Studies on gastrointestinal nematodes ("worms") of alpacas

by Jane Vaughan, Mohammed Rashid and Abdul Jabbar
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Foreword

Alpacas are increasingly popular as a commercial livestock species due to their soft, light and fine fibre, lean meat and hides as well as their ability to adapt to diverse climatic conditions across Australia. The health and productivity of alpacas can be compromised by gastrointestinal nematodes (GINs), resulting in substantial economic losses. Although Australia has the largest alpaca population outside South America, very limited information is available on the GINs of Australian alpacas.

The present project aimed to (i) assess the worm control practices used by Australian alpaca farmers, (ii) determine the prevalence of GINs of alpacas in various climatic zones in Australia and (iii) undertake field efficacy studies to determine the status of anthelmintic resistance in GINs of alpacas so that novel information generated in the project would help Australian alpaca farmers and veterinarians in controlling GINs of alpacas.

The worm control practices survey provided insights into the current husbandry and worm control practices used by Australian alpaca farmers of different herd sizes as producers' knowledge about GINs of alpacas, their diagnosis, treatment and control, and grazing management are important in the sustainable control of worms in alpacas.

Epidemiological studies revealed that Australian alpacas are affected by camelid-specific worm species, and sheep and cattle worms. Alpacas of all ages are affected by worms in all climatic zones of Australia, across all farm sizes, throughout the year.

The project showed that there is a widespread resistance to commonly used dewormers in GINs of Australian alpacas. This study established a new high-throughput, rapid and cost-effective DNA-based test for the accurate diagnosis of the GINs of alpacas which is available to alpaca farmers and veterinarians in Australia.

The project has resulted in recommendations to optimise health and production of alpacas and has also identified future research directions for the Australian Alpaca Association.

This project was funded from industry revenue (Australian Alpaca Association) which was matched with funds provided by the Australian Government (AgriFutures Australia).

This report is an addition to AgriFutures Australia's diverse range of over 2000 research publications and it forms part of our Emerging Industries arena, which aims to support early stage establishment of high potential rural industries.

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John Harvey
Managing Director
AgriFutures Australia

About the Authors

Dr Jane Vaughan graduated with a Bachelor of Veterinary Science (Honours) from the University of Melbourne in 1988, gained membership to the Australian and New Zealand College of Veterinary Scientists in the Ruminant Nutrition Chapter in 1998 and completed a Doctor of Philosophy in the control of alpaca ovarian function in 2002. Jane performs commercial embryo collection and transfer in alpacas throughout Australia, New Zealand and the United Kingdom. She also provides nutritional advice and Johne's disease Market Assurance and Q-Alpaca programs to alpaca breeders in Australia. Jane lectures regularly to farmers, veterinarians and veterinary students on alpaca reproduction, nutrition and husbandry in both Australia and internationally, and has co-authored a number of papers in refereed journals.

Mohammed Haronur Rashid is a PhD candidate at The University of Melbourne. He worked on gastrointestinal nematodes of alpacas in Australia during his PhD research. He has over 18 years of experience working in the agriculture and livestock sector in Australia and Bangladesh. In his professional career, Harun has worked as a veterinarian for veterinary pharmaceutical and nutraceutical companies, and poultry, dairy and pig farms.

The principal investigator of this project, Associate Professor Abdul Jabbar, has more than 15 years experience in veterinary parasitology, particularly in anthelmintic resistance in sheep, goats, cattle, alpacas, *in vitro* and *in vivo* efficacy studies of anthelmintics against gastrointestinal nematodes (GINs) of socioeconomic importance and the molecular diagnosis of animals as well as human parasites. Over the past 11 years while he has been at the Melbourne Veterinary School as a PhD student, postdoctoral fellow and an academic, A/Prof Jabbar's contribution to Veterinary Parasitology includes 137 research publications, 85 conference presentations at national and international scientific meetings and a research funding of more than $2 million for various research projects from the AgriFutures Australia, the Australian Research Council, the Australian Centre for International Agricultural Research and other government and industry organisations. A/Prof Jabbar's national and international standing as a parasitologist is reflected in having been invited 15 times to present his work in national and international conferences/meetings/workshops. In recognition of his research achievements, A/Prof Jabbar has received 15 awards, prizes and scholarships. In 2013, he received the national Dairy Science and Innovation Award for the Young Scientists from the Department of Agriculture and Water Resources, Canberra, for the development of next-generation molecular tools for the diagnosis of bovine theileriosis. In 2015, he was awarded the International Odile Bain Memorial Prize as an outstanding Early Career Researcher in Veterinary Parasitology, sponsored by *Merial* and the journal *Parasites & Vectors*. In 2017, A/Prof Jabbar received the Peter Nansen Young Scientist Award from the *World Association for the Advancement of Veterinary Parasitology*.

Acknowledgments

This project would not have been possible without the significant contribution of time, energy and enthusiasm by alpaca farmers around Australia. The authors are very grateful for all the assistance that facilitated such a large body of research to be completed in this study.

We are deeply indebted to Fiona Vanderbeek (Australian Alpaca association, AAA) for her constant support during the course of this project. We are also grateful to Ian Frith, Steve Chiodo and Chris Williams (AAA) for the provision of invaluable samples for the study. We are indebted to the support of Julie Bird (AgriFutures), Michelle Malt (AAA) and Scott Williams (Forest Hill Consulting) during the project development.

We are thankful to the project team for their invaluable support. Professor Ian Beveridge's insightful discussions, input and feedback during the course of the project were vital, particularly for the total worm count part of the project. Professor Mark Stevenson's help in study designs, and data analysis and interpretation are gratefully acknowledged. We are also grateful to Dr Angus Campbell for his support during the development and execution of the project.

Technical assistance provided by Ms Christine Andersen, Dr Lea Indjein and Dr Hagos Gebrekidan are gratefully acknowledged.

Abbreviations

AAA = Australian Alpaca Association

FEC = Faecal egg count

EPG = Eggs per gram of faeces

FECRT = Faecal egg count reduction test

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Executive Summary

What the report is about

The report describes how we have improved our understanding of the worms and worm control practices of Australian alpacas. This information improves our ability to control worm infestation in alpacas.

Who is the report targeted at?

This report is targeted at Australian alpaca farmers/managers, veterinarians, and biosecurity, policy makers and stakeholders.

Where are the relevant industries located in Australia?

The members of the Australian Alpaca Association (AAA) located in the south-eastern states of New South Wales and Victoria, with fewer in Queensland, Western Australia, South Australia and Tasmania will benefit from this report. Members of the AAA participated in various parts of this project.

Background

Gastrointestinal nematode (GIN) infections are considered as one of the important challenges alpaca farmers face globally, causing diarrhoea, reduced growth rate, anaemia and mortality. For instance, a wide range of GINs have been recorded in alpacas from Australia, Europe, New Zealand, the UK and the USA. 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.

Currently, the control of nematode infections in alpacas relies mainly on the use of chemicals (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 globally as limited information is available on appropriate dose rates and routes of administration of anthelmintics used in alpacas.

Although Australia has the largest alpaca population (>450,000) outside South America, very little is known about the epidemiology and control of GINs in alpacas. Furthermore, there is no information available on parasite control practices used by Australian alpaca farmers.

Aims

The project aimed to (i) assess the worm control practices used by alpaca farmers in Australia by conducting a questionnaire survey, (ii) determine the prevalence of gastrointestinal nematode of alpacas in various climatic zones in Australia, using traditional and the latest molecular diagnostic methods, (iii) undertake field efficacy studies to determine the status of anthelmintic resistance in gastrointestinal nematodes of alpacas, and (iv) train a research higher degree (MPhil/PhD) student.

Methods used

A questionnaire was conducted using an online programme, Research Electronic Data Capture to assess worm control practices used by Australian alpaca farmers. To determine the prevalence of GINs of alpacas in Australia three types of epidemiological studies were conducted. In addition, a new DNA-based test was developed to detect nematodes DNA in the alpaca faeces. 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, faecal egg count reduction trials were performed.

Key findings

1. The worm control practices survey provided insights into the current husbandry and worm control practices used by Australian alpaca farmers of different herd sizes. Farmers' knowledge about GINs of alpacas, their diagnosis, treatment and control, and grazing management are important in the sustainable control of worm control in alpacas.
2. This study generated new knowledge about the GINs in Australian alpacas. Australian alpacas are affected by camelid-specific worm species, and sheep and cattle worms. Parasitic gastroenteritis can lead to diarrhoea and/or anaemia, illthrift, loss of production (reduced growth, less wool production, poor fertility) and death of alpacas. Alpacas of all ages are affected by worms in all climatic zones of Australia, across all farm sizes, throughout the year.
3. There is a widespread AR in GINs of Australian alpacas. The field efficacy study of dewormers against GINs of alpacas revealed that monepantel (Zolvix®) and a dewormer containing four active ingredients (Q Drench®) were effective whereas fenbendazole, ivermectin, moxidectin and closantel were ineffective dewormers when used on their own.
4. This study established a new high-throughput, rapid and cost-effective DNA-based test for the accurate diagnosis of the GINs of alpacas. Alpaca farmers can use this service either through The University of Melbourne or other commercial diagnostic laboratories offering this service.
5. A conventional McMaster technique for assessing worm burden in alpacas was compared with a new diagnostic test $FECPAK_{G2}$® which revealed that the latter technique is user-friendly and can be used by farmers to perform FECs on their farms.
6. This project supported Australia's capacity building in science by the training of a new scientist in one of the emerging animal industries of Australia.
7. This project resulted in the publication of seven original peer-reviewed scientific papers which have been/will be published in peer-reviewed scientific journals.
8. Findings of the project were presented at national and international meeting/conferences. In addition, regular presentations were delivered at regional and national meetings of the AAA.
9. A summary of the key findings of this project was published in the AAA magazine in August 2018.

Recommendations

1. To optimise health and production of alpacas, farmers should monitor worm burdens in their herds by:
    a. regularly performing FECs, particularly in weaners and tuis or when alpacas lose weight/decrease body condition/exhibit diarrhoea or anaemia.
    b. identifying worm species on each farm using larval culture or DNA testing of alpaca faeces.
    c. performing FECs in co-grazing cattle, sheep and goats simultaneously as they share many worm species.
    d. interpreting FEC results in conjunction with respect to individual farm management (stocking rates, season, pasture length, body condition, age of alpacas).
    e. using alternate methods (such as FAMACHA®) to assess the severity of anaemia caused by blood-sucking nematodes (e.g. Barber's pole worm)
2. Currently, no dewormer is registered for use in alpacas in Australia, so all use is off-label and must be used with caution. Withholding periods from other livestock species do not necessarily apply to alpacas.

3. If using veterinary chemicals Alpaca farmers should seek veterinary advice to ensure legislative compliance.
4. Many dewormers used to treat alpacas are ineffective (likely due to resistance of worms to the active ingredients). Farmers are encouraged to use dewormers having at least two chemicals/actives when treating alpacas for worms. Seek veterinary guidance as necessary.
5. Weigh alpacas to determine appropriate dose of a dewormer.
6. Calibrate drench guns to ensure accurate dosage.
7. Farmers should monitor efficacy of dewormers by performing FECs 10-14 days after deworming to ensure efficacy (≥ 95% reduction in FECs).
8. Newly introduced alpacas should receive an effective dewormer ("quarantine drench") prior to entry into the herd.
9. Grazing management and pasture spelling are important adjuncts to worm control programs to minimise the need for deworming and delay the development of AR.
10. Alpaca farmers are encouraged to modify current practices to improve the health and welfare of alpacas in their care and reduce the risk of selecting worms for their resistance to commonly used dewormers on their farms by following the above recommendations.
11. More research is required to determine cut-off values for FECs when treatment with a dewormer is indicated.
12. More research is needed to determine the 'correct' dose of dewormers to be used in alpacas. Furthermore, alternative control strategies such as the use of available vaccine (i.e. Barbervax®) against Barber's pole worm and the use of Bioworma® for a biological control against GINs of alpacas should be investigated.
13. In order to effectively share the findings of the project, a proposal for holding extension workshops, webinars and seminars have been submitted to the AgriFutures Australia and the AAA for funding.
14. Paraboss is an invaluable resource for the control of worms, flies and lice in sheep and goats in Australia. Alpaca farmers are encouraged to regularly seek information from Wormboss (www.wormboss.com.au) as alpacas share a number of GINs with sheep and goats. In addition, efforts will be made to make findings of this project available through Wormboss.

