Severity and prevalence of small lungworm infection on three South Australian farms and associations with sheep carcass characteristics

Jenny E. Hanks*[a], Angus J. D. Campbell[b], John W. A. Larsen[a]

[a] Mackinnon Project, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, 250 Princes Highway, Werribee Victoria 3030, Australia

[b] Nossal Institute for Global Health, Melbourne School of Population & Global Health, University of Melbourne, Level 5, 333 Exhibition St, Melbourne Victoria 3010, Australia

* Corresponding author: E-mail: jenny.hanks@unimelb.edu.au, Tel: +61 428 358 805

Abstract

This field and abattoir study assessed the association of the severity and prevalence of small lungworm lesions with the carcass characteristics of 1332 lambs and adult sheep bred on three farms in southeast SA. Liveweight and measures of lungworm infection were measured on farm, then lung lesions and carcass characteristics assessed at slaughter. The overall prevalence of small lungworm lesions at slaughter was 79% (928/1177; 95% CI 76, 81), with a prevalence of 87% (569/658; 95% CI 84, 89) in lambs, and 69% (359/519; 95% CI 65, 73) in adults, respectively. Small lungworm infected lambs and adults had a similar hot standard carcass weight and dressing percentage compared to non-infected animals, both overall and within their respective cohort. Overall, the mean carcass weight for non-infected and infected lambs was 23.4 kg (95% CI 18, 29), and 23.6 kg (95% CI 18, 29), respectively, with a mean difference of 0.2 kg (95% CI -0.4, 0.8; P = 0.5). Mean carcass weight for non-infected and infected adults was 21.3 kg (95% CI 15, 28), and 21.5 kg (95% CI 15, 28), with a mean difference of 0.2 kg (95% CI -0.5, 0.9; P = 0.5).

This study confirmed a very high prevalence of small lungworm lesions in sheep bred on farms in this region of SA, but their hot standard carcass weights were not reduced by these lesions. Additional information to compare the presence of lesions with productivity within an individual was collected at slaughter which provided more detailed information than is currently collected by routine abattoir surveillance. The limitations of the currently available diagnostic tests for small lungworm were also demonstrated. This indicated a need for the development of more sensitive tests to assess lungworm infections both on farm and at the abattoir. Currently, farmers in this region are concerned about the very high prevalence of small lungworm in their sheep, but this study provides reassurance that the presence of lesions do not reduce production.

Keywords

small lungworm, sheep, prevalence, carcass characteristics, age

1 Introduction

A very high prevalence of infection with small lungworms has been reported in recent years in sheep from farms in the southeast of South Australia (SA), an area with more than three million sheep (ABS, 2017; Dal Grande et al., 2019). The most recent data suggests that, in southeast SA, there is a very high rate of infection of both adult sheep and lambs, with 20-35% of consignments infected based on abattoir surveillance (Dal Grande et al., 2019). Further, lungworm was the most frequent condition (of 8 common conditions monitored) in a case control study at an abattoir in South Australia, making up 29% of all conditions detected (Nielsen et al., 2020). The impact of these high prevalences on sheep production in SA is currently unknown, although lungworms do cause significant economic loss by contributing to morbidity and mortality of sheep in parts of Europe and Africa (Pandey et al., 1984; Vina et al., 2013). [1]

Small lungworms affect sheep by causing damage to the lung parenchyma and bronchioles (Cole, 1986; Soulsby, 1965). Nodules form, which generally contain adults, as well as first stage larvae within larger nodules (Rose, 1965). The pathogenicity of the two small lungworm species which infect sheep in Australia (*Muellerius capillaris* and *Protostrongylus rufescens)* is similar, with both causing a massive inflammatory response and chronic eosinophilic granulomatous pneumonia (Mansfield and Gamble, 1995). Clinical signs are often absent except for occasional coughing (Berrag and Cabaret, 1996; Cole, 1986; Rose, 1959; Seddon, 1967). In contrast, the large lungworm, *Dictyocaulus filaria*, found in Australia, frequently results in clinical respiratory signs and associated production loss has been previously demonstrated (Beveridge et al., 1985; Seddon, 1967). Consequently, small lungworm diagnosis relies on detection of lung lesions post-mortem at an abattoir—which may indicate current/active or historical infections—or from faecal samples taken ante-mortem using the Baermann method. The rapid speed at which sheep are processed at an abattoir means that relatively quick and straightforward scoring systems are required to record lung lesions (McRae et al., 2016),

[1] GINs: gastrointestinal nematodes, WEC: worm egg count, epg: eggs per gram, lpg: larvae per gram, rpm: revolutions per minute, EID: electronic identification ear tag, SA: South Australia

which may limit the likelihood and/or accuracy of detection. Although offered commercially, the Baermann method, which isolates larvae from faeces, is infrequently performed as part of routine parasitological monitoring on commercial sheep farms and is also relatively insensitive (Lopez et al., 2010; Regassa et al., 2010). Therefore, the high prevalence of small lungworm reported from sheep in the southeast of SA may, in fact, still be an underestimate of the true prevalence.

Small lungworm has a significant impact on small ruminants in many parts of the world, with economic losses resulting from reduced growth and carcass weights, contribution to other disease processes, and rejection of offal at slaughter (Larsen, 2018; Pandey et al., 1984; Rose, 1959). For example, in the United Kingdom reduced growth rates of 4.3 kg were attributed to the presence of small lungworm in lambs artificially infected with *M. capillaris* (Rose, 1959). Similar reductions in weight and growth rates due to small lungworm infection may occur in sheep in Australia (Cole, 1986), although no detailed investigations have followed these preliminary studies that were undertaken in the 1950s and 1960s. In an unpublished study in 2019 in southeastern Australia, 942 ewes were categorised as either light or heavy (602 and 340, respectively), based on their liveweight measured at the farm. At the abattoir, lungs were scored on a 0–3 scale, where 0 = no lesions present and 3 = the most severely affected lungs. There were more lungs with a score of 2 or 3 in the light group of ewes, compared to the heavy group (403/602 and 168/340, respectively; Webb Ware unpublished data). Meanwhile, in New Zealand the carcass weights of several goat breeds were compared with a quantitative measure of *M. capillaris* lesions, finding goats with more than ten small nodular lesions per 10 cm^2 were 0.75 kg lighter than those without the lesions, although the pathological effects of small lungworm are considered to be more severe in goats than sheep (Valero et al., 1992). The lack of detailed Australian studies, combined with these more recent observations, indicate a need for further investigation and quantification of production losses associated with small lungworm in sheep.

Production losses may also occur from the condemnation of lungs at abattoirs, although this depends on abattoir regulations within specific countries. In Australia, the presence of small lungworm lesions does not usually result in condemnation because lungs are infrequently sold for human consumption.

