Parasitology Research (2019) 118:901–911
https://doi.org/10.1007/s00436-019-06236-7

HELMINTHOLOGY - ORIGINAL PAPER

Epidemiology of gastrointestinal nematodes of alpacas in Australia: II. A longitudinal study

Mohammed H. Rashid[1] **· Mark A. Stevenson**[1] **· Jane L. Vaughan**[2] **· Muhammad A. Saeed**[1] **· Angus J. D. Campbell**[1] **· Ian Beveridge**[1] **· Abdul Jabbar**[1]

Received: 3 September 2018 / Accepted: 24 January 2019 / Published online: 9 February 2019

Abstract

We conducted a longitudinal survey on 13 alpaca farms in four climatic zones of Australia to understand the epidemiology of gastrointestinal nematodes (GINs) of alpacas. A total of 1688 fresh faecal samples were collected from both sexes of alpacas from May 2015 to April 2016 and processed for faecal egg counts (FEC) and molecular identification of eggs using the multiplexed-tandem PCR assay. Based on egg morphology, the overall prevalence of GINs was 61% while that for strongyles was 53%. The overall mean FEC was 168 eggs per gram (EPG) of faeces, with the highest count of 15,540 EPG. Weaners had the highest prevalence (73%) and mean FEC (295 EPG) of GINs followed by tuis, crias and adults. Alpacas in the winter rainfall zone had the highest prevalence (68%) as well as FEC (266 EPG) followed by Mediterranean-type, non-seasonal and summer rainfall zones. *Trichostrongylus* spp. (83%, 89/107), *Haemonchus* spp. (71%, 76/107) and *Camelostrongylus mentulatus* (63%, 67/107) were the three most common GINs of alpacas across all climatic zones. The mixed-effects zero-inflated negative binomial regression model used in this study showed that it could help to design parasite control interventions targeted at both the herd level and the individual alpaca level. The findings of this study showed that the epidemiology of GINs of alpacas is very similar to those of cattle and sheep, and careful attention should be paid when designing control strategies for domestic ruminants co-grazing with alpacas.

Keywords Longitudinal · Gastrointestinal · Nematodes · Prevalence · Climatic · Burden · *Camelostrongylus* · Alpacas · Australia

Introduction

Gastrointestinal nematode infections are one of the most severe disease problems in domesticated South American camelids (SACs), alpacas (*Vicugna pacos*) and llamas (*Lama glama*), leading to substantial economic losses due to lowered production of fibre and meat, and death (Ballweber 2009; Leguía 1991; Windsor et al. 1992a, b). In regions where SACs are imported outside South America, animals are kept under intensive to semi-intensive conditions (in contrast to extensive management in South America), with/without other domestic livestock, thereby exposing them to high infection pressures of gastrointestinal nematodes (GINs) (Ballweber 2009; Leguía 1991). Although SACs have their specific GINs (such as *Graphinema auchenia*, *Lamanema chavezi*), they are often infected with GINs of domestic ruminants (Ballweber 2009; Franz et al. 2015; Hill et al. 1993; Rashid et al. 2019a).

In the last three decades, our knowledge on the epidemiology and control of GINs in SACs has gradually been increasing due to a number of reports from Argentina, Australia, Bolivia, Chile, Ecuador, England, Japan, Libya, New Zealand, Peru, Switzerland and the USA (Abdouslam et al. 2003; Alcaino et al. 1991; Beltrán-Saavedra 2015; Cebra and Stang 2008; Dittmer et al. 2018; Hertzberg and Kohler 2006; Hill et al. 1993; Hyuga and Matsumoto 2016; Jabbar et al. 2013; Masson et al. 2016; Mitchell et al. 2016; Moreno et al. 2013; Rashid et al. 2018c; Rickard 1993; Robayo 2015; Tait et al. 2002; Welchman et al. 2008). These studies

Section Editor: Dante Zarlenga

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00436-019-06236-7) contains supplementary material, which is available to authorized users.

✉ Abdul Jabbar
jabbara@unimelb.edu.au

1 Department of Veterinary Biosciences, Melbourne Veterinary School, The University of Melbourne, Werribee, Victoria 3030, Australia

2 Cria Genesis, PO Box 406, Ocean Grove, Victoria 3226, Australia

mostly used convenient sampling methods such as the collection of faecal samples and focused only on one or a few regions in a country rather than conducting a national study to understand the epidemiology of GINs.

Recently, Rashid et al. (2019a) conducted the first national cross-sectional survey of GINs of alpacas to establish baseline data of the epidemiology of GINs of alpacas in Australia. This study showed that Australian alpacas were commonly infected with cattle and sheep nematodes (e.g. *Cooperia* spp., *Haemonchus* spp., *Nematodirus* spp., *Oesophagostomum* spp., *Ostertagia ostertagi*, *Teladorsagia circumcincta, Trichostrongylus* spp. and *Trichuris* spp.), and weaners had the highest prevalence whereas tuis had the highest faecal egg counts (FECs). It was also found that alpacas in the summer rainfall zone had the highest prevalence as well as FECs. Given that Australia has the largest alpaca population outside South America (Clarke 2016) and alpacas are reared in various climatic zones, it is crucial to understand better the effect of climatic conditions, seasons, gender, age, deworming history and other factors related to the epidemiology of GINs of alpacas. Therefore, we logically extended our studies to undertake a longitudinal survey of GINs of alpacas to understand the epidemiology of GINs of alpacas in Australia. In addition, we used a mixed-effects zero-inflated negative binomial regression model to design parasite control interventions.

Materials and methods

Selection of farms

This was a longitudinal study of monthly FECs in Australian alpacas for the period May 2015 to April 2016. The study included alpaca herds registered with the Australian Alpaca Association (AAA; www.alpaca.asn.au) on 1 January 2015. The herd had ≥ 50 alpacas on 1 January 2015. Alpaca farmers were contacted via the AAA as well as veterinarians to participate in this study during another study on worm control practices of Australian alpacas (Rashid et al. 2019b), and the herd managers of 13 alpaca farms consented to take part in the study in March/April 2015 (Fig. 1). Based on the distribution and density of alpaca populations in different climatic zones of Australia, the selected 13 alpaca farms were distributed in Mediterranean-type, non-seasonal, summer and winter rainfall zones in this study (Fig. 1). The details of these zones have already been described in Rashid et al. (2019a). The annual rainfalls for the farms located in these climatic zones from 2013 to 2017 are presented in Fig. 2. The average annual minimum and maximum temperatures for the farms located in four climatic zones from 2013 to 2017 are presented in Fig. 3.