Introduction

The national quality assurance and biosecurity program for alpacas, *Q-Alpaca*, was introduced by the Australian Alpaca Association (AAA) in conjunction with Animal Health Australia, and state and territory departments of agriculture in March 2005 to provide passive surveillance on causes of death in alpacas to (a) assist farmers with immediate husbandry needs, (b) provide assurance of the health of the national herd to allow both national and international movement of alpacas and (c) provide direction for research to optimise health and welfare in the national alpaca herd.

Every annual audit of *Q-Alpaca*[1] has shown gastrointestinal parasitism to be one of the major causes of death in alpacas in Australia since inception of the program. Consequently, a major research project was undertaken in 2015-2018 by the University of Melbourne in conjunction with AgriFutures Australia, the AAA and Cria Genesis. This booklet documents the findings of the project which will assist Australian alpaca farmers improve production and welfare of alpacas in their care through a better understanding of worm species and their behaviour, and control through grazing management and strategic use of effective anthelmintics or dewormers.

This booklet focuses on gastrointestinal nematodes (GINs), hereafter referred to as "worms". It does not address cestodes such as tapeworms, trematodes such as liver fluke or protozoa such as coccidia and sarcocysts as they fell beyond the scope of this project. Details of seven individual studies conducted under this project have been published in scientific journals. However, we are presenting a simplified version of the key findings and recommendations coming out of this project targeted at alpaca producers and veterinarians.

Initially, a survey of Australian alpaca farmers was undertaken to describe current alpaca herd demographics and animal husbandry and worm control practices. The survey results are reported in detail for the benefit of contributors, and to clarify the level of understanding of worm control the industry has attained over the first three decades of alpaca farming in Australia.

Project field work entailed collection of alpaca gastrointestinal tracts to identify worm species found in Australian alpacas, and collection of faecal samples from mixed ages and sexes of alpacas at different locations and times of the year to describe worm distribution, growth and behaviour in different climatic zones. Based on survey results, efficacy of commonly used anthelmintics was determined to assist with worm control in the national alpaca herd. Additionally, new methods of faecal egg counting and worm species identification were validated against currently available methods.

[1] Find Q-Alpaca annual reports at https://www.alpaca.asn.au/component/phocadownload/category/24-q-alpaca-annual-reports

Objectives

1. To assess the worm control practices used by alpaca farmers in Australia by conducting a questionnaire survey

2. To determine the prevalence of gastrointestinal nematode of alpacas in various climatic zones in Australia, using traditional and the latest molecular diagnostic methods

3. To undertake field efficacy studies to determine the status of anthelmintic resistance in gastrointestinal nematodes of alpacas

4. To train a research higher degree (MPhil/PhD) student

Methodology

This section briefly outlines methods used for various components of the project. Detailed methods used for each study can be found in individual chapters.

Assessment of worm control practices used by Australian alpaca farmers:

A questionnaire was conducted using an online programme, Research Electronic Data Capture. The questionnaire contained questions about farm demography and general husbandry practices of alpacas, farmers' knowledge about GINs and their importance, the use of worm control strategies and anthelmintics, and grazing management. The questionnaire was first validated using a pilot survey before conducting the definitive survey. The participants of the survey were registered members of the AAA and their participation in the study was entirely voluntary. The questionnaire survey was approved by the Human Ethics Committee of the University of Melbourne (UoM).

Epidemiology of gastrointestinal nematodes of Australian alpacas:

To determine the prevalence of GINs of alpacas in Australia three types of studies were conducted. In addition, a new DNA-based test was developed to detect nematodes DNA in the alpaca faeces. The collection of samples from alpacas was approved by the Animal Ethics Committee of the UoM.

i. **Cross-sectional epidemiological studies:** This study involved a national cross-sectional survey of GINs of alpacas to establish baseline data on their epidemiology in Australia. A total of 1,545 fresh faecal samples from 92 farms were collected from both sexes of alpacas and processed for faecal egg counts (FECs) and identification of nematodes using a newly developed DNA-based test.

ii. **Longitudinal epidemiological studies:** This study involved a longitudinal coproscopical study on 13 alpaca farms in four climatic zones (summer rainfall, winter rain fall, non-seasonal rainfall and Mediterranean-type rainfall) of Australia to understand the epidemiology of 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 FECs and identification of nematodes using a newly developed DNA-based test.

iii. **Examination of gastrointestinal tracts of alpacas:** In this study, one hundred gastrointestinal tracts of alpacas were examined to assess the burden and to identify the species of nematodes present in Australian alpacas. Faecal samples were collected from 97 alpacas and processed for FECs. For identification of nematodes, both DNA-based test and morphological technique were used.

iv. **Development of a new diagnostic tool:** This study involved a modification of two existing DNA-based tests (multiplexed-tandem polymerase chain reaction (MT-PCR) assays), originally developed for the GINs of sheep and cattle, to reliably detect and differentiate the common genera/species of GINs in the faeces of alpacas.

Efficacy of commonly used dewormers against 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.

Training of a research higher degree student:

Mohammed Haronur Rashid completed his PhD thesis by undertaking his research project on gastrointestinal nematodes of Australian alpacas.

Chapter 1 – Survey of worm control practices used by Australian alpaca farmers

Key findings

The survey provides insights into current husbandry and worm control practices in Australian alpacas of different herd sizes. Alpaca farmers are encouraged to modify current practices to improve the health and welfare of alpacas in their care and reduce the risk of development of anthelmintic resistance on their farms by assessing worm burdens prior to treatment, weighing alpacas and deworming to the heaviest in the group and calibrating the drench gun to check it is delivering the correct volume of dewormer.

Methods and results

The first stage of the project involved a voluntary, online survey of alpaca farmers to describe farm demography and determine general husbandry, worm problems and worm control practices. All active members (*n* = 954) of the AAA were invited to participate in the survey in July 2015. The response rate for the survey was 25% (239/954).

Figure 1 shows the distribution of alpaca farmers who responded to the online questionnaire survey. The highest response rates were from New South Wales (44%) and Victoria (30%), where the majority of Australian alpacas are farmed.

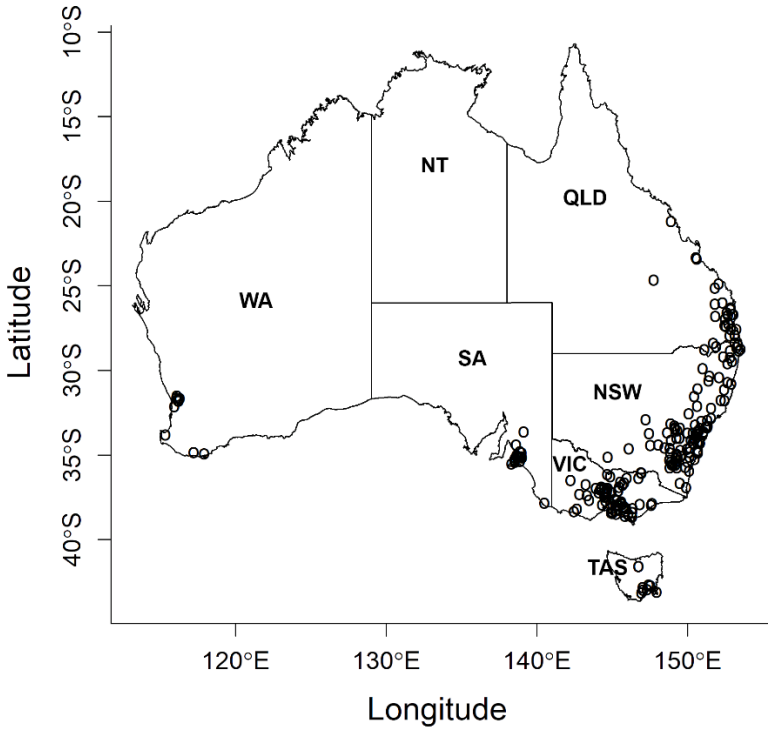

Fig. 1. Distribution of alpaca farms (*n* = 239) that participated in the survey based on farm address or post code submitted with survey. States and territories of Australia: NSW, New South Wales; NT, the Northern Territory; QLD, Queensland; SA, South Australia; TAS, Tasmania; VIC, Victoria; WA, Western Australia.

The average farming experience of respondents was 10.5 years (range 0-28 years). The primary purpose for farming alpacas was fibre production (78%), followed by breeding (77%), guard animals (42%), hobby farming (40%) and meat production (16%).

Table 1 summarises the demographic information of Australian alpaca farms. Overall, the average herd size was 57 alpacas. Small, medium and large herds were categorised as ≤50, 51-100 or >100 alpacas, respectively. There were 67% Huacaya-only herds, 13% Suri-only herds and 20% combined Huacaya/Suri herds represented in the survey. Around 75% (9,730/12,917) of alpacas were female, and 68% (8,800/12,917) of alpacas were females over one year of age.

Table 2 summarises the husbandry practices carried out at Australian alpaca farms. The most common weaning age was ≥ 5 months of age. Almost half of respondents across all herd sizes farmed sheep, cattle, or other domestic livestock species, including horses, donkeys and pigs on the same farm as their alpacas. Frequency of dung removal from paddocks was more common in small (79%) and medium (75%) herds than large (39%) herds, and varied from weekly (13%), to once every 2-6 months (15%), whereas 27% of respondents never removed dung from pastures. Pasture harrowing to spread dung across paddocks was practised by 22% (49/227) of respondents.

Table 1. Demographic information of alpaca farms that participated in the survey.

	Small herd (≤ 50)	**Medium herd (51-100)**	**Large herd (> 100)**
Number of respondents (%)	153 (64)	57 (24)	29 (12)
Number of alpacas			
Mean	24	72	199
Range	2 - 50	51 - 100	105 - 1150
Alpaca breed(s)			
No. of Huacayas (%)	102 (64)	41 (26)	17 (10)
No. of Suris (%)	24 (77)	3 (10)	4 (13)
No. of Huacayas & Suris (%)	26 (55)	13 (28)	8 (17)
Grazing area (ha) of farm			
Mean	43	60	123
Range	0.04 - 3440	2 - 931	14 - 696

Table 2. Farm husbandry and management practices used by Australian alpaca farmers based on herd size.

Husbandry practice	**Small herd (≤ 50 alpacas)**	**Medium herd (51-100)**	**Large herd (> 100)**
On-farm birth of crias (%)	127/146 (87)	47/52 (90)	25/28 (89)
Keeping agisted alpacas (%)	12/152 (8)	13/57 (22)	10/29 (34)
Supplementary feed (%)	142/150 (95)	47/52 (90)	23/29 (79)
Supplementation due to insufficient feed in winter (%)	80/142 (56)	32/47 (68)	12/23 (52)
Supplementation to lactating females as extra diet (%)	78/142 (55)	28/47 (60)	16/23 (70)
Keeping alpacas with other livestock species (%)	70/148 (47)	28/52 (54)	23/29 (79)
Co-grazing of alpacas with other livestock species (%)	46/68 (68)	20/28 (71)	15/23 (65)
Removal of alpaca dung from paddocks (%)	131/166 (79)	47/63 (75)	13/33 (39)

Table 3 shows that approximately 50% of respondents perceived worms as an important health problem in their herd. Regardless of herd size, the majority of respondents use faecal egg counts to monitor worm burdens in their alpaca herds, with 29% of respondents performing on-farm testing, and 69% sending faeces to a diagnostic laboratory. Barber's pole worm (*Haemonchus*) was apparently the most commonly diagnosed worm in alpaca herds in the survey, despite only 26% of farms using larval culture to identify worm species in their herds.