However, some South Australian processors exporting lungs for human consumption have discarded a high proportion of lungs from the southeast of SA due to lungworm lesions (JBS Australia, pers comm.). This, combined with the low profit margin for the product, meant that marketing of this product was discontinued. In an abattoir study of indigenous sheep and goat breeds in Tanzania a high proportion of lungs were condemned (17% in sheep, 15% in goats) due to calcified cysts, presumed to be from lungworms (Mellau et al., 2010). Thus, production losses potentially reduce profits for both sheep producers and meat processors.

In addition to direct production losses, small lungworm may exacerbate other respiratory diseases. For example, it has been suggested that small lungworm infection can result in secondary pneumonia or bronchitis by facilitating bacterial or viral infection of the lungs (Hansen and Perry, 1994; Rose, 1959; Vina et al., 2013). Bacterial or viral pneumonia has a negative impact on production, such as reduced growth rates in lambs and increased abattoir condemnations and mortalities, resulting in significant costs to processors and producers (Lacasta et al., 2019; Lima et al., 2020). Thus, direct and indirect losses are likely to be incurred in Australia as a result of active or historical small lungworm infections, consistent with evidence that small lungworm is a significant problem in many parts of the world that have a substantial sheep industry and similar climatic conditions to southeastern Australia. Additionally, whether small lungworm decreases the productivity of lambs or adult sheep has not yet been quantified, despite reports that heavier infections occur in older animals (Alemu et al., 2006; Lopez et al., 2011; McCraw and Menzies, 1986; Regassa et al., 2010; Rose, 1965).

Consequently, the aim of this study was to quantify the association between measures of current or historical lungworm infection by post-mortem visual observation of lung lesions, with the carcass characteristics of lambs and adult sheep from farms in southeast SA, to assess the impact of lungworm on sheep productivity under commercial farm conditions.

2 Materials and Methods

2.1 Farms

The study was carried out in a high rainfall region of Australia, in southeast SA, where sheep flocks are typically extensively grazed on improved perennial pastures. Between January 2017 and March 2019 sheep were monitored on three farms (Farm A, B and C), located from 50 to 60 km west or south-west of Naracoorte in SA. Farm B and C had a self-replacing Merino enterprise producing fine wool (17-18 micron). Farm A and B had prime lamb enterprises, producing crossbred lambs from either Merino or Merino-cross dams, with the lambs sold for meat at 6 - 12 months of age. The ewes and lambs grazed the same pasture together until the weaning of lambs at approximately 3 months of age, after which time the dams and lambs grazed separate pastures.

Farms were selected based on a known high prevalence of small lungworm lesions and the presence of intermediate host mollusc species (including *Prietocella barbara, Cernuella virgate* and *Theba pisana*), either within the past five years (Farm A and C; Webb Ware unpublished data; Hanks et al unpublished data; Trengove, 2018) or based on their proximity to these farms and the presence of high populations of the intermediate host snail species (Farm B). The location and characteristics of the three study farms are described in Table 1.

2.2 Study design

This study was approved by the University of Melbourne Animal Ethics Committee (Reference number 1814480, Melbourne, Australia). A total of 6 cohorts were monitored from the three study farms: 3 cohorts from Farm A, 2 from Farm B and 1 from Farm C, with each cohort being part of a separate consignment of sheep to the abattoir. Two age categories were included: prime lambs (8-11 months old; Farm A and B) and ewes which were culled from the flock based on their age ('cast for age' (CFA) ewes, typically 4-6 years old; Farm A, B and C). Each of the farms sold sheep directly to the abattoir according to their routine farm management practices and so the time of sale and monitoring differed for each cohort. Cohorts were monitored on each study farm (two visits, except for cohort 6) and then at the abattoir (Table 2). A unique electronic identification (EID) ear-tag was used to individually identify sheep to be monitored in each cohort. The pasture grazed during the farm monitoring period and the most recently administered anthelmintics are shown in Table 2.

The sample size for the cohorts to be monitored was calculated based on the largest abattoir consignment within the study of 1300 sheep. This required a sample size of 259 to estimate the prevalence of small lungworm when this was assumed to be 30%, with a desired confidence of 95% and precision of 5% (Sergeant, 2018). For simplicity, a standard logistically manageable sample size of approximately 250 sheep was used where possible. This sample size enabled detection of a difference in carcass weight of 800 g between infected and non-infected sheep, assuming prevalence of 30%, variance of 2.89, power of 80%, and desired confidence of 95% (Sergeant, 2018). For the smallest consignment within the study of 108 sheep, all sheep were monitored.

2.3 Measurements

2.3.1 Live weights and body condition scores

Sheep were weighed at the commencement of the study on each farm (farm visit 1) and prior to consignment to the abattoir (farm visit 2), using electronic scales accurate to 0.5 kg (Tru-Test MP600 loadbars, Tru-Test P/L). At each farm visit, body condition score (BCS) was assessed in adult sheep on a scale of 1 - 5, where 1 was emaciated and 5 was very fat. This score was assessed in increments of 0.25 by palpation of the spinous and transverse processes of each animal (Anonymous, 2011)).

2.3.2 Faecal samples

Faecal samples were collected from a subsample of 50 of the sheep within the cohort at farm visit 1, to assess the prevalence and severity of gastro-intestinal nematodes (GINs) and lungworm infections by worm egg count (WEC) and Baermann method, respectively. Faecal samples were collected directly from each sheep *per rectum* and stored in plastic 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 method, respectively.

2.3.2.1 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 describe by Andersen and Walters (1973). 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. They were 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 larval population (Lopez et al., 2011; Vina et al., 2013). 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).

2.3.2.2 Worm egg counts

Bulk WECs were performed using a modified McMaster technique (Anderson et al., 1991). Briefly, 5 g of faeces (1 g from each sample) were homogenised in 45 mL of water. The sample was transferred into a 10 ml test tube and allowed to sediment at 4°C for at least two hours. The supernatant was poured off the sample, and the plug of faecal debris was resuspended in a saturated solution of sodium chloride. After thorough mixing, two chambers of the McMaster slide were filled, and all strongyle-type eggs counted under x40 magnification. Using these dilutions, each egg counted was equivalent to 10 eggs per gram of faeces (epg). The mean WEC for the 10 pooled samples was calculated to determine the overall average for the 50 sheep monitored.

2.3.3 Measurements at slaughter

Sheep were killed at a commercial abattoir at a processing speed of about 10 sheep/minute. Because the ears were removed from each carcass early in the processing chain, each EID was scanned immediately after slaughter to establish the sequence of processing, and a numbered visual tag, linked to the EID, was attached to the carcass so that subsequent measurements could be matched to the EID of each sheep. When the viscera were removed from the carcass on the processing chain, the lungs were placed on a separate metal tray on the processing line, which kept them in the carcass processing sequence. The lungs were placed in a standardised dorsoventral position, photographed, and inspected for lungworm and other conditions. Other gross pathology observations were noted and recorded during the examination of the carcass.