Collection of faecal samples

Each alpaca farm manager was sent the sample collection kits containing submission forms, faecal collection bags, gloves, ice-packs for shipment and overnight courier envelopes. The instructions for the collection (via rectum) and shipping of alpaca faecal samples have previously described by Rashid et al. (2019a).

Faecal egg count

Following receipt of the overnight courier envelopes by the research team, faecal samples were refrigerated at 4 °C until processing which occurred within 7 days of the date of faeces collection. Each faecal sample was tested using a modified McMaster technique with a saturated sugar solution (specific gravity 1.27), as described previously (Rashid et al. 2018a). The estimated limit of detection of this modified McMaster method was 15 EPG.

Molecular identification of nematodes

Following the processing of fresh faecal samples ($n = 10–20$) for the FECs from each farm per month, as described above, 1–2 mL of the suspension containing a saturated sugar solution from each sample were transferred to a 50-mL Falcon tube® to extract eggs as previously described (Roeber et al. 2013a). The washed eggs in each pooled sample were transferred into a microcentrifuge tube and stored at − 20 °C until DNA isolation. Eggs in faecal samples were identified using a newly established molecular diagnostic kit for the identification of common GINs of alpacas (Rashid et al. 2018b).

Statistical analyses

The prevalence of parasitism in a herd for a given sampling event was calculated as the number of alpacas with nematodes of a particular type divided by the number of individual alpaca sampled expressed as a percentage (Margolis et al. 1982). The Kruskal–Wallis rank sum and Wilcoxon rank sum tests were used to statistically test for differences in FEC for different age groups and different climatic zones. A P value < 0.05 was used to declare statistically significant differences in FECs.

We used a mixed-effects zero-inflated negative binomial (ZINB) regression model (Nodtvedt et al. 2002; Denwood et al. 2005) to quantify the influence of herd-level and individual alpaca-level characteristics on FEC at the time of sampling. A zero-inflated negative binomial model addressed over-dispersion in the data arising from the relatively large number of zero FECs while at the same time allowing us to quantify the effect of factors influencing FEC when nematodes were actually present. A multilevel approach was used because of our study design. Faecal samples were collected

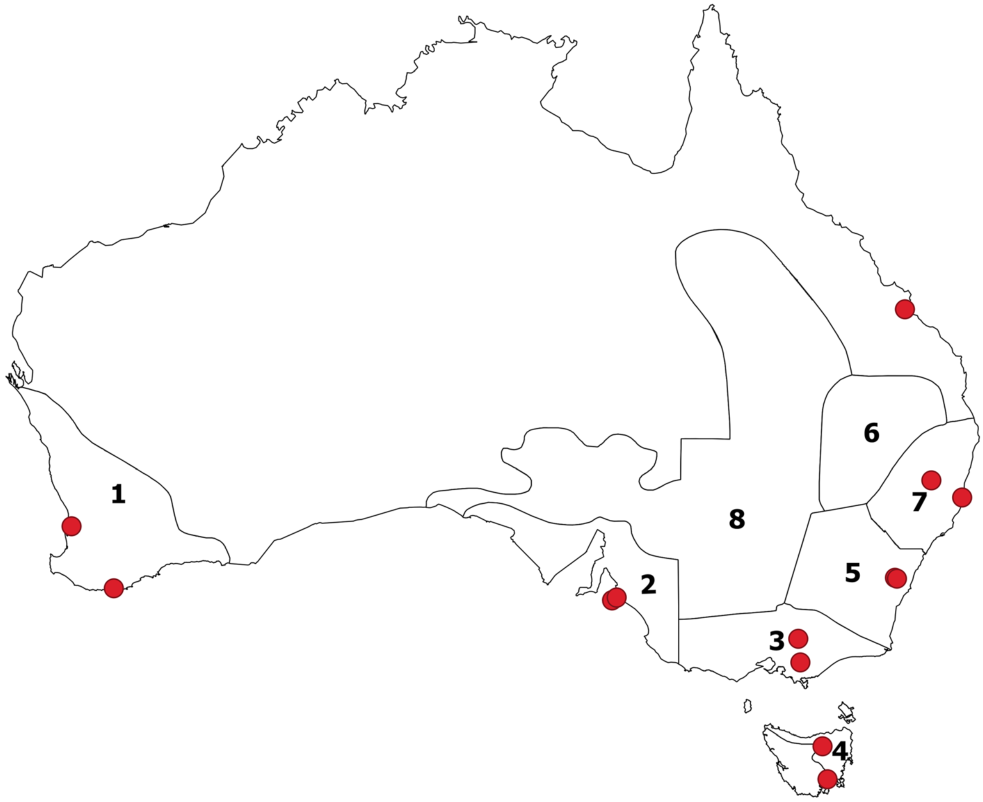

Fig. 1 Map of Australia showing the locations of alpaca farms enrolled in longitudinal epidemiological study. Each red circle represents one alpaca farm. The boundaries of different zones are drawn based on climatic zones described by www.wormboss.com.au. 1, Western Australian winter rainfall; 2, South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

from the same alpacas within individual herds over time (giving rise to repeated measures) and individual alpacas were clustered within herds.

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

$$Z_{ijk} \sim \begin{cases} \text{Bernoulli } (p) \text{ with probability } p_{ijk} \textit{ if } O_{ijk} = 0, \\ \text{Negative binomial } \left(\mu_{ijk}, s\right) \end{cases} \quad (1)$$

In Eq. 1, O_{ijk} was an independent Bernoulli random variable with a mean of p_{ijk} if $O_{ijk} = 0$; otherwise, O_{ijk} was assumed to follow a negative binomial distribution defined by a mean μ_{ijk} and a shape parameter s. Thus,

$$\text{logit}\left(p_{ijk}\right) = \alpha_0 + \alpha_1 x_{1ijk} + zH_i + zA_{jk} \quad (2)$$

and

$$\log\left(\mu_{ijk}\right) = \beta_0 + \beta_1 x_{1ijk} + \ldots + \beta_m x_{mijk} + nbA_{jk}. \quad (3)$$