Table 3. Knowledge of worms by Australian alpaca farmers based on herd size.

Worm control issue	**Small herd (≤ 50)**	**Medium herd (51-100)**	**Large herd (> 100)**
Worms are an important health issue of alpacas (%)	65/142 (46)	32/51 (63)	17/27 (63)
Diagnostic method(s) used:			
Faecal egg count (FEC) (%)	56/65 (86)	27/32 (84)	15/17 (88)
Larval culture (%)	15/65 (23)	9/32 (28)	6/17 (35)
Post mortem (%)	8/65 (12)	5/32 (16)	7/17 (41)
Other method[1] (%)	6/65 (9)	4/32 (13)	2/17 (12)
Identified worms on alpaca farms:			
Barber's pole (*Haemonchus* spp.) (%)	39/53 (74)	20/24 (83)	11/13 (85)
Black scour (*Trichostrongylus* spp.) (%)	15/53 (28)	9/24 (38)	6/13 (46)
Brown stomach (*Ostertagia* spp.) (%)	10/53 (19)	11/24 (46)	4/13 (31)
Other[2] (%)	13/53 (25)	6/24 (25)	3/13 (23)

[1]Body condition scoring, anaemia, diarrhoea, tapeworm segments in faeces; [2]Coccidia, tapeworm

Table 4 shows that more than 75% of respondents used dewormers to control worms in their alpaca herds, based on visual appraisal of poor body condition or recent faecal egg count result (112/174, 65%). The type and frequency of clinical signs in alpacas reported by famers assumed to be associated with parasitic gastroenteritis included weight loss, anaemia, weakness, death and scouring. Virtually all the signs are non-specific, but anaemia can be associated with barber's pole worm.

Table 4. Management practices used by Australian alpaca farmers based on herd size.

Management practice	**Small herd (≤ 50)**	**Medium herd (51-100)**	**Large herd (> 100)**
Use of anthelmintics (de-wormers) (%)	107/144 (74)	45/51 (88)	22/25 (88)
Simultaneous deworming of mixed livestock species (%)	25/66 (38)	9/27 (33)	4/19 (21)
Anthelmintics* (%):			
Macrocyclic lactones (ML or "mectins")	74/189 (39)	35/97 (36)	18/45 (40)
Combination of 4 actives (closantel, BZ, LEV, ML)	47/189 (25)	17/97 (18)	8/45 (18)
Monepantel	27/189 (14)	16/97 (17)	7/45 (16)
Benzimidazoles (BZ)	15/189 (8)	13/97 (13)	3/45 (7)
Combination of 2 (BZ, ML) & 3 (BZ, LEV, ML)	10/189 (5)	5/97 (5)	6/45 (13)
Levamisole (LEV)	9/189 (5)	5/97 (5)	1/45 (2)
Closantel	7/189 (4)	6/97 (6)	2/45 (4)
Rotation of anthelmintics (%)	62/103 (60)	35/45 (78)	15/22 (68)
Quarantine dewormer (%)	98/138 (78)	44/50 (71)	24/26 (92)
Deworming and moving of alpacas to clean pasture (%)	23/103 (22)	10/44 (23)	6/22 (27)
Quarantine for introduced alpacas (%)	46/137 (34)	46/51 (90)	21/25 (84)
"Drench resistance test" (%)	7/105 (7)	12/45 (27)	2/22 (9)

*No anthelmintic is registered for use in alpacas in Australia.

Regardless of herd size, farmers showed a preference for macrocyclic lactones (MLs or "mectins"), 4-way combination and monepantel dewormers. Historically, macrocyclic lactones have been used for worm control widely and for more than two decades in the Australian alpaca industry due to (a) the convenience of administration by subcutaneous injection rather than oral deworming, (b) the perceived broad-spectrum of efficacy against internal and external parasites and, (c) the perceived extended duration of action of some of the products in this group of anthelmintics.

No anthelmintic is registered for use in alpacas so all use is off-label. Farmers with small herds tended to use a single sheep or cattle dose of anthelmintic, whereas those with medium and large herds tended to use 1.5-2 times sheep dose. This discrepancy may be because there is no alpaca guide on any dewormer label. The majority of respondents (123/174, 71%) calculated the dose of anthelmintic based on visual estimation of body weight. Approximately half of respondents (81/169, 48%) used a drenching gun of which only 14% (11/81) of respondents calibrated the drench gun prior to use to ensure accurate volume delivery.

Whilst 78% of respondents used a quarantine deworming treatment, the majority of respondents (143/239, 60%) were unaware of anthelmintic resistance. Only 12% (21/172) of respondents assessed the status of anthelmintic resistance on their farms by assessing pre- and post-treatment faecal egg counts. Only a third of respondents performed simultaneous deworming of mixed livestock species and a quarter moved stock onto a "clean" pasture after deworming.

Table 5 indicates that Australian alpaca farmers obtain worm control information primarily from their veterinarian or other alpaca farmers. Nevertheless, only 33% (57/174) respondents follow veterinary recommendations for deworming and 8% (14/174) use a strategic deworming program.

Table 5. Source of deworming advice used by Australian alpaca farmers in survey based on herd size.

Source of advice on deworming	**Small herd (≤ 50)**	**Medium herd (51-100)**	**Large herd (> 100)**
Veterinarian (%)	98/153 (64)	36/53 (68)	20/29 (69)
Fellow farmers (%)	91/153 (59)	25/53 (47)	9/29 (31)
Journals/Magazines (%)	33/153 (12)	15/53 (11)	8/29 (21)
Australian Alpaca Association newsletter (%)	38/153 (25)	13/53 (25)	4/29 (14)
Online (e.g. wormboss.com) (%)	25/153 (16)	14/53 (26)	4/29 (14)

Chapter 2 – Worm species found in Australian alpacas

Key findings

Alpacas are affected by camelid-specific worm species, and sheep and cattle worms. Parasitic gastroenteritis can lead to diarrhoea and/or anaemia, illthrift, loss of production (reduced growth, less wool production, poor fertility) and death.

Methods and results

Gastrointestinal tracts from approximately 100 alpacas of varying breed, age and sex were collected from sites in New South Wales, Victoria and South Australia during the project. Total worm counts were performed to identify the gastrointestinal nematodes, or worm, species found in the stomach and intestines of Australian alpacas. The mean worm burden was 1,280 adult worms, with the highest burden of 28,640 worms in one alpaca. The mean faecal egg count (FEC) of guts examined was 501 eggs per gram (epg); maximum FEC was 3,495 spg.

Nineteen different species of nematodes were identified by examining adult worms. The main genera/species were camelid-specific (*Camelostrongylus mentulatus*, *Graphinema aucheniae* and Trichuris tenuis) and worms which commonly infect sheep and cattle (*Haemonchus contortus*, *Cooperia* spp., *Ostertagia ostertagi*, *Teladorsagia circumcincta*, *Trichostrongylus* spp., and Nematodirus spp). Appendix 1 tabulates the full list of worms identified in the project.

Figure 2 describes which worms live where in alpacas. Worm species denoted by (s) lay "strongyle" eggs which look similar microscopically.

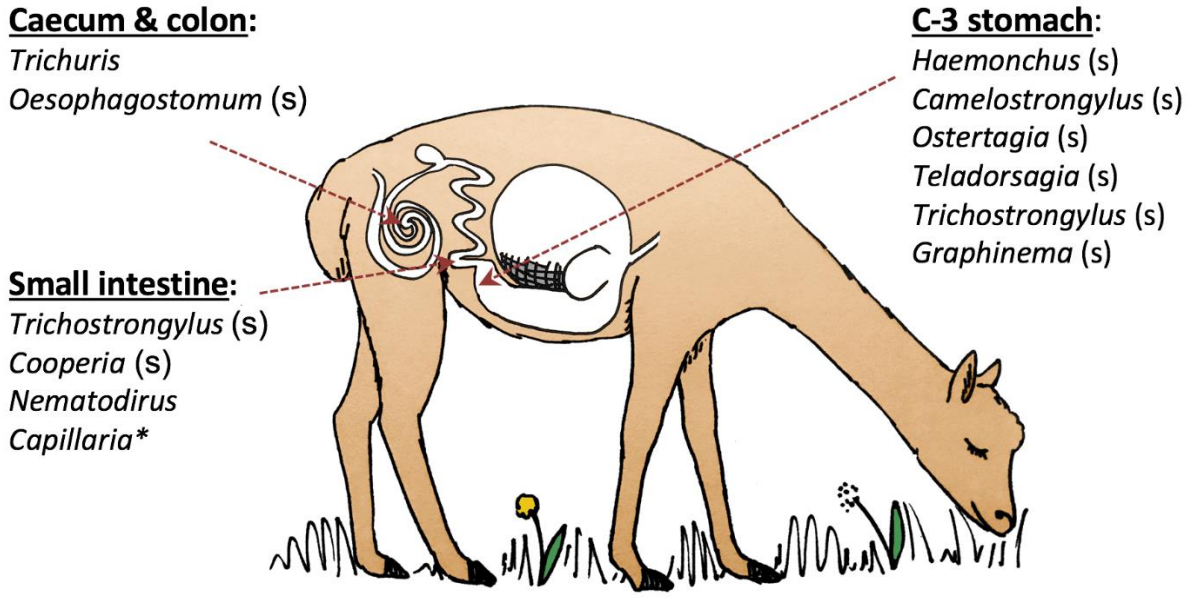

Fig. 2. Locations of gastrointestinal nematodes or "worms" found in Australian alpacas during total worms counts. All worms denoted with an (s) lay a typical "strongyle" egg. *Note that *Capillaria* spp. eggs were found during faecal egg counting but no adult worms were identified during total worm counts. (Picture courtesy of Dr Zoe Vogels)

Of all the worms that lay strongyle-type eggs, the majority of adult worms attach to the gut lining and feed on tissue fluids of alpacas. Heavy burdens of these worms can lead to illthrift, weight loss, diarrhoea ("scours") and death and are colloquially referred to as scour worms.

Haemonchus contortus, or barber's pole worm (BPW), on the other hand, sucks blood from alpacas and causes anaemia, illthrift, weight loss and death (Fig. 3). These worms are known as barber's pole worms because in fresh specimens of the adult female, the blood-filled gut is wrapped around with the white, egg-laden uterus to form a red and white spiral like a barber's pole. The worms are approximately 20-30 mm long and can be seen with the naked eye in the third compartment of the stomach.

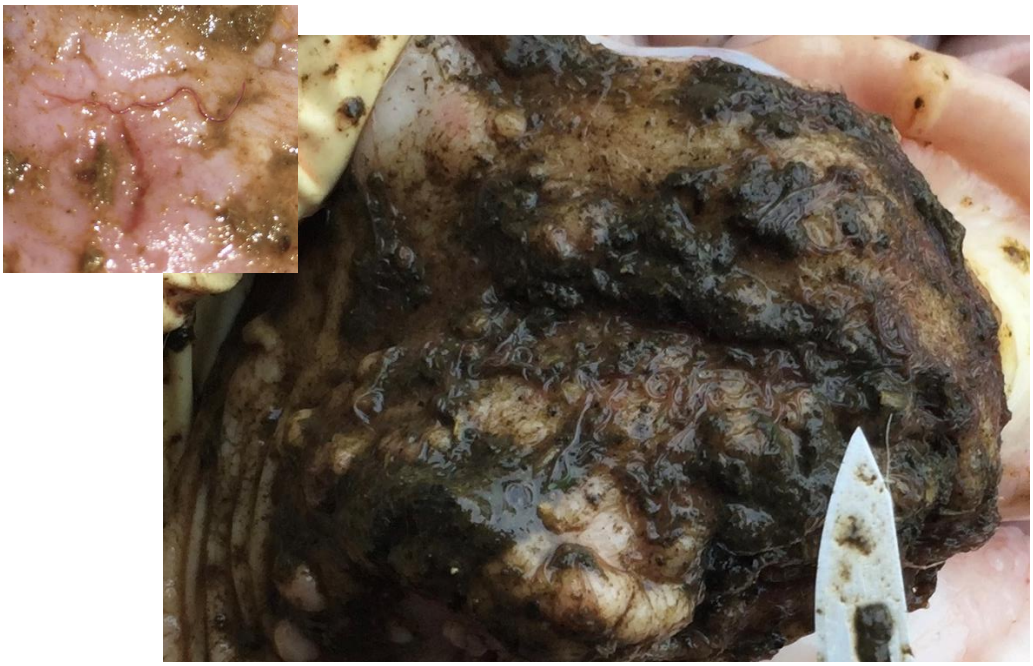

Fig. 3. *Haemonchus contortus* worms in the third compartment of the stomach of an alpaca. They are known as "barber's pole worms" because in the female worm, the blood-filled gut wraps around the white, egg-laden uterus to form a red and white spiral like a barber's pole (inset). The worms are approximately 20-30 mm long and can be seen with the naked eye.

