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HELMINTHOLOGY - SHORT COMMUNICATION

# Worm burdens and associated histopathological changes caused by gastrointestinal nematodes in alpacas from Australia

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## Abstract

In this study, 100 gastrointestinal tracts of Australian alpacas were examined to assess the worm burden and to identify the species of nematode present. Faecal samples were collected from 97 alpacas and processed for faecal egg counts (FECs). For identification of the species, both molecular (multiplexed-tandem polymerase chain reaction [MT-PCR]) and morphological techniques were used. Total worm counts (TWCs) revealed a mean burden of 1300 worms, with the highest burden of 29,000 worms. The average egg count was 501 eggs per gram of faeces (EPG), with the highest count of 3500 EPG. Nineteen different species of gastrointestinal nematodes (GINs) were identified, and *Graphinema auchenia*, *Camelostrongylus mentulatus* and *Trichuris tenuis* were recovered from Australian alpacas for the first time. *Haemonchus contortus* was the most prevalent nematode (81%) followed by *C. mentulatus* (60%). The majority of the nematodes found are shared with sheep, goats and cattle. Findings of this study provide useful insights into the spectrum of GINs and their burden in Australian alpacas.

**Keywords** Gastrointestinal nematodes · Alpaca · Total worm count · *Camelostrongylus mentulatus* · *Graphinema auchenia* · *Haemonchus contortus*

## Introduction

A wide range of gastrointestinal nematodes (GINs) can infect alpacas and llamas, many of which also affect sheep, goats and cattle (Ballweber 2009; Hill et al. 1993; Rashid et al. 2019a, 2019b; Schmäschke 2015). However, the parasitological status of alpacas and llamas in non-native countries such as Australia is unknown.

To date, few total worm count (TWC) studies have been conducted in Australia to assess the nematode burdens of alpacas, and there is a lack of information about the spectrum of GINs occurring in alpacas. *Haemonchus* spp. and other strongylid nematodes (*Cooperia*, *Chabertia*, *Oesophagostomum*, *Nematodirus*, *Ostertagia*/*Teladorsagia* and *Trichostrongylus*) have been diagnosed by larval culture from Australian alpacas (Carmichael 1999; Presidente 2007) and using molecular methods (Jabbar et al. 2013). Globally, there is also a scarcity of TWC data from South American camelids (SACs). One study in the UK reported the recovery of *Capillaria* spp., *Nematodirus* spp. and *Trichuris* spp. from three alpacas (Tait et al. 2002); however, a worm count was not performed on these animals. In addition, the molecular method developed so far, to detect and differentiate GINs in the faces of alpacas (Rashid et al. 2018a), can only detect seven common nematodes; thereby, they are also unable to provide the full spectrum of GINs infecting SACs.

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

## Materials and methods

### Collection and processing of gastrointestinal tracts of alpacas

Alpacas used in this study were either killed in abattoirs for meat or were culled on a farm. Hence, no institutional animal

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**Table 1** Worm burden caused by gastrointestinal nematodes in Australian alpacas

| Method | No. of samples | Mean ± SD | 95% confidence interval | Median | Range |
|---|---|---|---|---|---|
| Faecal egg count (EPG) | 97 | 501 ± 609 | 381–622 | 255 | 0–3495 |
| Total worm counts | | | | | |
| Third compartment of the stomach | 98 | 617 ± 867 | 445–789 | 313 | 0–4400 |
| Small intestine | 98 | 689 ± 2974 | 99–1279 | 70 | 0–28,530 |
| Overall | 100 | 1280 ± 3020 | 681–1879 | 660 | 0–28,640 |

SD standard deviation, EPG eggs per gram of faeces

ethics approval was required. The alpacas were of mixed sex and were mature before slaughter. More than 100 alpaca gastrointestinal tracts were collected. The majority of the samples (86) were collected from New South Wales, followed by Victoria (11) and South Australia (6). Fresh alpaca gastrointestinal tracts were processed immediately after killing. Total worm counts were performed, following Hutchinson (2009) with minor modifications. After killing, the third compartment (C3) of the stomach was isolated by ligating the two ends using cotton thread, separated from the second compartment and the small intestine and collected into a plastic bag. Similarly, small and large intestines were also collected into other plastic bags and stored on ice. About 20 g of faeces was also collected from the rectum of each animal and stored on ice for faecal egg counts and the molecular identification of nematodes. All samples were transported to the University of Melbourne and stored at 4 °C until processing.

