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Factors associated with small lungworm infections in heavily infected sheep in southeast South Australia

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2 Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that influenced the work reported in this paper.

3 Abstract and key words

This field observational study describes the seasonal pattern of small lungworm infections under different grazing management from August 2018 to March 2019. Liveweight, lungworm and gastrointestinal nematode infection, as well as pasture type grazed and snail density, were measured at 5 farm visits. Across all visits and mobs about one quarter to one half of sheep were positive for small lungworm, although prevalence was as low as 0% and as high as 78%. The density of the intermediate host molluscs was greater than 1600 snails/ m² in irrigated perennial lucerne pasture when it was grazed ('Pasture A'), but was low (< 300) in non-irrigated perennial pasture ('Pasture B') and non-irrigated forage crop ('Pasture C'). Overall, non-infected lambs had a similar liveweight compared to the small lungworm infected lambs (mean difference -0.6 kg; 95% CI -1.6, 0.2; P = 0.1). The odds ratio of small lungworm infection associated with a two-fold increase in worm egg count was 1.7 (95% CI 1.1, 2.7; P = 0.02).

Rather than a distinct seasonal pattern of infection, we found that small lungworm can occur throughout the year, with prevalence most influenced by pasture type (irrigated vs. dryland), grazing

management and the population density of the intermediate hosts. Importantly, this study suggested that small lungworm infection did not reduce lamb liveweights. It reinforced that to improve sheep productivity well-established determinants of production, such as correct grazing management to optimise pasture quality and strategies to reduce infections with gastrointestinal nematodes should be the priority of farm managers.

Keywords

small lungworm, sheep, snail density, gastrointestinal nematodes, liveweight

4 Introduction

Small lungworm is highly endemic in sheep in southeast South Australia (SA), with recent abattoir surveillance indicating very high rates of infection in consignments of adult sheep (35-40%) and lambs (20-25%)¹. Entire consignments of sheep are often found to be infected¹, and despite there being no clear evidence that small lungworm infections are associated with decreased production, many producers in this region are concerned that this may not always be the case. Consequently, further information is needed on the epidemiology of infection and the effect on sheep production in southeastern Australia. Understanding the seasonal patterns of infection can help to identify factors that increase the likelihood of infection and contribute to the development of more effective or appropriate management strategies in grazing sheep.

In Australia, two species of small lungworm, both with similar indirect lifecycles, infect sheep and goats: *Muellerius capillaris* and *Protostrongylus rufescens*^{2, 3}. The prepatent period (PPP), when development occurs within the definitive host sheep, ranges from 4-9 weeks^{4, 5}. However, larvae can be inhibited in the lungs then resume development when climatic conditions are favourable⁴. Patent infection, where larvae are shed in the faeces, can persist for up to four and a half years in sheep⁶. Development outside of the host includes the free-living stage on the pasture and then stages within intermediate host snails (molluscs). Here, larvae can survive for several months and so cause a

carryover of infection between consecutive years⁷. Sheep become infected through ingestion of the intermediate host snail containing infective stage small lungworm larvae⁸. Consequently, sheep can become infected throughout the year, although seasonal variation in the rate of infection of sheep is often reported.

Lungworm is most commonly found in regions with cool temperatures and high rainfall⁹, and in southeastern Australia infections generally occur during autumn or winter which corresponds with high rainfall and cooler temperatures¹⁰. Despite these general assertions for Australia, the prevalence of small lungworm infections does not always follow this seasonal pattern in other parts of the world with similar climates. In northwest Spain, small lungworm was most prevalent in Galician sheep from mid-spring to mid-autumn¹¹. This area of Spain has high temperatures and low rainfall during summer, similar to southeast SA. In addition to rainfall and temperature, the seasonal pattern may be influenced by irrigation. This favours breeding of the intermediate host¹² and is a common practice in southeast SA. Given the slightly conflicting observations made in similar climates it is important to describe the epidemiology of this parasite in SA.

The interaction of many factors, including season, species, age, sex, irrigation, and grazing management, influences the overall risk of small lungworm infection^{13, 14}. For example, grazing management is an important determinant of the exposure of sheep to the intermediate host snails and infective larvae (L3) on pasture, although this is often not reported in the literature¹⁴⁻¹⁶. Consequently, knowledge of the snail density in different seasons of the year, and the type and irrigation status of pastures grazed, is needed to determine which periods may pose a greater risk for infection of sheep¹¹. Understanding how the factors that influence exposure to L3, hence the prevalence of small lungworm, also provides an opportunity to develop more appropriate or effective control strategies.

The need for control strategies is dependent upon the association of small lungworm with productivity and other diseases, such as gastrointestinal nematode (GIN) infection. Lungworms make important

contributions to the overall effects of nematodes on mortality and morbidity of sheep in parts of Europe and Africa and can cause economic loss^{17, 18}, although recent studies in the southeast of SA found that carcass weights were not affected by mild small lungworm infections¹⁹. In addition, the conditions which predispose sheep to small lungworm and GIN infections are likely to be quite similar^{2, 7}. Control strategies for GINs are well described for southeastern Australia, hence identifying any similarities in their patterns of infection may assist the management of small lungworm if this was thought to be necessary.

