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Epidemiology of gastrointestinal nematodes of alpacas in Australia: I. A cross-sectional study

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Abstract

This study involved a national cross-sectional survey of gastrointestinal nematodes (GINs) of alpacas in Australia. A total of 1545 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 characterisation 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.

Keywords Gastrointestinal nematodes · Prevalence · Worm burden · *Camelostrongylus* · Alpacas · Australia

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 peruviana*, have also been reported (Cafrune et al. 2001; Guerrero and Chávez 1967; Mitchell et al. 2016).

GINs 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

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(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 remains 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.

Materials and methods

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. 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 2 months in autumn; although, this practice varies among farms. Crias are weaned at an average age of 5 to 6 months.

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.

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 the 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 were selected for this study (Fig. 1).

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 4 to 6 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.

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 (Rashid et al. 2018a). The minimum detectable limit of the McMaster technique was 15 eggs per gram (EPG) of faeces.

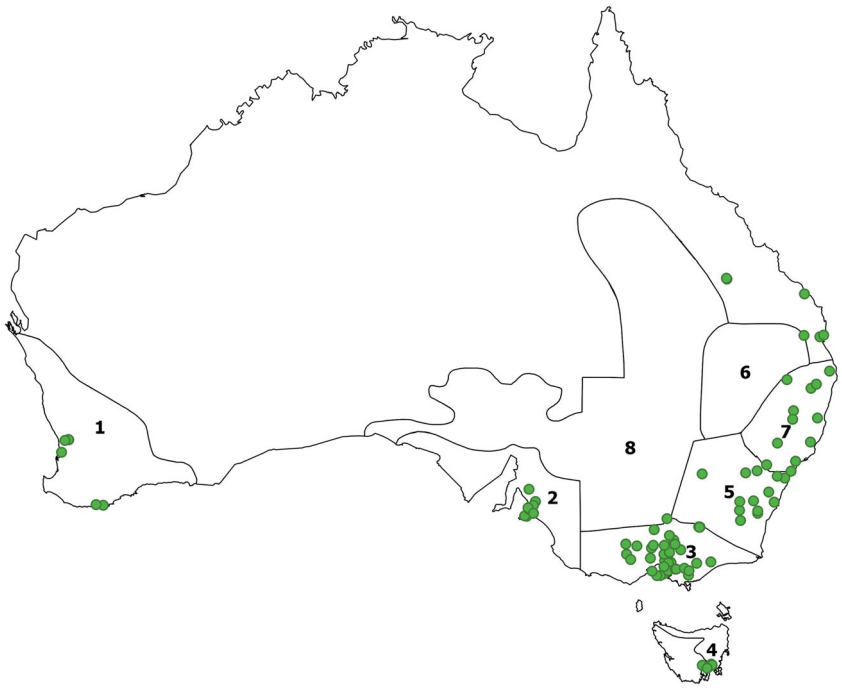

Fig. 1 Map of Australia showing the locations of alpaca farms included in the cross-sectional epidemiological study. Each green circle represents one alpaca farm. The climatic zones are drawn based on climatic zones described by www.wormboss.com.au. 1 Western Australian winter rainfall, 2 South Australian 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

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, we used a newly established molecular diagnostic test, the multiplexed-tandem polymerase chain reaction (MT-PCR) (Rashid et al. 2018b).

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.

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 to 565 ha, with a mean size of 66 ha; however, the mean grazing area for alpacas was only 59 ha. 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 (Rashid et al. 2018c).

Of 1545 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 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 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 1 Prevalence and faecal egg counts of gastrointestinal nematodes in Australian alpacas

	% Prevalence (proportion)	EPG (mean ± SE)	95% CI		Range
Sex of alpacas					
Female	63 (602/954)	227 ± 26a	176	277	0–10,800
Male	68 (334/490)	328 ± 60b	210	447	0–17,415
Type of nematode egg					
Strongyle	59 (916/1545)	276 ± 28a	220	332	0–17,400
Nematodirus spp.	17 (261/1545)	12 ± 1b	10	14	0–600
Trichuris spp.	6 (103/1545)	3 ± 1b	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 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 ($P < 0.05$).

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 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 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 4). All age groups of alpacas had the highest prevalence (except cria) as well as mean FECs in the summer rainfall zone (Table 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 6).

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. 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 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 7).

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

Table 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 ± 26a,d	107	211	(0–2490)
Weaners	80 (165/206)	331 ± 58b,c	218	445	(0–12,390)
Tuis	74 (157/211)	402 ± 63a,b	278	526	(0–9490)
Adults	58 (547/936)	214 ± 31d	152	275	(0–17,425)

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

Table 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 ± 101a	235	631	(0–14,355)
Winter rainfall	58 (411/703)	104 ± 11b	83	125	(0–3015)
Non-seasonal rainfall	64 (151/236)	165 ± 50b	65	264	(0–10,980)
Summer rainfall	77 (293/383)	630 ± 89c	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

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 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 we collected 1545 faecal samples from 92 alpaca farms across six states compared with 1030 faecal samples from South Australia and Victoria only. Furthermore, a countrywide 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 et al. 2014; Masson et al. 2016). Similarly, we observed a prevalence of 6% for *Trichuris* spp. in this study 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 the following: (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).