The C3 was placed onto a tray, opened and its contents washed into a graduated bucket using water. The volume of the washed contents was made up to 1 to 2 L based on the amount of content. The content was mixed, and then, 5–10% aliquots were drawn into a 100-mL sieve-top (38-μm mesh) glass jar. The contents were washed with water and were fixed by adding 70% ethanol.

Six meters of the duodenum was separated from their mesenteric attachments and about 20 mL of water passed through the intestine three times. The content was made up to 1 L and an aliquot of 100 mL was transferred into a sieve-top jar for washing. The content was preserved as described earlier.

The entire caecum and colon were opened and examined for the presence of nematodes which were preserved using 70% ethanol.

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

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

## Faecal egg count

Faecal samples were examined using the modified McMaster technique (Gordon and Whitlock 1939; Rashid et al. 2018b). The minimum detection limit using this method was 15 EPG.

**Table 2** Species of gastrointestinal nematodes found in Australian alpacas based on morphological examination of adult male worms

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

[a] Detected by faecal examination only

### Molecular identification of common nematodes

DNA was extracted from the nematode eggs present in the faeces of alpacas using a method described previously with few modifications (Roeber et al. 2013; Rashid et al. 2018a). Following the processing of fresh faecal samples for the faecal egg counts (FECs) of GINs in alpacas by employing the McMaster technique (Rashid et al. 2018b), 5 mL of the suspension containing the saturated sugar solution form each sample was drawn and transferred to a 50-mL Falcon tube to extract eggs of GINs as previously described (Roeber et al. 2013). The washed eggs in each sample were transferred into a microcentrifuge tube and stored at − 20 °C until further use. Following thawing, 250 μL of the concentrated eggs were used to extract and isolate DNA using Powersoil® DNA Isolation Kit (MO BIO Laboratories Inc., West Carlsbad, CA, USA) as per manufacturer's recommended protocol.

A multiplexed-tandem PCR (MT-PCR) assay was used as per the protocol described by Rashid et al. (2018a). The assay was capable of identifying *Camelostrongylus mentulatus*, *Cooperia* spp., *Haemonchus* spp., *Ostertagia ostertagi*, *Oesophagostomum* spp., *Teladorsagia circumcincta* and *Trichostrongylus* spp. by targeting the second internal transcribed spacer of these nematodes. Randomly selected amplicons representing each nematode genus/species were subjected to sequencing to verify the target nematodes.

### Statistical analysis

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

## Results and discussion

Ninety-three percent (93/100) of the gastrointestinal tracts examined were infected with nematodes. The mean worm burden was 1280, with a maximum of 28,640 worms. The mean