Here we report an observational study of small lungworm infection on a farm in southeast SA known to have a high prevalence of small lungworm. The seasonal prevalence of small lungworm infection was compared in three mobs of sheep subjected to different grazing management. We also a) assessed the association of small lungworm infection with the productivity of lambs, and b) compared the presence of small lungworm infection with the severity of GIN infections.

5 Methods and materials

5.1 Farm description

The farm selected for this study was 55 km west of Naracoorte in SA (37.001 °S, 140.085 °E), with an average annual rainfall of 602 mm (1879-2020) that followed a winter dominant pattern of hot dry summers and cool wet winters, typical for a 'Mediterranean' climate. The useable area of the farm was 2758 hectares (ha), stocked with 3000 Merino and first-cross (Border Leicester x Merino) ewes at an annual average stocking rate of 6 dry sheep equivalents (DSE)/ha. The farm was selected because recent abattoir monitoring had detected a high prevalence of small lungworm infections in lambs, with all of a consignment of 200 lambs infected in 2018 (Supplementary Figure S1). There was also a very high prevalence of a known intermediate host conical snail on the farm (*Prietocella Barbara*; Supplementary Figure S2).

5.2 Study design

This was an observational study measuring sheep liveweight, lungworm and GIN infections, pasture grazed and pasture snail density from August 2018 to March 2019. The total population available was a flock of 1500 first cross (Border Leicester x Merino) lambs and Merino ewes, from which 25 wether (castrate male) lambs, 25 ewe (female) lambs and 25 ewes were randomly selected. The effect size based on groups of sheep (mobs) of 25 allowed a detection of difference in prevalence of 35% between the mobs, with a power of 80%, and confidence of 95%²⁰. These 75 sheep were monitored for an eight-month period, from prior to weaning until wether lambs were sold for slaughter. Ewes and ewe lambs were retained for breeding on the farm. The lambs were born over 6 weeks from 14 May to 1 July 2018. As part of routine farm management all sheep were shorn on 7 December 2018.

Three pastures were grazed by the monitor mobs (ewes, ewe lambs and wether lambs) during the study (Table 1; Figure 1). From birth until weaning ewes and lambs grazed an irrigated perennial lucerne pasture (*Medicago sativa*) ('Pasture A'). Following weaning until November 2018, wether and ewe lambs grazed a non-irrigated perennial pasture of perennial ryegrass (*Lolium perenne*), barley (*Hordeum vulgare*), and clover (*Trifolium subterranean*) ('Pasture B'). Subsequently, from November 2018 until the completion of the study, wether lambs were returned to the previously grazed Pasture A. Flood irrigation was applied to this pasture from late spring through summer. In contrast, ewe lambs continued to graze Pasture B until February 2019, then grazed a non-irrigated crop of forage rape (*Brassica napus*) and millet (*Panicum miliaceum*) ('Pasture C') until the completion of the study. Meanwhile, from weaning until February 2019, ewes grazed Pasture C, and then Pasture B from February 2019 until the completion of the study (Figure 1).

Anthelmintics were administered for routine control of GINs on three occasions (Table 1). First, at lamb marking, adult ewes received a commercial double-active combination anthelmintic (DuoCare® (Virbac Australia P/L)). Second, at weaning, ewes and lambs received the same combination anthelmintic, and lambs were vaccinated against *Corynebacterium pseudotuberculosis*, *Clostridium perfringens* type D and *Clostridium tetani* (Glanvac® 3 in 1 B12; Zoetis Australia Pty Ltd). Third, at the

‘first summer drench’ ewes and ewe lambs received a commercial triple-active combination anthelmintic (Hat-Trick® (Ancare, Merial Australia P/L)), whilst wether lambs received a commercial single-active anthelmintic (Cydectin LV® (Virbac Australia P/L)).

5.3 Measurements

Animal measurements were performed on the farm on five occasions at approximately 6-8 weekly intervals coinciding with key husbandry activities (Table 1). The first visit (day 0) was three weeks prior to weaning in August 2018, at which monitor lambs and ewes were randomly selected. The second visit was at weaning (day 20) in September 2018 and the third visit occurred at day 69 in November 2018. The fourth visit was at the time of the strategic ‘first summer drench’ for lambs at day 133 in January 2019, and the final visit was one day prior to wether lamb consignment to the abattoir (day 208) in March 2019. These samplings were used to detect current infection with small lungworm and GINs, which were acquired from either the current or previously grazed pasture. A numbered ear tag was used to individually identify monitor sheep.

5.3.1 Liveweights

At each visit all trial sheep were weighed with electronic scales accurate to 0.5 kg (Tru-Test MP600 loadbars, Tru-Test P/L).

5.3.2 Faecal samples

Faecal samples were collected from trial sheep at each visit, to assess the prevalence and severity of GIN and lungworm infections by worm egg count (WEC) and Baermann method, respectively. Faecal samples were not collected from ewes at measurement 4 because an anthelmintic had been administered 14 days prior. Faecal samples were collected from each sheep *per rectum* and stored in plastic faecal collection trays in a chilled, portable cooler during sample collection and transport to the laboratory. At the laboratory, samples were refrigerated at 4°C and processed within one and three days from collection for WEC and Baermann tests, respectively.

