10	QDR	452 ± 1039	0-3315	2 ± 5	0-15
		10	CLO	604 ± 1031	0-3435	6 ± 14	0-45
		10	FBZ	81 ± 140	0-450	358 ± 728	0-2400
		10	CYD	861 ± 2562	0-8145	2 ± 5	0-15
		10	CON	78 ± 69	0-165	118 ± 142	0-420
15	TAS	10	MON	81 ± 129	0-360	0	0
		10	QDR	126 ± 189	0-570	0	0
		10	CLO	111 ± 209	0-675	54 ± 96	0-315

		10	FBZ	122 ± 150	15-510	32 ± 37	0-105
		10	CYD	22 ± 29	0-75	0	0
		10	CON	184 ± 187	0-630	106 ± 119	0-345
16	NSW	10	MON	130 ± 175	0-525	0	0
		10	QDR	300 ± 288	0-855	16 ± 47	0-150
		10	CLO	243 ± 297	0-930	9 ± 19	0-45
		10	FBZ	208 ± 186	0-435	124 ± 250	0-780
		10	CYD	147 ± 165	0-435	246 ± 588	0-1905
		10	CON	339 ± 504	0-1620	249 ± 395	0-1305
17	VIC	10	MON	83 ± 118	0-315	0	0
		9	QDR	55 ± 70	0-165	0	0
		9	CLO	143 ± 208	0-640	176 ± 323	0-945
		9	FBZ	94 ± 93	0-225	54 ± 45	0-120
		9	CYD	268 ± 480	0-1440	7 ± 20	0-60
		9	CON	84 ± 163	0-480	60 ± 56	0-150
18	NSW	7	MON	1589 ± 2127	90-5840	0	0
		10	QDR	658 ± 437	200-1640	0	0
		10	CLO	1000 ± 1156	0-3800	44 ± 133	0-400
		10	FBZ	658 ± 588	0-1632	256 ± 301	0-816
		10	CYD	1846 ± 3844	20-12280	114 ± 232	0-672
		10	CON	1406 ± 3623	0-11700	1178 ± 2781	0-9048
19	VIC	10	MON	124 ± 137	0-450	2 ± 5	0-15
		10	QDR	348 ± 265	105-855	2 ± 5	0-15
		10	CLO	622 ± 508	135-1440	118 ± 152	0-465
		10	FBZ	412 ± 422	0-1065	258 ± 343	0-1080
		10	CYD	218 ± 185	15-495	0	0
		10	CON	369 ± 548	15-1755	104 ± 160	0-520
20	VIC	5	MON	312 ± 150	195-540	0	0
		5	QDR	760 ± 930	90-2360	0	0
		6	CLO	378 ± 499	30-1350	318 ± 614	0-1560
		5	FBZ	312 ± 698	0-1560	191 ± 256	0-570
		6	CYD	842 ± 1161	0-2820	0	0
		5	CON	45 ± 92	0-210	162 ± 354	0-795

*MON- monepantel, QDR- Q-drench (contains levamisole, closantel, albendazole, abamectin), CLO- closantel, FBZ- fenbendazole, IMV- ivermectin, CYD- Cydectin (moxidectin), CON- control.

NSW, New South Wales; QLD, Queensland; SA, South Australia; TAS, Tasmania; VIC, Victoria; WA, Western Australia.

Table 8.4. Effect of different anthelmintics on the common gastrointestinal nematodes before and after treatment in naturally infected alpacas on 20 alpaca farms in Australia.

Farm No.	Anthelmintic	Treatment	*Camelostrongylus*	*Cooperia*	*Haemonchus*	*Oesophagostomum*	*Ostertagia*	*Teladorsagia*	*Trichostrongylus*
1	**MON**	**Pre**	**NT**	**NT**	**NT**	**NT**	**NT**	**NT**	**NT**
		Post	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	CLO	**Pre**	**NT**	**NT**	**NT**	**NT**	**NT**	**NT**	**NT**
		Post	**1**	**0**	**1**	**0**	**1**	**0**	**1**
	IVM	**Pre**	**NT**	**NT**	**NT**	**NT**	**NT**	**NT**	**NT**
		Post	**0**	**0**	**1**	**0**	**0**	**0**	**0**
	FBZ	**Pre**	**NT**	**NT**	**NT**	**NT**	**NT**	**NT**	**NT**
		Post	**0**	**0**	**1**	**0**	**0**	**0**	**0**
	CON	**Pre**	**NT**	**NT**	**NT**	**NT**	**NT**	**NT**	**NT**
		Post	**0**	**0**	**1**	**0**	**0**	**0**	**1**
2	**MON**	**Pre**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
		Post	**0**	**0**	**0**	**0**	**0**	**0**	**1**
	CLO	**Pre**	**1**	**0**	**1**	**0**	**1**	**0**	**1**
		Post	**1**	**1**	**0**	**0**	**1**	**0**	**1**
	IVM	**Pre**	**1**	**0**	**1**	**0**	**1**	**0**	**1**
		Post	**0**	**1**	**1**	**0**	**0**	**0**	**1**
	FBZ	**Pre**	**1**	**0**	**1**	**0**	**1**	**0**	**1**
		Post	**0**	**1**	**1**	**0**	**0**	**0**	**1**
	CON	**Pre**	**0**	**0**	**1**	**0**	**0**	**0**	**1**
		Post	**1**	**1**	**1**	**0**	**0**	**0**	**1**
3	**MON**	**Pre**	**1**	**0**	**1**	**0**	**1**	**0**	**0**
		Post	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	QDR	**Pre**	**1**	**0**	**1**	**0**	**1**	**0**	**1**
		Post	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	CLO	**Pre**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
		Post	**1**	**0**	**1**	**0**	**1**	**0**	**1**
	IVM	**Pre**	**1**	**0**	**1**	**0**	**0**	**0**	**1**

		Post	0	0	1	0	0	0	0
	CON	Pre	1	0	1	0	0	0	1
		Post	1	0	1	0	1	0	1
4	MON	Pre	0	0	0	0	0	0	0
		Post	0	0	0	0	0	0	1
	QDR	Pre	0	0	1	0	0	0	0
		Post	0	0	0	0	0	0	0
	CLO	Pre	1	1	1	0	0	0	1
		Post	0	0	0	0	0	0	0
	FBZ	Pre	0	0	1	0	0	0	1
		Post	1	0	1	0	1	0	1
	IVM	Pre	0	1	1	0	1	0	0
		Post	0	0	1	0	0	0	1
	CON	Pre	1	1	1	0	1	0	1
		Post	1	0	1	0	1	0	1
5	MON	Pre	1	1	1	0	1	0	1
		Post	1	0	1	0	1	0	1
	QDR	Pre	1	1	1	0	1	0	1
		Post	1	0	1	0	1	0	1
	CLO	Pre	1	0	1	0	0	0	1
		Post	1	0	1	0	1	0	1
	FBZ	Pre	0	1	1	0	0	0	1
		Post	1	0	1	0	1	0	1
	IVM	Pre	1	1	1	0	1	0	1
		Post	1	1	1	0	1	0	1
	CON	Pre	1	1	1	0	1	0	1
		Post	1	0	1	0	0	0	1
6	MON	Pre	1	1	1	1	1	0	1
		Post	1	0	0	0	1	0	0
	QDR	Pre	1	1	1	0	1	0	1
		Post	0	0	0	0	0	0	0

	CLO	**Pre**	**1**	**1**	**1**	**0**	**1**	**0**	**1**
		Post	**1**	**1**	**0**	**0**	**0**	**0**	**1**
	FBZ	**Pre**	**1**	**1**	**1**	**0**	**1**	**0**	**1**
		Post	**1**	**0**	**1**	**0**	**1**	**0**	**1**
	IVM	**Pre**	**1**	**1**	**1**	**0**	**1**	**0**	**1**
		Post	**0**	**0**	**1**	**0**	**0**	**0**	**0**
	CON	**Pre**	**1**	**1**	**1**	**0**	**1**	**0**	**1**
		Post	**1**	**0**	**1**	**0**	**1**	**0**	**1**
7	**MON**	**Pre**	**1**	**1**	**1**	**0**	**0**	**0**	**1**
		Post	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	QDR	**Pre**	**0**	**0**	**1**	**1**	**0**	**0**	**1**
		Post	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	CLO	**Pre**	**0**	**0**	**1**	**0**	**0**	**0**	**1**
		Post	**0**	**0**	**0**	**0**	**0**	**1**	**1**
	FBZ	**Pre**	**1**	**0**	**1**	**0**	**0**	**0**	**1**
		Post	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	IVM	**Pre**	**0**	**1**	**1**	**1**	**0**	**0**	**1**
		Post	**0**	**0**	**1**	**0**	**0**	**0**	**0**
	CON	**Pre**	**0**	**0**	**1**	**0**	**0**	**0**	**1**
		Post	**1**	**1**	**1**	**0**	**0**	**0**	**1**
8	**MON**	**Pre**	**1**	**0**	**0**	**0**	**1**	**0**	**1**
		Post	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	QDR	**Pre**	**1**	**1**	**1**	**0**	**1**	**0**	**1**
		Post	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	CLO	**Pre**	**1**	**1**	**1**	**0**	**1**	**0**	**1**
		Post	**1**	**1**	**0**	**0**	**1**	**0**	**1**
	FBZ	**Pre**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
		Post	**1**	**0**	**1**	**0**	**1**	**0**	**1**
	IVM	**Pre**	**1**	**1**	**0**	**1**	**0**	**0**	**1**
		Post	**0**	**0**	**1**	**0**	**0**	**0**	**0**
	CON	**Pre**	**1**	**0**	**1**	**1**	**1**	**0**	**1**

		Post	1	1	1	1	1	0	1
9	MON	Pre	0	1	1	0	0	0	1
		Post	0	0	0	0	0	0	0
	QDR	Pre	0	1	1	0	0	0	0
		Post	0	0	0	0	0	0	0
	CLO	Pre	0	1	1	0	1	0	1
		Post	0	1	0	0	0	0	0
	FBZ	Pre	0	1	1	0	0	0	0
		Post	0	0	1	0	0	0	0
	IVM	Pre	0	1	1	0	0	0	0
		Post	0	1	1	0	0	0	0
	CON	Pre	0	1	1	0	0	0	0
		Post	0	1	1	0	0	0	0
10	MON	pre	1	0	1	0	1	0	1
		Post	0	0	1	0	0	0	0
	QDR	pre	1	0	1	0	1	0	1
		Post	0	0	0	0	0	0	0
	CLO	pre	1	0	1	0	1	0	1
		Post	1	0	1	0	1	0	1
	FBZ	pre	1	0	1	0	1	0	1
		Post	1	0	1	0	1	0	1
	IVM	pre	1	0	1	0	1	0	1
		Post	1	0	1	0	1	0	1
	CYD	pre	1	0	1	0	1	0	1
		Post	0	0	1	0	0	0	1
	CON	pre	1	0	1	0	1	0	1
		Post	1	0	1	0	1	0	1
11	MON	Pre	1	0	1	0	1	0	1
		Post	0	0	0	0	0	0	0
	QDR	Pre	1	0	1	0	1	0	1
		Post	0	0	0	0	0	0	0