The existence of high numbers of *Camelostrongylus mentulatus* in the third compartment of the stomach was associated with changes to the epithelial lining, colloquially termed "Moroccan leather" appearance (Fig. 4). The worm was likely imported into Australia in camels in 19th century, along with *Trichuris tenuis*, the camel whip worm. The relatives of this parasite in sheep (*Teladorsagia circumcincta*) and cattle (*Ostertagia ostertagi*) are known to cause significant morbidity and mortality in south-eastern Australia.

Graphinema aucheniae is the only South American camelid-specific nematode to be identified in the study. All other worms are found in Australian cattle, sheep and/or goats (Appendix 1).

The camelid-specific, small intestinal nematode, *Lamanema chavezi*, was not found in any gut samples. Nevertheless, it has been identified in New Zealand camelids so could exist in Australia too. Farmers and veterinarians are advised to keep looking for the presence of this parasite in Australia because (a) its hepatic migration can be associated with illthrift and death, and (b) post-mortem examinations of Australian alpacas regularly reveal granulomatous lesions in the liver consistent with parasitic migration.

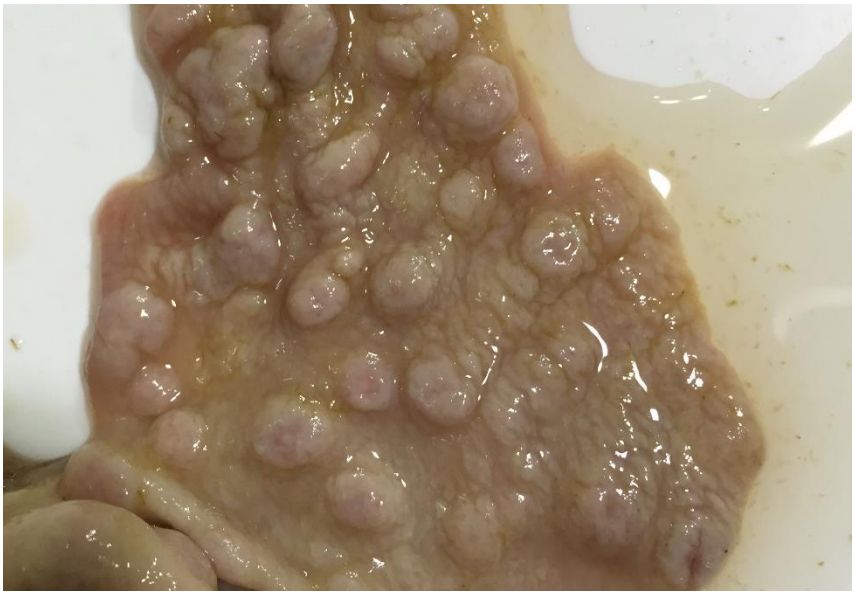

Fig. 4. Damage to the lining of the third compartment of the stomach ("Moroccan leather" appearance) was associated with high numbers of adult *Camelostrongylus mentulatus* worms in the study.

Lifecycle of worms and faecal egg counts

A typical strongyle worm lifecycle is simple (Fig. 5). An alpaca ingests worm larvae from the pasture which burrow into the gut wall and develop into adult worms over a period of around 3 weeks. Adult female worms lay eggs which pass out onto the pasture in alpaca faecal pellets. Eggs hatch under favourable conditions. Larvae take days to weeks to develop, moult twice, migrate away from alpaca faeces and ascend moist pasture leaves ready to be ingested by a grazing alpaca.

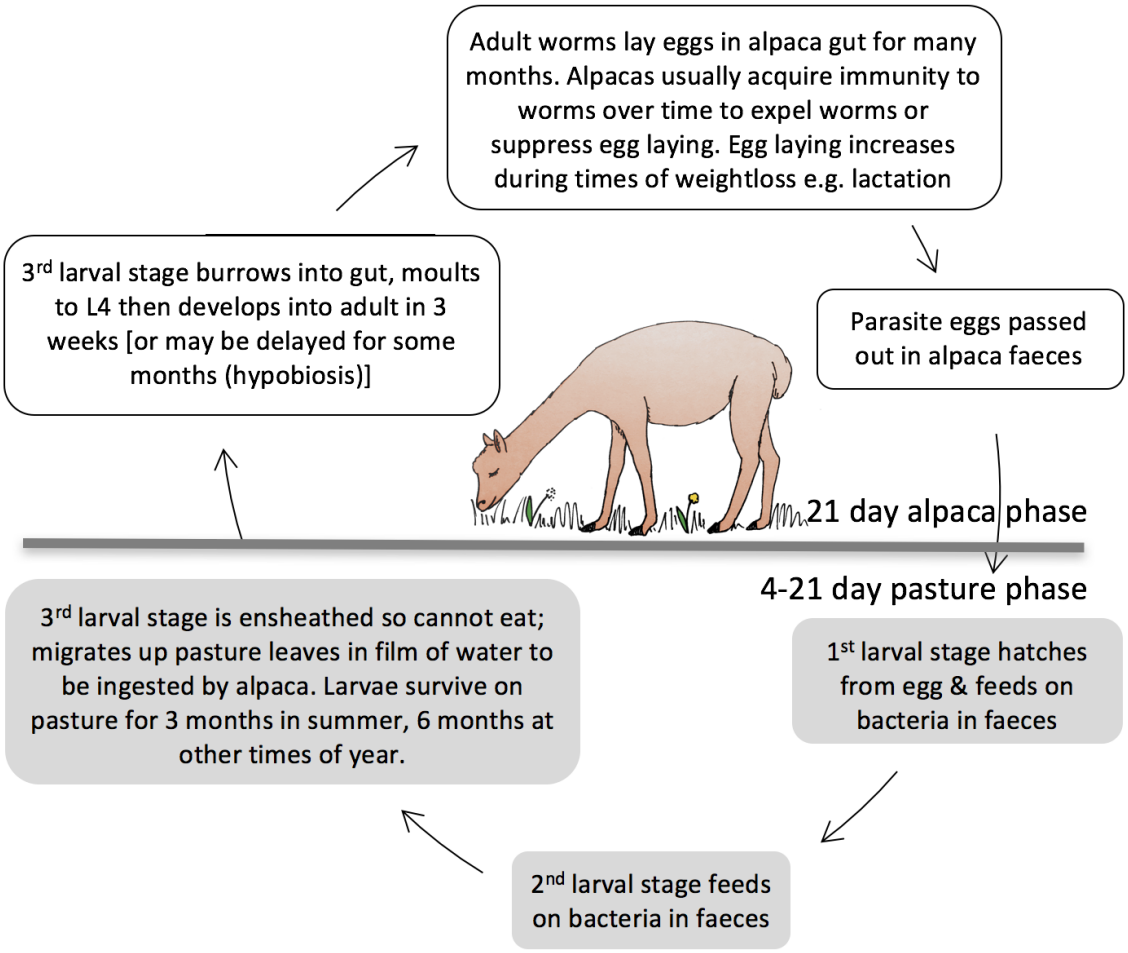

Fig. 5. A typical strongyle worm lifecycle. Note that Nematodirus and Trichuris spp. larvae develop inside their thick-shelled eggs and can therefore survive dry pasture conditions for many months. (Picture courtesy of Dr Zoe Vogels)

How to identify worm eggs

It is possible to collect, prepare and examine alpaca faeces microscopically to count worm eggs per gram (EPG) of faeces based on shape, form and size. This quantitative procedure is known as a faecal egg count (FEC) or a worm egg count (WEC; interchangeable with FEC; term used depends on laboratory; FEC will be used in this booklet). The procedure is described in full in Appendix 2.

As a generalisation, most of the worm species found in alpacas produce similar eggs that cannot be differentiated from one another under the microscope and are collectively referred to as strongyle eggs (Fig. 6). Other common worms that lay distinctive eggs are *Nematodirus* spp. and *Trichuris* spp.

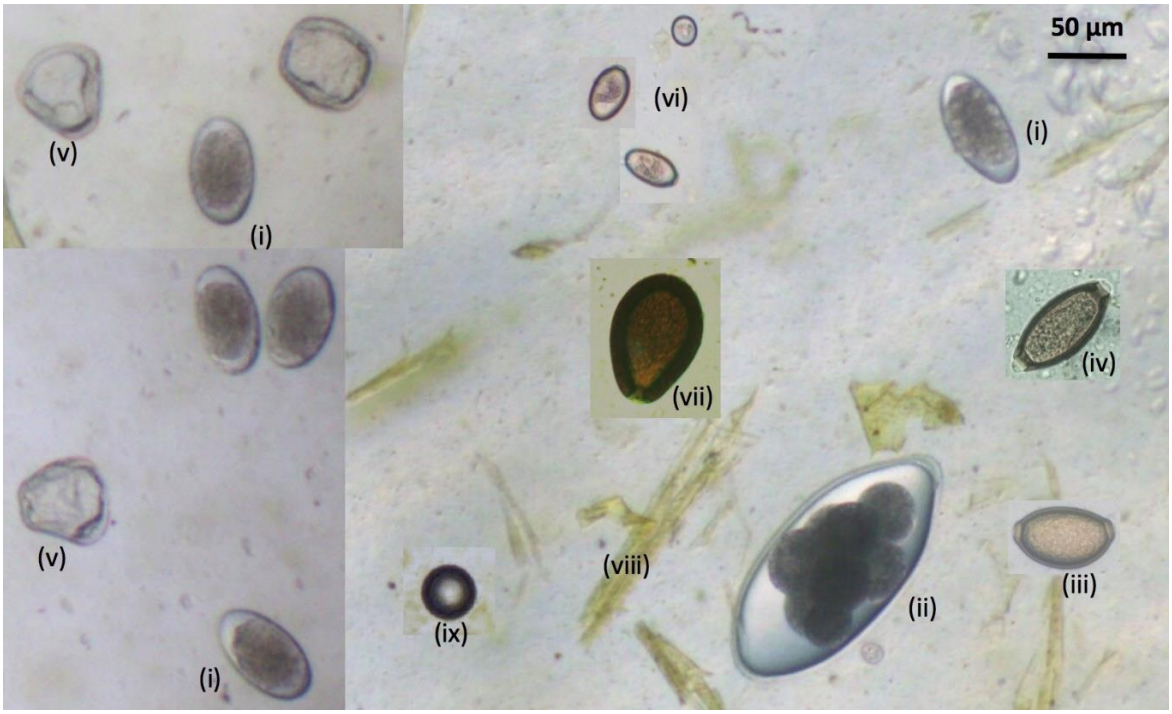

Fig. 6. Composite picture showing the relative sizes and shapes of various gastrointestinal parasite eggs: (i) strongyle eggs, (ii) *Nematodirus* egg, (iii) *Capillaria* egg, (iv) *Trichuris*/whip worm egg, (v) tapeworm eggs, (vi) small coccidial oocysts, (vii) large coccidial oocyst (*Eimeria macusaniensis*), (viii) plant matter, (ix) air bubble.