We observed an overall mean FEC of 291 EPG, with the highest count of 17,415 EPG (Table 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 4700 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

Table 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 ± 30a	78	198	(0–1980)
	Winter rainfall	58 (249/433)	98 ± 13a	72	124	(0–3015)
	Non-seasonal rainfall	60 (91/152)	109 ± 25a	59	159	(0–3090)
	Summer rainfall	76 (183/242)	577 ± 92b	396	759	(0–10,800)
Male	Mediterranean type	75 (40/53)	293 ± 105a,d	83	503	(0–4575)
	Winter rainfall	58 (124/212)	102 ± 17b,c	67	136	(0–2730)
	Non-seasonal rainfall	71 (60/84)	266 ± 134d	0	532	(0–10,980)
	Summer rainfall	78 (110/141)	720 ± 185a,b	355	1085	(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 Prevalence and faecal egg counts 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 ± 130b	− 89	470	(0–1845)
	Winter rainfall	61 (33/54)	92 ± 21a	50	134	(0–705)
	Non-seasonal rainfall	89 (8/9)	238 ± 99a	15	461	(0–885)
	Summer rainfall	75 (6/8)	469 ± 300b	− 222	1161	(0–2490)
Weaner	Mediterranean type	81 (26/32)	262 ± 66c	128	395	(0–1395)
	Winter rainfall	77 (60/78)	144 ± 25b,c	95	193	(0–1125)
	Non-seasonal rainfall	72 (33/46)	155 ± 42b,c	70	239	(0–1260)
	Summer rainfall	93 (46/50)	833 ± 275a,c	280	1385	(0–12,390)
Tuis	Mediterranean type	65 (20/31)	401 ± 180a	35	768	(0–4575)
	Winter rainfall	66 (65/98)	130 ± 35a	60	199	(0–2265)
	Non-seasonal rainfall	86 (25/29)	76 ± 17a	4	110	(0–360)
	Summer rainfall	89 (47/53)	1083 ± 261b	559	1607	(0–9480)
Adult	Mediterranean type	62 (64/103)	93 ± 24a	45	140	(0–1980)
	Winter rainfall	50 (204/409)	61 ± 7a	47	75	(0–1650)
	Non-seasonal rainfall	56 (85/152)	180 ± 77a	28	332	(0–10,980)
	Summer rainfall	71 (194/272)	509 ± 102b	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

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. We acknowledge that 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, we assume that 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

Table 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 ± 49a	255	448	(0–17,415)
Medium	65 (319/488)	177 ± 30a	119	235	(0–10,980)
Large	70 (248/352)	328 ± 65a	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

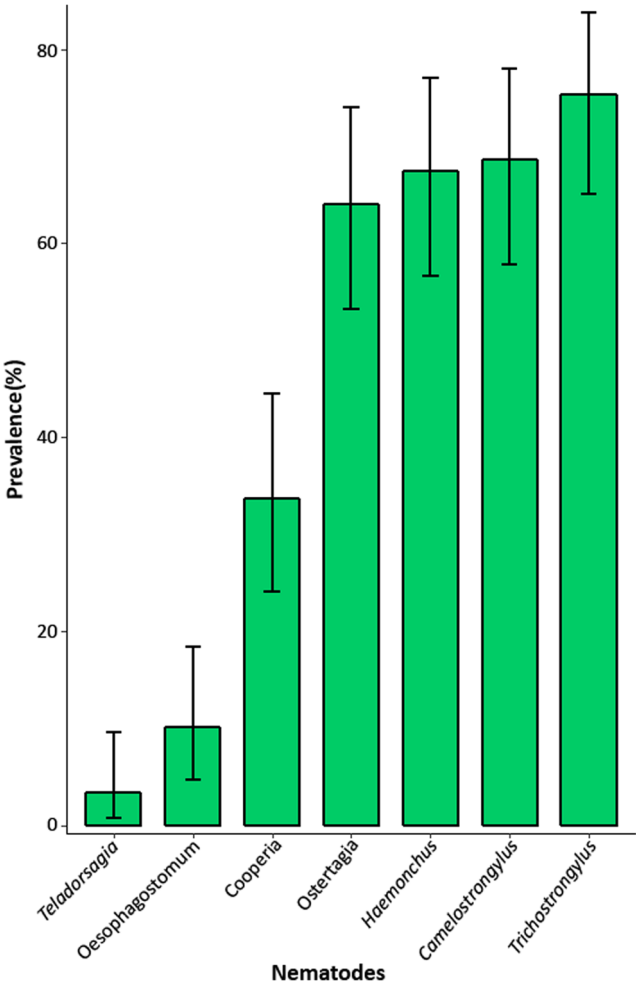

Fig. 2 Overall prevalence of gastrointestinal nematodes of alpacas in Australia. Each green bar shows the percentage of the farms found positive for a nematode genus/species using the multiplexed tandem PCR assay, and each vertical line shows upper and lower 95% confidence intervals

determine the threshold of FECs for various GINs in alpacas using experimental infections with monospecific infections.

We found that tuis had the highest FEC (402 EPG) followed by weaners (331 EPG), adults (214 EPG) and crias (159 EPG) (Table 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 the following: (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 (Rashid et al. 2018c).

In this study, we found seven GINs, including *C. mentulatus*, *Cooperia* spp., *Haemonchus* spp., *Oesophagostomum* spp., *O. ostertagi*, *T. circumcincta* and *Trichostrongylus* spp. (see Fig. 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. was the most common parasite of alpacas living in this climatic zone (see Table 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 (van 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).

Table 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. Lowercase 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

Conclusion

We undertook a national cross-sectional survey 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.

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Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethics approval The questionnaire was approved by the Human Ethics Committee (Ethics ID 1443529) of the University of Melbourne. The participants of the survey were registered members of the Australian Alpaca Association (AAA), and their participation in the study was entirely voluntary. All active members of the AAA received an invitation through email from the head office of the AAA to participate in the

survey. The use of alpacas in this study was approved by the Animal Ethics Committee (AEC no. 1413412.1) of the University of Melbourne. A plain language statement about the project was provided to participating alpaca farmers before obtaining their written consent.

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