	CLO	Pre	1	0	1	0	1	0	1
		Post	1	1	1	0	1	0	1
	FBZ	Pre	1	0	1	0	1	0	1
		Post	1	0	1	0	1	0	1
	CYD	Pre	1	0	1	0	1	0	1
		Post	0	0	1	0	0	0	0
	CON	Pre	1	0	1	0	0	0	1
		Post	1	0	1	0	1	0	0
12	MON	Pre	1	0	1	0	1	0	1
		Post	0	0	0	0	0	0	0
	QDR	Pre	1	1	1	0	1	0	0
		Post	0	0	0	0	0	0	0
	CLO	Pre	1	1	1	0	1	0	1
		Post	1	1	1	0	1	0	0
	FBZ	Pre	1	1	1	0	1	0	0
		Post	1	0	1	0	1	0	1
	CYD	Pre	1	1	1	0	1	0	0
		Post	0	0	1	0	0	0	0
	CON	Pre	1	0	1	0	1	0	1
		Post	1	0	1	0	1	0	0
13	MON	Pre	1	0	1	0	1	0	1
		Post	0	0	0	0	0	0	0
	QDR	Pre	1	0	1	0	1	0	1
		Post	0	0	0	0	0	0	0
	CLO	Pre	1	0	1	0	1	0	1
		Post	0	0	1	0	0	0	0
	FBZ	Pre	1	0	1	0	1	0	1
		Post	0	0	1	0	0	0	1
	CYD	Pre	1	0	1	0	0	0	1
		Post	0	0	0	0	0	0	0
	CON	Pre	0	0	1	0	0	0	0

		Post	0	0	1	0	0	0	1
14	MON	Pre	0	1	1	0	0	0	1
		Post	0	0	1	0	0	0	0
	QDR	Pre	0	0	1	0	0	0	0
		Post	0	0	1	0	0	0	0
	CLO	Pre	0	0	1	0	0	0	1
		Post	0	0	0	0	0	0	1
	FBZ	Pre	0	1	1	0	0	0	1
		Post	1	0	1	0	1	0	1
	CYD	Pre	0	0	1	0	0	0	0
		Post	0	0	0	0	0	0	1
	CON	Pre	0	0	1	0	0	0	0
		Post	1	1	1	0	1	0	1
15	MON	Pre	1	0	0	0	1	0	1
		Post	0	0	0	0	0	0	0
	QDR	Pre	1	0	1	0	1	0	1
		Post	0	0	0	0	0	0	0
	CLO	Pre	1	0	0	0	1	0	1
		Post	1	0	0	0	0	0	0
	FBZ	Pre	1	0	1	0	1	0	0
		Post	0	0	0	0	0	0	0
	CYD	Pre	1	0	0	0	1	0	1
		Post	0	0	0	0	0	0	0
	CON	Pre	1	0	0	0	1	0	0
		Post	1	0	0	0	1	0	0
16	MON	Pre	1	0	1	0	1	0	1
		Post	0	0	0	0	0	0	0
	QDR	Pre	1	0	1	0	0	0	1
		Post	0	0	0	0	0	0	0
	FBZ	Pre	0	0	1	0	0	0	0
		Post	0	0	1	0	0	0	0

	CLO	**Pre**	**0**	**0**	**1**	**0**	**0**	**0**	**1**
		Post	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	CYD	**Pre**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
		Post	**0**	**0**	**1**	**0**	**0**	**0**	**0**
	CON	**Pre**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
		Post	**0**	**0**	**1**	**0**	**0**	**0**	
17	**MON**	**Pre**	**1**	**1**	**1**	**0**	**1**	**0**	**1**
		Post	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	QDR	**Pre**	**1**	**0**	**1**	**0**	**1**	**0**	**1**
		Post	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	CLO	**Pre**	**1**	**1**	**1**	**0**	**1**	**0**	**1**
		Post	**1**	**1**	**1**	**0**	**1**	**0**	**1**
	FBZ	**Pre**	**1**	**1**	**1**	**0**	**1**	**0**	**0**
		Post	**0**	**1**	**1**	**0**	**0**	**0**	**0**
	CYD	**Pre**	**1**	**1**	**1**	**0**	**1**	**0**	**1**
		Post	**0**	**1**	**1**	**0**	**0**	**0**	**0**
	CON	**Pre**	**1**	**1**	**1**	**0**	**1**	**0**	**0**
		Post	**0**	**0**	**1**	**0**	**0**	**0**	**1**
18	**MON**	**Pre**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
		Post	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	QDR	**Pre**	**1**	**0**	**1**	**0**	**1**	**0**	**1**
		Post	**0**	**0**	**1**	**0**	**0**	**0**	**0**
	CLO	**Pre**	**1**	**0**	**1**	**0**	**1**	**0**	**0**
		Post	**0**	**0**	**1**	**0**	**0**	**0**	**0**
	FBZ	**Pre**	**1**	**0**	**1**	**0**	**0**	**0**	**0**
		Post	**0**	**0**	**1**	**0**	**0**	**0**	**0**
	CYD	**Pre**	**1**	**0**	**1**	**0**	**1**	**0**	**1**
		Post	**0**	**0**	**1**	**0**	**0**	**0**	**1**
	CON	**Pre**	**1**	**0**	**1**	**0**	**1**	**0**	**0**
		Post	**1**	**0**	**1**	**0**	**1**	**0**	**0**
19	**MON**	**Pre**	**0**	**0**	**1**	**0**	**1**	**0**	**1**

		Post	0	0	0	0	0	0	0
	QDR	Pre	0	0	1	0	0	0	1
		Post	0	0	0	0	0	0	0
	CLO	Pre	0	1	1	0	1	0	1
		Post	1	0	0	0	1	0	1
	FBZ	Pre	1	1	1	0	1	0	1
		Post	1	0	1	0	0	0	1
	CYD	Pre	0	0	1	0	1	0	1
		Post	0	0	0	0	0	0	0
	CON	Pre	1	1	1	0	0	0	1
		Post	1	0	1	0	1	0	1
20	MON	Pre	1	0	1	0	1	0	1
		Post	0	0	0	0	0	0	0
	QDR	Pre	1	0	1	0	1	0	1
		Post	0	0	0	0	0	0	0
	CLO	Pre	1	1	1	0	1	0	0
		Post	1	0	0	0	1	0	1
	FBZ	Pre	1	0	1	0	1	0	0
		Post	0	0	1	0	0	0	1
	CYD	Pre	1	1	1	0	1	0	1
		Post	0	0	0	0	0	0	0
	CON	Pre	1	0	1	0	1	0	1
		Post	1	0	1	0	1	0	1

NT, Not -tested; 0, the nematode was not detected; 1, the nematode was detected.
*MON- monepantel, QDR- Q-drench (contains levamisole, closantel, albendazole, abamectin), CLO- closantel, FBZ- fenbendazole, IMV- ivermectin, CYD- Cydectin (moxidectin), CON- control.

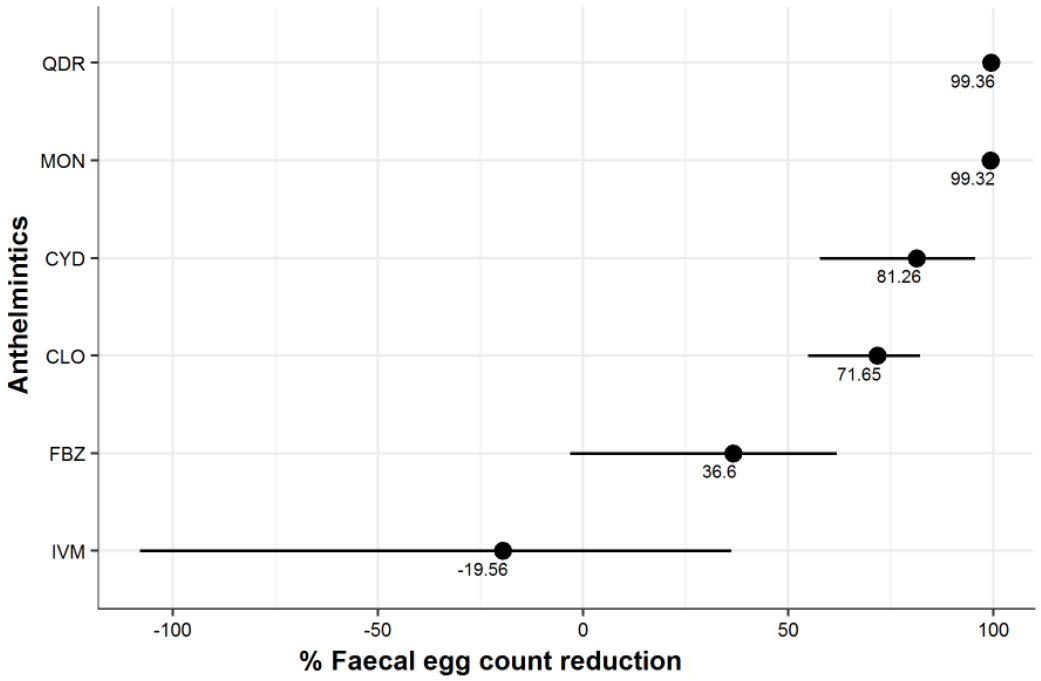


Fig. 8.2. Overall efficacy of six anthelmintics against gastrointestinal nematodes of alpacas on 20 farms in Australia. Each circle shows percentage of the faecal egg count reduction while each horizontal line shows upper and lower 95% confidence intervals. CLO, closantel; CYD, cydectin; IVM, ivermectin; FBZ, fenbendazole; QDR, Q-drench (a combination of abamectin, albendazole, closantel and levamisole); MON, monepantel.

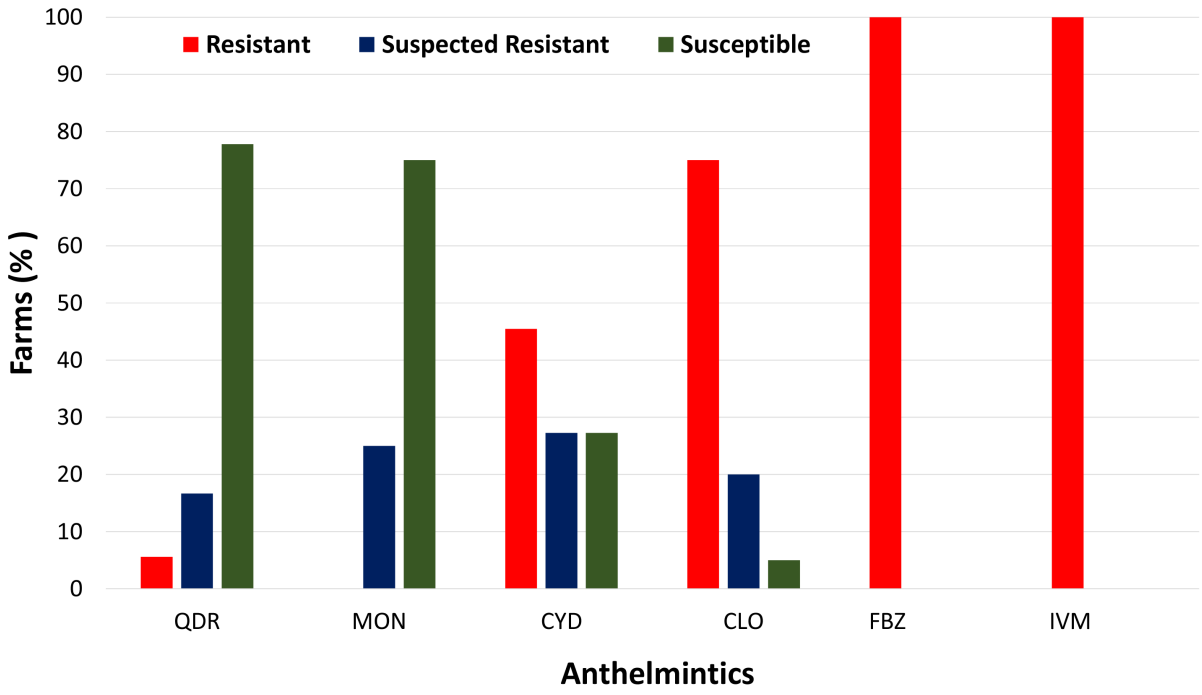


Fig. 8.3. The proportion of farms with resistance, suspected resistance and susceptibility of gastrointestinal nematodes of alpacas to six anthelmintics on 20 farms in Australia. Abbreviations: CLO, closantel; CYD, cydectin; IVM, ivermectin; FBZ, fenbendazole; QDR, Q-drench (a combination of abamectin, albendazole, closantel and levamisole); MON, monepantel.