5.3.2.1 Modified Baermann method

Each individual 5 g faecal sample was formed into a small pat and suspended in a plastic funnel fitted with a short tube and clamp according to the method described by Andersen and Walters²¹. The funnel was filled with tepid water to just cover the faecal sample and incubated at 25°C for 8 hours. After 8 hours a 5 mL subsample was collected into a test tube by releasing the clamp and then either processed immediately or stored at 4°C for up to 7 days. Samples were centrifuged for 3-4 minutes at 2000 revolutions per minute, and 4.5 mL supernatant removed to leave 0.5 mL of sediment containing the larvae^{14, 18}. This was mixed thoroughly and two 50 µL aliquots (total of 100 µL) examined at x40 magnification over two microscope slides with cover slips. Using these dilutions, each larva counted was equivalent to the total number of larvae per gram of faeces (lpg).

5.3.2.2 Worm Egg Counts

Samples were either processed for an individual WEC (visit 3,4 and 5) or bulk WEC (visit 1 and 2). Bulk and individual WECs were performed using a modified McMaster technique²². Briefly, 5 g of faeces (1 g from each sample) were homogenised in 45 mL of water for bulk WECs, whereas 3 g of faeces (1 g minimum) were homogenised in 42 mL of water for individual WECs. Using these dilutions, each egg counted was equivalent to 15 eggs per gram of faeces (epg) for individual WECs and 10 epg for bulk WECs. The mean WEC for each of the 5 pooled samples was calculated to determine the overall average for each monitor mob (visit 1 and 2). Individual WECs were used to calculate the overall average for each monitor mob (visit 3, 4 and 5).

5.3.3 Snail monitoring

The number of snails on pastures where a monitor mob had grazed immediately prior to the visit date was estimated four times (at each visit, excluding visit 2). At each time point, all snails were collected by hand from within 0.0625 m² (0.25 m x 0.25 m) quadrats placed at four separate locations in each paddock. Snails were then sorted into conical shelled (Cochlicellidae (*P. barbara*)) and round shelled (Hygromiidae (*Cerņuella virgata*) and Helicidae (*Theba pisana*)) before counting²³.

5.4 Statistical analysis

Statistical analyses were carried out using R version 3.6²⁰. Arithmetic mean larval count and worm egg count were calculated with basic nonparametric bootstrap 95% confidence limits. At each visit, the proportion of each mob positive for small lungworm on Baermann test were compared using Fisher's exact test. A mixed effects logistic regression was used to model the association of small lungworm infection status (positive or negative) and GIN infection. WEC data was log transformed for use in the model and 12 zero values were replaced with half the dilution factor (7.5). Sheep was fitted as a random effect, and log WEC as a fixed effect.

Liveweights were approximately normally distributed. For lambs a total average fleece weight of 1250 g was subtracted incrementally from liveweight 1, 2 and 3 measurements to account for shearing between visits 3 and 4²⁴. Similarly, to account for the shearing of ewes between visits 3 and 4 a total average fleece weight of 3500 g was subtracted incrementally.

There were three linear mixed effects models used to compare lamb liveweights. First, liveweights were compared between small lungworm infected and non-infected lambs. Small lungworm infection status, and sex were included as fixed effects, and interactions between all variables and visit were fitted. Second, liveweights were compared between wether and ewe lambs. Sex was included as a fixed effect, and interactions with visit was fitted. Third, liveweights were compared between lambs with a WEC ≤ 150 and lambs with a WEC > 150 epg. Instead of small lungworm infection status, WEC status was included as a fixed effect. Sheep was fitted as a random effect in all three models.

The study was approved by the University of Melbourne Animal Ethics Committee (Reference number 1814480, Melbourne, Australia).

6 Results

6.1 Visits and summary

A total of 75 sheep were monitored at 5 farm visits from August 2018 to March 2019. A total of 75, 75, 75, 42 and 67 sheep were weighed, and faecal samples assessed from 49, 55, 69, 40 and 64 sheep for WECs and Baermann tests, at visits 1-5, consecutively.

The grazing patterns, pasture snail density, small lungworm prevalence, GIN burden and anthelmintic treatment for the monitor mobs are summarised in Figure 2 and detailed results are provided below. Monitoring of sheep and pastures commenced at the end of winter and continued until the beginning of the following autumn. Across all visits and mobs, generally about one quarter to one half of sheep were positive for small lungworm, although prevalence was as low as 0% and as high as 78%. Females (ewes and ewe lambs) predominantly grazed pastures with low snail densities, whilst males (wether lambs) predominantly grazed pasture A which had a high snail density. Infection may have been acquired from the pasture grazed at the time of sampling or from the pasture grazed prior to sampling. The average WEC was above the threshold of 150 epg²⁵ for all mobs at each visit, other than ewes at visits 2 and 3.

6.2 Snail densities for pastures grazed

The density of *P. barbara* was greater than 1600 snails/ m² in pasture A (irrigated perennial lucerne pasture) each time it was grazed (visits 1, 4 and 5; Table 2). In pastures B (non-irrigated perennial pasture) and C (non-irrigated forage crop) the density of *P. barbara* was relatively low (<300) at visits 3, 4, and 5. The density of *T. pisana* and *C. virgata* was relatively low at each visit in each pasture grazed.