To identify which worms are laying the typical strongyle-type eggs, it is necessary to hatch out the eggs under controlled laboratory conditions and examine the larvae. Larval culture may take 10-12 days. A newly validated method of worm egg differentiation through DNA testing is discussed later in this booklet (see page 28).

With respect to interpreting strongyle faecal egg counts, the "scour worms" tend to produce tens to hundreds of eggs per gram of alpaca faeces, whereas *Haemonchus contortus*, or barber's pole worm is a prolific egg layer so FECs may be in the thousands to tens of thousands of eggs per gram of faeces.

Chapter 3 – Distribution of worms in Australian alpacas

Key findings

Alpacas of all ages are affected by worms in all climatic zones of Australia, across all farm sizes, throughout the year. To optimise health and production of alpacas, farmers should monitor worm burdens in their herds. Interpretation of FECs must be done with respect to individual farm management (stocking rates, season, pasture length, body condition, age of alpacas). More research is required to determine cut-off values for FECs when treatment with a dewormer is indicated.

Methods and results

There is a paucity of information regarding the behaviour of worms in alpacas in Australia and to date, farmers and veterinarians have relied on small studies performed in alpacas in south-eastern Australia and extrapolation of worm behaviour data from other domestic livestock species.

In order to describe the growth and survival of worms in alpacas at different locations across the continent and throughout the year, farmers collected and posted 15 alpaca faecal samples from different climatic zones either as a one-off event (*n* = 92 farms) or on a monthly basis (where possible; *n* = 13 farms) to monitor numbers and types of worm eggs being shed in alpaca faeces. The map below shows sites where faeces was collected, with regions initially based on WormBoss sheep zones.[2] Zones were combined to enable statistical comparisons amongst regions of differing rainfall and temperature (Fig. 7).

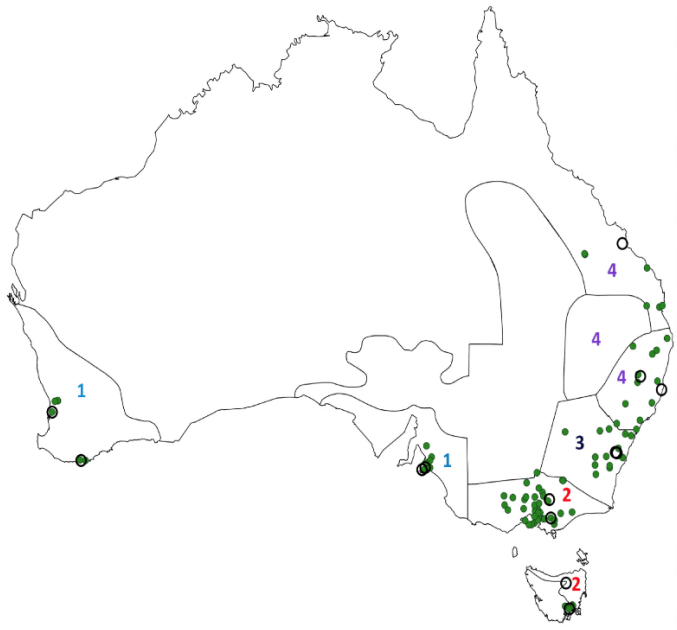

Fig. 7. Locations of farms that contributed faecal samples to the study included the Mediterranean-type rainfall zone (1) of Western and South Australia, the winter rainfall zone (2) of Tasmania and southern Victoria, the non-seasonal rainfall zone (3) of southern New South Wales, and the summer rainfall zone (4) of Queensland and northern New South Wales. Farms contributed alpaca faecal samples on a monthly basis (*n* = 13 farms; O) or a one-off basis (*n* = 92 farms; ●).

[2] See WormBoss sheep zone map at http://www.wormboss.com.au/programs/sheep.php

Prevalence of worms in different alpaca herds

Approximately two-thirds of all faecal samples contained worm eggs, regardless of farm size. There was no statistical difference in average FECs among different herd sizes but there is vast variation in individual FEC (Table 6). Average FEC ranged from 177-352 epg, individual samples contained 0-17,000 epg.

Table 6. Prevalence of infection and mean faecal egg counts (eggs per gram) of gastrointestinal nematodes in Australian alpacas in small (n = 42 farms), medium (n = 31 farms) and large herds (n = 19 farms) ($P > 0.05$). Different superscripts denote significant differences.

Herd size (no. alpacas)	**% Prevalence (proportion)**	**Mean FEC (epg)**	**FEC range (epg)**
Small (≤50)	63 (445/705) [a]	352 [a]	(0 – 17415)
Medium (51-100)	65 (319/488) [a]	177 [a]	(0 – 10980)
Large (>100)	70 (248/352) [a]	328 [a]	(0 – 14355)

When a farmer submits alpaca faeces to a laboratory for faecal egg counting, the typical report comes back with eggs per gram of "strongyle", "*Nematodirus*" and sometimes "*Trichuris*" (whip worm) eggs, as this is the degree of differentiation of worm species possible with this test. Across many farms, and across different seasons, 53-57% of faecal samples contained strongyle eggs, 15-18% contained *Nematodirus* eggs and 6-11% contained *Trichuris* eggs (Tables 7 & 8).

Table 7. Prevalence of infection and mean faecal egg counts (eggs per gram) of gastrointestinal nematodes from a single sampling of alpacas from 92 farms across Australia.

Type of nematode	**% Prevalence (proportion)**	**Mean FEC (epg)**	**FEC range (epg)**
Strongyle	59 (916/1545)	276	0 - 17400
Nematodirus spp.	17 (261/1545)	12	0 - 600
Trichuris spp.	7 (103/1545)	3	0 - 420
Overall	66 (1012/1545)	291	0 - 17415

Table 8. Prevalence of infection and mean faecal egg counts (eggs per gram) of gastrointestinal nematodes in alpacas sampled monthly for a year from 13 farms across Australia.

Type of nematode	% Prevalence (proportion)	Mean FEC (epg)	FEC range (epg)
Strongyle	53 (876/1,666)	151	0 – 15,540
Nematodirus spp.	18 (305/1,666)	12	0 – 615
Trichuris spp.	11 (180/1,665)	6	0 – 1,275
Overall	61 (1,037/1,688)	168	0 – 15,630

Note that many samples contained 0 eggs per gram strongyle eggs, but individual FECs contained up to 17,000 epg. Whilst *Nematodirus* and *Trichuris* eggs are relatively rare in alpacas and are shed in faeces in low numbers, the eggs are very resistant in the environment and may pose a hazard to alpacas particularly after long periods of hot, dry weather/drought conditions where strongyle eggs hatch and larvae desiccate, but where *Nematodirus* and *Trichuris* eggs survive and accumulate in the environment.

Prevalence of worms in alpacas of different ages

As a generalisation, around two-thirds of all faecal samples contained worm eggs, regardless of age (Tables 9 & 10). Table 9 illustrates what was anticipated to occur in alpacas when parasite data are extrapolated from other domestic livestock.

- Crias (<6 mo) with relatively low average and individual FECs as they are deriving nutrition from milk and supplementing diet with grazing so have limited worm pickup.
- Weaners (6-12 mo) with highest average FEC (295 epg) and very high individual FEC (15,000 epg) as they are deriving all nutrition from grazing but have had little time to develop resistance to worms.
- Tuis (1-2 yo) and adults (> 2yo) develop resistance to worms with age so average FEC declines, and individual FECs are much lower than those seen in weaners.

Nevertheless, Table 10 clearly illustrates across many farms, that FEC can be very high in tuis and adults, with individual FECs reaching > 17,000 epg.

Table 9. Prevalence of infection and mean faecal egg counts (eggs/gram) of gastrointestinal nematodes in alpacas sampled monthly for a year from 13 farms across Australia.

Age group	% Prevalence (proportion)	Mean FEC (epg)	FEC range (epg)
Crias (<6 mo)	59 (58/98)	68	0 – 870
Weaners (6-12 mo)	73 (233/321)	295	0 – 15,630
Tuis (1-2 yo)	69 (240/346)	187	0 – 4,635
Adults (> 2yo)	55 (502/919)	126	0 – 4,770

Table 10. Prevalence of infection and mean faecal egg counts (eggs/gram) of gastrointestinal nematodes in different age groups of alpacas from a single sampling of alpacas from 92 farms across Australia.

Age group	% Prevalence (proportion)	Mean FEC (epg)	FEC range (epg)
Crias (<6 mo)	66 (56/85)	159	(0 - 2490)
Weaners (6-12 mo)	80 (165/206)	331	(0 - 12390)
Tuis (1-2 yo)	74 (157/211)	402	(0 - 9490)
Adults (> 2yo)	58 (547/936)	214	(0 - 17425

Prevalence of worms in alpacas in different climatic zones

The four main climatic zones where alpaca farming occurs in Australia, based on sheep WormBoss worm control regions[3], are represented in Fig. 7 as:

1. The Mediterranean-type rainfall zone of Western and South Australia
2. The winter rainfall zone of Tasmania and southern Victoria
3. The non-seasonal rainfall zone of southern New South Wales
4. The summer rainfall zone of Queensland and northern New South Wales.

More than three-quarters of all faecal samples contained worm eggs in the summer rainfall zone and alpacas had highest average (630 epg) and individual (11,000 epg) FECs (Table 11). In the other 3 zones around two-thirds of all samples contained worm eggs and average and individual FECs were lower.

Table 11. Prevalence of infection and mean faecal egg counts (epg) of gastrointestinal nematodes in Australian alpacas in four different climatic zones (different superscripts denote significant differences among groups).

Climatic zone	% Prevalence (proportion)	Mean FEC (epg)	FEC range (epg)
Mediterranean-type	70 (157/223)	433 [a]	(0 - 14355)
Winter rainfall	58 (411/703)	104 [b]	(0 - 3015)
Non-seasonal rainfall	64 (151/236)	165 [b,c]	(0 - 10980)
Summer rainfall	77 (293/383)	630 [d]	(0 - 17415)

[3] See WormBoss sheep zone map at http://www.wormboss.com.au/programs/sheep.php

Regardless of seasonal rainfall pattern, *Haemonchus*, *Camelostrongylus* and *Ostertagia* are prevalent in the C-3 stomach and *Trichostongylus* spp. in the small intestine of alpacas in all rainfall zones (Table 12). Nevertheless, summer rainfall tends not to favour the proliferation of *Camelostrongylus* and *Ostertagia.*

Table 12. Farm prevalence of alpaca worms in four climatic zones of Australia.

	Farm prevalence (%) of worms in alpacas			
Nematode/ worm	**Mediterranean-type rainfall (*n* = 12)**	**Winter rainfall (*n* = 42)**	**Non-seasonal rainfall (*n* = 14)**	**Summer rainfall (*n* = 21)**
C-3 stomach				
Haemonchus spp.	67	55	79	86
Camelostrongylus mentulatus	92	79	79	29
Ostertagia ostertagi	92	69	71	33
Teladorsagia circumcincta	0	7	0	0
Small intestine				
Trichostrongylus spp.	83	69	93	71
Cooperia spp.	33	24	43	48
Large intestine				
Oesophagostomum spp.	8	14	7	5

Figure 8 demonstrates that the prevalence of worms tends to increase during wetter seasons. In the winter rainfall zone, though the highest FEC was observed from late winter to early spring, a second peak was seen unexpectedly in summer. The usual trend in this zone is peak prevalence in winter followed by a decrease in spring to the lowest in summer. However, the second peak in the prevalence of GINs in the winter rainfall zone (Fig. 8) was most likely associated with higher rainfall (876 mm) during the summer season in 2016 (Fig. 9) as the average annual rainfall in this zone prior to and after 2016 was lower. In the Mediterranean-type environment, the highest FEC was observed in winter whereas that in the non-seasonal and summer rainfall zones was in late summer to early autumn.