Molecular detection of seven common GINs in pre- and post-treatment pooled faecal samples of alpacas revealed that monepantel and a combination of anthelmintics were most successful in eliminating all the GINs (i.e. *C. mentulatus*, *Cooperia* spp., *Haemonchus* spp., *O. ostertagi*, *Oesophagostomum* spp. *T. circumcincta* and *Trichostrongylus* spp.) prevalent on 20 alpaca farms (Fig. 8.4; Table 8.3). Fenbendazole had no effect on *Haemonchus* spp. and *Trichostrongylus* spp., with very little effect on *C. mentulatus*, *O. ostertagi* and *Cooperia* spp. Likewise, ivermectin showed no efficacy against *Haemonchus* spp. with variable effect on other GINs (Fig. 8.4). Moxidectin seemed to have the highest efficacy against *C. mentulatus* and *O. ostertagi* while it was moderately effective against other five GINs (Fig. 8.4). Closantel is a narrow-spectrum anthelmintic and known to be effective against blood feeding nematodes such as *Haemonchus* spp. However, it was not effective against this important nematode in all alpaca farms studied herein (Fig. 8.4).

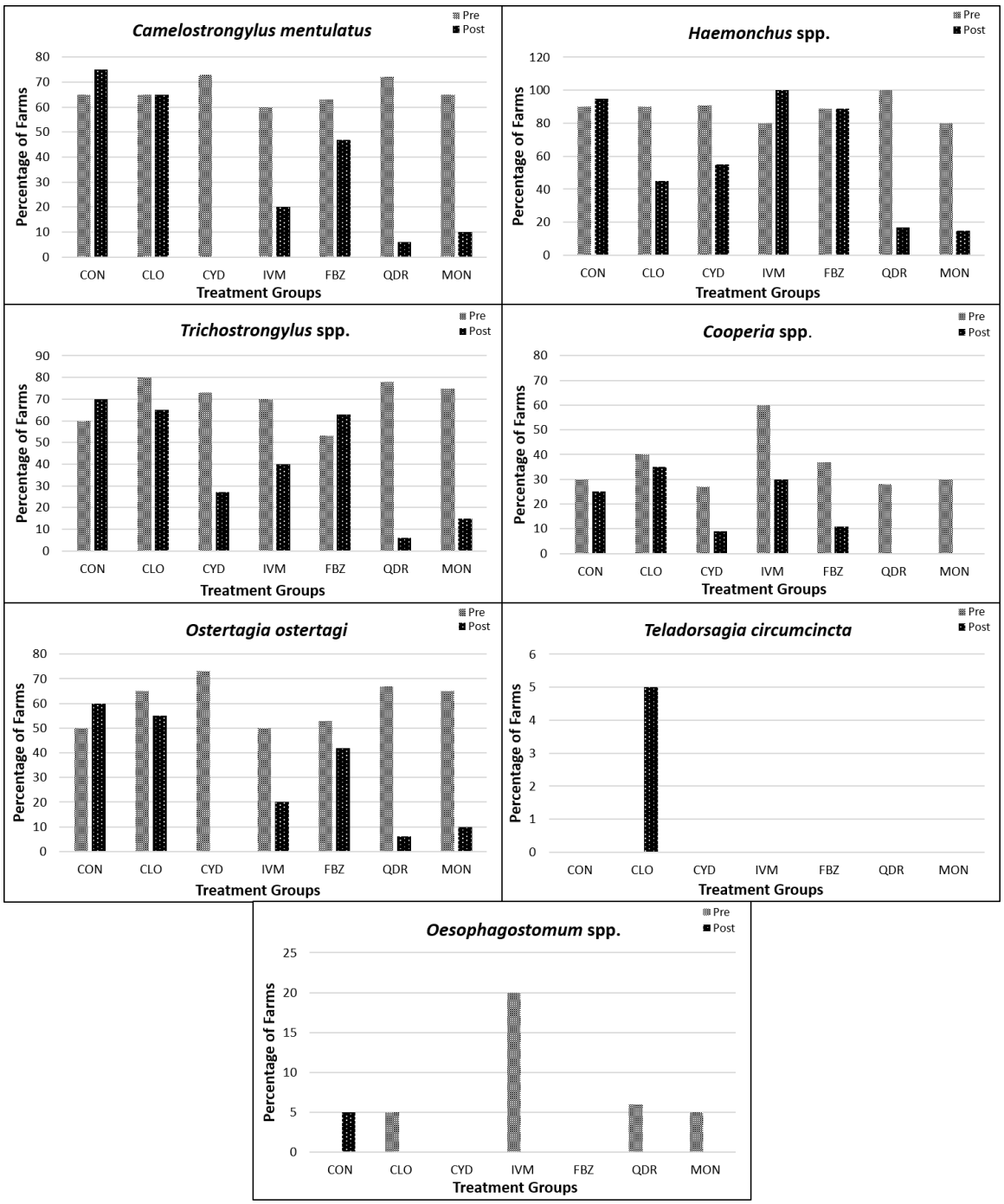


Fig. 8.4. Gastrointestinal nematodes found in faeces of alpacas pre- and post-treatment with six anthelmintics on alpaca farms in Australia. The nematodes were identified using the multiplexed-tandem PCR. CLO, closantel; CON, negative control; CYD, cydectin; IVM, ivermectin; FBZ, fenbendazole; QDR, Q-drench (a combination of abamectin, albendazole, closantel and levamisole); MON, monepantel.

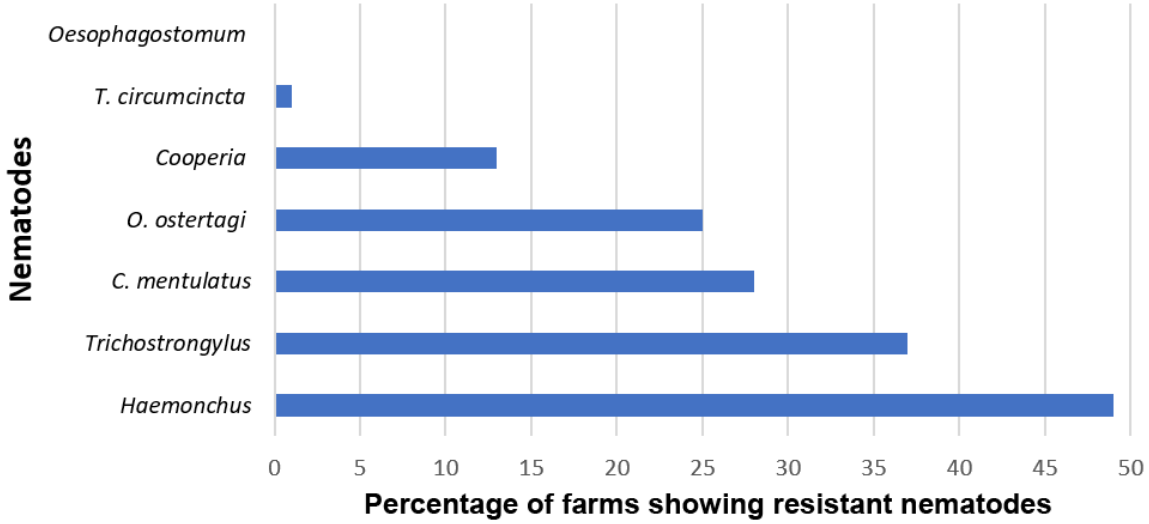


Fig. 8.5. Percentage of resistant gastrointestinal nematodes against commonly used anthelmintics on 20 alpaca farms in Australia.

8.5. Discussion

Gastrointestinal nematodes are a major clinical and economic threat to SACs throughout the world. Since there are no anthelmintics registered for use in SACs, limited information is available on their efficacy and safety. This is the first study to comprehensively study the efficacy of commonly used anthelmintics against GINs of alpacas and llamas. The combination of levamisole, closantel, albendazole and abamectin was the most effective dewormer on the 20 Australian alpaca farms in this study, followed by monepantel, moxidectin, closantel, fenbendazole and ivermectin. *Haemonchus* spp. were the most commonly resistant nematodes followed by *Trichostrongylus* spp., *C. mentulatus*, *O. ostertagi* and *Cooperia* spp. Previously, AR in GINs of alpacas and llamas had been reported from Australia (Jabbar et al., 2013), Belgium (Sarre et al., 2012), Canada (Galvan et al., 2012) and the USA (Gillespie et al., 2010) against two commonly used classes of anthelmintics, benzimidazoles and macrocyclic lactones in *H. contortus*. However, almost all of these studies were case reports, including the one from Australia (Jabbar et al., 2013) as opposed to this study that provides insights into the problem of AR in GINs of alpacas at a national level across the alpaca industry in Australia - the country which has the highest number of alpacas outside South America.

In this study, the questionnaire survey revealed invaluable information about the husbandry practices on Australian alpaca farms. Over 50% of farmers kept alpacas with sheep and cattle as well as allowing them to graze with domestic ruminants that can expose alpacas to shared GINs such as *Haemonchus* spp. (Rickard and Bishop, 1991; Hill et al., 1993; Rickard, 1994; Ballweber, 2009; Lambacher et al., 2016), sometimes resulting in fatal infections (Rickard, 1993; Jabbar et al., 2013). Similarly, keeping different livestock species that can harbour similar GINs on the same property, can increase the transmission of resistant GINs among ruminants. Previously, Edwards et al. (1986) found the highest prevalence of levamisole resistant *Trichostrongylus* sp. in sheep where sheep and cattle had grazed together on the same paddock in Western Australia; however, such studies have not been undertaken for SACs. Furthermore, it was found that agistment of alpacas occurred on 25% of Australian alpaca farms surveyed. This practice may further increase the risk of introducing resistant GINs to a herd if proper quarantine deworming and/or procedure(s) are not followed.