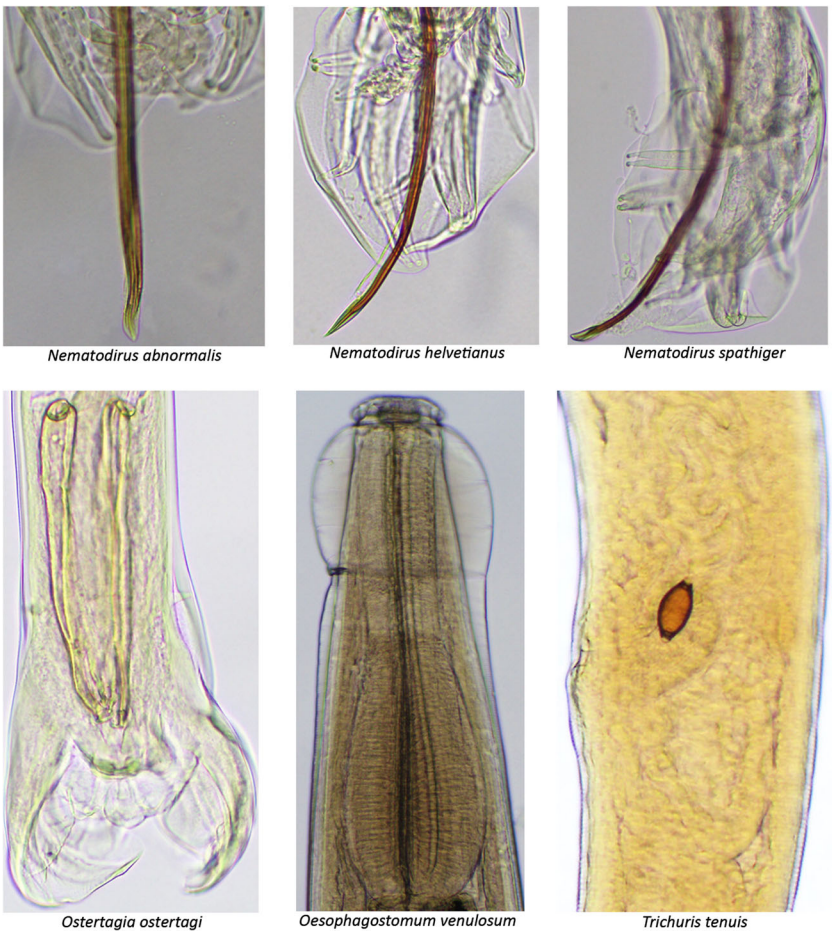


**Fig. 1** Gastrointestinal nematodes found in Australian alpacas based on morphological examination of spicules of male worms