6.3 Small lungworm and GIN infection

The proportion of each mob infected with small lungworm was similar, except at visits 2 and 4 (Table 3). At visit 2, more ewe lambs were infected than wether lambs and no adult ewes were detected with small lungworm (52% (11/21) ewe lambs vs. 25% (4/16) wether lambs vs. 0% (0/18) ewes; $P < 0.001$).

At visit 4, more wether lambs were infected than ewe lambs (78% (14/18) wether lambs vs. 5% (1/22) ewe lambs; $P < 0.001$). From visit 2 onwards the proportion of ewe lambs infected decreased, whereas the proportion of wether lambs infected mostly increased. No individual sheep was excreting larvae at every visit. At visit 2, 1/4 (25%) of sheep remained positive since visit 1. At visit 3, 6/19 (32%) of sheep remained positive, despite anthelmintic treatment given following visit 2. At visit 4, 4/15 (27%) of sheep remained positive since visit 3. At visit 5, 4/7 (57%) of sheep remained positive despite anthelmintic treatment given following visit 4.

The larval lungworm count was relatively low (mean ≤ 17 lpg for each mob at each visit), except for one ewe lamb which had a count of 111 lpg at visit 2 (Table 4). WECs were quite high throughout the study, usually above a threshold of 150 epg²⁵. WECs were particularly high in both lamb mobs at visit 4 immediately prior to administration of the 'first summer drench'.

There were 161 paired measurements for individuals when the mean larval count and mean WEC were measured concurrently. The median log WEC was higher in sheep which were concurrently positive for small lungworm infection compared to those which were negative for small lungworm infection (Figure 3). The odds ratio of small lungworm infection associated with a two-fold increase in WEC was 1.7 (95% CI 1.1, 2.7; $P = 0.02$).

6.4 Lamb liveweights

A total of 189 lamb liveweight measurements had a corresponding Baermann test result indicating if a lamb was infected with small lungworm or not at that visit. The statistical model with infection status and sex included as fixed effects indicated that non-infected lambs were 3.1 kg heavier than infected lambs at visit 4 ($P = 0.01$), but non-infected and infected lambs were a similar weight at all other visits ($P \geq 0.3$; Table 5). Overall, non-infected lambs had a similar liveweight compared to the infected lambs (mean difference -0.6 kg; 95% CI -1.6, 0.2; $P = 0.1$).

The statistical model with sex included as a fixed effect indicated that wether lambs and ewe lambs were a similar weight at visit 1, 2 and 3 ($P = 0.1-0.3$), whilst at visit 4 and 5 wether lambs were heavier

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than ewe lambs ($P < 0.001$; Table 6). Overall, wether lambs were 5.6 kg heavier than ewe lambs (95% CI 2.8, 8.5; $P < 0.001$). Conversely, the statistical model with WEC as a fixed effect, indicated that there was no weight difference between lambs with WECs ≤ 150 epg compared to those lambs with a WEC > 150 epg for visits 3 to 5 ($P = 0.3\text{--}0.7$; Supplementary Table S1).

6.5 Rainfall and irrigation

The rainfall was estimated from the closest Bureau of Meteorology (BoM) recording station, Lucindale Post Office (BoM site 026016) which was 26 km north-east of the farm. In late winter/ spring (August–November 2018) rainfall was average, whilst in summer/ early autumn (December 2018– March 2019) rainfall was 40% above average (Supplementary Table S2). The irrigated lucerne pasture was flood irrigated from November 2018 to March 2019 with approximately 1500 mm of water applied when a paddock was irrigated.

7 Discussion

This longitudinal observational study was conducted in response to producer concerns about the high prevalences of small lungworm infection detected by abattoir surveillance of sheep in the southeast of SA. It provides the first description of patterns of infection under different grazing management for this region. We found no clear seasonal pattern to small lungworm infections, but rather that patent infections appeared to be influenced more by where sheep grazed, and most likely the snail populations on these pastures. Lamb liveweights also appeared to be most significantly influenced by the pastures grazed, rather than small lungworm infections. Sheep with a greater GIN burden, as indicated by WEC, were more likely to be infected with small lungworm.

The lack of a seasonal pattern in small lungworm infection may be because pasture management, particularly irrigation, had a greater influence on the persistence and availability of small lungworm larvae and/or their intermediate hosts on specific pastures, rather than the prevailing weather conditions. Particularly high small lungworm prevalences occurred during both spring and summer in lambs grazing pasture A (ewe lambs in spring at visit 2 and wether lambs in summer at visit 4). This

lucerne pasture had the highest snail density of the pastures grazed and was irrigated during summer, providing favourable conditions for snails to breed¹². Additionally, certain pasture types may be more favourable for snails, although this has not been specifically determined for lucerne²⁶.

In contrast, pastures B and C were not irrigated and therefore provided a clearer indication of any seasonal patterns of infection risk. Both pastures had low snail densities but still had a moderate prevalence of small lungworm in grazing sheep. When ewe lambs grazed pasture B from late summer to early autumn, the mob prevalence gradually decreased from around 50% (which was presumably acquired grazing pasture A beforehand) to 1%, suggesting few additional infections, if any, were acquired grazing this non-irrigated perennial pasture. On the other hand, ewes grazing a non-irrigated annual fodder crop (pasture C) at the same time had a steady infection prevalence of about 20%. The infections detected in the ewes may have been confounded by their prior acquisition of infections from Pasture A (or earlier)⁶, increasing immunity²⁷, and the sensitivity of the Baermann test (which is 90% and cannot detect non-patent infections)²⁸. Nevertheless, this study indicates that infection with small lungworm can occur at any time of the year when environmental conditions are favourable, either due to seasonal effects (rainfall) or management (irrigation).