Photographs were assessed to semi-quantitatively score the prevalence (*M. capillaris* and *P. rufescens*) and severity (*M. capillaris* only) of lungworm lesions, with animals categorised as 'infected' or 'non-infected' with small lungworm based on observation of typical *M. capillaris* or *P. rufescens* lesions (Rose, 1959; Stockdale, 1976). Nodular lesions indicative of *M. capillaris* were scored on a 0–3 scale (Rose, 1959; Sauerlander, 1988; Valero et al., 1992) where 0 = no lesions present; 1 = small superficial purple or grey nodules on the dorsal aspects of the caudal lung lobes; 2 = increased number and size of nodules on the dorsal aspects of the caudal lung lobes and 3 = nodules covered most of the dorsal aspects of the caudal lung lobes and extended into other lung lobes. *P. rufescens* was assessed as present if 1-4 cm diameter, grey or white plaques were observed in the dorsal aspects of the caudal lung lobes (Mansfield and Gamble, 1995; Stockdale, 1976)**.**

The presence and severity of pleurisy or pneumonia was assessed based on the presence of connective tissue on the pulmonary pleura and/or consolidated dark-purple areas in any lung lobes. The degree of lung consolidation was scored from 0 to 2, where 0 = no lesion present, 1 = any individual lobe with up to 50% of the lobe affected, and 2 = greater than 50% of the lobe affected (McRae et al., 2018).

A sub-sample of approximately 20 lungs from each cohort which were representative of the range of lesions observed were collected. Standard clinical diagnosis of *D. filaria* was used by dissecting the trachea and primary bronchi the following day. Lesions were incised to determine if they were suppurative or parasitic. To validate *M. capillaris* lung scores based on photographs, a sub-sample of lungs were re-scored at the laboratory using gross palpation and visual assessment. Sections were taken from 25 lungs with lesions (5 from each cohort, cohort 3 omitted due to an absence of lesions suitable for sampling) and submitted for microbial culture and sensitivity. Lesions visually categorised as *M. capillaris* were dissected from 9 lungs and adults were extracted for microscopic identification.

2.4 Statistical analyses

Statistical analyses were carried out using R version 3.6 (R, 2019). Proportion positive on Baermann method, and arithmetic mean worm egg count with basic nonparametric bootstrap 95% confidence limits were calculated.

Models were used to assess associations between the presence of small lungworm lesions and age, and small lungworm and pneumonia. A generalised estimating equation using a binomial distribution with a logit link function was used to model small lungworm lesions detected at slaughter as a measure of active and/or historical small lungworm infection. Cohort was fitted as a cluster variable and age was included as a fixed effect. A generalised estimating equation using a binomial distribution with a logit link function was used to model association of pneumonia with small lungworm lesions. Cohort was fitted as a cluster variable, and small lungworm, age and their interaction were included as fixed effects. *M. capillaris* photography lung scores were validated by comparing a sub-sample from cohorts 2, 3 and 6 which were using photographic and laboratory examination. The correlation coefficient was calculated between the two methods using a weighted Cohen's Kappa.

Liveweights, BCS and carcass weights were excluded from cohort 5 because small lungworm lesions detected at slaughter was unable to be matched to an individual sheep due to a disruption of the order of sheep within the processing chain. This did not affect any other cohorts. BCS 1 and liveweight 1 from cohort 6 were compared with BCS 2 and liveweight 2 measurements from other cohorts to compare all measurements which were made close to slaughter date. Liveweights and carcass weights were approximately normally distributed. Carcasses which were trimmed, condemned, or had pneumonia present in the lungs were excluded from the carcass weight and dressing percentage analysis. The dressing percentage was calculated for each cohort based on carcass weight and liveweight measurement 2, except for cohort 6 (liveweight 1 was used), and cohort 5 (not calculated).

Associations between small lungworm lung lesions and animal production measurements on-farm and at slaughter were modelled. Liveweight 2, BCS 2, hot standard carcass weights (HSCW) and dressing percentages were compared between infected and non-infected sheep using a linear mixed effects model including small lungworm lesions, age, and their interaction as fixed effects. Cohort was fitted as a random effect. Additionally, carcass weights and dressing percentages were compared between moderate/severe lesions and no/mild lesions using a linear mixed effects model with the same effects.

3 Results

3.1 Measurements

A total of 1332 sheep were monitored from the 6 cohorts included in the study. At slaughter, carcass measurements were made on 1271 sheep, and lung photo observations collected from 1177 sheep. There were 756 carcass weight measurements that could be paired with lung photos from the same individual, from 536 lambs and 220 adults, respectively. The number of sheep from each cohort, at each measurement is provided in Supplementary Table 1.

3.2 Small lungworm infection

3.2.1 On farm measurements of lungworm and GIN infection

In each cohort, the proportion of sheep with a positive Baermann result was relatively low (< 15%), except in cohort 2 (76%; Table 3). Similarly, the larval count was low, except for lambs in cohort 2 where the larval count was greater than 50 lpg in four lambs. Based upon their morphology, 94% of the lungworm larvae isolated were classified as *P. rufescens* (597 of 635) and 5% were *M. capillaris* (34 of 635), with four larvae unable to be classified. Except for cohort 2, the worm egg counts for GIN were generally < 150 epg (Table 3), which is below the threshold for additional anthelmintic treatments in this environment (Jacobson et al., 2009).

3.2.2 Abattoir measurements of post-mortem lungworm and pneumonia infection

3.2.2.1 Prevalence of small lungworm

The overall prevalence of small lungworm detected from lung lesions was 79% (928/1177; 95% CI 76, 81), with a prevalence of 87% (569/658; 95% CI 84, 89) in lambs, and 69% (359/519; 95% CI 65, 73) in adults, respectively. The generalised estimating equation model showed no statistically significant difference in prevalence between lambs and adults (odds ratio = 2.9; 95% CI 0.8, 10.3; P = 0.1). There was a high prevalence of small lungworm lesions in each cohort (59% - 100%), with these visually classified as being predominantly mild *M. capillaris* (Table 4). *D. filaria* was not detected grossly in the trachea or primary bronchi of the 115 lungs which were examined further by detailed dissection and was not detected in any of the 1177 lungs assessed using photography.

3.2.2.2 Association of small lungworm with pneumonia

The overall prevalence of pneumonia detected visually at post-mortem was 12% (139/1177; 95% CI 10, 14), with a prevalence of 13% (82/658; 95% CI 10, 15) in lambs and 11% (57/519; 95% CI 8, 14) in adults. A similar proportion of lambs and adults were affected with pneumonia, regardless of whether they also had small lungworm lesions (Table 5). The presence of small lungworm lesions did not statistically change the odds of a lamb having pneumonia (odds ratio 0.7; 95% CI 0.4, 1.1; $P = 0.1$), but did reduce the odds of an adult having pneumonia (odds ratio = 0.7; 95% CI 0.6, 0.8; $P < 0.001$). A range of bacteria were cultured from lungs showing evidence of pneumonia in adults and lambs (Supplementary Table S2).