The FECRT results of this study should be interpreted carefully because this study tested various anthelmintics in alpacas at 1.5 times the dose rate recommended for sheep, as no information is available on the therapeutic doses of commonly used anthelmintics in SACs. Furthermore, the number of animals per group of treatment for the FECRT on some farms was less (see Table 8.2) than that (10-15 animals per group) recommended by the WAAVP for evaluating the efficacy of anthelmintics against GINs of ruminants (Wood et al., 1995) due to lack of the required number of animals per farm. Additionally, two MLs, ivermectin and moxidectin were not tested on all farms because the study aim was to assess the efficacy of both a less potent (ivermectin) as well as a more potent (moxidectin) ML drug in alpacas. Furthermore, these two drugs were not able to be tested together on any farm due to the small number of alpacas available.

Fenbendazole and albendazole are widely used broad-spectrum dewormers in sheep and cattle (Leathwick and Besier, 2014). Anthelmintic resistance to BZs was reported in GINs of sheep from Australia soon after the introduction of thiabendazole (Smeal et al., 1986). Recently, Playford et al. (2014) conducted a national survey to assess the prevalence of AR in GINs of sheep in Australia and they found that 96% of farms in Australia had resistant *H. contortus*, *T. circumcincta* and *Trichostrongylus* spp. Similarly, this study found that none of the 20 alpaca farms included in this study had susceptible worms (as per guidelines of the WAAVP) against fenbendazole at 7.5 mg/kg body weight, with an overall FECR of 36% (see Fig. 8.2, Table 8.2). It completely failed to reduce the number of *Haemonchus* spp. and *Trichostrongylus* spp., and was only partially successful in reducing the numbers of *C. mentulatus*, *Cooperia* spp., *O. ostertagi* and *Oesophagostomum* spp. (see Fig. 8.4). Previously, BZ resistance in GINs of alpacas has been reported from the USA (Galvan et al., 2012) where no FECR (-111%) was observed when fenbendazole was given orally at 10 mg/kg compared with 59% when albendazole was used at the same dose rate. The second BZ used in this study was albendazole in combination with other anthlemintics, levamisole, closantel and abamectin, which proved to be one of the most effective anthelmintics against GINs of alpacas. The efficacy of BZ in this combination formulation cannot be evaluated separately as the high efficacy of this group might be due to the presence of effective anthelmintic(s) when two or more classes of anthelmintics are used (Bartram, et al., 2012).

In this study, ivermectin failed to reduce FECs (-20% see Fig. 8.2) of nematodes when given orally at 300 μg/kg. In addition, it was unsuccessful in reducing the numbers of

Haemonchus spp. but was partially effective against *C. mentulatus*, *Cooperia* spp., *O. ostertagi*, *Oesophagostomum* spp. and *Trichostrongylus* spp. (see Fig. 8.4). Similar results were found in alpacas and llamas from the USA by Gillespie et al. (2010) when they used the same dose rate and the route of administration of ivermectin against *H. contortus*. However, they found better efficacy of ivermectin in llamas on two farms (FECR 22-37%) than on one alpaca farm (FECR -65%). In a previous study from Australia, Jabbar et al. (2013) also found *H. contortus* in alpacas resistant to ivermectin (FECR 35%) when given orally at 200 µg/kg. Ivermectin resistance in strongyles of alpacas has also been reported from Peru (Leathwick, 2012). The questionnaire survey results of this study revealed that MLs were the most commonly used anthelmintics by Australian alpaca farmers, and the high frequency of the use of dewormers have been found to be associated with the development of AR in sheep GINs (Jabbar et al., 2006); hence, this might also be the case for the development of AR in GINs of SACs.

The second ML, moxidectin, used in this study was 81% effective against GINs of alpacas at farm level, and resistance was reported on 46% farms, with 27% farms susceptible and 27% suspected for resistance. This is the first report of moxidectin resistance against GINs in alpacas. However, Gillespie et al. (2010) reported moxidectin resistance against *H. contortus* in llamas from the USA. The third ML used in this study was abamectin in combination with other anthlemintics, albendazole, levamisole and closantel which proved to be one of the effective anthelmintics against GINs of alpacas.

Closantel is a narrow spectrum anthelmintic and is recommended for *H. contortus* in small ruminants in Australia. Findings of this study showed that 75% of the 20 alpaca farms had resistant populations of GINs. However, this result should be interpreted carefully as closantel is not claimed to be effective against the majority of GINs (*C. mentulatus*, *Cooperia* spp., *O. ostertagi*, *Oesophagostomum* spp. *T. circumcincta* and *Trichostrongylus* spp.) found in alpacas on the 20 farms. Regarding its efficacy against *Haemonchus* spp., this nematode was found on 18 (out of 20) farms but closantel was effective only on 50% (9/18) farms. Similar results were found by Playford et al. (2014) where they reported 43% (23/53) prevalence of closantel resistance in *H. contortus* of sheep from Australia. Conversely, Jabbar et al. (2013) found that an ivermectin-resistant population of *H. contortus* in alpacas was 99% susceptible to closantel. Given that *H. contortus* can lead to fatal infections in alpacas and it is resistant to most of the commonly used anthelmintics, closantel should be used very carefully in areas/farms where its

resistance has not been reported, thereby delaying the development of AR against specific, narrow spectrum anthelmintics.

This is the first report documenting the efficacy of monepantel against GINs in alpacas. This study did not find monepantel resistance in GINs of alpacas when used at 3.75 mg/kg body weight. However, as per the guidelines of the WAAVP, monepantel resistance was suspected on five farms (see Fig. 8.2). Previously, Dadak et al. (2013) undertook a study aimed at establishing an effective dose rate of monepantel for treating GINs in llamas in Austria, and found that three dose rates of 2.5 mg/kg, 5.0 mg/kg and 7.5 mg/kg of monepantel were able to reduce FECs by 84%, 93%, and 100%, respectively. However, 1.5 times the dose rate recommended for sheep were used herein as it was the most commonly used dose by Australian alpaca farmers as well as veterinarians. In addition, this study found that monepantel at this dose rate was 100% effective against GINs on 13 alpaca farms while 95-99% effective on seven farms (see Table 8.2). These differences in the efficacy of monepantel in two species of SACs using different doses might be associated with differences in the pharmacokinetic properties of the drug in alpacas and llamas. However, this proposal warrants further investigation. Although monepantel is a relatively new drug, GINs of sheep resistant to this drug have been reported from Australia (Sales and Love, 2016), Brazil (Cintra et al., 2016), New Zealand (Scott et al., 2013), Netherlands (Van den Brom et al., 2015) and Uruguay (Mederos et al., 2014). Similarly, the injudicious and frequent use of monepantel can also lead to the development of AR in GINs of alpacas. Therefore, care should be taken when using monepantel to prolong its efficacy against GINs of alpacas as this is one of the two dewormers found to be effective herein.

This study reports multiple AR in GINs of alpacas for the first time as most cases were of resistance to fenbendazole, closantel and ivermectin on different Australian alpaca farms (see Fig 8.4). Multiple anthelmintic resistance occurs when two or more classes of anthelmintics are unable to control GIN populations that were previously susceptible (more than 95% killed) to anthelmintics at their therapeutic doses (Taylor et al., 2009). Previously, Gillespie et al. (2010) documented *H. contortus* resistance to ivermectin, fenbendazole and moxidectin in llamas from the USA. Multiple resistance to anthelmintics is very common among the main GINs that infect sheep ((Taylor et al., 2009), goats (Saeed et al., 2010) and cattle (Ramos et al., 2016). Given that Australia is among the world leaders for the high prevalence of multiple AR in GINs of small

ruminants (Playford et al., 2014), more sustainable strategies to control GINs in alpacas are required to control the problem of multiple AR.

This study utilised a newly established molecular diagnostic technique (MT-PCR) to identify nematode species in alpacas in pre- and post-treatment pooled faecal DNA samples (see Chapter 4). Traditionally, larval cultures (LC) are used to identify nematodes. However, this procedure is time-consuming and lacks sensitivity and specificity. In addition, the LC requires experienced personnel for accurate identification of the third-stage larvae (L3s) as many nematode species are difficult to distinguish morphologically (Roeber and Kahn, 2014). The MT-PCR assay used in this study also allowed an accurate identification of one of the common nematodes of alpacas, *C. mentulatus* (see Chapter 4) as LC does not allow its reliable identification due to unavailability of morphological keys of the third-stage larvae. Therefore, the testing of pre- and post-treatment pooled faecal DNA samples from 20 alpaca farms allowed us, for the first time, to ascertain the efficacy of closantel, fenbendazole, ivermectin, monepantel moxidectin and a combination of four anthelmintics against *C. mentulatus* as well as other GINs (see Fig. 8.4). Hence, the MT-PCR assay should be used in future epidemiological and AR studies to accurately identify the common GINs of alpacas as well as llamas.

The efficacy of a combination of four classes of anthelmintics were assessed for the first time in alpacas and it was found to be the most effective dewormer in alpacas in this study. Recently, there has been growing interest in the use of combinations of anthelmintic classes for the control of GINs of ruminants as multiple AR is an emerging threat for the control of nematode parasites (Bartram et al., 2012; Leathwick et al., 2012; Kotze et al., 2018). Given that this study found multiple AR for all anthelmintics when used as a single dewormer, the use of combinations of two or more anthelmintics with good efficacy as single dewormer(s) could be an effective means of delaying the development of AR in GINs of alpacas and llamas. However, future large-scale studies will be required to test a variety of combinations of anthelmintics against GINs of SACs.

Given that no anthelmintics are registered for use against GINs in SACs and very little is known about pharmacokinetic properties of the commonly used anthelmintics in alpacas and llamas, appropriate dose rates and the route(s) of administration for various anthelmintics in these animals are unknown. For example, Guerden and Hemelrijk (2005) found that ivermectin reduced 100% FECs of GINs in both alpacas and llamas when used subcutaneously at a dose rate of 200 µg/kg body weight. However, Windsor

et al. (1992) reported that subcutaneous administration of ivermectin reduced but did not completely eliminate GIN infections in alpacas. Conversely, the oral administration of ivermectin at a dose rate of 300 µg/kg body weight did not result in the reduction of GINs of alpacas in the USA (FECR -65%; (Gillespie et al., 2010) or in Australia (FECR -20%, this study). These differences in the efficacy of ivermectin in the above four studies might be due to the different route of administration (oral vs subcutaneous) as the serum concentration of ivermectin was lower in llamas following its administration at a dose rate of 200 µg/kg body weight orally (less than 2 ng/ml) than subcutaneously (3 ng/ml) (Harris et al., 2009). However, Burkholder *et* al. (2004) were not able to find detectable level of ivermectin in serum of llamas when ivermectin was injected subcutaneously at a dose rate of 200 µg/kg body weight. The pharmacokinetics of ivermectin have not been studied in alpacas but this study cautiously expects that the serum concentration of ivermectin in alpacas after the administration via various routes will be very similar to those found in llamas. Such limited but conflicting reports on the pharmacokinetics of anthelmintics in SACs complicate the situation and do not provide sound evidence-based practice for using an accurate dose rate and route(s) of administration for different anthelmintics in alpaca and llama medicine. Therefore, large-scale pharmacokinetic studies are needed to understand the pharmacokinetic properties, appropriate dose rate(s) and the route(s) of administration of the commonly used anthelmintics in alpacas and llamas.