Non-infected and infected lambs grew at similar rates (non-infected lambs were 0.6 kg heavier, $P = 0.1$). This lack of association with patent infections may be because we detected only light infections (≤ 17 lpg for each mob at each visit), much lower than 150 lpg which Soulsby³ suggested was a pathological burden. Instead, lamb liveweight appeared to be more significantly influenced by the pasture grazed, as indicated by higher growth rates in wether lambs at visits 4 and 5 ($P < 0.001$), after they returned to the higher quality irrigated lucerne pasture. WECs were always above 150 epg in the lamb mobs, the threshold when anthelmintic treatment would often be recommended for weaned lambs in this environment, and thus likely to be impacting growth rates²⁵. These results remind us that well described determinants of production should be managed first, because the effect of GIN

burdens or the quality of the pasture grazed may have obscured any association of small lungworm infection with liveweight.

The likelihood of small lungworm infection was greater when there was a doubling of the GIN burden. This association is not surprising, given that similar climatic conditions favour the acquisition of both infections^{2,7}. However, this association can only occur where there are intermediate host snails of small lungworm present. Lambs may be more susceptible to infection with both parasites. Lambs generally had higher WECs and larval counts than adults, with the peak prevalence of small lungworm recorded in wether lambs (78%) in January coinciding with their maximum WEC of 780 epg. Lambs are more susceptible to GIN infections because their acquired immunity is not fully developed²⁷. This study and recent findings in the southeast of SA that the odds of infection in adults and lambs were similar¹⁹ suggests this may also be the case for small lungworm, although higher burdens in adults have also been reported⁶.

The anthelmintics used for the routine treatment of GIN infections on our study farm did not appear to control small lungworm. Small lungworm prevalence increased in ewes and wether lambs after anthelmintic treatment at visit 2, with animals still positive 7 weeks later at visit 3 (within the PPP of 4-6 weeks for *M. capillaris* and 4-9 weeks for *P. rufescens*)^{4,5}. Previously acquired infections were likely to contribute to the infections detected at visit 3 because larval output typically increases after the PPP²⁹. Persistent infection of some sheep may have occurred, suggesting that using anthelmintics at the dose rates which effectively treated GINs did not completely eliminate small lungworm infections. Previous studies have shown small lungworm infections must be treated with higher anthelmintic doses than those used for the GINs³⁰ or additional anthelmintic treatments⁴. Furthermore, anthelmintic treatment does not remove the intermediate host which facilitates re-infection. Since small lungworm infection did not appear to impact production in our study on a commercial farm, we recommend that anthelmintic treatment need only be targeted to GINs, avoiding over-treatment and its associated costs, and the increased risk of anthelmintic resistance⁸.

When small lungworm infections reduce productivity, such as in parts of Europe and Africa ²⁹, or in more susceptible species, such as goats, ^{31, 32} improved diagnostic methods are warranted. Currently, it is difficult to monitor small lungworm infections because the Baermann method is not performed as part of routine commercial parasitology testing. It is also relatively insensitive and time consuming ^{8, 16, 18, 28}. In contrast, GIN infections are readily and routinely monitored during farm management ⁸. Consequently, WECs could be used as a screening test to identify sheep which are more susceptible to small lungworm in regions where the intermediate host snails are present. Faeces from sheep with high WECs could then be bulked for the Baermann test, increasing the number of sheep screened for lungworm ¹⁸, although this approach would need to be validated.

This observational study was performed on an operating commercial farm, which always introduces some constraints. First, monitoring was only conducted during the eight months of lamb production, hence there were no measurements in late autumn or early winter. Although this captured the critical time for monitoring growth in lambs ³³. Second, due to logistical constraints the sample size was relatively small. This highlights the need for more sensitive and labour efficient techniques for the diagnosis of small lungworm, which would increase laboratory throughput and enable larger sample sizes ¹⁸. Nevertheless, our sample size still allowed for the detection of a difference in small lungworm prevalence of 35% between the mobs, which was a reasonable expectation given the high prevalence in this region ¹⁹. Third, the density of snails observed on pasture may have been influenced by the location and thus topography, drainage, soil type and plant species of the quadrat measurements. In addition, we did not measure alive versus dead snails, nor immatures versus adults. This would have provided a more thorough description of the snail populations present and their infectivity, with adults typically being more severely parasitised ^{11, 34}. Consequently, further studies would need additional resources to assess the distribution and infectivity of snails on pasture, then relate these observations to the prevalence of small lungworm in sheep ^{3, 35}.