Overall, this study revealed that the temporal distribution of different GINs of alpacas in various climatic zones follow the patterns of those previously described for sheep and cattle GINs in Australia.

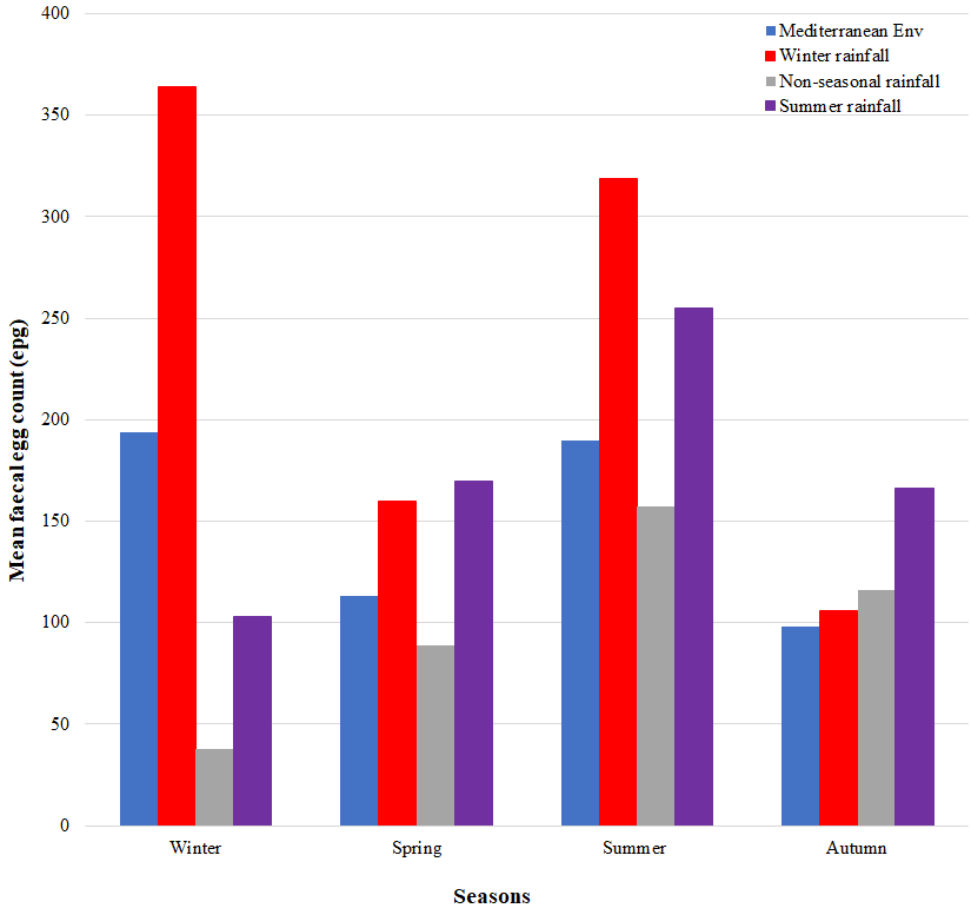

Fig. 8. Mean faecal egg counts (epg) of gastrointestinal nematodes in alpacas by season in different climatic zones of Australia between May 2015 and April 2016.

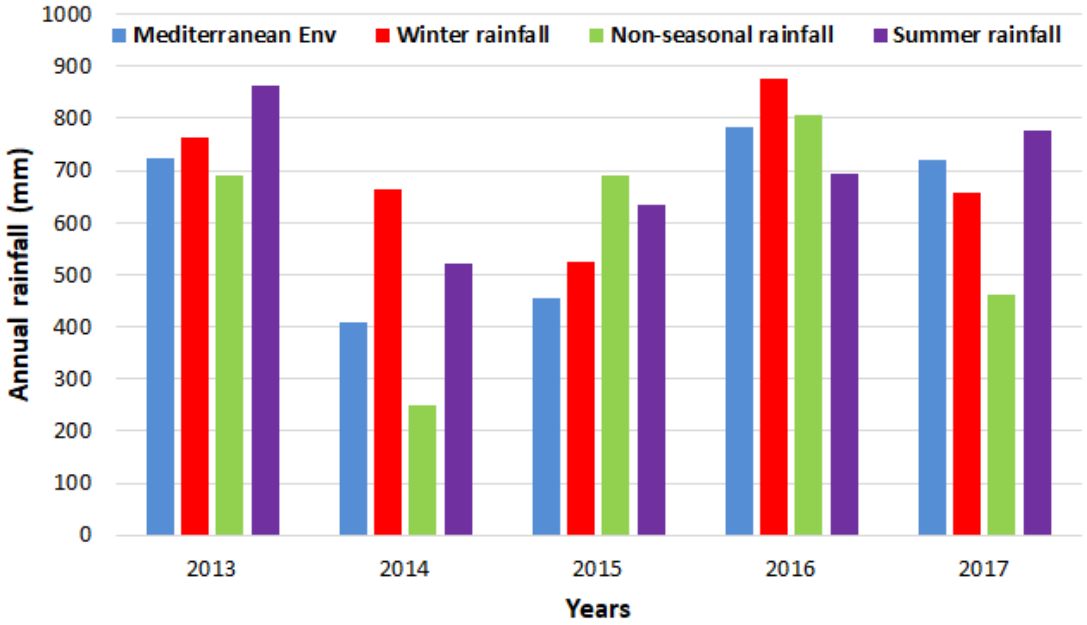

Fig. 9. Annual rainfall (mm) of the farms located in four selected climatic zones of Australia from 2013 to 2017 (collected from the Bureau of Meteorology (www.bom.gov.au) stations nearest to the farms).

Chapter 4 – Efficacy of dewormers in Australian alpacas

Key findings

Effective dewormers reduce faecal egg counts by ≥ 95% 10-14 days after treatment. In this study, effective dewormers included monepantel (Zolvix®) and a 4-active dewormer (Q Drench®). Ineffective dewormers included fenbendazole, ivermectin, moxidectin and closantel. Farmers should monitor efficacy of dewormers by performing FECs 10-14 days after treatment to ensure > 95% reduction in FEC.

Methods and results

Anthelmintics are compounds that are used to kill worms in domestic livestock. The term "drench" refers to products that are administered orally, however there are injectable anthelmintics available as well. Therefore, in this discussion, products used to control worms in alpacas will be referred to as "dewormers".

There are a number of dewormers that have been registered for use in sheep and cattle in Australia. A major issue of worm control in alpacas is the lack of understanding of how these dewormers function in alpacas with respect to absorption, distribution in body tissues and duration of action, and this is reflected in the vast range of dose rates that are recommended in alpacas, and the discrepancy in dose rates used within the Australian alpaca industry.

Dewormer families/actives

Dewormers contain chemicals or active ingredients ("actives") that belong to specific chemical classes or families depending on molecular makeup (Table 13). Dewormers in any one family have a similar molecular structure and range of toxicity against worms. In sheep and cattle, controlled trials have determined dose rates of various dewormers that effectively kill all susceptible worms. Worms may possess genetic mutations that allow them to survive a normal dose of dewormer, known as "drench/anthelmintic resistance".

Dose rates of dewormers for alpacas are unknown, but have been extrapolated from sheep and cattle doses (Appendix 3). No dewormers are registered for use in alpacas in Australia so must be used with caution and preferably under veterinary advice.

The project undertook dewormer efficacy trials on 20 alpaca farms located in all climatic zones (Fig. 10). Participating farms had (a) 40-60 mixed-age and sex alpacas, (b) not dewormed in the last 8 weeks, (c) an average FEC ≥ 150 epg, and (d) had used dewormer/s on the farm in the last 5 years. More than 50 farms were tested to obtain 20 suitable farms.

Based on available literature and the industry survey, it was decided to test the efficacy of the dewormers in Table 14 in alpacas administered at 1.5 times the on-label sheep dose in faecal egg count reduction tests (FECRTs) on 20 farms spread over the 4 climatic zones. Animals were dosed individually based on body weight using scales where available.

Table 13. The active ingredients contained in different chemical classes of dewormers.

Active	Chemical class	Common name	Target parasites
BZ	Benzimidazoles	**"White drench"**	Scour worms & BPW (± whip worms)
LEV	Imidazothiazoles = levamisole, morantel	**"Clear drench"**	Scour worms, BPW
ML	Macrocyclic lactones	**Avermectins & milbemycins**	Scour worms & BPW (including inhibited L4), whip worms
OP	Organophosphates/ anticholinesterases	**"OP drench"** e.g. Naphthalophos	Scour worms & BPW
CLO	Salicylanilides	Closantel	BPW
MPL/ AAD	Neonicotinoids/ aminoacetonitrile derivatives	**"Orange drench"**: Monepantel (+ abamectin = Zolvix Plus®)	Scour worms & BPW (including inhibited L4); (whip worms by ML)
SPI	Spiroindoles	Derquantel (+ abamectin = Startect®)	Scour worms & BPW (including inhibited L4), whip worms

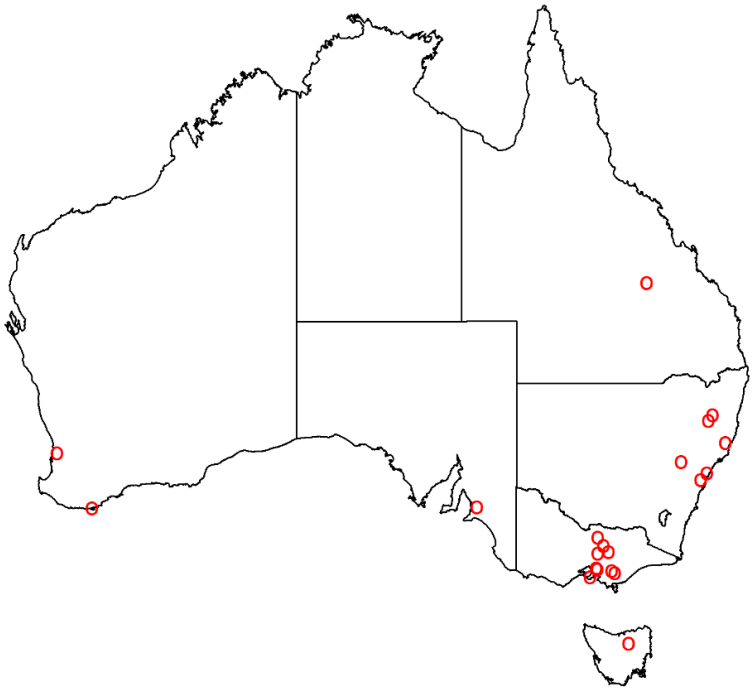

Fig. 10. Location of alpaca farms (*n* = 20) that participated in anthelmintic/dewormer efficacy trials.

Table 14. Dewormers used in faecal egg count reduction tests on 20 farms across Australia.

Active	**Chemical (abbreviation)**	**Brand name**	**Treatment route**
BZ	fenbendazole (FBZ)	Panacur 25®	Oral
ML (avermectins)*	ivermectin (IVM)	Ivomec®	Oral
ML (milbemycins)	moxidectin (MOX)	Cydectin®	injected SC
CLO	closantel (CLO)	Closicare®	Oral
MPL	monepantel (MPL)	Zolvix®	Oral
4-actives:			
BZ	albendazole	Q-Drench®	Oral
ML	abamectin		
CLO	closantel		
LEV	levamisole		

*Ivermectin was found to be ineffective in the first 9 trials so was replaced with a different ML, moxidectin, for the remaining trials.

The FECRTs were conducted as per guidelines of the World Association for the Advancement of Veterinary Parasitology. Individual FECs were performed in alpacas before each trial to establish baseline epg (method used in Appendix 2). Animals were then assigned to a treatment group to receive one of the actives listed above, or to remain as a control animal and receive no dewormer. Faeces were collected from all animals 11-14 days later to ascertain FECs. Pre- and post-treatment FECs were compared to calculate dewormer efficacy.