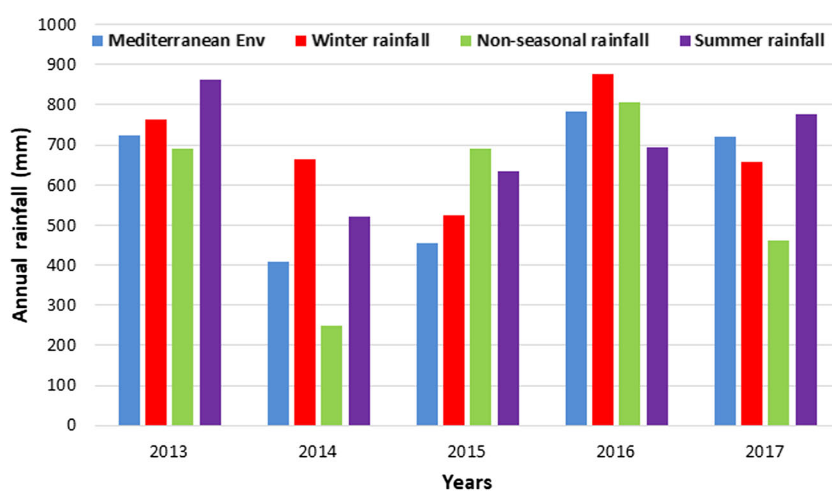

Fig. 2 Annual rainfall (mm) of the farms located in four selected climatic zones of Australia from 2013 to 2017 (collected from the Bureau of Meteorology (www.bom.gov.au) stations nearest to the farms)

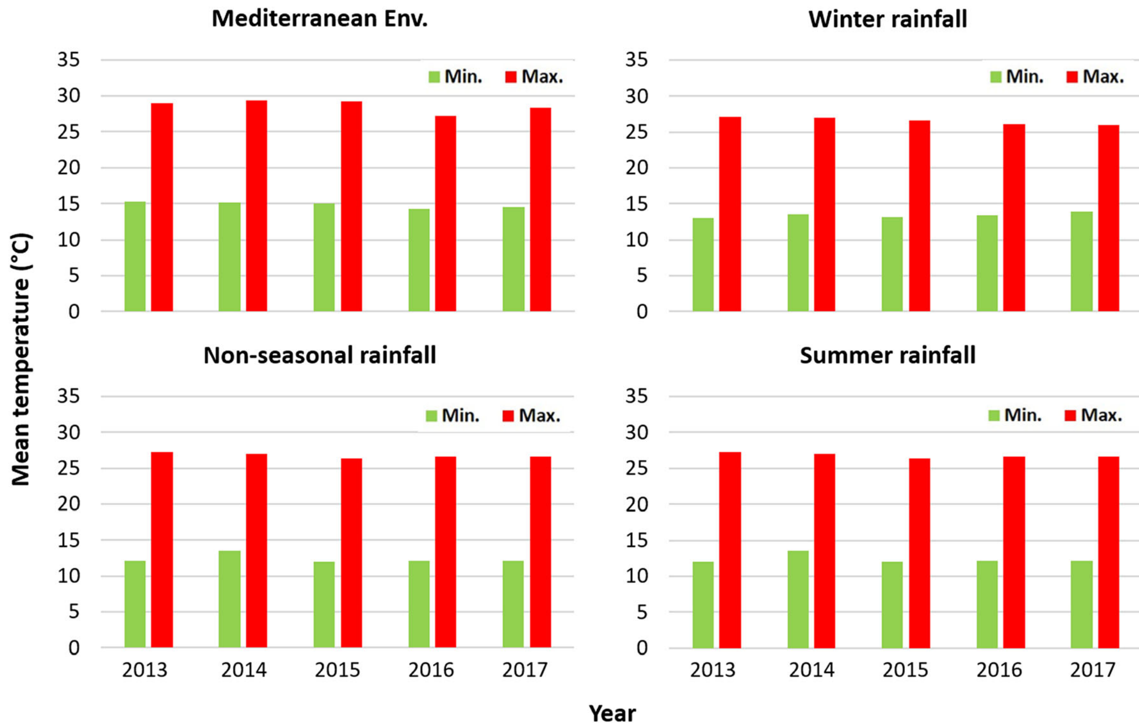

Fig. 3 Average minimum and maximum annual temperatures of the farms located in four selected climatic zones of Australia from 2013 to 2017 (collected from the Bureau of Meteorology (www.bom.gov.au) stations nearest to the farms)

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

$$zH_i = (zH_1, \ldots zH_N) \sim N(0, \sigma_{zH}) \quad (4)$$

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

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

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

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

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

Results

Based on the alpaca population in different climatic zones, the highest number of faecal samples were collected from the summer rainfall zone (475 individual samples from 3 farms) followed by Mediterranean-type environment (417 samples from 4 farms), winter rainfall zone (330 faecal samples from 4 alpaca farms) and non-seasonal rainfall zone (299 samples from 2 farms) during the study period. Of 1688 alpaca faecal samples tested using the McMaster technique, 61% had at least one type of GIN egg. Overall, the mean FEC (i.e. EPG ± standard error of the mean) was 168 ± 14 EPG (Table 1). Based on egg morphology, strongyle nematodes were the

Table 1 Prevalence and faecal egg counts gastrointestinal nematodes in Australian alpacas

Type of nematode egg	% Prevalence (proportion)	EPG (mean ± SE)	95% CI		Range
Strongyle	53 (876/1666)	151 ± 14b	123	179	(0–15,540)
Nematodirus spp.	18 (305/1666)	12 ± 1a	10	14	(0–615)
Trichuris spp.	11 (180/1665)	6 ± 1a	4	8	(0–1275)
Overall	61 (1037/1688)	168 ± 14	140	196	(0–15,630)

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

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

most prevalent (53%, 876/1666) followed by *Nematodirus* spp. (18%) and *Trichuris* spp. (11%) and this difference was statistically significant ($P < 0.05$) (Table 1).

Based on the age of alpacas, the highest prevalence and mean FEC were recorded for weaners (73%, 233/321) followed by tuis (69%, 240/346), crias (59%, 58/98) and adults (55%, 502/919). Similarly, the mean FEC was highest in weaners (295 ± 60 EPG) followed by tuis (187 ± 23 EPG), adults (126 ± 13 EPG) and crias (68 ± 13 EPG) (Table 2). Furthermore, the prevalence of GINs was significantly different between weaners and crias ($P < 0.05$) and adults ($P < 0.05$) and tuis and crias ($P < 0.05$) and adults ($P < 0.05$).