8 Conclusion

This study found that small lungworm can occur throughout the year, with its prevalence most influenced by pasture type (irrigated vs. dryland), grazing management and the population density of the intermediate host snails. We suggest that to effectively improve sheep productivity well-established determinants of production, such as pasture quality and GIN infections, must be managed before small lungworm infections. If, once other key production factors are controlled, small lungworm appears to reduce liveweight gain or affect sheep health, then targeted small lungworm control may be warranted. Targeting control options towards sheep grazing high risk pastures, such as those with high snail populations, could be an important component of an integrated parasite management strategy^{36, 37}. Producers should be reassured that the current high rates of small lungworm infections from abattoir surveillance are unlikely to be impacting productivity.

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10 Tables

Table 1 Farm grazing and anthelmintic management according to observational farm visits and monitor mobs

Visit (day)	Date	Management activity	Lamb Age (months)	Grazing management	Anthelmintic administered
1 (0)	29-Aug-18	Pre-weaning	2 to 3	ewes and lambs grazed together	nil
2 (20)	18-Sep-18	Weaning	3 to 4	ewes and lambs separated	Benzimidazole and Levamisole (all mobs) [†]
3 (69)	6-Nov-18	Post-weaning	5 to 6	wether and ewe lambs separated	nil
4 (133)	9-Jan-19	"First summer drench"	7 to 8	each monitor mob separate	Macrocyclic lactone (wether lambs) [‡] ; Macrocyclic lactone, Levamisole, Benzimidazole (ewes and ewe lambs) [§]
5 (208)	25-Mar-19	1 day prior to sale (wether lambs)	9 to 10	each monitor mob separate	nil

[†] Levamisole 40 g/L and Fenbendazole 25 g/L (DuoCare® at 8 mg/kg Levamisole, 5 mg/kg Fenbendazole; Virbac Australia Pty Ltd)

[‡] Moxidectin 1 g/L (Cydectin LV® at 0.2 mg/kg Moxidectin; Virbac Australia Pty Ltd)

[§] Abamectin 1 g/L, Levamisole 33.9 g/L, Oxfendazole 22.7g/L (Hat-Trick® at 0.2 mg/kg Abamectin, 6.8 mg/kg Levamisole, 4.5 mg/kg Oxfendazole; Ancare Australia Pty Ltd)

Table 2 Median (interquartile range) mollusc density (number of snails per m²) in each pasture grazed at each visit according to snail category

Visit (day)	Date	Mob grazing pasture (since previous monitoring)			<i>P. barbara</i> (median (IQR))			<i>T. pisana and C. virgata</i> (median (IQR))		
		A	B	C	A	B	C	A	B	C
1 (0)	29-Aug-18	E, EL, WL			3048 (1664,3696)	N/A	N/A	216 (128,328)	N/A	N/A
2 (20)	18-Sep-18	E, EL, WL			not measured			not measured		
3 (69)	6-Nov-18		EL, WL	E	N/A	56 (48,72)	0 (0,0)	N/A	0 (0,0)	0 (0,0)
4 (133)	9-Jan-19	WL	EL	E	1616 (128,4280)	0 (0,0)	0 (0,0)	416 (184,704)	0 (0,0)	0 (0,0)
5 (208)	25-Mar-19	WL	EL/ E [†]	E/ EL [†]	2136 (1752,3280)	296 (280,336)	0 (0,0)	680 (320,952)	16 (16,32)	0 (0,0)

A: irrigated perennial lucerne pasture, B: non-irrigated perennial pasture, C: non-irrigated forage crop, N/A: not applicable because pasture was not grazed

E: ewes, EL: ewe lambs, WL: wether lambs

[†] Ewes moved from C to B, whilst ewe lambs moved from B to C in February 2019

Table 3 Proportion of monitor mob positive for small lungworm on Baermann test (denominator is the number of usable faecal samples at each visit), comparison between mob prevalences (P value), and number of individuals positive at consecutive visits

Visit (day)	Date	Small lungworm prevalence				Positive at consecutive visits		
		(N/N sheep positive/tested (%))			P value	(N positive at prior and current visit/ N positive at current visit)		
		Ewe	Ewe lamb	Wether lamb			Ewe	Ewe lamb
1 (0)	29-Aug-18	5/23 (22%)	IF	5/19 (26%)	0.61	N/A	N/A	N/A
2 (20)	18-Sep-18	0/18 (0%)	11/21 (52%)	4/16 (25%)	<0.001	0	IF	1/4 [†]
3 (69)	6-Nov-18	4/22 (18%)	7/24 (29%)	8/23 (35%)	0.47	0/4	4/7	2/8
4 (133)	9-Jan-19	NF	1/22 (5%)	14/18 (78%)	<0.001	NF	0/1	4/14 [†]
5 (208)	25-Mar-19	6/25 (24%)	2/21 (1%)	5/18 (28%)	0.33	N/A	0/2	4/5

IF insufficient faeces for analysis; NF no faeces for analysis; N/A not applicable because no samples collected at prior visit

[†] 2 positive lambs not sampled at prior sampling

Table 4 Arithmetic mean lungworm larvae per gram (lpg) faeces and arithmetic mean strongyle egg per gram (epg) faeces