3.2.2.3 Validation of lung scores

The accuracy of *M. capillaris* lesion categorisation based on photographic lung scores was validated by comparing 47 lungs which were scored using both photographic assessment and detailed visual examination back in the laboratory (Table 6). The kappa value for this comparison was 0.51 (95% CI 0.22, 0.80; $P < 0.001$), suggesting moderate agreement between the two methods. Differences were predominantly by one score of categorisation. For example, 4 lungs which were negative (score 0) based on photography were judged to be mildly infected (score 1) based on laboratory examination, suggesting that one or more *M. capillaris* nodules were not detected when using photographs (Table 6). All 24 adult worms extracted from lung lesions classified as *M. capillaris* were identified as such.

3.3 Production characteristics

3.3.1 Liveweight and body condition score

Small lungworm infected lambs and adults, as measured by lung post-mortem lesions, had a similar liveweight at farm visit 2 compared to non-infected animals (Table 7). Mean liveweight for non-infected and infected lambs at farm visit 2 was 53.2 kg (95% CI 39, 67), and 53.7 kg (95% CI 40, 68), respectively, with a mean difference of 0.5 kg (95% CI -0.7, 1.7; $P = 0.4$). Mean liveweight for non-infected and infected adults at farm visit 2 was 57.5 kg (95% CI 40, 75), and 58.8 kg (95% CI 42, 76), respectively, with a mean difference of 1.3 kg (95% CI -0.2, 3.0; $P = 0.07$). Mean BCS for non-infected and infected adults at measurement 2 was 3.2 (95% CI 2, 4), and 3.4 (95% CI 2, 5), respectively, with a mean difference of 0.2 (95% CI 0.0, 0.4; $P = 0.04$).

3.3.2 Carcass characteristics

3.3.2.1 Carcass weight and dressing percentage

The median and inter-quartile HSCW of infected and non-infected sheep within each cohort (as categorised by inspection of lungs for typical small lungworm lesions) were similar (Figure 1). Small lungworm infected lambs and adults had a similar hot standard carcass weight and dressing percentage compared to non-infected animals overall (Table 7). Mean HSCW for non-infected and infected lambs was 23.4 kg (95% CI 18, 29), and 23.6 kg (95% CI 18, 29), respectively, with a mean difference of 0.2 kg (95% CI -0.4, 0.8; P = 0.5). Mean HSCW for non-infected and infected adults was 21.3 kg (95% CI 15, 28), and 21.5 kg (95% CI 15, 28), with a mean difference of 0.2 kg (95% CI -0.5, 0.9; P = 0.5). Mean dressing percentage for non-infected and infected lambs was 44.0% (95% CI 41, 47), and 44.0% (95% CI 41, 47), respectively, with a mean difference of 0% (95% CI -0.8, 0.8; P = 1). Mean dressing percentage for non-infected and infected adults was 37.3% (95% CI 33, 41), and 36.9% (95% CI 33, 41), respectively, with a mean difference of -0.4% (95% CI -1.5, 0.6; P = 0.4).

Because of the imperfect agreement between the semi-quantitative lung score methods, a comparison of the HSCW and dressing percentage was made between score 0/1 lungs and score 2/3 lungs. Score 0 and 1 were categorised together as non-infected/mildly infected to reflect the subtle difference between these scores and compared with those sheep which were more obviously infected with moderate or severe lesions (score 2 or 3). The HSCW and dressing percentage was similar between lambs and adults with no or mild lesions compared to lambs and adults with moderate or severe small lungworm lesions.

3.3.2.2 Condemned and trimmed carcasses

A total of 150 carcasses were trimmed and 4 were condemned (Table 8). Lungs were examined on 72% (111/154) of these carcasses. In many carcasses where the ribs were trimmed the lungs could not be examined, resulting in the exclusion of 10/17 (59%) lamb and 10/41 (24%) adult carcasses (excluding cohort 5). Rib trimming is likely to have occurred due to pleurisy or pneumonia, leg trimming due to arthritis and abdominal trimming due to a variety of infectious and non-infectious causes, such as caseous lymphadenitis and grass seed infestations. The presence or absence of trimming was not observed for two carcasses from cohort 6.

4 Discussion

This study was carried out in response to producer concerns about the very high prevalence of small lungworm infection in the southeast of SA (Dal Grande et al., 2019; Nielsen et al., 2020), and its impact on sheep productivity. In many countries, small lungworm has been associated with reduced growth and carcass weights, increased lung diseases and rejection of offal at slaughter (Larsen, 2018; Pandey et al., 1984; Rose, 1959). Consequently, producers in the southeast of SA were concerned about potential economic loss from the high prevalence of small lungworm lesions in their sheep reported in abattoir surveillance. We detected a very high prevalence of small lungworm infection, particularly when measured by the presence of lung lesions at slaughter (indicating current and/or historical infection). Nevertheless, liveweight and carcass weights were not reduced in infected (current or historical) sheep and infection did not appear to increase the risk of a sheep having pneumonia. This suggests, at least for farmers in this region with similar production systems, that additional control measures for small lungworm, such as additional anthelmintic treatments beyond those required to control GINs or control of intermediate host molluscs, are unlikely to be necessary or cost-effective.

There was no strong association between small lungworm prevalence and the age of the sheep (lambs versus adults), and no demonstrable effect of small lungworm lesions (indicative of current or historical infection) on carcass weight in either age class. Factors contributing to this result are discussed in more detail below. Additionally, the study found no association between the presence of small lungworm lesions and pneumonia. It also highlighted the limitations of currently available diagnostic tests for small lungworm on farm and for assessing infections at the processing speeds of a commercial abattoir (McRae et al., 2016; Pyziel et al., 2015; Vina et al., 2013). A previous unpublished study in southeast SA had suggested small lungworm lesions reduced carcass weight (Webb Ware unpublished data). However, the current study used additional individual animal slaughter information and more detailed diagnosis (Edwards et al., 1999), to demonstrate that carcass weight was not reduced in sheep infected with small lungworm.

The presence of small lungworm lesions was 18% higher in lambs compared to adults (OR 2.9) in this study. This is likely to reflect a true difference because the overall sample size was large enough to detect a difference of 7%. However, it was not statistically significant, due mainly to the variability between cohorts. In contrast, most reports in the literature suggest that 'infection' rate increases with age (Alemu et al., 2006; Lopez et al., 2011; McCraw and Menzies, 1986; Regassa et al., 2010; Rose, 1965)—this likely reflects the steady accrual of focal lung calcification or scarring that does not resolve and thus represents a cumulative measure of exposure but not necessarily an increase of active infections in sheep. Additionally, the interaction of numerous risk factors, including species, age, breed, sex and grazing management, influences the overall risk of small lungworm infection, and may have influenced prevalence during this study (Berrag and Urquhart, 1996; Lopez et al., 2011). For example, grazing management is an important determinant of the exposure of adults and lambs to the intermediate host mollusc and infective larvae of small lungworm (L3) but is often not described in the literature (Alemu et al., 2006; Lopez et al., 2011; Regassa et al., 2010). Therefore, higher prevalences may be due to increased exposure to the parasite, which may occur intermittently or sporadically in association with high populations of molluscs, rather than simply the age of the host. In this study, there was a known high density of molluscs on the pasture grazed by two lamb cohorts (1 and 2), which most likely increased their exposure to L3. Lambs are also more likely to be more indiscriminate grazers than adult sheep, potentially increasing their exposure to L3 regardless of the density of molluscs (Larsen, 2018). Differences in grazing management are also likely to have been a significant risk factor which influenced the prevalence of small lungworm and GINs in lambs and adults during this study (Larsen, 2018).