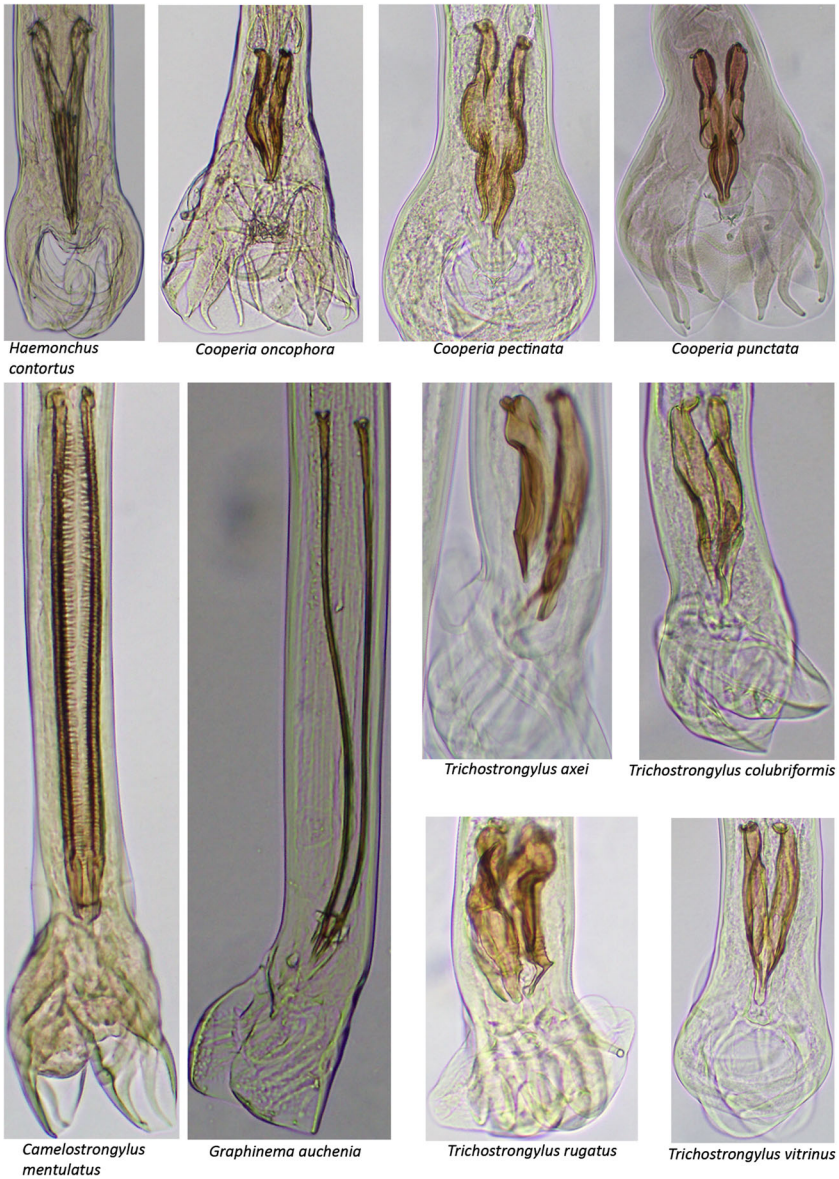


**Fig. 1** (continued)

worm counts in the C3 and the small intestine were 617 and 689, with maxima of 4400 and 28,530, respectively (Table 1). Similar studies of naturally infected alpacas are lacking globally. Previously, Carmichael (1999) reported TWCs of 14,500 and 23,500 in two healthy alpacas from Victoria. In Australia, a total of 460 *H. contortus* was recovered from the C3 of a 1-year-old alpaca that was recently treated with abamectin. No other nematodes were found in the C3 and small intestine (Jabbar et al. 2013). A necropsy of a llama found a total of 6510 adult nematodes in the C3 (Rickard 1993).

Morphological examination revealed 19 different nematodes species (Table 2 and Fig. 1). *Camelostrongylus mentulatus*, *G. auchenia* and *Tr. tenuis* are primarily camelid nematodes whilst the remaining species are shared with cattle and sheep. The shared species have been reported previously in sheep, goats and cattle in Australia (Anderson 1972, 1973; Barger 1993). Moreover, this study reports the occurrence of significant nematodes of alpacas such as *C. mentulatus*, *C. pectinata*, *G. auchenia*, *Oe. venulosum* and *Tr. tenuis* for the first time in Australia.

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

**Table 3** Prevalence of gastrointestinal nematodes of alpacas in Australia using morphological examination of adult worms and the identification of eggs in the alpaca faeces using MT-PCR

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

Agreement (%) and kappa values of both techniques for the identification of gastrointestinal nematodes of alpacas are also presented

This disagreement between two diagnostic methods for *T. circumcincta* could be due to its low prevalence detected by both morphological (1%, 1/97) and molecular (3%, 3/86) methods.

The nematode species identified from the C3 herein have been previously documented in alpacas and llamas apart from *G. auchenia* which has not been reported outside South American countries (Ballweber 2009). *Haemonchus contortus* was found to be the cause of death in alpacas in Australia (Jabbar et al. 2013). The prevalence of *C. mentulatus* was 60% and 48% using morphological and molecular techniques, respectively. Similar prevalences of *C. mentulatus* were observed in cross-sectional (69%) and longitudinal (68%) surveys of GINs in Australian alpacas (Rashid et al. 2019a, 2019b). *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). To the best of our knowledge, the prevalence of *C. mentulatus* in alpacas is being reported for the first time in Australia.

In this study, the prevalence of *O. ostertagi* by morphology (3%, 3/97) was lower compared with MT-PCR (31%, 27/86). There was only a slight agreement between two methods which may be due to very low abundance of this species. The prevalence of *Te. circumcincta* (1–3%) found herein is similar to that (3%) was found in a recent cross-sectional study (Rashid et al. 2019a) but lower than that (8%) reported in the longitudinal epidemiological studies on GINs of alpacas in Australia (Rashid et al. 2019b).

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

In the small intestine, three species of *Trichostrongylus* spp., four species of *Nematodirus* spp. and three species of *Cooperia* spp. were identified (Table 2). These species, except *C. pectinata*, have previously been identified in SACs from Australia (Carmichael 1999) and elsewhere (Ballweber 2009). *Trichostrongylus rugatus*, *T. colubriformis* and *T. vitrinus* occur in sheep in Australia (Beveridge and Ford 1982; Beveridge et al. 1989), and in this study, they were also encountered in the small intestine of alpacas. Few reports of occurrence of the *Nematodirus* spp. in alpacas are available. The prevalence of *Nematodirus* spp. (33%) found herein was lower than that reported from the UK (93%) in a necropsy of an alpaca (Tait et al. 2002). Three different species of *Cooperia* (*C. oncophora*, *C. punctata* and *C. pectinata*) found