8.6. Conclusions

This is the first study to assess the current efficacy status of anthelmintics used by alpaca farmers in Australia. The findings of this study indicate that a combination of four classes of anthelmintics is the most effective dewormer in alpacas. AR for commonly used anthelmintics (Ivermectin, benzimidazole and closantel) is prevalent when used as single dewormers at one-and-half times sheep dose. Multi-species GINs resistance is also prevalent in Australian alpacas.

8.7. References

Ballweber, L.R. (2009). Ecto- and endoparasites of New World Camelids. *Veterinary Clinics of North America: Food Animal Practice,* 25, 295-310.

Bartram, D.J., Leathwick, D.M., Taylor, M.A., Geurden, T., & Maeder, S.J. (2012). The role of combination anthelmintic formulations in the sustainable control of sheep nematodes. *Veterinary Parasitology,* 186, 151-158.

Burkholder, T.H., Jensen, J., Chen, H., Junkins, K., Chatfield, J., & Boothe, D. (2004). Plasma evaluation for ivermectin in llamas (*Lama glama*) after standard subcutaneous dosing. *Journal of Zoo and Wildlife Medicine,* 35, 395-396.

Cintra, M.C.R., Teixeira, V.N., Nascimento, L.V., & Sotomaior, C.S. (2016). Lack of efficacy of monepantel against *Trichostrongylus colubriformis* in sheep in Brazil.

Clarke, M. (2018). Market Assessment - New and Emerging Animal Industries Tranche 1: Mohair, Alpaca and Camel Milk. NSW, Australia2016. http://www.agrifutures.com.au/publications/ market-assessment-new-and-emerging-animal-industries-tranche-1-mohair-alpaca-and-camel-milk. Accessed 15 Jan 2018.

Coles, G.C., Bauer, C., Borgsteede, F.H.M., Geerts, S., Klei, T.R., & Taylor, M.A. (1992). World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology,* 44, 35-44.

Dadak, A.M., Asanger, H., Tichy, A., & Franz, S. (2013). Establishing an efficacious dose rate of monepantel for treating gastrointestinal nematodes in llamas under field conditions. *Veterinary Record,* 172, 155.

Edwards, J.R., Wroth, R., de Chaneet, G.C., Besier, R.B., Karlsson, J., & Morcombe, P.W. (1986). Survey of anthelmintic resistance in Western Australian sheep flocks. 2. Relationship with sheep management and parasite control practices. *Australian Veterinary Journal,* 63,139-44.

Falzon, L.C., van Leeuwen, J., Menzies, P.I., Jones-Bitton, A., Sears, W., & Jansen, J.T. (2014). Comparison of calculation methods used for the determination of anthelmintic resistance in sheep in a temperate continental climate. *Parasitology Research,* 113, 2311-2322.

Falzon, L.C., O'Neill, T.J., Menzies, P.I., Peregrine, A.S., Jones-Bitton, A., & vanLeeuwen, J. (2014). A systematic review and meta-analysis of factors associated with anthelmintic resistance in sheep. *Preventive Veterinary Medicine,* 117, 388-402.

Franz, S., Wittek, T., Joachim, A., Hinney, B., & Dadak, A.M. (2015). Llamas and alpacas in Europe: Endoparasites of the digestive tract and their pharmacotherapeutic control. *Veterinary Journal,* 204 3, 255-62.

Galvan, N., Middleton, J.R., Nagy, D.W., Schultz, L.G., & Schaeffer, J.W. (2012). Anthelmintic resistance in a herd of alpacas (*Vicugna pacos*). *Canadian Veterinary Journal,* 53, 1310-1313.

Gerken, M., & Renieri, C. (2004). South American camelids research. In: 4th European Symposium on South American Camelids and DECAMA European Seminar; Göttingen, Germany, p7-8.

Geurden, T., & Van Hemelrijk, K. (2005). Ivermectin treatment against gastrointestinal nematodes in New World camelids in Belgium. *Small Ruminant Research,* 58, 71-73.

Gillespie, R.A.M., Williamson, L.H., Terrill, T.H., & Kaplan, R.M. (2010). Efficacy of anthelmintics on South American camelid (llama and alpaca) farms in Georgia. *Veterinary Parasitology,* 172, 168-171.

Harris, P.A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N., & Conde, J.G. (2009). Research electronic data capture (REDCap) - a metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of Biomedical Informatics,* 42, 377-381.

Hennessy, D.R., Sangster, N.C., Steel, J.W., Collins, G.H. (1993). Comparative pharmacokinetic behaviour of albendazole in sheep and goats. *International Journal for Parasitology,* 23, 321-325.

Hunter, R.P., Isaza, R., Koch, D.E., Dodd, C.C., & Goately, M.A. (2004). Moxidectin plasma concentrations following topical administration to llamas (*Lama glama*) and alpacas (*Lama pacos*). *Small Ruminant Research,* 52, 275-279.

Jabbar, A., Campbell, A.J.D., Charles, J.A, & Gasser, R.B. (2013). First report of anthelmintic resistance in *Haemonchus contortus* in alpacas in Australia. *Parasites and Vectors,* 6, 243.

Jabbar, A., Iqbal, Z., Kerboeuf, D., Muhammad, G., Khan, M.N., & Afaq, M. (2006). Anthelmintic resistance: the state of play revisited. *Life Science,* 79, 2413-2431.

James, C.E., Hudson, A.L., & Davey, M.W. (2009). Drug resistance mechanisms in helminths: is it survival of the fittest? *Trends in Parasitology,* 225, 328-335.

Kotze, A.C., Ruffell, A., Lamb, J., & Elliott, T.P. (2018). Response of drug-susceptible and -resistant *Haemonchus contortus* larvae to monepantel and abamectin alone or in combination *in vitro*. *Veterinary Parasitology,* 249, 57-62.

Lambacher, B., Wittek, T., Joachim, A., Dadak, A., Stanitznig, A., & Hinney, B. (2016). From the New World to the Old World: endoparasites of South American Camelids in Austria. *Wiener Tierärztliche Monatsschrift,* 103, 33-42.

Leathwick, D.M. (2012). Modelling the benefits of a new class of anthelmintic in combination. *Veterinary Parasitology*, 186, 93-100.

Leathwick, D.M., & Besier, R.B. (2014). The management of anthelmintic resistance in grazing ruminants in Australasia-strategies and experiences. *Veterinary Parasitology,* 204, 44-54.

Leguia, G. (1991). The epidemiology and economic impact of llama parasites. *Parasitology Today,* 7, 54-55.

Mederos, A.E., Ramos, Z., & Banchero, G.E. (2014). First report of monepantel *Haemonchus contortus* resistance on sheep farms in Uruguay. *Parasites and Vectors,* 7, 598.

Playford, M.C., Smith, A.N., Love, S., Besier, R.B., Kluver, P., & Bailey, J.N. (2014). Prevalence and severity of anthelmintic resistance in ovine gastrointestinal nematodes in Australia (2009-2012). *Australian Veterinary Journal,* 92, 464-471.

Ramos, F., Portella, L.P., Rodrigues, F.D., Reginato, C.Z., Potter, L., & Cezar, A.S. (2016). Anthelmintic resistance in gastrointestinal nematodes of beef cattle in the state of Rio Grande do Sul, Brazil. *International Journal for Parasitology: Drugs and Drug Resistance,* 6, 93-101.

Rickard, L.G., & Bishop, J.K. (1991). Helminth parasites of llamas (*Lama glama*) in the Pacific Northwest. *Journal of Helminthological Society of Washington,* 58, 110-115.

Rickard, L.G. (1994). Parasites. *Veterinary Clinics of North America: Food Animal Practice,* 102, 239-47.

Rickard. L.G. (1993). Parasitic gastritis in a llama (*Lama glama*) associated with inhibited larval *Teladorsagia* spp. (Nematoda: Trichostrongyloidea). *Veterinary Parasitology,* 45, 331-335.

Roeber, F., & Kahn, L. (2014). The specific diagnosis of gastrointestinal nematode infections in livestock: larval culture technique, its limitations and alternative DNA-based approaches. *Veterinary Parasitology*, 205, 619-628.

Roeber, F., Jex, A.R., & Gasser, R.B. (2013). Comparative evaluation of two DNA isolation techniques for PCR-based diagnosis of gastrointestinal nematode infections in sheep. *Molecular and Cellular Probes,* 27, 153-157.

Sales, N., & Love, S. (2016). Resistance of *Haemonchus* sp to monepantel and reduced efficacy of a derquantel/abamectin combination confirmed in sheep in NSW, Australia. *Veterinary Parasitology,* 228, 193-196.

Saeed, M., Iqbal, Z., Jabbar, A., Masood, S., Babar, W., & Saddiqi, H.A. (2010). Multiple anthelmintic resistance and the possible contributory factors in Beetal goats in an irrigated area (Pakistan). *Research in Veterinary Science,* 88, 267-272. *Veterinary Parasitology,* 216, 4-6.

Sarre, C., Claerebout, E., Vercruysse, J., Levecke, B., Geldhof, P., & Pardon, B. (2012). Doramectin resistance in *Haemonchus contortus* on an alpaca farm in Belgium. *Veterinary Parasitology,* 185, 346-351.

Scott, I., Pomroy, W.E., Kenyon, P.R., Smith, G., Adlington, B., & Moss, A. (2013). Lack of efficacy of monepantel against *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. *Veterinary Parasitology,* 198, 166-171.

Smeal, M.G., Gough, P.A., Jackson, A.R., & Hotson, I.K. (1986). The occurrence of strains of *Haemonchus contortus* resistant to thiabendazole. *Australian Veterinary Journal,* 44, 108-109.

Taylor, M.A., Learmount, J., Lunn, E., Morgan, C., & Craig, B.H. (2009). Multiple resistance to anthelmintics in sheep nematodes and comparison of methods used for their detection. *Small Ruminant Research,* 86, 67-70.

Torgerson, P.R., Paul, M., & Furrer, R. (2014). Evaluating faecal egg count reduction using a specifically designed package "eggCounts" in R and a user friendly web interface. *International Journal for Parasitology,* 44, 299-303.

Van den Brom, R., Moll, L., Kappert, C., & Vellema, P. (2015). *Haemonchus contortus* resistance to monepantel in sheep. *Veterinary Parasitology,* 209, 278-280.

Windsor, R.H.S., Teran, M., & Windsor, R.S. (1992). Effects of parasitic infestation on the productivity of alpacas (*Lama pacos*). *Tropical Animal Health and Production,* 24, 57-62.

Wood, I.B., Amaral, N.K., Bairden, K., Duncan, J.L., Kassai, T., & Malone, J.B. (1995). World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). *Veterinary Parasitology,* 58, 181-213.

CHAPTER 9. GENERAL DISCUSSION

9.1. Goals and achievements

This thesis aimed to: (i) assess the worm control practices used by Australian alpaca farmers (Chapter 2); (ii) assess the performance of a new diagnostic tool, FECPAK^{G2} for estimating worm burden (faecal egg counts, FEC) in alpacas (Chapter 3); (iii) validate a molecular diagnostic tool to detect the common gastrointestinal nematodes (GINs) of alpacas in Australia (Chapter 4); (iv) determine the epidemiology, and worm burden and associated pathology in alpacas (Chapters 5-7); and (v) assess the efficacy of commonly used anthelmintics against GINs of alpacas in Australia (Chapter 8). All of the aims set at the beginning of this PhD project were achieved. This chapter discusses the significant findings and limitations of this thesis, shows how the results of this project fill knowledge gaps and suggests future research directions in the field of parasites and South American camelids (SACs).