HSCW and carcass dressing percentage were similar between sheep infected with small lungworm and non-infected sheep, regardless of age class. Infected adults had a slighter higher BCS but this small difference in BCS is unlikely to be biologically or economically significant, with infected adults still considered to be in good condition for sale (Abbott, 2018). Regardless of whether the small lungworm lesions observed represented current or historical infections, the lack of association with productivity suggests that sheep had either not been significantly affected by infection when it was active or had had sufficient time for compensatory growth once infections and/or host response subsided. The majority of *M. capillaris* lesions were mild and so this may have influenced the lack of effect on carcass weight. Rose described 'light' infections (as indicated by lung lesions) having little impact on the health of sheep, whereas 'medium' and 'heavy' infections compromised a considerable amount of the lung tissue and were thus more likely to affect the health of sheep (Rose, 1959). A similar scoring system was used during this study for *M. capillaris* only (because it has not been described for *P. rufescens*), with scores referred to as mild, moderate and severe.

Our study provides evidence that in Australia small lungworm is unlikely to directly cause extensive production losses when lesions are predominantly mild, as previously asserted by Cole (1986) and (Seddon, 1967) (although without supporting data). Our study found that there was no impact of small lungworm on productivity in both age classes, despite a high prevalence of small lungworm in all the cohorts studied. GINs often have a greater production effect in young sheep, which have had less opportunity to develop protective immunity (Barger, 1993). In our study, the prevalence and impact of small lungworm lesions may have been influenced by the age of each cohort and its relationship to duration of exposure to small lungworm and acquisition of host immunity. Lambs from Farm A cohort 1 had an additional 2-3 months grazing pastures compared to cohort 2, increasing their time for exposure to L3, which may have contributed to all lambs from cohort 1 being infected with small lungworm. Thus, small lungworm may have reduced carcass weights or liveweights in cohort 1, but this could not be compared to non-infected lambs because all lambs within the cohort were infected. GIN infections might have also confounded the lack of effect of lungworm on production observed in our study, but WECs were low and unlikely to have caused noticeable production loss in all groups except cohort 2 in our study, making this less likely.

The presence of small lungworm did not appear to change the risk of lambs also having pneumonia, in fact in adults it reduced the risk (odds ratio = 0.7). However, when ribs from a carcass were trimmed because of lung or pleural adhesions, these lungs could not be assessed. Thus, the presence of small lungworm lesions could not be ascertained and included in odds ratio calculations. It is likely that these lungs had pneumonia which developed to pleurisy resulting in adhesion of the lungs to the chest wall (McRae et al., 2016). Other studies have suggested that small lungworm infection can result in secondary pneumonia or bronchitis by facilitating bacterial or viral infection of the lungs (Rose, 1959; Vina et al., 2013). For example, in Great Britain, all lamb cohorts (12/30 consignments) which had lungworm lesions detected at the abattoir, also had pneumonia and/ or pleurisy lesions (Edwards et al., 1999). Should small lungworm infection contribute to secondary pneumonia and pleurisy, infection would increase the likelihood of carcass trimming. Significant costs to meat processors and sheep producers would then be incurred (Lacasta et al., 2019; Lima et al., 2020). The present study suggests that this is not the case, but more detailed monitoring to ascertain whether there is an association between patent small lungworm infection and pneumonia is warranted.

The present study allowed for the small lungworm status and carcass characteristics of an individual to be correlated, compared to the method used for enhanced abattoir surveillance in SA where an overall estimate of the prevalence for a consignment is based on a rapid assessment by meat inspectors (Matthews and Dickason, n.d.). Similarly, in Great Britain flock level monitoring has been used at the farm and abattoir, but discrimination between those individuals with and without abnormalities was not possible and thus relied on averaging information across the flock (Edwards et al., 1999). This would have influenced the association made between small lungworm and pneumonia described above. The accurate matching of each individual sheep with measurements made at the abattoir and at the farm provided the confidence that this current study accurately represented the association, or lack thereof of small lungworm lesions with productivity.

Data accuracy was likely further improved during our study by increasing the time for assessment of individual lungs. However, whilst photographs allowed more time for examination of lungs and likely improved diagnosis, limitations of using a semi-quantitative scoring system remained. This was evident based on the imperfect agreement between photographic and laboratory lung assessments (Watson and Petrie, 2010), although the imperfect agreement between the methods was unlikely to have altered the overall comparison between lesions and productivity parameters of sheep. The subsample of lungs which were further examined in the laboratory grossly and via lesion incision suggested that some lesions could not be distinguished grossly to be the result of *P. rufescens* or pneumonia. Misdiagnosis is further exacerbated when scoring at speed, requiring simplified methods which may not capture the presence of abscesses resulting from pneumonia (McRae et al., 2016). Thus, bacterial pneumonia may have been misdiagnosed as small lungworm infection in previous studies involving semi-quantitative scoring at abattoir processing speed. If misdiagnosed the association of small lungworm infection with carcass characteristics would have been confounded (McRae et al., 2016). This study reduced the likelihood of this occurrence through longer lung examinations.

In this study there was a significant discrepancy between the species identified on Baermann test, predominantly *P. rufescens* and the species identified by lung examination, predominantly *M. capilliaris*. Further research is needed to improve diagnosis which can be used at abattoir processing speed and prior to slaughter. The potential for inaccuracy using a semi-quantitative score suggests that an objective measurement would be useful, such as quantifying the number of small lungworm lesions within a discrete area of the caudo-dorsal lung lobes as used by Valero et al (1992) in their assessment of goats in New Zealand abattoirs. However, this method was not validated, cannot be performed at abattoir processing speed, does not allow for diagnosis of the species present and must occur post-mortem. Alternatively, a genus- or species-specific molecular test could be developed for sheep lungworms (Pyziel et al., 2015), which would also allow for improved testing whilst sheep were on the farm. It would also provide a better indication of the effect of a current patent infection on liveweight as patent infections may have a more significant effect on sheep productivity (Sauerlander, 1988).