The highest prevalence (68%, 233/330) and mean FEC (266 ± 59 EPG) were recorded in the winter rainfall zone followed by the Mediterranean type (65%, 270/417; 149 ± 19 EPG). However, the prevalence of GINs in the non-seasonal rainfall zone was higher (60%, 179/299) than that in the summer rainfall (54%, 258/475) but the mean FEC was higher (167 ± 21 EPG) in the summer rainfall zone than the non-seasonal rainfall zone (101 ± 17) (Table 3). The prevalences of GINs were significantly different between Mediterranean-type and winter rainfall ($P < 0.05$) and summer rainfall ($P < 0.05$) zones and winter rainfall and summer rainfall ($P < 0.05$) and non-seasonal rainfall ($P < 0.05$) zones.

The effect of climatic conditions (rainfall and temperature) on nematodes of alpacas in the four climatic zones was studied over the study period (i.e. May 2015 to April 2016). In the winter rainfall zone, though the highest mean FEC (364 ± 119 EPG) was observed from late winter to early spring (i.e. August–September), a second peak (319 ± 111 EPG) was seen unexpectedly in summer (i.e. December–February) (Fig. 4). Within the winter rainfall zone, there was a statistical difference in the prevalence of GINs between winter and spring ($P < 0.05$), summer ($P < 0.05$) and autumn ($P < 0.05$) seasons. In the Mediterranean-type environment, the highest mean FEC (194 ± 36 EPG) was noted in winter (June–August) followed by a second peak (190 ± 74 EPG) in summer, and there was no difference in the prevalence of GINs across different seasons in this zone. Conversely, the highest mean FEC was recorded in late summer to early autumn (January–March) in both non-seasonal (157 ± 45 EPG) and summer rainfall (255 ± 52 EPG) zones (Fig. 4). Furthermore, there was a statistical difference in the prevalence of GINs between summer and winter seasons in non-seasonal ($P < 0.009$) and summer rainfall ($P < 0.004$) zones.

The identification of nematode genera/species using MT-PCR assay revealed that overall farm-level prevalence of *Trichostrongylus* spp. was the highest (83%, 89/107) followed by *Haemonchus* spp. (71%), *C. mentulatus* (63%), *O. ostertagi* (53%), *Cooperia* spp. (11%), *T. circumcincta* (8%) and *Oesophagostomum* spp. (6%) (Fig. 5). *Trichostrongylus* spp. were the most common nematodes in both the Mediterranean-type environment (89%, 25/28) and the summer rainfall zone (83%, 30/36) followed by *Haemonchus* spp. (57% and 69%, respectively) and *C. mentulatus* (50% and 39%, respectively) (Fig. 6; Supplementary Table 1). *Camelostrongylus mentulatus* was the most common nematode in the winter rainfall (81%, 17/21) and non-seasonal rainfall (100%, 22/22) zones followed by *Trichostrongylus* spp. (76% and 72%, respectively) and *Haemonchus* spp. (71% and 91%, respectively). *Ostertagia ostertagi* was the fourth most common nematode among all climatic zones (Fig. 6; Supplementary Table 1). There was a statistical difference in the prevalence of *C. mentulatus* between the non-seasonal rainfall zone and the summer rainfall zone ($P < 0.05$) and the Mediterranean-type environment

Table 2 Prevalence and faecal egg counts of gastrointestinal nematodes in different age groups of Australian alpacas

Age group	% Prevalence (proportion)	EPG (mean ± SE)	95% CI		Range
Crias	59 (58/98)	68 ± 13a	42	94	(0–870)
Weaners	73 (233/321)	295 ± 60b	178	413	(0–15,630)
Tuis	69 (240/346)	187 ± 23b	142	231	(0–4635)
Adults	55 (502/919)	126 ± 13a	101	151	(0–4770)

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

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

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	65 (270/417)	149 ± 19a	111	188	(0–4635)
Winter rainfall	68 (223/330)	266 ± 59b	151	382	(0–15,630)
Non-seasonal rainfall	60 (179/299)	101 ± 17a,c	68	134	(0–3690)
Summer rainfall	54 (258/475)	167 ± 21c	126	207	(0–4695)

Similar letters in a column indicate that no significant difference was shown among climatic zones

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

($P < 0.05$) and between the winter rainfall and summer rainfall ($P < 0.05$) zones. Similarly, there was a statistical difference in the prevalence of *O. ostertagi* between the non-seasonal rainfall zone and the summer rainfall zone ($P < 0.05$) and the Mediterranean-type environment ($P < 0.05$) and between the winter rainfall and summer rainfall ($P < 0.05$) zones.

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

Estimated regression coefficients and their standard errors for the mixed-effects ZINB model are presented in Table 4. Alpacas on farms in the winter rainfall zone, while holding all other variables in the model constant, had FECs 4.93 (95% CI 0.87 to 28.1) times greater than alpacas on farms in the Mediterranean-type environment. Male alpacas, while holding all other variables in the model constant, had FECs 1.38 (95% CI 1.01 to 1.87) times greater than female alpacas. For alpacas in herds where deworming was carried out in the month prior to sampling, the odds of having a zero faecal egg count was increased by a factor of 6.6 (95% CI 4.2 to 10) compared with alpacas in herds where de-worming was not carried out in the month prior to sampling (Table 4). The variance of the herd- and alpaca-level random effect terms was similar for both the negative binomial component (herd 0.4938; alpaca 0.4961) and the zero-count component (herd 0.6378; alpaca 0.7790) of the model (Table 4). This means that after controlling for each of the fixed effects included in the model, the variation in the residual (unexplained) component of FEC was similar at both the herd and individual alpaca level.