In Chapter 2, an online questionnaire survey was conducted to assess the worm control practices used by Australian alpaca farmers. Results of the survey revealed that more than 50% of respondents perceived that GINs were an important health problem of alpacas. However, previous epidemiological surveys of GINs of alpacas showed a higher prevalence (70 to 100%) of GINs in alpacas in various countries around the world (Ballweber, 2009; Contreras et al., 2014; Franz et al., 2015; Hertzberg and Kohler, 2006; Leguia, 1991). These differences could be due to farmers' lack of knowledge of parasites and their impact on the health of alpacas. Most of the alpaca farmers were unaware of pasture spelling and 32% of respondents had shared paddocks with sheep and cattle. These practices may facilitate pasture contamination and cross-transmission of GINs among livestock species, particularly when GINs are shared between domestic ruminants and South American camelids (Ballweber, 2009; Franz et al. 2015). This survey revealed that macrocyclic lactones (MLs) were the most commonly used anthelmintics in alpacas (38% of respondents) followed by a commercial combination of four anthelmintics (abamectin, albendazole, closantel and levamisole; 22%) and monepantel (15%). Almost half of the respondents (47%) used anthelmintics at the dose rate recommended for sheep, although some used 1.5 (31%) and 2 (13%) times the dose rate recommended for sheep. As no anthelmintics are registered for use in alpacas around the world, various studies have recommended different anthelmintic dose rates to treat GINs in alpacas (Dadak et

al., 2013; Geurden and Hemelrijk, 2005; Gillespie et al., 2010; Jabbar et al., 2013) but commonly used dose rates in alpacas in Australia are unknown and veterinarians have been recommending 1.5 times the dose rate recommended for sheep (J. Vaughan, CriaGenesis, Australia). Sixty percent of respondents were unaware of anthelmintic resistance (AR) and most of them had never performed a faecal egg count reduction test (FECRT) on their farms. This shows that Australian alpaca farmers do not seem to have a sound understanding of the FECRT for assessing the effectiveness of dewormers on their properties. The findings of Chapter 2 emphasized the need to (i) gain insights into the epidemiology of GINs of alpaca in Australia and (ii) assess the efficacy of commonly used anthelmintics for designing a sustainable worm control program for GINs of Australian alpacas.

An accurate diagnosis of GINs is vital in epidemiological studies. As limited information was available on the diagnosis of GINs of SACs, a study first compared a conventional technique with a novel diagnostic method for FECs (Chapter 3) and then a molecular test was established to accurately identify common GINs of alpacas (Chapter 4). Specifically, Chapter 3 aimed to compare a newly developed diagnostic method, the FECPAK^{G2} and a routinely used the McMaster technique for counting GIN eggs in the faeces of alpacas using two flotation solutions (saturated sodium chloride and sucrose solutions). Data revealed moderate to good agreement between the two methods, with better agreement achieved when saturated sugar is used as a flotation fluid, particularly when FECs are less than 1000 eggs per gram of faeces. There was a significant difference ($P = 0.03$) in performance between two techniques when salt solution was used. This was the first study to assess agreement of measurements between McMaster and FECPAK^{G2} methods for estimating faecal eggs in South American camelids. Hence, this device can be used as an alternative diagnostic tool to estimate FEC in alpacas and other SACs. Similarly, FECPAK^{G2} can also be used in other livestock species as it offers several advantages over the McMaster technique. For example, setting up the FECPAK^{G2} test does not require specialised laboratory equipment or technical skills, and samples can be processed easily in the field by a lay operator. Furthermore, it involves capturing digital images of samples without the use of a microscope. Digital images of samples are stored, ready for assessment by trained technicians for identification and counting nematode eggs (Sowerby et al., 2016). Each digital image remains available for reference and auditing purposes.

In Chapter 4, to reliably detect and differentiate seven common GINs of alpacas, a multiplexed-tandem PCR (MT-PCR) was developed employing the second internal transcribed spacer of the nuclear ribosomal DNA. This molecular diagnostic tool is much faster than the conventional larval culture method used for the identification of nematode genera/species and also has higher sensitivity and specificity as previously demonstrated for cattle and sheep GINs (Roeber et al., 2017a, b). Another advantage of using MT-PCR in identifying GINs of nematodes of alpacas is that it allows the reliable detection of *Camelostrongylus mentulatus* which was not possible using the larval culture method.

To understand the epidemiology of GINs of alpacas in Australia, three studies were undertaken by conducting national cross-sectional (Chapter 5) and longitudinal (Chapter 6) epidemiological surveys to count GIN eggs (FEC), and the examination GI tracts of alpacas (Chapter 7) to count and morphologically identify adult worms. A total of 1,545 and 1,688 fresh faecal samples were collected from alpacas of both sexes and different age groups across the country for cross-sectional (Chapter 5) and longitudinal (Chapter 6) surveys, respectively. The findings of both surveys were comparable in terms of the overall prevalence (cross-sectional [CS]: 66%; longitudinal [LS] 61%), susceptibility of different age groups, intensity of worm burden (CS: 17,415 eggs per grams of faeces [EPG]; LS: 15,540 EPG], spectrum of GINs (*C. mentulatus, Cooperia* spp., *Haemonchus* spp., *Oesophagostomum* spp., *Ostertagia ostertagi*, *Teladorsagia circumcincta* and *Trichostrongylus* spp.), climatic zones and seasonal variation. As the prevalence and intensity of GINs of alpacas may vary depending on age, season and climatic zone, the ZINB model used in the longitudinal study (Chapter 6) was found to be useful in estimating effects of various factors such as age, seasons and deworming history on worm burdens while considering variation at animal and herd levels. Given this model was found to be suitable for over-dispersed FEC data inflated with zeros (Nodtvedt et al., 2002; Denwood et al., 2008), it can also be used in analysing FEC data of nematodes from other host species.

FECs may not be able to provide a precise estimation of the actual number of adult worms present in the GI tract. To get a better understanding of GIN population present in the GI tracts of Australian alpacas, 100 GI tracts were examined for total worm counts and nematode identification (Chapter 7). Results revealed a mean burden of 1,300 worms, with the highest burden of 29,000 worms. Nineteen different species of GINs were identified from Australian alpacas, some for the first time by using MT-PCR and morphological techniques. The majority of the nematodes recovered from alpacas were

shared-GIN, most of them share with cattle. Three camelid specific nematodes: *Graphinema auchenia*, *C. mentulatus* and *Trichuris tenuis* were recovered from Australian alpacas for the first time. *Haemonchus contortus* was the most prevalent nematode (81%) followed by *C. mentulatus* (60%). The findings of Chapters 5 to 7 indicated that alpacas harbour many of the same nematodes as sheep and cattle and their role in the transmission of these parasites where they co-graze should be considered in management and control practices. Furthermore, the patterns of seasonal variation in the epidemiology of GINs of alpacas in various climatic zones are very similar to those of cattle and sheep, and careful attention should be paid when designing control strategies for domestic ruminants co-grazing with alpacas and vice versa.

In Chapter 8, the efficacy of commonly used anthelmintics against GINs of alpacas was assessed. An online questionnaire survey was conducted to assess current worm control practices on 97 Australian alpaca farms enrolled in CS study, with an emphasis on the use of anthelmintics. Of this group of 97 alpaca farms, 20 were selected to assess the efficacy of eight anthelmintics and/or their combinations (closantel, fenbendazole ivermectin, monepantel, moxidectin and a combination of levamisole, closantel, albendazole, abamectin) using the FECRT. A multiplexed-tandem PCR (MT-PCR) was used to identify the prevalent nematode genera/species. Although only 63% respondents perceived worms to be an important health concern for alpacas, the majority of respondents (89%) used anthelmintics to control GINs of alpacas. The commonly used anthelmintics were macrocyclic lactones, monepantel, benzimidazoles, levamisole, closantel and their combinations, and they were typically administered at the dose rate recommended for sheep. The FECRT results showed that a combination of levamisole, closantel, albendazole and abamectin was the most effective dewormer followed by single anthelmintic, either monepantel, moxidectin, closantel, fenbendazole or ivermectin. *Haemonchus* spp. were the most commonly resistant nematodes followed by *Trichostrongylus* spp., *C. mentulatus*, *O. ostertagi* and *Cooperia* spp. This was the first study to comprehensively investigate the efficacy of commonly used anthelmintics against GINs of alpacas and llamas. This study highlighted the need for future large-scale pharmacokinetic studies to understand pharmacokinetic properties, appropriate dose rate(s) and the route(s) of administration of the commonly used anthelmintics in SACs.

9.2. Implications, future research and conclusions

This thesis highlights the need for future research on many aspects of GINs of alpacas, including epidemiology, pathophysiology, effect on production and control using chemical and non-chemical methods. In-depth investigations of these aspects of the nematodes, host-parasite interactions, and pharmacokinetic studies should substantially improve understanding of parasitic gastroenteritis in alpacas, to ultimately develop and implement nematode control program(s) for reducing the economic impact of GINs.

The most commonly used technique for assessing worm burden in livestock species is the McMaster technique (Ballweber et al. 2014; Gordon and Whitlock 1939; Cebra and Stang 2008) as it is simple and user-friendly (Levecke et al., 2009). However, the findings of Chapter 3 show that the FECPAK^{G2} technique can be used as an alternative diagnostic tool for FECs in alpacas. In addition, two statistical techniques, Bland-Altman plot (Bland and Altman, 2010) and Lin's concordance correlation coefficient (Lin et al., 2002) were utilized for the first time to compare (Chapter 3) the performances of the two diagnostic techniques (FECPAK^{G2} and McMaster) and such methods could be used to compare the performance of other diagnostic test(s). Furthermore, results showed fair agreements and correlations between performances of the McMaster and FECPAK^{G2} techniques and a better agreement was observed when saturated sugar was used as a flotation solution and FECs were less than 1,000 EPG. However, it was also revealed that the results of FECPAK^{G2} may be affected by the type of flotation solution or the presence of high or low numbers of eggs in the faeces. Furthermore, the reliability of the results of the FECPAK^{G2} method can also be influenced by the area of the slide (or volume of the sample) counted (Bosco et al., 2014). Therefore, future studies targeted at the use of the FECPAK^{G2} technique in alpacas should undertake experiments using standardised faecal samples with known number of eggs per gram of faeces, with technical and biological replicates. The findings of Chapter 3 suggest that the FECPAK^{G2} technique can be used for assessing worm burden in other livestock species.