An effective dewormer is defined as one which reduces the FEC by ≥ 95%. Where dewormers reduced FEC by less than 95% in each trial, it was not possible to ascertain whether the lack of efficacy was due to inadequate dose rate or existence of resistance by worms to the dewormer.

In this study, monepantel (Zolvix®) and the 4-active combination dewormer (Q-Drench®) were effective on all farms because FEC reduction was ≥ 95% on all farms (Fig. 11). Ineffective dewormers included fenbendazole, ivermectin, moxidectin and closantel. (Note that closantel is only effective against BPW so efficacy trials using this dewormer must be performed in conjunction with larval culture.)

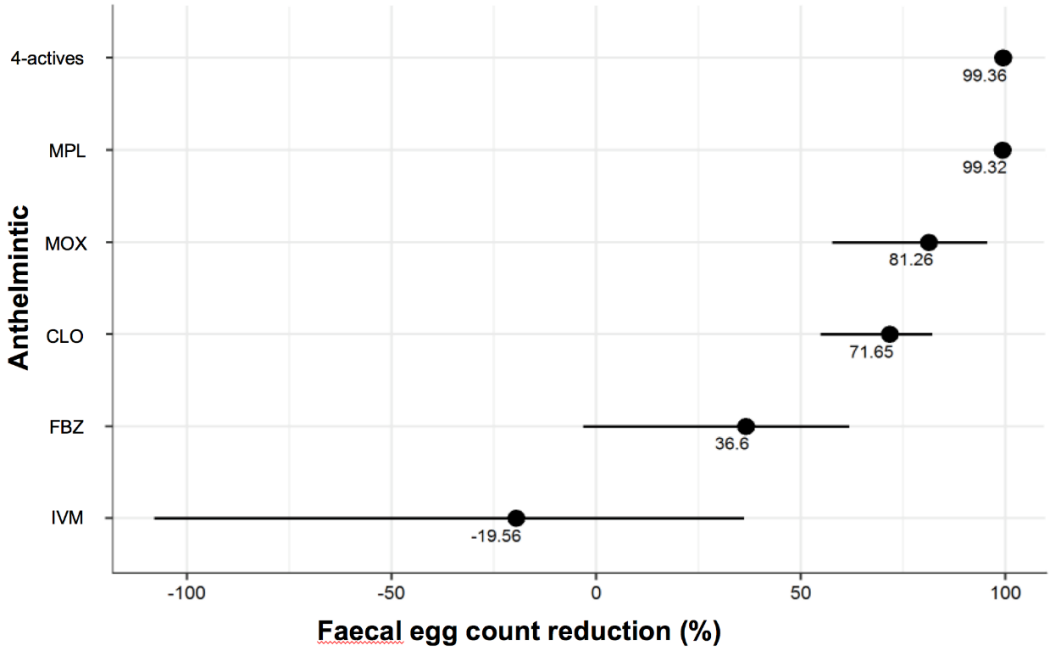

Fig. 11. Percent reduction in faecal egg count 10-14 days after treatment with ivermectin (IVM), fenbendazole (FBZ), closantel (CLO), moxidectin (MOX), monepantel (MPL) or 4-actives (albendazole, abamectin, closantel and levamisole in a commercial combination).

Nevertheless, efficacy of monepantel (Zolvix®) was questionable on 5 farms because FECs were reduced by approximately 95% rather than > 99%. Similarly, the 4-active drench (Q-Drench®) was suspected to be ineffective on 3 of 18 and ineffective on 1 of 18 farms for similar reasons (Fig. 12).

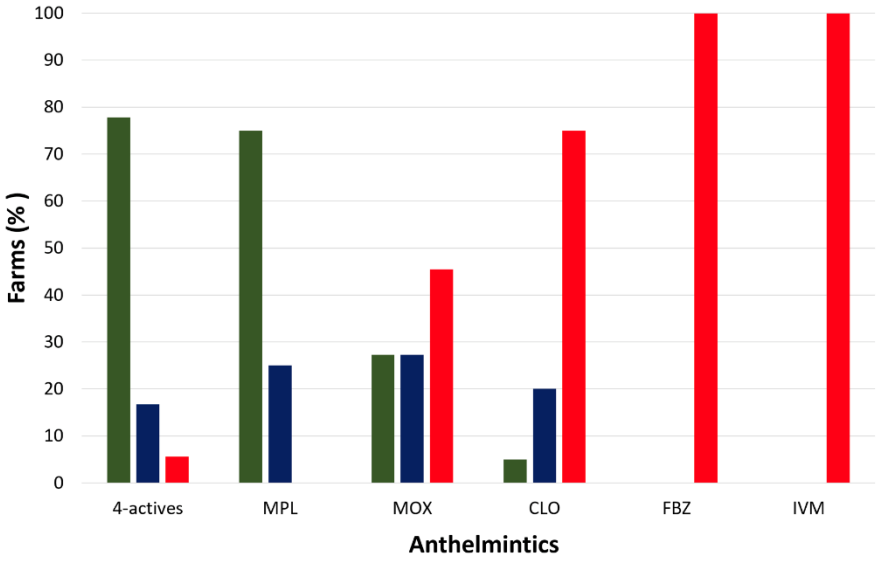

Fig. 12. Percentage of farms where dewormers were effective (■), marginally ineffective (■) or ineffective (■) to a 4-actives dewormer (albendazole, abamectin, closantel and levamisole in a commercial combination), monepantel (MPL), moxidectin (MOX), closantel (CLO), fenbendazole (FBZ) or ivermectin (IVM).

Figure 13 demonstrates that there are many worm genera/species on many alpaca farms across Australia that did not respond to 1.5 times the on-label sheep dose of dewormer. This could be due to inadequate dose rate or development of resistance by worms to the active ingredient in the dewormer. Barber's pole worm persisted after treatment on almost 50% of farms regardless of dewormer administered; other worms persisted on fewer farms. Appendix 4 shows the percentage of farms on which worm genera/species persisted after treatment with the different dewormers used in the FECRTs.

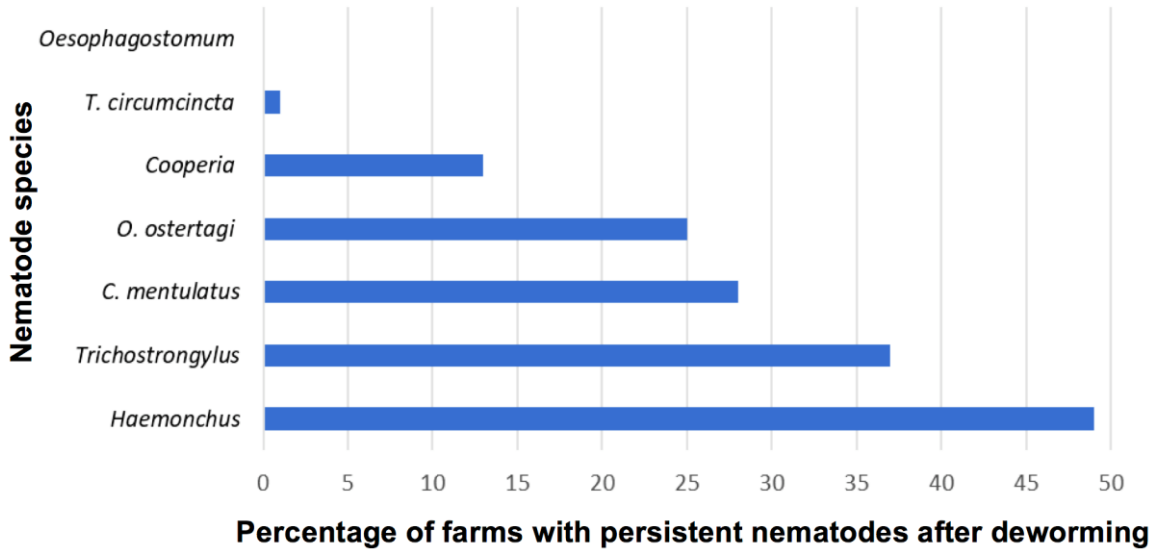

Fig. 13. Percentage of farms with persistent worms after treatment with 1.5 times on-label sheep dose of dewormer.

Unfortunately, FECRT results do not suggest an effective dose rate for treating worms in alpacas. It is apparent that 1.5 times the on-label sheep dose of ivermectin, moxidectin, fenbendazole and closantel failed to effectively reduce worm burdens in Australian alpacas. Every farm is different, so it is essential to perform FECs 10-14 days after any and every dewormer treatment regardless of active used to monitor dewormer efficacy.

Determination of dewormer dose rates in alpacas was beyond the scope of this project but needs to be performed in future research to assist with integrated worm management.

Chapter 5 - Validation of new diagnostic techniques for worms in alpacas

Key findings

There are two new methods that complement long-used techniques to monitor worm burdens in alpaca herds. Regular FECs and identification of worm species are crucial to optimise health and production on individual farms and the new methods complement the old methods.

Validation of FECPAK^{G2} with the modified McMaster method of faecal egg counting

The McMaster technique of estimating parasite egg numbers in host faeces has been used in livestock species for decades to monitor worm burdens (method in Appendix 2). It has a minimum detection limit of 15 epg. The test floats parasite eggs out of diluted faeces using a saturated sugar (or salt) solution to allow identification and counting of eggs under a light microscope. The technique is simple and cheap to perform but sometimes it is hard to see parasite eggs if faeces are thick and dark, and technical skill is required to identify and count eggs.

The FECPAK^{G2} method is a modified version of the McMaster technique with a minimum detection limit of 30-35 epg. It uses a floatation-dilution approach but involves taking digital images of samples without the use of a microscope. Preparation can be performed in the field by a lay operator as no specialised laboratory equipment is required, and images can be viewed by a trained technician off-site and stored permanently.

Faecal egg count estimates were compared using the McMaster and the FECPAK^{G2} methods in 94 alpacas from Victoria, New South Wales and Queensland. There was moderate to good agreement between the two methods and the new technique offers an alternate to Australian alpaca farmers for doing on-farm FECs.

Validation of a DNA test to identify worm species in alpaca faeces

The historical method to identify which worms are laying the typical strongyle-type eggs is to hatch and culture worm eggs from alpaca faeces under controlled laboratory conditions for 7-10 days, then use a taxonomic key to identify 3rd-stage larvae under the microscope. This method has low sensitivity and specificity.

Two existing DNA-based tests [called semi-quantitative multiplexed-tandem polymerase chain reaction (MT-PCR) assays] originally developed for sheep and cattle were modified and validated to reliably detect and differentiate the common species of worm DNA in the faeces of alpacas (Table 15). This test only takes 24-48 hours to perform and is more sensitive and specific.

There was good agreement between the DNA test and morphological identification of adult worms collected from the same alpacas. The DNA-based test can be used as a substitute for larval culture to identify common worms.

Table 15. Prevalence of and, agreement (%) between the identification of gastrointestinal nematodes of alpacas using morphological identification of adult worms and the DNA-based (MT-PCR) assay.