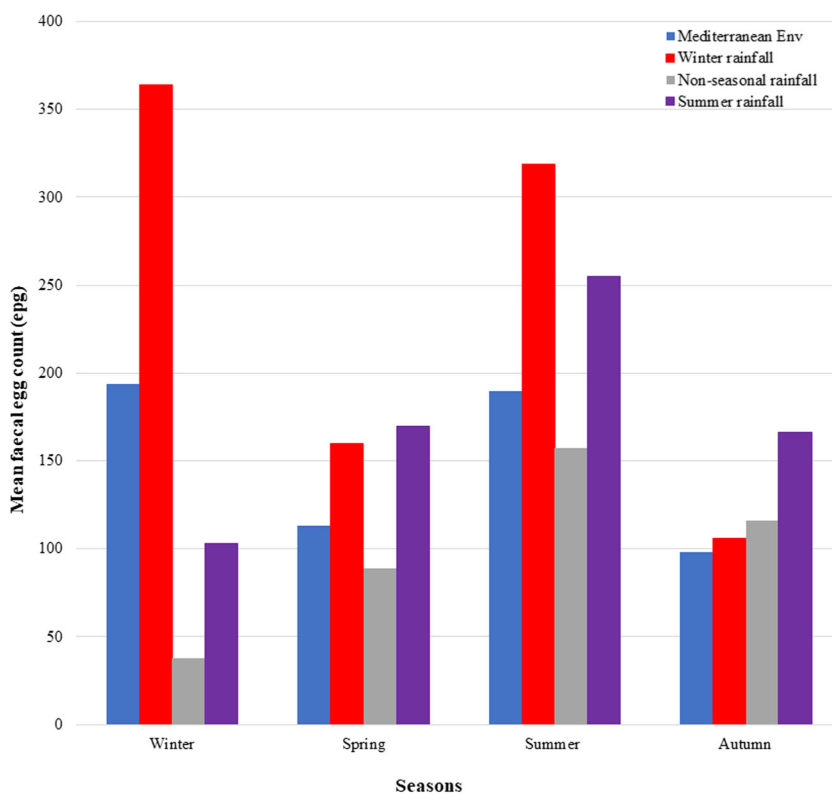

Fig. 4 Seasonal variation in the mean faecal egg counts (i.e. eggs per gram [epg] of faeces) of gastrointestinal nematodes in alpacas from the four climatic zones of Australia during the study period (May 2015 to April 2016)

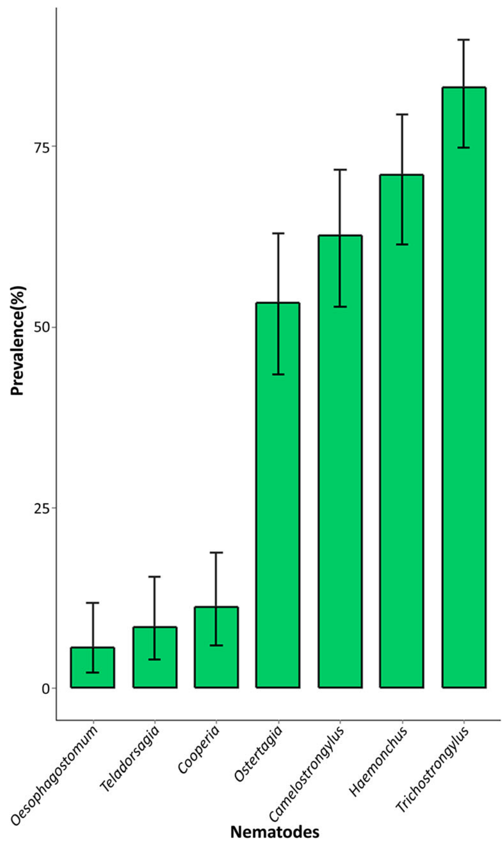

Fig. 5 Overall prevalence (%) of gastrointestinal nematodes of alpacas in Australia. Each green bar shows percentage of the farms found positive for a nematode genus/species using the multiplexed-tandem PCR assay, and each vertical line shows upper and lower 95% confidence intervals

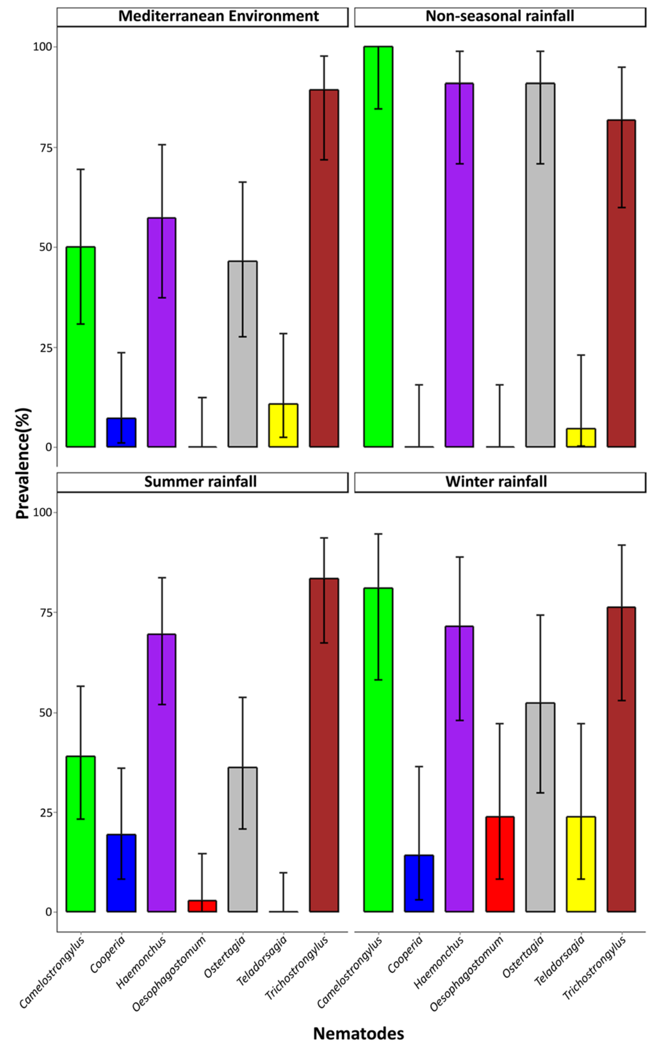

Fig. 6 Prevalence (%) of seven common gastrointestinal nematodes of alpacas in the four climatic zones of Australia. Nematodes were identified in pooled DNA samples using the multiplexed-tandem PCR assay. Each vertical line shows upper and lower 95% confidence intervals

Discussion

This is the first national longitudinal survey to determine the prevalence and worm burden of GINs in alpacas. *Camelostrongylus mentulatus*, *Haemonchus* spp. and *Trichostrongylus* spp. were the three most common GINs. Weaners were found to be the most susceptible age group as they harboured the highest prevalence as well as FECs. Alpacas in the winter rainfall zone had the highest prevalence and FECs. Overall, the epidemiology of GINs of Australian alpacas determined in this study is very similar to that was observed in a recent cross-sectional survey of GINs of alpacas (Rashid et al. 2019a).