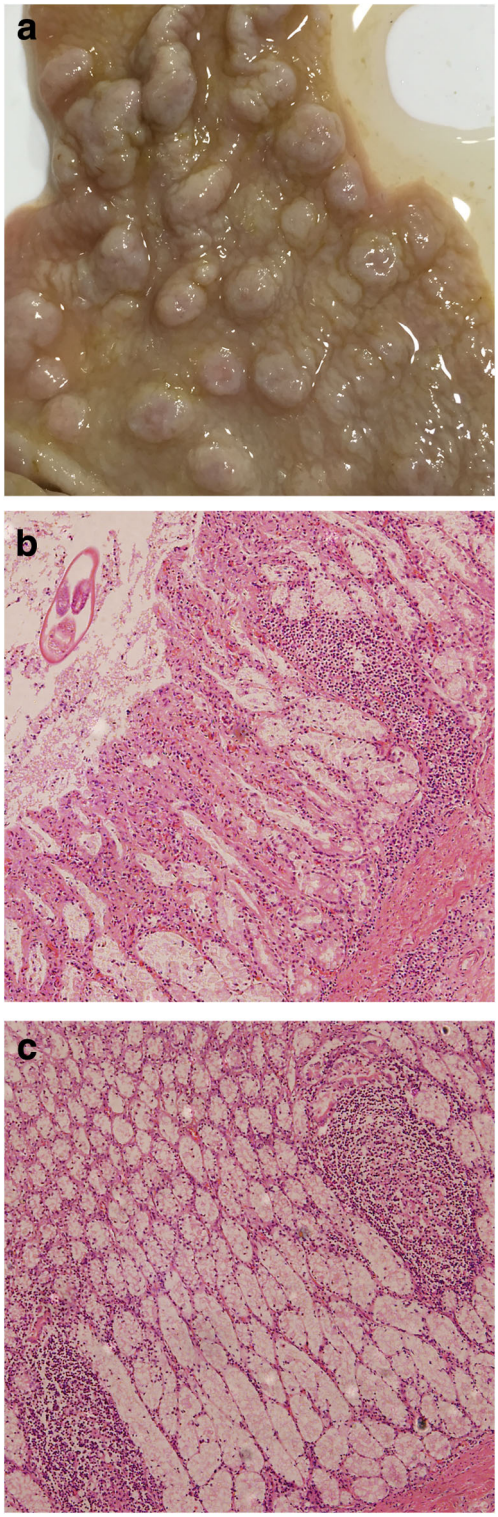


**Fig. 2** Gross and histopathological changes in the third compartment of the stomach (C3) of alpacas caused by gastrointestinal nematodes. **a** Multiple raised nodular lesions ("Morocco leather" appearance) on the mucosal surface of the C3 (black arrow) caused by high numbers of *Camelostrongylus mentulatus*. **b** Mucosal surface of the C3 showing a transverse section of a nematode (cross), mucosal and crypt necrosis (triangle) and mononuclear cellular infiltrations (star). **c** Histological section of tumour-like lesion of the third compartment showing aggregation of mononuclear inflammatory cells (rectangle)

in our study have been reported previously in alpacas from Peru (Contreras et al. 2014) and New Zealand (Hill et al. 1993). We also found *Capillaria* type eggs in the faecal samples of alpacas for the first time, but no adults of this genus were encountered. *Capillaria* has previously been reported in SACs in the UK (Tait et al. 2002) and Japan (Hyuga and Matsumoto 2016).

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

A total of 97 individual faecal samples was processed for FECs. The mean FEC for 97 samples was 501 EPG, with the highest count of 3495 eggs in one alpaca (Table 1), which is higher than recently found in cross-sectional (291 EPG) and longitudinal (168 EPG) studies of GINs in Australian alpacas (Rashid et al. 2019a, 2019b). It was also higher than levels reported from other countries (Tait et al. 200; Dittmer et al. 2018; Hertzberg and Kohler 2006). There was a low correlation ($r = 0.23$) between faecal egg counts and total worm count. Earlier studies have shown a moderate to high relationship between FEC and worm burden (Bryan and Kerr 1989; McKenna 1981; Roberts 1957; Thomas and Boag 1973) (for fecund genera of nematodes such as *Haemonchus* spp. and *Oesophagostomum* spp.), whereas other studies found poor correlation between FEC and worm burden (Brunsdon 1971; Michel 1969a, 1969b; Rose and Small 1980; Rubin 1967) (for less fecund genera such as *Nematodirus* spp., *Cooperia* spp., *O. ostertagia* and *Trichostrongylus axei*).

## Conclusion

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

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interests.

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