Nematode genera/species	**% prevalence identified by morphology (proportion)**	**% prevalence identified by MT-PCR (proportion)**	**Agreement (%)**
Haemonchus spp.	81 (78/96)	78 (67/86)	80
Camelostrongylus mentulatus	60 (58/96)	48 (41/86)	64
Trichostrongylus spp.	47 (46/97)	56 (48/86)	58
Ostertagia ostertagi	3 (3/97)	31 (27/86)	69
Teladorsagia circumcincta	1 (1/97)	3 (3/86)	96
Oesophagostomum spp.	8 (8/97)	12 (10/86)	85
Cooperia spp.	38 (37/97)	17 (15/86)	64

Conclusions and recommendations

- Australian alpacas are affected by camelid-specific worm species, and sheep and cattle worms.
- Parasitic gastroenteritis can lead to diarrhoea and/or anaemia, illthrift, loss of production (reduced growth, less wool production, poor fertility) and death. If an alpaca dies, check the third compartment of the stomach for barber's pole worms and Moroccan leather appearance (see page 16).
- Alpacas of all ages are affected by worms in all climatic zones of Australia, across all farm sizes, throughout the year. Worm burdens tend to increase during months/seasons of higher rainfall as moisture assists with survival of worm larvae on pasture.
- To optimise health and production of alpacas, farmers should monitor worm burdens in their herds by:
    - Regularly performing FECs, particularly in weaners and tuis or when alpacas lose weight/decrease body condition/exhibit diarrhoea or anaemia.
    - Identifying worm species on each farm using larval culture or DNA testing of alpaca faeces.
    - Performing FECs in co-grazing cattle, sheep and goats simultaneously as they share many worm species.
    - Interpreting FEC results in conjunction with respect to individual farm management (stocking rates, season, pasture length, body condition, age of alpacas).
- Many dewormers used to treat alpacas are ineffective (likely due to resistance of worms to the active ingredients). Farmers are encouraged to use at least 2 actives when treating alpacas for worms. Seek veterinary guidance as necessary.
- Weigh alpacas to determine appropriate dose of dewormer.
- Calibrate drench guns to ensure accurate dosage.
- Farmers should monitor efficacy of dewormers by performing FECs 10-14 days after treatment to ensure efficacy (≥ 95% reduction in FEC).
- Newly introduced alpacas should receive an effective dewormer with ≥ 2 actives ("quarantine drench") prior to entry into the herd.
- Grazing management and pasture spelling are important adjuncts to worm control programs to minimise the need for deworming.
- More research is required to determine FEC cut-off levels and effective dose rates of dewormers in alpacas based on experimental studies.

Publications arising from this research

- Rashid MH, Vaughan JL, Stevenson MA, Campbell AJD, Beveridge I, Jabbar A, 2018. Anthelmintic resistance in gastrointestinal nematodes of alpacas (*Vicugna pacos*) in Australia. Parasitites & Vectors, 11:388. (open access)
- Rashid MH, Gebrekidan H, Jabbar A, 2018. Multiplexed-tandem PCR (MT-PCR) assay to detect and differentiate gastrointestinal nematodes of alpacas. Parasites & Vectors, 11:370. (open access)
- Rashid MH, Stevenson MA, Waenga S, Mirams G, Campbell AJD, Vaughan JL, Jabbar A, 2018. Comparison of McMaster and FECPAK$_{G2}$ methods for counting nematode eggs in the faeces of alpacas. Parasites & Vectors, 11:278. (open access)
- Rashid MH, Stevenson AM, Campbell AJD, Vaughan JL, L, Beveridge I, Jabbar A, 2018. An assessment of worm control practices used by alpaca farmers in Australia, submitted.
- Rashid MH, Vaughan JL, Stevenson AM, Campbell AJD, Saeed MA, Indjein L, Beveridge I, Jabbar A, 2018. Epidemiology of gastrointestinal nematodes of alpacas in Australia I: Cross-sectional studies, submitted.

- Rashid MH, Stevenson AM, Vaughan JL, Saeed MA, Campbell AJD, Beveridge I, Jabbar A, 2018. Epidemiology of gastrointestinal nematodes of alpacas in Australia II: Longitudinal studies, submitted.
- Rashid MH, Beveridge I, Vaughan JL, Jabbar A, 2018. Worm burdens and associated histopathological changes caused by gastrointestinal nematodes in alpacas from Australia, submitted.

Appendix 1: Gastrointestinal nematodes ("worms") identified during total worm counts of Australian alpacas

Table A1. Gastrointestinal nematodes identified during total worm counts of Australian alpacas.

Nematode species identified during total worm counts	Common name	Egg type	Main host
C-3 stomach			
Haemonchus contortus	barber's pole worm	strongyle	sheep
Camelostrongylus mentulatus	striated spicules worm	strongyle	camel
Teladorsagia circumcincta	brown stomach worm	strongyle	sheep
Ostertagia ostertagi	brown stomach worm	strongyle	cattle
Trichostrongylus axei	stomach hair worm	strongyle	sheep, cattle
Graphinema aucheniae	long prongs worm	strongyle	SAC*
Small intestine			
Trichostrongylus rugatus	black scour worm	strongyle	sheep
Trichostrongylus colubriformis	black scour worm	strongyle	sheep
Trichostrongylus vitrinus	black scour worm	strongyle	sheep
Nematodirus spathiger	thin-necked intestinal worm	nematodirus	sheep
Nematodirus filicollis	thin-necked intestinal worm	nematodirus	sheep
Nematodirus helvetianus	thin-necked intestinal worm	nematodirus	cattle
Nematodirus abnormalis	thin-necked intestinal worm	nematodirus	sheep
Cooperia oncophora	small intestinal worm	strongyle	cattle
Cooperia punctate	small intestinal worm	strongyle	cattle
Cooperia pectinate	small intestinal worm	strongyle	cattle
Capillaria spp.	small intestinal worm	capillaria	Sheep/cattle/ goat/camelids
Large intestine			
Trichuris tenuis	whip worm	trichuris	camel
Oesophagostomum venulosum	large bowel worm	strongyle	sheep

*South American camelids

Note that *Capillaria* sp. eggs found during faecal egg counting but no adult worms were identified during total worm counts.

Appendix 2: Faecal egg count method used in this project

Collecting faeces

Equipment

- Gloves (latex, disposable)
- Zip-lock bags
- Permanent marker pen
- Water-based lubricant

Method

- Insert lubricated, gloved finger into rectum
- Hook faeces out carefully: do not damage rectal wall
- Place into zip-lock bag & exclude air from bag when sealing
- Refrigerate samples until processing so eggs do not hatch

Counting parasite eggs (not suitable for liver fluke eggs)

Equipment

- 10 faecal samples
- Scales suitable to weigh 4g faeces
- 60 mL cylinder/syringe
- Saturated sugar solution (SG 1.27)
- (130 g sugar/100 mL HOT water)
- 2 mixing bowls > 100 mL
- Spatula for mixing
- Small strainer
- Small syringe or pipette
- Compound microscope with x10 eyepiece(s) & x4 objective
- Whitlock Universal FEC slide (www.whitlock.com.au)
- Fresh water
- Paper towel
- Recording sheet

Method

- Weigh 4g faeces, place into mixing bowl, add 11 mL water and soak for 5-30 min
- Mix well then make up to 60 mL with saturated sugar solution
- Let sit for 30-45 min
- Pour through strainer to remove excess plant matter into 2nd bowl
- Stir sample (north/south & east/west not in circle)
- Immediately pipette solution into *wet* Whitlock slide chamber
- Wait 5 min to allow worm eggs to float to top of slide
- Count eggs & record
- Clean microscope with 50/50 Windex® & 70% ethanol after use to protect from corrosion
- Discard unused sugar solution & rinse all gear well

$$\text{Number of eggs/g faeces} = \frac{\text{number eggs x total volume of mix (mL)}}{\text{volume of slide chamber (mL) x weight of faeces (g)}}$$

(minimum detection limit of this technique is 15 epg of faeces)

Appendix 3: Estimated dewormer dose rates

The doses listed in Table A3 are derived from scientific literature, where available, and are approximately 1.5 times on-label sheep dose rates. When FECs indicate treatment is warranted (a) use at least 2 actives, (b) administer a single dose of each active, and (c) monitor FECs 10-14 days later to check treatment has been effective (≥ 95% reduction in FEC).

Table A3. Estimated anthelmintic/dewormer dose rates for alpacas.

Drug	Active class	Dose*	Route of admin
Albendazole	BZ	10-15 mg/kg	oral, not if pregnant
Fenbendazole	BZ	20 mg/kg	oral (3 d for whip worms)
		50 mg/kg	
Levamisole	LEV	5-8 mg/kg	oral; beware low toxicity threshold of 22 mg/kg
		(6 mg/kg SC)	
Closantel	CLO	7.5-10 mg/kg	oral (barber's pole-specific)
Ivermectin^	ML	0.3 mg/kg	oral, SC
Moxidectin^	ML	0.3-0.4 mg/kg	oral
		1 mg/kg	SC
Doramectin^	ML	0.3-0.4 mg/kg	SC
Monepantel	AAD/MPL	5.0-7.5 mg/kg	oral
Derquantel	SPI	3 mg/kg	oral

^ Avoid pour-on products in alpacas as efficacy is unpredictable. SC = subcutaneous

Appendix 4: Persistence of worms after deworming

Molecular (DNA) testing was used to detect seven worm genera/species in pre- and post-treatment samples on the 20 farms that participated in the FECRTs to assess persistence of worm genera/species and efficacy of dewormers (Fig. A4).

- Monepantel (Zolvix®) and the 4-active combination (Q-Drench®) were most effective at reducing worm species after treatment on farms participating in the trials. Nevertheless, *Haemonchus contortus* (BPW), *Camelostrongylus mentulatus*, *Trichostrongylus* spp. and *Ostertagia ostertagi* persisted on a small percentage of farms after treatment with either product.
- Fenbendazole did not remove *Haemonchus contortus* (BPW) or *Trichostrongylus* spp. from any farms and had little effect on reducing *C. mentulatus*. *O. ostertagi* and *Cooperia* spp. from farms.
- Ivermectin did not remove *H. contortus* from any farms and exhibited variable efficacy on other worms.
- Moxidectin injection was successful at removing *C. mentulatus* and *O. ostertagi* from all farms but exhibited variable efficacy on other worms.
- Closantel is only indicated for treatment of *Haemonchus* spp. but failed to remove this worm from all farms.

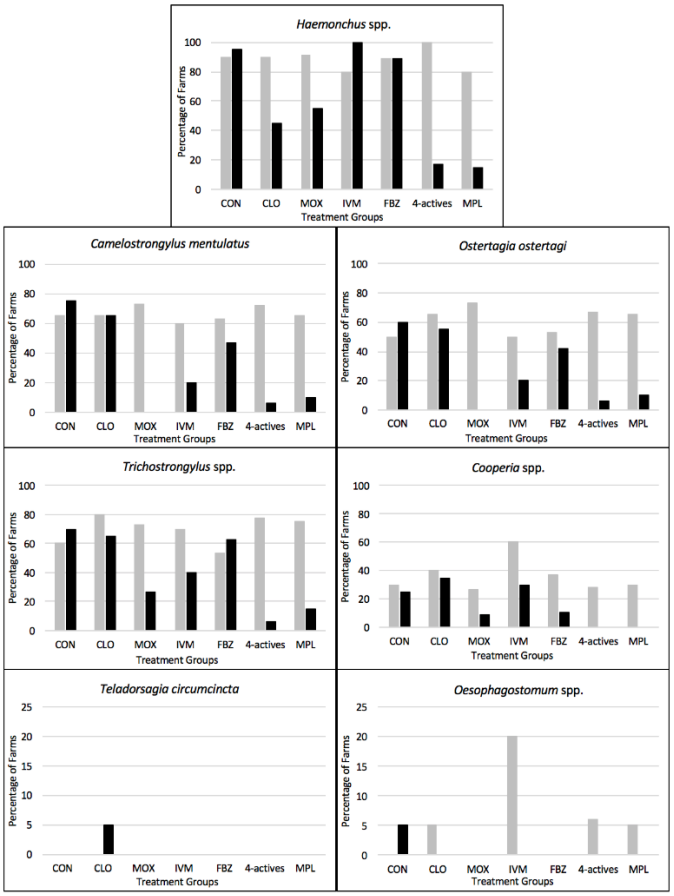

Fig. A4. Percentage of farms on which gastrointestinal nematode ("worm") genera/species persisted 10-14 days after no treatment (CON) or after treatment with closantel (CLO), moxidectin (MOX), ivermectin (IVM), fenbendazole (FBZ), 4-actives (albendazole, abamectin, closantel and levamisole in a commercial combination) or monepantel (MPL) (pre-treatment % ■, post-treatment % ■).

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Studies on gastrointestinal nematodes ("worms") of alpacas

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