The prevalence of GINs (61%) found in this study was comparable to that (66%) recently reported in a cross-sectional survey of GINs in Australian alpacas (Rashid et al. 2019a) but higher than those (48% and 20%) reported by Carmichael (1999) and Presidente (2007), respectively, previously from Australia. However, the latter studies were either based on the opportunistic collection of alpaca faecal samples (Presidente 2007) or were of limited scope (only South Australia and some parts of Victoria were included) (Carmichael 1999) as opposed to this study being a longitudinal survey across six states of Australia, involving a higher number of alpaca faecal samples.

We found that weaners had the highest prevalence (73%) of GINs followed by tuis, crias and adults which are concordant with findings of Rashid et al. (2019a). Similarly, the highest FECs were recorded in weaners whereas that was observed in tuis (402 EPG) in the cross-sectional study. The prevalences of strongyles (53%), *Nematodirus* spp. (18%) and *Trichuris* spp. (11%) were also very similar to those found in a cross-sectional study (Rashid et al. 2019a). These differences in GIN prevalence between both studies might be due to geographical variations and the total number of faecal samples analysed, i.e. higher number ($n = 1688$) of faecal samples from 13 alpaca farms across six states (New South Wales, Queensland, South Australia, Tasmania, Victoria and Western Australia) were analysed in this study. Other important factors underlying substantial differences in GIN

Table 4 Coefficients and standard errors from a mixed-effects zero-inflated negative binomial model of faecal egg counts

Negative binomial component:				
Variable	Coefficient (SE)	*z* value	Pr (> |*z*|)	IRR 95% CI
Intercept	5.024 (0.4708)	10.67	< 0.01	
Climatic zone:				
Mediterranean-type	Reference	–	–	
Winter rainfall	1.596 (0.8877)	1.80	0.07	4.93 (0.87, 28.1)
Non-seasonal rainfall	− 0.7269 (0.7187)	− 1.01	0.31	0.48 (0.12, 1.98)
Summer rainfall	− 0.6577 (0.6719)	− 0.98	0.33	0.52 (0.14, 1.93)
Gender:				
Female	Reference	–	–	
Male	0.3204 (0.1571)	2.04	0.04	1.38 (1.01, 1.87) [a]
Age (years):	− 0.0179 (0.0194)	− 0.92	0.35	0.98 (0.95, 1.02)
Deworming:				
No	Reference	–	–	
Yes	− 0.8277 (0.1912)	− 4.33	< 0.01	0.44 (0.30, 0.64)
Sample month × herd interaction:				
Month × herd 1	Reference			
Month × herd 2	− 0.0048 (0.0013)	− 3.63	< 0.01	–
Month × herd 3	− 0.0057 (0.0013)	− 4.34	< 0.01	–
Month × herd 4	0.0009 (0.0012)	0.73	0.46	–
Month × herd 5	0.0016 (0.0008)	2.02	0.04	–
Month × herd 6	0.0068 (0.0019)	3.52	< 0.01	–
Month × herd 7	0.0026 (0.0011)	2.36	0.02	–
Month × herd 8	− 0.0001 (0.0018)	− 0.05	0.96	–
Month × herd 9	0.0005 (0.0008)	0.65	0.52	–
Month × herd 10	− 0.0107 (0.0052)	− 2.07	0.04	–
Random effects:	Variance			
Herd	0.4938			
Alpaca	0.4961			
Zero-count component:				
Variable	Coefficient (SE)	*z* value	Pr (> |*z*|)	OR (95% CI)
Intercept	− 0.8412 (0.2750)	− 3.06	< 0.01	–
Deworming:				
No	Reference	–	–	
Yes	1.8907 (0.2338)	8.08	< 0.01	6.6 (4.2 to 10) [b]
Random effects:	Variance			
Herd	0.6378			
Alpaca	0.7790			

SE standard error, *IRR* incidence rate ratio, *OR* odds ratio

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

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

prevalence in alpacas could be mixed grazing (with domestic ruminants) practices, intensive commercial farming and the year in which the sampling occurred (Rashid et al. 2018a, 2019b; Saeed et al. 2018).

The overall mean FEC (168 EPG) recorded in this study appears lower than those (291 and 200 EPG) observed in Australia (Rashid et al. 2019a) and the UK (Tait et al. 2002), respectively. The main distinguishing feature of this study is that it revealed the temporal distribution of FECs. The acquisition and frequency of GIN infections in a camelid herd could vary at different times of the year due to variable temperatures and humidity levels throughout the year which ultimately affect larval growth on the pasture (Hill et al. 1993). Thus, the temporal distribution of GIN burdens in alpacas would be more informative in interpreting the epidemiological data. Importantly, the mean FEC for strongyle eggs (151 EPG) in this study was comparable to the overall mean FEC (168 EPG) in alpacas which indicates that alpacas were commonly infected with strongyle nematodes as are other domestic livestock in Australia (Roeber et al. 2011, 2013b) and elsewhere (Ballweber 2009; Franz et al. 2015).

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

As with infections in other domestic ruminant species, the distribution of GINs in alpacas is considerably impacted by climatic conditions such as rainfall and temperature which ultimately affects the prevalence and burden of these parasites in camelids and domestic ruminants across different climatic zones worldwide. Therefore, we assessed the impact of climatic conditions on the prevalence and FECs in alpacas from different climatic zones of Australia throughout 1 year. The highest prevalence (68%) and FECs (266 EPG) in alpacas were observed in the winter rainfall zone which encompasses the south-eastern part of the continent. For instance, in the winter rainfall zone, we found the peak prevalence in winter, which decreased in spring and increased again in the summer months. The usual trend in this zone is peak prevalence in winter followed by a decrease in spring to the lowest in summer (Roeber et al. 2013b). However, the second peak in the prevalence of GINs in the winter rainfall zone (Fig. 3) was most likely associated with higher rainfall (876 mm) during the summer season in 2016 (see Fig. 2) as the average annual rainfall in this zone prior to (2013 764 mm; 2014 664 mm; 2015 523 mm) and after (2017 658 mm) 2016 was lower. Overall, this study revealed that the temporal distribution of different GINs of alpacas in various climatic zones follows the patterns of those previously described for sheep and cattle GINs in Australia (Roeber et al. 2013b).