To understand the epidemiology of GINs of alpacas, cross-sectional and longitudinal surveys (Chapters 5 and 6) and examination of GI tracts of alpacas (Chapter 7) were undertaken. This three-pronged approach is complementary and provided detailed information about GINs as the cross-sectional survey provided information on the worm burden and prevalence of GINs of alpacas across Australia (Chapter 5) whereas the longitudinal survey allowed insights into the seasonal variation in the prevalence of GINs

in various climatic zones of Australia (Chapter 6). In addition, the examination of 100 GI tracts of alpacas (Chapter 7) allowed an assessment of worm burden (based on total worm counts) and the type of nematodes (based on morphological characterisation) infecting Australian alpacas. These studies provide a solid framework for undertaking similar studies to understand epidemiology of GI parasites in other livestock species. However, in order to gain better insights into the epidemiology of GINs of alpacas, further longitudinal surveys for an extended period of three to five years should be conducted as the longitudinal survey data presented in this thesis was limited to only one year. As Australia is a diverse country with various climatic zones, annual weather patterns can influence the epidemiology of GINs of alpacas and such changes have been shown for GINs of sheep in South Australia (Beveridge et al. 1985) where the mortality of sheep varied among five consecutive years due to differences in the availability of third-stage larvae of GINs on pastures depending on the annual rainfall and temperature.

Pathophysiology of parasitic gastroenteritis in sheep and cattle are well characterised (e.g., Besier et al., 2016), however very little is known about the pathophysiology of GINs in alpacas. The findings of Chapter 7 revealed that Australian alpacas can be infected with at least 19 different nematode species and these worms were isolated from GI tracts of apparently healthy animals. Furthermore, 'Moroccan leather' type lesions were found in the third compartment of the stomach of some alpacas which were infected with high numbers of *C. mentulatus*. Previously, Hilton et al. (1978) suggested that *C. mentulatus* could be a potential pathogen of alpacas as it can behave like *T. circumcincta* and *O. ostertagi* in sheep and cattle, respectively. However, such hypothesis has not been proven using experimental studies. Therefore, future studies should focus on understanding the pathophysiology of not only *C. mentulatus* but also the other commonly prevalent GINs (see Chapters 5-7). This will also help to estimate worm burdens that can cause production losses and mortalities in alpacas. Furthermore, such studies will provide data for establishing the threshold of FECs for various GINs in alpacas.

The FECRT was used to assess the efficacy of six different anthelmintics at one-and-half time dose recommended for sheep against GINs of alpacas and the majority of them were found to be ineffective (Chapter 8). Based on the results of this study, it was not possible to conclude whether the nematodes were resistant or the dose rate was inadequate to kill GINs of alpacas, as very little is known about pharmacokinetic properties of the commonly used anthelmintics in SACs. For example, ivermectin, a

macrocyclic lactone (ML) was found 100% effective in reducing FECs of GINs in both alpacas and llamas when used at 200 µg/kg body weight when administered subcutaneously (Geurden et al., 2005) whereas another study showed partial reduction at the same dose rate (Windsor et al., 1992). Conversely, in the USA, Gillespie et al. (2010) showed that ivermectin was ineffective in alpacas when given orally at 300 µg/kg body weight. Moxidectin, another ML, was effective when administered orally at 200 µg/kg bodyweight but ineffective on another farm (Gillespie et al., 2010). These discrepancies in the efficacy of MLs could be associated with difference in routes of administration as subcutaneous administration of moxidectin at 200 µg/kg bodyweight showed higher bioavailability compared to oral administration (Cocquyt et al., 2016). Similarly, doramectin when applied topically at 0.5 mg/kg bodyweight, showed slow absorption in alpacas and llamas (Hunter et al., 2004b) which could be due to the nature of the fibre/wool of these animals as compared to cattle. Similarly, fenbendazole, a member of the benzimidazoles, showed similar absorption rates and metabolites in llamas and alpacas as those in sheep, goats and cattle but it had a prolonged elimination rate in SACs (Beier et al., 1999; Lakritz et al., 2015). Like MLs, fenbendazole has been reported to be effective when given orally at 5 mg/kg (Beier et al., 2000) as well as 10mg/kg (Shaw, 2001) in alpacas and llamas. Dadak et al. (2013) reported that monepantel was 100% effective GINs of SACs when used at 3-times the dose recommended for sheep.

These differences in the efficacy of different anthelmintics in llamas and alpacas could be due to the differences in the route of administration or other factors such as location of the studies, age of animals, nematode species etc. Furthermore, the off-label use of anthelmintics and possible low bioavailability in SACs may result in under-dosing, leading to parasite exposure to suboptimal drug concentrations which may result in the development of AR (Chapter 8; Jabbar et al., 2013). Similarly, the frequent overuse of an anthelmintic drug/class without rotation could also contribute to AR (Williamson, 2014; Gomez-Puerta et al., 2017). For instance, ivermectin resistance was observed on an alpaca farm where only MLs had been used for more than six years (Jabbar et al., 2013). Furthermore, hybridisation of different species within a genus (e.g. *Haemonchus contortus* and *H. placei*) can have important implications for the transmission of AR genes (Chaudhry et al., 2015) and this could be important in nematodes of alpacas as they can be infected with both sheep and cattle GINs (Chapters 5-7). Occurrence of interspecies hybridisation of nematodes has been experimentally demonstrated (Chaudhry et al., 2015; Le Jambre, 1981; Le Jambre and Royal, 1980; Isenstein, 1971),

therefore careful attention should be paid to the nematode genus/species on farms when managing inefficacy and/or AR in GINs of alpacas. Overall, large-scale pharmacokinetic studies are required to understand pharmacokinetic properties, appropriate dose rate(s) and the route(s) of administration of the commonly used anthelmintics in alpacas and llamas.

Owing to the widespread resistance in GINs of small ruminants worldwide, alternate controlling methods such as biological control and vaccination have recently been commercialised in Australia to control GINs of sheep. Barbervax®, a commercial vaccine manufactured from native gut membrane proteins of *H. contortus*, is available for use in sheep in Australia and the field trials of this vaccine have shown a significant success in reducing morbidities and mortalities associated with *H. contortus* in the summer rainfall zone of Australia (Besier et al., 2015; de Matos et al., 2017; Smith et al., 2013). Recently, Barbervax® vaccine was used first time in alpacas in the USA and demonstrated little success in the production of antibodies against *H. contortus* (VanHoy et al., 2018) due to the failure of an establishment of experimental infection with *H. contortus* in alpacas. Furthermore, the study was conducted on only a limited number of animals (12 alpacas). Given that Barbervax® has proven to be successful in reducing the burden of Barber's pole worm in sheep, further well-designed, controlled studies are required to assess the immune responses induced by this vaccine in alpacas before undertaking field studies.

Another biological mean of controlling GINs of ruminants is using predaceous fungi which has become into commercial use recently in Australia. BioWorma®, a commercial product containing the chlamydospores of the nematophagous fungus, *Duddingtonia flagrans* strain IAH 1297 is being used as a supplement feed to reduce larval contamination on pasture. Studies have found that BioWorma® was effective in reducing worm burdens in sheep, goats, cattle and horses (Knox et al., 2013; Healey et al., 2018a, b). Given that no anthelmintic is registered for use in alpacas and very little information of available on the pharmacokinetic properties of currently used anthelmintics in SACs, it is prudent to explore other methods to control GINs of alpacas and llamas, and the efficacy of BioWorma® should be assessed against GINs of alpacas.

In conclusion, the present study has contributed filling some significant gaps in the knowledge of GINs of alpacas in Australia. The findings of this thesis emphasize the need to future intensive research on the pathophysiology GINs of alpacas, host-parasite interactions, pharmacokinetic studies of anthelmintics and the validation of non-chemical

control methods to treat and control GINs of alpacas and llamas, thereby aiding in using anthelmintics judiciously and developing integrated parasites control programs for SACs in Australia and elsewhere.

9.3. References

Ballweber, L. R., Beugnet, F., Marchiondo, A. A., & Payne, P. A. (2014). American Association of Veterinary Parasitologists' review of veterinary fecal flotation methods and factors influencing their accuracy and use-Is there really one best technique? *Veterinary Parasitology*, 204, 73-80.

Besier, B., Kahn, L., Dobson, R., & Smith, D. (2015, May). Barbervax− a new strategy for *Haemonchus* management. In *Proceedings of the Australian Veterinary Association (AVA) Annual Conferences, Pan Pacific (NZVA and AVA) Veterinary Conference 2015, Combined proceedings*, pp. 168-174.

Besier, R. B., Kahn, L. P., Sargison, N. D., & Van Wyk, J. A. (2016). The pathophysiology, ecology and epidemiology of *Haemonchus contortus* infection in small ruminants. *Advances in Parasitology*, 93, 95-143.

Beier III, E. (1999). *Oral pharmacokinetic and clinical efficacy profiles of fenbendazole in Llamas, South American Camelids* (Doctoral dissertation, Oklahoma State University).

Beier III, E., Lehenbauer, T. W., & Sangiah, S. (2000). Clinical efficacy of fenbendazole against gastrointestinal parasites in llamas. *Small Ruminant Research*, 36, 17-23.

Beveridge, I., Barker, I. K., Rickard, M. D., & Burton, J. D. (1974). Experimental infection of sheep with *Camelostrongylus mentulatus* and associated gastritis. *Australian Veterinary Journal*, 50, 36-37.

Beveridge, I., & Ford, G. E. (1982). The trichostrongyloid parasites of sheep in South Australia and their regional distribution. *Australian Veterinary Journal*, 59, 177-179.

Beveridge, I., Brown, T. H., Fitzsimons, S. M., Ford, G. E., Judson, G. J., Martin, R. R., & Miller, D. W. (1985). Mortality in weaner sheep in South Australia under different regimes of anthelmintic treatment. *Australian Journal of Agricultural Research*, 36, 857-865.

Beveridge, I., Pullman, A. L., Henzell, R., & Martin, R. R. (1987). Helminth parasites of feral goats in South Australia. *Australian Veterinary Journal*, 64, 111-112.

Bland, J. M., & Altman, D. G. (2010). Statistical methods for assessing agreement between two methods of clinical measurement. *International Journal of Nursing Studies*, 47, 931-936.

Bosco, A., Rinaldi, L., Maurelli, M., Musella, V., Coles, G., & Cringoli, G. (2014). The comparison of FLOTAC, FECPAK and McMaster techniques for nematode egg counts in cattle. *Acta parasitologica*, 59, 625-628.

Brunsdon, R. V. (1971). Trichostrongyle worm infection in cattle: further studies on problems of diagnosis and on seasonal patterns of occurrence. *New Zealand Veterinary Journal*, 19, 203-212.

Bryan, R. P., & Kerr, J. D. (1989). The relation between the natural worm burden of steers and the faecal egg count differentiated to species. *Veterinary Parasitology*, 30, 327-334.

Cebra, C. K., & Stang, B. V. (2008). Comparison of methods to detect gastrointestinal parasites in llamas and alpacas. *Journal of the American Veterinary Medical Association*, 232, 733-741.

Chaudhry, U., Redman, E. M., Abbas, M., Muthusamy, R., Ashraf, K., & Gilleard, J. S. (2015). Genetic evidence for hybridisation between *Haemonchus contortus* and *Haemonchus placei* in natural field populations and its implications for interspecies transmission of anthelmintic resistance. *International Journal for Parasitology*, 45, 149-159.

Cocquyt, C. M., Van Amstel, S., Cox, S., Rohrbach, B., & Martín-Jiménez, T. (2016). Pharmacokinetics of moxidectin in alpacas following administration of an oral or subcutaneous formulation. *Research in Veterinary Science*, 105, 160-164.