Visit (day)	Date	Lungworm larval count (mean lpg (95% CI)) [†]			GIN worm egg count (mean epg (95% CI)) [†]		
		Ewe	Ewe lamb	Wether lamb	Ewe	Ewe lamb	Wether lamb
1 (0)	29-Aug-18	4 (2,7)	IF	17 (0,35)	400 (274,566)	572 (416,734)	480 (358,602)
2 (20) [‡]	18-Sep-18	0 (0,0)	12 (2,33)	11 (5,15)	108 (50,178)	428 (340,573)	295 (213,350)
3 (69)	6-Nov-18	9 (1,24)	8 (4,16)	5 (1,10)	143 (53,258)	246 (81,521)	524 (223,922)
4 (133) [‡]	9-Jan-19	NF	5 (5,5)	9 (4,15)	NF	1193 (880,1588)	780 (621,953)
5 (208)	25-Mar-19	2 (1,3)	2 (1,2)	3 (1,6)	346 (259,445)	200 (154,247)	488 (342,627)

IF insufficient faeces for analysis; NF no faeces for analysis

[†] Basic nonparametric bootstrap 95% confidence limits

[‡] Anthelmintic treatment (measurements made prior to treatment)

Table 5 Mean liveweight (95% CI) of lambs (wether & ewe) according to small lungworm infection status at each visit, and mean difference in liveweight between infected and uninfected lambs (95% CI)

Visit (day)	Date	Lamb Age (months)	Mean liveweight (kg)		Mean difference [†]	P value
			Non-infected lamb	Infected lamb		
1 (0)	29-Aug-18	2 to 3	27.9 (26.1,29.8)	27.7 (25.6,29.9)	-0.2 (-2.3, 1.8)	0.8
2 (20)	18-Sep-18	3 to 4	32.3 (30.6,33.9)	33.2 (31.4,35.1)	1.0 (-0.7, 2.7)	0.3
3 (69)	6-Nov-18	5 to 6	43.2 (41.7,44.8)	43.0 (41.2,44.8)	-0.3 (-1.8, 1.3)	0.7
4 (133)	9-Jan-19	7 to 8	53.1 (51.3,54.9)	50.0 (48.0,52.0)	-3.1 (-5.4, -0.7)	0.01
5 (208)	25-Mar-19	9 to 10	57.6 (56.1,59.2)	56.9 (54.7,59.2)	-0.7 (-2.8, 1.3)	0.5

[†] Means and mean differences from mixed model, positive mean difference indicates that there was a higher mean for infected mob, adjusted for sex

Table 6 Mean liveweight and mean daily liveweight gain (95% CI) of wether and ewe lambs, mean difference in liveweight between wether and ewe lambs (95% CI)

Visit (day)	Date	Lamb Age (months)	Mean liveweight (kg (95% CI))		Mean difference [†]	P value	Mean daily liveweight gain since previous sampling (g/day (95% CI))	
			Ewe lamb [‡]	Wether lamb [§]			Ewe lamb	Wether lamb
1 (0)	29-Aug-18	2 to 3	26.3 (23.7,28.9)	29.4 (27.2,31.6)	3.1 (-0.3,6.5)	0.07	N/A	N/A
2 (20)	18-Sep-18	3 to 4	31.8 (29.7,34.0)	33.4 (31.2,35.6)	1.6 (-1.5,4.7)	0.3	213 (173,254)	194 (160,229)
3 (69)	6-Nov-18	5 to 6	41.9 (39.8,44.0)	44.4 (42.2,46.5)	2.5 (-0.5,5.5)	0.1	205 (183,228)	231 (212,250)
4 (133)	9-Jan-19	7 to 8	48.9 (46.8,51.1)	54.8 (52.6,57.0)	5.8 (2.7,8.9)	<0.001	109 (91,126)	173 (153,194)
5 (208)	25-Mar-19	9 to 10	51.2 (49.1,53.4)	63.8 (61.6,66.0)	12.6 (9.5,15.6)	<0.001	29 (10,48)	120 (100,140)

[†] Means and mean differences from mixed model, positive mean difference indicates that there was a higher mean for wether lambs

[‡] n = 25 at visit 1, 2, 3, and 5, n = 24 at visit 4; [§] n = 25 at visit 1,2, and 3, n = 18 at visit 4 and 5