Producers need to know the full extent of economic loss associated with a disease, rather than just the prevalence, in order to make appropriate management decisions. Given the high prevalence of small lungworm, there was concern that by simply reporting this, it implied that some management was required on the farm. This may have resulted in increased unnecessary use of anthelmintics that are routinely used in to control gastro-intestinal nematodes on farms (Spark pers comms.), thus potentially contributing to increased anthelmintic resistance in GINs (Larsen, 2014). However, this study strongly suggests that there is no requirement for additional anthelmintic use, nor the control of intermediate host molluscs for indirect management of small lungworm in this region. Nevertheless, molluscs control is still required during establishment of new pastures when they cause significant pasture loss (Baker and Hawke, 1990; Micic et al., 2008). Mild small lungworm lesions are unlikely to incur significant costs to producers and processors because there is no reduction in growth nor any association with pneumonia. This is a crucial finding for producers in this region, and should prevent unnecessary, and potentially adverse, additional anthelmintic use.

5 Conclusion

This study helps address an important concern of producers and processors in southeastern Australia about the effect of a high prevalence of small lungworm lesions on sheep productivity. Whilst we did find a very high prevalence of small lungworm, carcass weights were not reduced in sheep with lung lesions at slaughter, and lesions did not appear to increase the risk of a sheep having pneumonia. Our results also suggest that there was no difference in the prevalence or effect of small lungworm between adults and lambs. This means that additional control measures for small lungworm are unlikely to be needed, which reduces the need for additional anthelmintic treatments above that required to control GINs. This study made use of additional information available at slaughter, but also demonstrated the limitations of the currently available diagnostic tests. This highlights the need for better tests for small lungworm which are more sensitive, practical, and feasible for use on farms and in abattoirs. The evidence that small lungworm lesions have little or no effect on the productivity of sheep provides a strong assurance to producers that economic losses from small lungworm are unlikely, at least in the southeast of SA. This is also likely to be the case in areas with similar climatic and environmental conditions, provided that infected lungs are not condemned for human consumption and that small lungworm does not predispose sheep to pneumonia.

6 References

Abbott, K., 2018. The sheep farm as a production system, In: Abbott, K. (Ed.) The Practice of Sheep Veterinary Medicine. University of Adelaide Press, Adelaide.

ABS 2017. Sheep numbers- as at June 2016 In Natural resource management (North Sydney, Meat and Livestock Australia Limited).

Alemu, S., Leykun, E.G., Ayelet, G., Zeleke, A., 2006. Study on small ruminant lungworms in northeastern Ethiopia. Vet. Parasitol. 142, 330-335.

Andersen, F.L., Walters, G.T., 1973. Efficacy of baermann technique for recovery of *Dictyocaulus viviparus* larvae from bovine feces. Am. J. Vet. Res. 34, 39-40.

Anderson, N., Martin, P.J., Jarrett, R.G., 1991. The efficacy of mixtures of albendazole sulfoxide and levamisole against sheep nematodes resistant to benzimidazole and levamisole Aust. Vet. J. 68, 127-132.

Anonymous 2011. Condition Scoring of Sheep. In Lifetme Wool (http://www.lifetimewool.com.au/conditionscore.aspx (accessed 01/05/2018)).

Baker, G.H., Hawke, B.G., 1990. Life history and population dynamics of *Theba pisana* (mollusca, helicidae) in a cereal pasture rotation. J. Appl. Ecol. 27, 16-29.

Barger, I.A., 1993. Influence of sex and reproductive status on susceptibility of ruminants to nematode parasitism. Int. J. Parasit. 23, 463-469.

Berrag, B., Cabaret, J., 1996. Impaired pulmonary gas exchange in ewes naturally infected by small lungworms. Int. J. Parasit. 26, 1397-1400.

Berrag, B., Urquhart, G.M., 1996. Epidemiological aspects of lungworm infections of goats in Morocco. Vet. Parasitol. 61, 81-95.

Beveridge, I., Brown, T.H., Fitzsimons, S.M., Ford, G.E., Judson, G.J., Martin, R.R., Miller, D.W., 1985. Mortality in weaner sheep in South-Australia under different regimes of anthelmintic treatment. Aust. J. Agric. Res. 36, 857-865.

Cole, V.G., 1986. Animal Health in Australia- Helminth Parasites of Sheep and Cattle, Vol 8. Conmonwealth of Australia, Canberra, Australia.

Dal Grande, E., Nielsen, T., O'Handley, R., 2019. Endemic parasitic conditions in sheep and association with other endemic conditions using the South Australian Enhanced Abattoir Surveillance (EAS) Program. In: Australian Parasitology Conference, Adelaide.

Edwards, D.S., Christiansen, K.H., Johnston, A.M., Mead, G.C., 1999. Determination of farm-level risk factors for abnormalities observed during post-mortem meat inspection of lambs: A feasibility study. Epidemiol. Infect. 123, 109-119.

Hansen, J., Perry, B. 1994. The Epidemiology, Diagnosis and Control of Helminth Parasites of Ruminants (Nairobi, Kenya, International Laboratory for Research on Animal Diseases).

Jacobson, C., Pluske, J., Besier, R.B., Bell, K., Pethick, D., 2009. Associations between nematode larval challenge and gastrointestinal tract size that affect carcass productivity in sheep. Vet. Parasitol. 161, 248-254.

Lacasta, D., Gonzalez, J.M., Navarro, T., Saura, F., Acin, C., Vasileiou, N.G.C., 2019. Significance of respiratory diseases in the health management of sheep. Small Ruminant Res. 181, 99-102.

Larsen, J., 2018. Helminth diseases of sheep, In: Abbott, K. (Ed.) The Practice of Sheep Veterinary Medicine. University of Adelaide Press, Adelaide.

Larsen, J.W.A., 2014. Sustainable internal parasite control of sheep in Australia. Small Ruminant Res. 118, 41-47.

Lima, E., Lovatt, F., Green, M., Roden, J., Davies, P., Kaler, J., 2020. Sustainable lamb production: Evaluation of factors affecting lamb growth using hierarchical, cross classified and multiple memberships models. Prev. Vet. Med. 174, 12.

Lopez, C.M., Cienfuegos, S., Dacal, V., Vazquez, L., Panadero, R., Fernandez, G., Diaz, P., Lago, N., Diez-Banos, P., Morrondo, M.P., 2010. Efficacy of anthelminthic control programs against natural *Muellerius capillaris* infection in sheep in the north-west of spain: Effect on blood gases and ph in venous blood samples. Parasite-J. Soc. Fr. Parasitol. 17, 167-171.

Lopez, C.M., Fernandez, G., Vina, M., Cienfuegos, S., Panadero, R., Vazquez, L., Diaz, P., Pato, J., Lago, N., Dacal, V., Diez-Banos, P., Morrondo, P., 2011. Protostrongylid infection in meat sheep from Northwestern Spain: Prevalence and risk factors. Vet. Parasitol. 178, 108-114.