In this study, we observed a high prevalence of *H. contortus* in spring and autumn which is consistent previous studies in sheep from Australia (Roeber et al. 2013b). *Camelostrongylus mentulatus* was the most frequently (81%) observed GIN followed by *Trichostrongylus* spp. (76%) in winter rainfall zone. *Camelostrongylus mentulatus* has been found in sheep, feral goats and also in camels in Australia (Beveridge et al. 1974, 1987; Beveridge and Ford 1982; Copland 1965; Rogers 1939). Hilton et al. (1978) suggested *C. mentulatus* be a potential pathogen as it behaves like *Teladorsagia* spp. in sheep. Outside South American countries, *C. mentulatus* has also been found in alpacas, llamas and zoo animals in New Zealand, the UK and the USA (Averbeck et al. 1981; Dwyer et al., 2014; Flach 2008; Hinkson 2015; Rickard 1993; Welchman et al. 2008). Recently, Rashid et al. (2019c) found four species of *Trichostrongylus* (*T. axei*, *T. colubriformis*, *T. rugatus* and *T. vitrinus*) in alpacas which are known to commonly infect sheep in the winter rainfall zone of Australia, exhibiting high pathogenicity and production losses in the south-eastern states of the country (Beveridge and Ford 1982; Roeber et al. 2013b). Given that alpacas share GINs with sheep and *C. mentulatus* is known to infect sheep, it is proposed that the presence of this parasite in sheep should be evaluated in Australia, particularly in those regions where mixed grazing is a common practice.

In a recent cross-sectional study, Rashid et al. (2019a) reported the highest prevalence (77%) and FECs (630 EPG) in alpacas were from the summer rainfall zone in Australia; however, we found herein that those (prevalence, 68%; FEC 266 EPG) were modestly higher in the winter rainfall zone. Similarly, other differences in the prevalence of *C. mentulatus*, *Cooperia* spp., *O. ostertagi* and *T. circumcincta* were recorded in Mediterranean zone between this study and the cross-sectional study by Rashid et al. (2019a). These differences in the prevalence and FECs in alpacas across different climatic zones could be attributed to different timing of the two studies (longitudinal 2015–2016 versus cross-sectional 2016–2017), variations in rainfall (see Fig. 2) and management practices of alpaca herds sampled. Further longitudinal epidemiological studies, spanning for 3–5 years, are required to further investigate the prevalence of different GINs of alpacas in different seasons across various climatic zones of Australia. Also, studies should focus on examining the gastrointestinal tracts of alpacas to precisely determine the type of GINs as we used FEC and the MT-PCR assay only to identify GIN genera/species in alpacas.

In our recent cross-sectional study (Rashid et al. 2019a) as well as this longitudinal epidemiological survey, we found that male alpacas had higher prevalence and burden (i.e. FECs) of GINs than females. Sex could be an important factor in the susceptibility of hosts for infection with GINs, though variable results have been reported previously. For example, male alpacas were found to have higher burden of *H. contortous* than females (Dittmer et al., 2018). Contrarily, in another study by Edwards et al. (2016), no association was found between the occurrence of *H. contortous* and sex of camelids. Further studies are required to investigate the precise effect of the sex on the susceptibility of alpacas and llamas for GINs.

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

Conclusion

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

Acknowledgments We are grateful to alpaca farmers across Australia who provided faecal samples for this study.

Funding The financial assistance for this project was provided by the AgriFutures Australia and the Australian Alpaca Association. M.H.R. is a grateful recipient of the Australian Postgraduate Award through the University of Melbourne and the PhD top-up scholarship from the AgriFutures Australia.

Compliance with ethical standards

Ethics approval The use of alpacas in this study was approved by the Animal Ethics Committee (AEC no. 1413412.1) of the University of Melbourne.

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

References

Abdouslam O, Al-Bassam L, Al-Izzi S, Azwai S (2003) Prevalence of external and internal parasites in llamas (*Lama glama*) at Surman park in Libya. J Camel Pract Res 10:61–65

Alcaino H, Gorman T, Burgos M (1991) Helmintiasis gastrointestinal en llamas (*Lama glama*) de la I Región de Chile. Parasitol Día 15:93–96

Anderson N (1972) Trichostrongylid infections of sheep in a winter rainfall region. 1. Epizootiological studies in the Western District of Victoria, 1966–67. Aust J Agric Res 23:1113–1129

Anderson N (1973) Trichostrongylid infections of sheep in a winter rainfall region. 2. Epizootiological studies in the Western District of Victoria, 1967–68. Aust J Agric Res 24:599–611

Averbeck GA, Scholtthauer JC, Hinueber JG (1981) *Camelostrongylus mentulatus* infection in a camel (*Camelus dromedarius*): a case report. J Zoo Anim Med 12:24–26

Ballweber LR (2009) Ecto- and endoparasites of new world camelids. Vet Clin North Am Food Anim Pract 25:295–310

Beltrán-Saavedra LF (2015) Health assessment of free-ranging vicuna of the National Integrated Management Natural Area Apolobamba, Bolivia. J Selva Andina Anim Sci 46:1–17

Beveridge I, Ford GE (1982) The trichostrongyloid parasites of sheep in South Australia and their regional distribution. Aust Vet J 59:177–179

Beveridge I, Barker I, Rickard M, Burton J (1974) Experimental infection of sheep with *Camelostrongylus mentulatus* and associated gastritis. Aust Vet J 50:36–37

Beveridge I, Pullman AL, Henzell R, Martin RR (1987) Helminth parasites of feral goats in South Australia. Aust Vet J 64:111–112

Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Maechler M, Bolker BM (2017) Modeling zero-inflated count data with glmmTMB. bioRxiv 1:132753 https://www.biorxiv.org/content/biorxiv/early/2017/05/01/132753.full.pdf

Carmichael I (1999) Internal parasitism in alpacas in southern Australia. In: Hack W, McGregor B, Ponzoni R, Judson G, Carmichael I, Hubbard D (eds) Australian alpaca fibre improving productivity and marketing. Rural Industries Research and Development Corporation, Australia, pp 92–130

Cebra CK, Stang BV (2008) Comparison of methods to detect gastrointestinal parasites in llamas and alpacas. J Vet Med Assoc 232:733–741

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

Copland JW (1965) *Ostertagia mentulata* recorded in New South Wales. Aust Vet J 41:27–27

Denwood MJ, Stear MJ, Matthews L, Reid SW, Toft N, Innocent GT (2005) The distribution of the pathogenic nematode *Nematodirus battus* in lambs is zero-inflated. Parasitology 135:1225–1235