Contreras, S., Chávez, V., Pinedo, V., Leyva, V., & Suárez, A. (2014). Helminthiasis in alpacas (*Vicugna pacos*) of two peasant communities in Macusani, Puno during the dry season. *Revista de Investigaciones Veterinarias del Perú (RIVEP)*, 25, 268-275.

Craig, T. M. (1986). Epidemiology and control of gastrointestinal nematodes and cestodes in small ruminants: Southern United States. *The Veterinary clinic of North America: Food Animal Practice*, 2, 367-72.

Dadak, A. M., Asanger, H., Tichy, A., & Franz, S. (2013). Establishing an efficacious dose rate of monepantel for treating gastrointestinal nematodes in llamas under field conditions. *Veterinary Record*, 172, 155-155.

Denwood, M. J., Stear, M. J., Matthews, L., Reid, S. W. J., Toft, N., & Innocent, G. T. (2008). The distribution of the pathogenic nematode *Nematodirus battus* in lambs is zero-inflated. *Parasitology*, 135, 1225-1235.

Galvan, N., Middleton, J. R., Nagy, D. W., Schultz, L. G., & Schaeffer, J. W. (2012). Anthelmintic resistance in a herd of alpacas (*Vicugna pacos*). *The Canadian Veterinary Journal*, 53, 1310

Gillespie, R. A. M., Williamson, L. H., Terrill, T. H., & Kaplan, R. M. (2010). Efficacy of anthelmintics on South American camelid (*llama and alpaca*) farms in Georgia. *Veterinary Parasitology*, 172, 168-171.

Godber, O. F., Phythian, C. J., Bosco, A., Ianniello, D., Coles, G., Rinaldi, L., & Cringoli, G. (2015). A comparison of the FECPAK and Mini-FLOTAC faecal egg counting techniques. *Veterinary Parasitology*, 207, 342-345.

Gordon, H. M., & Whitlock, H. V. (1939). A new technique for counting nematode eggs in sheep faeces. *Journal of the Council for Scientific and Industrial Research*, 12, 50-52.

Geurden, T., & Van Hemelrijk, K. (2005). Ivermectin treatment against gastrointestinal nematodes in New World camelids in Belgium. *Small Ruminant Research*, *58*, 71-73.

Guerrero, C. A., Rojas, M., & Alva, J. (1981). *Lamanema chavezi*, an enterohepatic nematode of South American Camelidae and its control using levamisole. *Revista Latinoamericana de Microbiologia*, 23, 121.

Gibbs, H. C. (1986). Hypobiosis and the periparturient rise in sheep. *The Veterinary Clinics of North America. Food animal practice*, 2, 345-353.

Gomez-Puerta, L. A., Yucra, D., Lopez-Urbina, M. T., & Gonzalez, A. E. (2017). The alpaca (*Vicugna* pacos) as a natural intermediate host of *Taenia omissa* (Cestoda: Taeniidae). *Veterinary Parasitology*, 246, 93-95.

Harris, P. A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N., & Conde, J. G. (2009). Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of Biomedical Informatics*, 42, 377-381.

Healey, K., Lawlor, C., Knox, M. R., Chambers, M., & Lamb, J. (2018a). Field evaluation of *Duddingtonia flagrans* IAH 1297 for the reduction of worm burden in grazing animals: tracer studies in sheep. *Veterinary Parasitology*, 253, 48-54.

Healey, K., Lawlor, C., Knox, M. R., Chambers, M., Lamb, J., & Groves, P. (2018b). Field evaluation of *Duddingtonia flagrans* IAH 1297 for the reduction of worm burden in grazing animals: pasture larval studies in horses, cattle and goats. *Veterinary Parasitology*, 258, 124-132.

Hertzberg, H., & Kohler, L. (2006). Prevalence and significance of gastrointestinal helminths and protozoa in South American camelids in Switzerland. *Berliner und Münchener Tierärztliche Wochenschrift*, 119, 291-294.

Hill, F. I., Death, A. F., & Wyeth, T. K. (1993). Nematode burdens of alpacas sharing grazing with sheep in New Zealand. *New Zealand Veterinary Journal*, 41, 205-208.

Hilton, R. J., Barker, I. K., & Rickard, M. D. (1978). Distribution and pathogenicity during development of *Camelostrongylus mentulatus* in the abomasum of sheep. *Veterinary Parasitology*, 4, 231-242.

Hunter, R. P., Isaza, R., Koch, D. E., Dodd, C. C., & Goatley, M. A. (2004). The pharmacokinetics of topical doramectin in llamas (*Lama glama*) and alpacas (*Lama pacos*). *Journal of Veterinary Pharmacology and Therapeutics*, 27, 187-189.

Isenstein, R. S. (1971). Hybridization of two species of nematodes parasitic in ruminants, *Cooperia oncophora* (Railliet, 1898) Ransom, 1907, and *Cooperia pectinata* Ransom, 1907. *The Journal of Parasitology*, 57, 320-326.

Jabbar, A., Campbell, A. J., Charles, J. A., & Gasser, R. B. (2013). First report of anthelmintic resistance in *Haemonchus contortus* in alpacas in Australia. *Parasites and Vectors*, 6, 243.

Knox, M., Healey, K., Lawlor, C., Lamb, J., Chambers, M., & Groves, P. (2013). BioWorma® for control of nematode parasites of livestock. In *Proceedings World Association for the Advancement of Veterinary Parasitology Congress*, Perth, Australia, pp. 25-29.

Lakritz, J., Linden, D., Anderson, D. E., & Specht, T. A. (2015). Plasma concentrations of fenbendazole (FBZ) and oxfendazole in alpacas (*Lama pacos*) after single intravenous and oral dosing of FBZ. *Veterinary Medicine: Research and Reports*, 6, 71-81.

Le Jambre, L. F. (1981). Hybridization of Australian *Haemonchus placei* (Place, 1893), *Haemonchus contortus cayugensis* (Das & Whitlock, 1960) and *Haemonchus contortus* (Rudolphi, 1803) from Louisiana. *International Journal for Parasitology*, 11, 323-330.

Le Jambre, L. F., & Royal, W. M. (1980). Meiotic abnormalities in backcross lines of hybrid Haemonchus. *International Journal for Parasitology*, 10, 281-286.

Leguía, G. (1991). The epidemiology and economic impact of llama parasites. *Parasitology Today*, 7, 54-56.

Levecke, B., De Wilde, N., Vandenhoute, E., & Vercruysse, J. (2009). Field validity and feasibility of four techniques for the detection of *Trichuris* in simians: a model for monitoring drug efficacy in public health? *PLoS Neglected Tropical Diseases*, 3, e366.

Lin, L., Hedayat, A. S., Sinha, B., & Yang, M. (2002). Statistical methods in assessing agreement: models, issues, and tools. *Journal of the American Statistical Association*, 97, 257-270.

Matos, A. F. I. M., Nobre, C. O. R., Monteiro, J. P., Bevilaqua, C. M. L., Smith, W. D., & Teixeira, M. (2017). Attempt to control *Haemonchus contortus* in dairy goats with Barbervax®, a vaccine derived from the nematode gut membrane glycoproteins. *Small Ruminant Research*, 151, 1-4.

Meier, L., Torgerson, P. R., & Hertzberg, H. (2016). Vaccination of goats against *Haemonchus contortus* with the gut membrane proteins H11/H-gal-GP. *Veterinary Parasitology*, 229, 15-21.

Michel, J. F. (1969a). Observations on the faecal egg count of calves naturally infected with *Ostertagia ostertagi*. *Parasitology*, 59, 829-835.

Michel, J. F. (1969b). The regulation of egg output by *Ostertagia ostertagi* in calves infected once only. *Parasitology*, 59, 767-774.

Newton, S. E. (1995). Progress on vaccination against *Haemonchus contortus*. *International Journal for Parasitology*, 25, 1281-1289.

Playford, M. C., Smith, A. N., Love, S., Besier, R. B., Kluver, P., & Bailey, J. N. (2014). Prevalence and severity of anthelmintic resistance in ovine gastrointestinal nematodes in Australia (2009–2012). *Australian Veterinary Journal*, 92, 464-471.

Roeber, F., Hassan, E. B., Skuce, P., Morrison, A., Claerebout, E., Casaert, S., ... & Larsen, J. (2017a). An automated, multiplex-tandem PCR platform for the diagnosis of gastrointestinal nematode infections in cattle: an Australian-European validation study. *Veterinary Parasitology*, 239, 62-75.

Roeber, F., Morrison, A., Casaert, S., Smith, L., Claerebout, E., & Skuce, P. (2017). Multiplexed-tandem PCR for the specific diagnosis of gastrointestinal nematode infections in sheep: an European validation study. *Parasites and Vectors*, 10, 226.

Roberts, J. L., & Swan, R. A. (1981). Quantitative studies of ovine haemonchosis. I. Relationship between faecal egg counts and total worm counts. *Veterinary Parasitology*, 8, 165-171.

Rogers, W. P. (1939). Nematode parasites of sheep in Western Australia. *Journal of Helminthology*, 17, 151-158.

Rose, J. H., & Small, A. J. (1980). Transmission of *Oesophagostomum* spp among sows at pasture. *The Veterinary Record*, 107, 223-225.

Rubin, R. (1967). Some observations on the interpretation of fecal egg counts. *American Journal of Veterinary Clinical Pathology*, 1, 145-148.

Shaw, G. C. (2001). *Most Efficient Single Dose of Fenbendazole to Control Gastrointestinal Parasites of Llamas and Alpacas* (Doctoral dissertation, Kalamazoo College).

Sarre, C., Claerebout, E., Vercruysse, J., Levecke, B., Geldhof, P., Pardon, B., ... & Geurden, T. (2012). Doramectin resistance in *Haemonchus contortus* on an alpaca farm in Belgium. *Veterinary Parasitology*, 185, 346-351.

Smith, W. D., Newlands, G. F., Fitzpatrick, J. L., & Besier, R. B. (2013). Barbervax, a potential commercial vaccine for *Haemonchus contortus*: field efficacy trials with sheep in a high-risk region of Australia. In *Proceedings World Association for the Advancement of Veterinary Parasitology Congress,* Perth, Australia. pp. 25-29.

Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M., & Jennings, F.W. (1996). Veterinary Helminthology – Phylum Nemathelminthes - Class Nematoda – Superfamily Trichostrongyloidea. In: GM, Armour J, Duncan JL, Dunn AM, Jennings FW, eds. *Veterinary Parasitology*, 2nd ed. Oxford. Blackwell Science Ltd. pp 4-17

VanHoy, G., Carman, M., Habing, G., Lakritz, J., Hinds, C. A., Niehaus, A., ... & Marsh, A. E. (2018). Safety and serologic response to a *Haemonchus contortus* vaccine in alpacas. *Veterinary Parasitology*, 252, 180-186.

Williamson, L. H. (2014). Anthelmintic resistance in camelid parasites. *Llama and Alpaca Care-E-Book: Medicine, Surgery, Reproduction, Nutrition, and Herd Health*, Elsevier Inc. pp 16-22.

Windsor, R. H. S., Teran, M., & Windsor, R. S. (1992). Effects of parasitic infestation on the productivity of alpacas (*Lama pacos*). *Tropical Animal Health and Production*, 24, 57-62.