Mansfield, L.S., Gamble, H.R., 1995. Alveolar mastocytosis and eosinophilia in lambs with naturally acquired nematode infections of *Protostrongylus rufescens* and *Haemonchus contortus*. Vet. Immunol. Immunopathol. 49, 251-262.

Matthews, E., Dickason, C., n.d. Ground truthing the enhanced abattoir surveillance program in South Australia. In: Australian Sheep Veterinarians Conference.

McCraw, B.M., Menzies, P.I., 1986. Treatment of goats infected with the lungworm *Muellerius capillaris*. Can. Vet. J.-Rev. Vet. Can. 27, 287-290.

McRae, K.M., Baird, H.J., Dodds, K.G., Bixley, M.J., Clarke, S.M., 2016. Incidence and heritability of ovine pneumonia, and the relationship with production traits in New Zealand sheep. Small Ruminant Res. 145, 136-141.

McRae, K.M., Rowe, S.J., Baird, H.J., Bixley, M.J., Clarke, S.M., 2018. Genome-wide association study of lung lesions and pleurisy in New Zealand lambs. J. Anim. Sci. 96, 4512-4520.

Mellau, L.S.B., Nonga, H.E., Karimuribo, E.D., 2010. A slaughterhouse survey of lung lesions in slaughtered stocks at Arusha, Tanzania. Prev. Vet. Med. 97, 77-82.

Micic, S., Hoffmann, A.A., Strickland, G., Weeks, A.R., Bellati, J., Henry, K., Nash, M.A., Umina, P.A., 2008. Pests of germinating grain crops in southern Australia: An overview of their biology and management options. Aust. J. Exp. Agric. 48, 1560-1573.

Nielsen, T., Schultz, S., Mazoudier, P., Caraguel, C., Dal Grande, E., 2020. Starting from the other end-sheep condition findings at abattoir. In: VETFEST, Australian Veterinary Association Virtual.

Pandey, V.S., Cabaret, J., Fikri, A., 1984. The effect of strategic anthelmintic treatment on the breeding performance and survival of ewes naturally infected with gastrointestinal strongyles and protostrongylids. Annales De Recherches Veterinaires 15, 491-496.

Pyziel, A.M., Laskowski, Z., Hoglund, J., 2015. Development of a multiplex PCR for identification of Dictyocaulus lungworms in domestic and wild ruminants. Parasitology Research 114, 3923-3926.

R 2019. RStudio: Integrated Development for R. (Boston, MA, RStudio, Inc.).

Regassa, A., Toyeb, M., Abebe, R., Megersa, B., Mekibib, B., Mekuria, S., Debela, E., Abunna, F., 2010. Lungworm infection in small ruminants: Prevalence and associated risk factors in Dessie and Kombolcha districts, northeastern Ethiopia. Vet. Parasitol. 169, 144-148.

Rose, J.H., 1959. Experimental infection of lambs with *Muellerius capillaris*. Journal of Comparitive Pathology and Therapeutics 69, 414-422.

Rose, J.H., 1965. Some observations on transmission of lungworm infection in a flock of sheep at pasture. Research in Veterinary Science 6, 189-195.

Sauerlander, R., 1988. Experimental infection of sheep and goats with *Muellerius capillaris* (protostrongylidae, nematoda). J. Vet. Med. Ser. B-Zent.bl. Vet. Med. Reihe B-Infect. Dis. Vet. Public 35, 525-548.

Seddon, H.R., 1967. Diseases of Domestic Animals in Australia Part 1- Helminth Infestations. Government Printing Office, Canberra, Australia.

Sergeant, E. 2018. Epitools epidemiological calculators (http://epitools.ausvet.com.au (accessed 14/09/2019), Ausvet).

Soulsby, E.J.L., 1965. Textbook of Veterinary Clinical Parasitology, Vol 1. Heminths. Blackwell Scientific Publications, Oxford, UK, 473-497 pp.

Stockdale, P.H.G., 1976. Pulmonary pathology associated with metastrongyloid infections British Veterinary Journal 132, 595-608.

Trengove, C.L. 2018. Investigating the impact and control of lungworm in lambs. In Australian Veteriary Association Annual Conference (Brisbane, Australia, Australian Veterinary Association).

Valero, G., Alley, M.R., Manktelow, B.W., 1992. A slaughterhouse survey of lung lesions in goats. N. Z. Vet. J. 40, 45-51.

Vina, M., Panadero, R., Diaz, P., Fernandez, G., Perez, A., Diez-Banos, P., Morrondo, P., Lopez, C.M., 2013. Evaluation of the use of pooled fecal samples for the diagnosis of protostrongylid infections in sheep. Vet. Parasitol. 197, 231-234.

Watson, P.F., Petrie, A., 2010. Method agreement analysis: A review of correct methodology. Theriogenology 73, 1167-1179.

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8 Tables

Table 1 Description of study farms

Farm	**Location** (longitude, latitude)	**Average annual rainfall** (mm)	**Land** (ha)	**Stocking rate** (DSE/ha)	**Time of lambing**	**No. of ewes**	**Breed**
A	37.001°S, 140.085°E	602	2878	6	June-July	3000	Merino, Border Leicester x Merino, Border Leicester x Merino x Dorset
B	37.243°S, 140.087°E	641	7440	14	April- May	25000	Merino, Merino x Poll Dorset
C	37.074°S, 140.085°E	640	3500	14	August-October	13800	Merino

Table 2 Description of the six cohorts monitored

Cohort	Farm	Number	Age	Age class	Sex	Breed	Farm visit 1	Farm visit 2	Most recent anthelmintic	Anthelmintic groups administered	Pasture grazed	Slaughter date
							(number of days prior to slaughter)					
1	A	249	11 months	lamb	mixed	Border Leicester x Merino x Dorset	16	2	35	Macrocyclic lactone†	irrigated lucerne (*Medicago sativa*)	17-May-18
2	A	229	9 months	lamb	wethers	Border Leicester x Merino	76	1	76	Macrocyclic lactone†	irrigated lucerne (*Medicago sativa*)	26-Mar-19
3	A	108	4-6 years	adult	ewes	Border Leicester x Merino	114	6	63	Macrocyclic lactone, Levamisole, Benzimidazole±	millet (*Panicum miliaceum*), forage rape (*Brassica napus*)	28-Feb-19
4	B	250	11 months	lamb	mixed	Merino x Poll Dorset	43	2	150	Macrocyclic lactone, Levamisole, Benzimidazole±	irrigated clover (*Trifolium subterranean*), phalaris (*Phalaris aquatica*), perennial ryegrass (*Lolium perenne*)	14-Jun-18
5	B	250	4-6 years	adult	ewes	Merino	62	9	124	Macrocyclic lactone, Levamisole, Benzimidazole±	clover (*Trifolium subterranean*), phalaris (*Phalaris aquatica*), tall fescue (*Festuca arundinacea*)	17-Jan-19
6	C	246	7-8 years	adult	ewes	Merino	7	N/A	45	Macrocyclic lactone, Spiroindole¥	irrigated clover (*Trifolium subterranean*), perennial ryegrass (*Lolium perenne*)	28-Feb-19