11 Figure legends

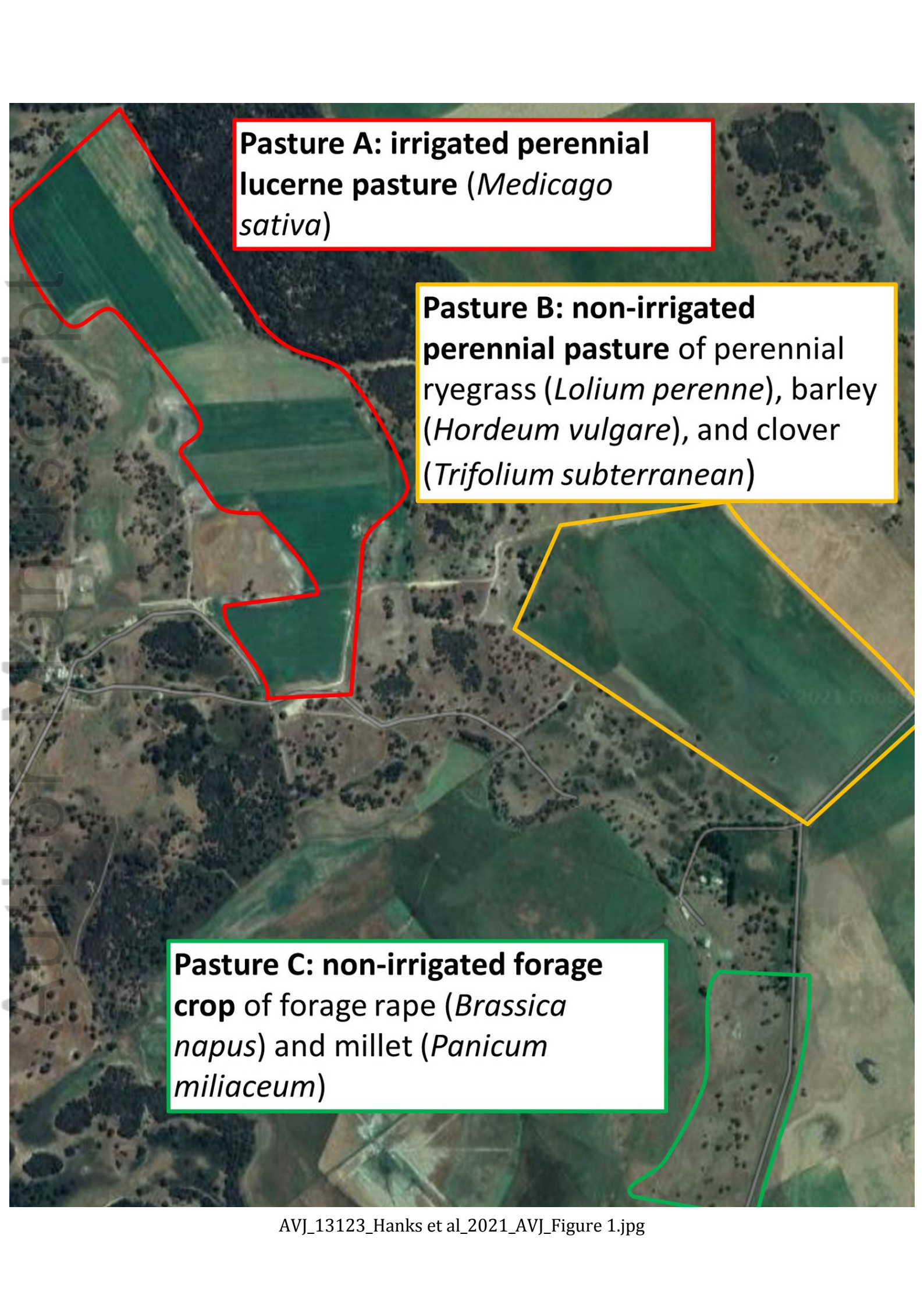
Figure 1 Location and species of pastures grazed by monitor mobs during the study

Figure 2 Grazing patterns, pasture snail density, gastrointestinal nematode burden (epg threshold), anthelmintic treatments (Tx), and small lungworm prevalence (%) at visits for monitor mobs during the study

Figure 3 Small lungworm infection status relative to worm egg count (log eggs per gram) from paired samples

12 Appendix A

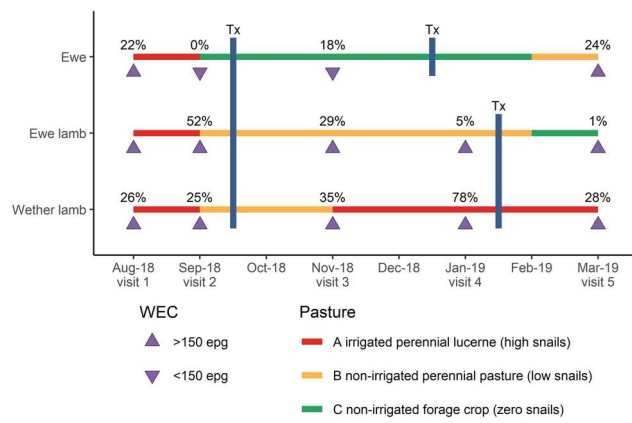
Supplementary material related to this article is contained in the Appendix A word document.



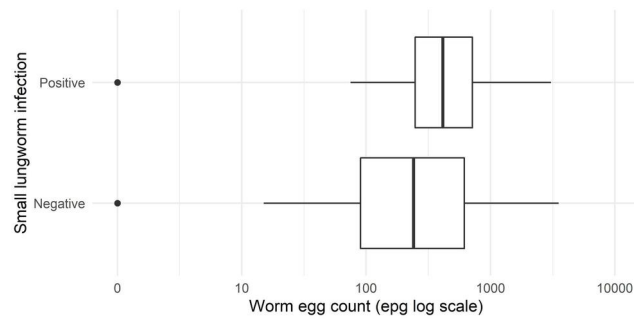
Pasture A: irrigated perennial lucerne pasture (*Medicago sativa*)

Pasture B: non-irrigated perennial pasture of perennial ryegrass (*Lolium perenne*), barley (*Hordeum vulgare*), and clover (*Trifolium subterranean*)

Pasture C: non-irrigated forage crop of forage rape (*Brassica napus*) and millet (*Panicum miliaceum*)



AVJ_13123_Hanks et al_2021_AVJ_Figure 2.jpg



AVJ_13123_Hanks et al_2021_AVJ_Figure 3.jpg