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

Dwyer C, et al. (2014) *Trichostrongylus askivali*—a new record for alpacas in New Zealand. Proceedings of the New Zealand Society for Parasitology: 42nd Conference, Wellington

Edwards EE, Garner BC, Williamson LH, Storey BE, Sakamoto K (2016) Pathology of *Haemonchus contortus* in New World camelids in the southeastern United States: a retrospective review. J Vet Diagn Investig 28:105–109

Flach E (2008) Alpaca and llama nematodes in Britain. Vet Rec 163:128–128

Franz S, Wittek T, Joachim A, Hinney B, Dadak AM (2015) Llamas and alpacas in Europe: endoparasites of the digestive tract and their pharmacotherapeutic control. Vet J 204:255–262

Hertzberg H, Kohler L (2006) Prevalence and significance of gastrointestinal helminths and protozoa in South American camelids in Switzerland. Berl Munch Tierarztl Wochenschr 119:291–294

Hill F, Death A, Wyeth T (1993) Nematode burdens of alpacas sharing grazing with sheep in New Zealand. N Z Vet J 41:205–208

Hilton RJ, Barker IK, Rickard MD (1978) Distribution and pathogenicity during development of *Camelostrongylus mentulatus* in the abomasum of sheep. Vet Parasitol l4:231–242

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

Hyuga A, Matsumoto J (2016) A survey of gastrointestinal parasites of alpacas (*Vicugna pacos*) raised in Japan. J Vet Med Sci 78:719–721

Jabbar A, Campbell AJD, Charles JA, Gasser RB (2013) First report of anthelmintic resistance in *Haemonchus contortus* in alpacas in Australia. Parasit Vectors 6:243

Leguía G (1991) The epidemiology and economic impact of llama parasites. Parasitol Today 7:54–56

Margolis L, Esch GW, Holmes JC, Kuris AM, Schad G (1982) The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). J Parasitol 68:131–133

Masson M, Gutiérrez G, Puicón V, Zárate D (2016) Helmintiasis y eimeriosis gastrointestinal en alpacas criadas al pastoreo en dos granjas comunales de la región Pasco, Perú, y su relación con el peso y condición corporal. Rev. Investig Vet Perú 27:805–812

Mitchell S, Hopkins B, Corfield C (2016) *Nematodirus lamae* identified in an alpaca in the UK. Vet Rec 178:271–272

Moreno PG, Eberhardt MAT, Lamattina D, Previtali MA, Beldomenico PM (2013) Intra-phylum and inter-phyla associations among gastrointestinal parasites in two wild mammal species. Parasitol Res 112: 3295–3304

Nodtvedt A, Dohoo I, Sanchez J, Conboy G, DesCoteaux L, Keefe G, Leslie K, Campbell J (2002) The use of negative binomial modelling in a longitudinal study of gastrointestinal parasite burdens in Canadian dairy cows. Can J Vet Res 66:249–257

Presidente PJA (2007) Alpaca parasites and their control: recent experiences. Australian Alpaca Veterinarians Annual Conference, Australia, pp 1–14

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

Rashid MH, Stevenson MA, Waenga S, Mirams G, Campbell AJ, Vaughan JL, Jabbar A (2018a) Comparison of McMaster and FECPAK^{G2} methods for counting nematode eggs in the faeces of alpacas. Parasit Vectors 11:278

Rashid MH, Gebrekidan H, Jabbar A (2018b) Multiplexed-tandem PCR (MT–PCR) assay to detect and differentiate gastrointestinal nematodes of alpacas. Parasit Vectors 11:370

Rashid MH, Vaughan JL, Stevenson MA, Saeed MA, Campbell AJD, Beveridge I, Jabbar A (2018c) Anthelmintic resistance in gastrointestinal nematodes of alpacas (*Vicugna pacos*) in Australia. Parasit Vectors 11:388

Rashid MH, Vaughan JL, Saeed MA, Stevenson MA, Indjein L, Campbell AJD, Beveridge I, (2019a) Epidemiology of gastrointestinal nematodes of alpacas in Australia: I. Cross sectional studies. Parasitol Res. https://doi.org/10.1007/s00436-019-06235-8

Rashid MH, Stevenson MA, Campbell AJD, Vaughan JL, Beveridge I, Jabbar A (2019b) An assessment of worm control practices used by alpaca farmers in Australia. Vet Parasitol 265:91–100

Rashid MH, Vaughan JL, Beveridge I, Jabbar A (2019c) Worm burdens and associated histopathological changes caused by gastrointestinal nematodes in alpacas from Australia. Parasitol Res. https://doi.org/10.1007/s00436-019-06237-6

Rickard LG (1993) Parasitic gastritis in a llama (*Lama glama*) associated with inhibited larval *Teladorsagia* spp.(Nematoda: Trichostrongyloidea). Vet Parasitol 45:331–335

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

Roeber F, Jex AR, Campbell AJ, Campbell BE, Anderson GA, Gasser RB (2011) Evaluation and application of a molecular method to assess the composition of strongylid nematode populations in sheep with naturally acquired infections. Infect Genet Evol 11:849–854

Roeber F, Jex AR, Gasser RB (2013a) Comparative evaluation of two DNA isolation techniques for PCR-based diagnosis of gastrointestinal nematode infections in sheep. Mol Cell Probes 27:153–157

Roeber F, Jex AR, Gasser RB (2013b) Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance—an Australian perspective. Parasit Vectors 6:153

Rogers WP (1939) Nematode parasites of sheep in Western Australia. J Helminthol 17:151–158

Saeed MA, Rashid MH, Vaughan J, Jabbar A (2018) Sarcocystosis in South American camelids: the state of play revisited. Parasit Vectors 11:146

Tait S, Kirwan J, Fair C, Coles G, Stafford K (2002) Parasites and their control in South American camelids in the United Kingdom. Vet Rec 150:637–638

Welchman DB, Parr J, Wood R, Mead A, Starnes A (2008) Alpaca and llama nematodes in Britain. Vet Rec 162:832–832

Windsor RS, Teran M, Windsor RH (1992a) Effects of parasitic infestation on the productivity of alpacas (*Lama pacos*). Trop Anim Health Prod 24:57–62

Windsor RS, Windsor RH, Teran M (1992b) Economic benefits of controlling internal and external parasites in South American camelids. Ann N Y Acad Sci 653:398–405