N/A not applicable; insufficient time for second farm visit to occur prior to consignment to the abattoir
†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)
¥Derquantel 10 mg/mL, Abamectin 1mg/mL (Startect® at 2 mg/kg Derquantel, 0.2 mg/kg Abamectin; Zoetis Australia Pty Ltd)

Table 3 Proportion of sheep positive for small lungworm on Baermann test, median lungworm larvae per gram faeces of positive sheep and arithmetic mean strongyle eggs per gram faeces in cohorts 1 - 6

Cohort	**Farm**	**Age class**	**Small lungworm** (N/N infected (%))	**Lungworm larval count** (median lpg (IQR))	**GIN worm egg count** (mean epg (95% CI))
1	A	lamb	N/A[†]	N/A[†]	21 (9,34)
2	A	lamb	38/50 (76%)	9 (2,18)	795 (643,928)
3	A	mutton	1/24 (4%)	5 (5,5)	40 (4,92)
4	B	lamb	N/A[†]	N/A[†]	97 (81, 119)
5	B	mutton	7/49 (14%)	1 (1,11)	8 (3,15)
6	C	mutton	5/49 (10%)	1 (1,7)	1 (0,3)

[†]Results excluded because key modifications to the Baermann technique were made after these samples were processed (incubation period changed from 15 to 8 hours)

Table 4 Prevalence of small lungworm lesions observed at slaughter in cohorts 1 - 6

Cohort	**Farm**	**Age class**	**Small lungworm** (N/N examined (%))	**Small lungworm species** (N/N infected lungs (%))		***M. capillaris* nodule severity** (N/N *M.capillaris* infected lungs (%))		
				P. rufescens†	*M. capillaris*†	Mild lesions	Moderate lesions	Severe lesions
1	A	lamb	235/235 (100%)	9/235 (4%)	235/235 (100%)	130/235 (55%)	70/235 (30%)	35/235 (15%)
2	A	lamb	177/202 (87%)	9/177 (5%)	176/177 (99%)	138/176 (78%)	33/176 (19%)	5/176 (3%)
3	A	mutton	69/98 (70%)	4/69 (6%)	69/69 (100%)	57/69 (83%)	12/69 (17%)	0
4	B	lamb	157/221 (71%)	6/157 (4%)	156/157 (99%)	149/156 (96%)	7/156 (4%)	0
5	B	mutton	116/196 (59%)	14/116 (12%)	113/116 (97%)	102/113 (90%)	9/113 (8%)	2/113 (2%)
6	C	mutton	174/225 (80%)	50/173 (29%)	169/174 (97%)	106/169 (63%)	42/169 (25%)	21/169 (12%)

†N with both small lungworm species present: cohort 1= 9, cohort 2 = 8, cohort 3 = 4, cohort 4 = 5, cohort 5 = 11, cohort 6 = 45

Table 5 Frequency of pneumonia and small lungworm lesions observed at slaughter in age classes (N; (%); percentage represents column totals)

Age class			**Small lungworm status**		**Totals**
			Infected	Non infected	
lamb	**Pneumonia status**	Affected	68 (12%)	14 (16%)	82
		Not affected	501 (88%)	75 (84%)	576
	Totals		569	89	658
adult	**Pneumonia status**	Affected	35 (10%)	22 (14%)	57
		Not affected	324 (90%)	138 (86%)	462
	Totals		359	160	519

Table 6 Contingency table of frequencies for *M. capillaris* score using photographs and laboratory assessments

		Laboratory score				Totals
		Non infected	Mild	Moderate	Severe	
Photo score	Non infected	1	4	0	0	5
	Mild	0	14	3	1	18
	Moderate	0	4	9	0	13
	Severe	0	3	5	3	11
Totals		1	25	17	4	47

Table 7 Mean liveweight, body condition score (BCS), hot standard carcass weight (HSCW) and dressing percentage (95% CI) of lamb and adult according to infection status, and mean difference between infected and uninfected sheep (95% CI)

Age class	Non-infected	Infected[¥]	Mean difference[‡]	P value
	BCS 2 (1-5)			
adult	3.2 (2,4)	3.4 (2,5)	0.2 (0.0,0.4)	0.04
	Liveweight 2 (kg)			
lamb	53.2 (39,67)	53.7 (40,68)	0.5 (-0.7,1.7)	0.4
adult	57.5 (40,75)	58.8 (42,76)	1.3 (-0.2,3.0)	0.07
	HSCW (kg)			
lamb	23.4 (18,29)	23.6 (18,29)	0.2 (-0.4,0.8)	0.5
adult	21.3 (15,28)	21.5 (15,28)	0.2 (-0.5,0.9)	0.5
	Dressing percentage (%)			
lamb	44.0 (41,47)	44.0 (41,47)	0.0 (-0.8,0.8)	1.0
adult	37.3 (33,41)	36.9 (33,41)	-0.4 (-1.5,0.6)	0.4

‡ Means and mean differences from mixed model, positive mean difference indicates that there was a heavier mean for infected group, adjusted for cohort

¥ Presence of small lungworm lesions at slaughter

Table 8 Proportion of carcasses trimmed and condemned in cohorts 1 - 6

Cohort	**Farm**	**Age class**	**Carcass trimmed** (N/N observed (%))					**Carcass condemned**[†]
			overall	rib	leg	abdomen	other	
1[₣]	A	lamb	13/239 (5.4%)	6/239 (2.5%)	3/239 (1.3%)	0	4/239 (1.7%)	1/240 (0.4%)
2[±]	A	lamb	23/213 (10.8%)	8/213 (3.8%)	6/213 (2.8%)	9/213 (4.2%)	0	0
3	A	mutton	29/104 (27.9%)	14/104 (13.5%)	7/104 (6.7%)	4/104 (3.8%)	4/104 (3.8%)	2/106 (1.9%)
4[₣]	B	lamb	14/246 (5.7%)	3/246 (1.2%)	5/246 (2.0%)	6/246 (2.4%)	0	0
5	B	mutton	14/222 (6.3%)	4/222 (1.8%)	10/222 (4.5%)	0	0	0
6[¥]	C	mutton	64/245 (26.1%)	27/245 (11.0%)	4/245 (1.6%)	17/245 (7.0%)	16/245 (6.5%)	1/246 (0.4%)

[₣] 1 carcass trimmed in 2 locations, [±]2 carcasses trimmed in 2 locations, [¥]3 carcasses trimmed in 2 locations

[†]Condemned because of jaundice (cohort 3), emaciation (cohort 3,6), unknown (cohort 1)

9 Figure captions

Figure 1 Carcass weight according to presence of small lungworm infection (lung lesions detected at slaughter) for cohort 1-6 (cohort 5 excluded; cohort 1 all lambs infected)

10 Appendix A Supplementary material

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