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PII: S0304-4017(09)00404-X
Reference: VETPAR 4922

To appear in: Veterinary Parasitology

Received date: 13-11-2008
Revised date: 8-6-2009
Accepted date: 6-7-2009

Please cite this article as: Jacobson, C., Bell, K., Forshaw, D., Besier, R.B., Association between nematode larvae and “low worm egg count diarrhoea” in sheep in Western Australia, Veterinary Parasitology (2008), doi:10.1016/j.vetpar.2009.07.018

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Title:
Association between nematode larvae and “low worm egg count diarrhoea” in sheep in Western Australia

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Abstract

Nine flocks of sheep with a high prevalence (>30%) of diarrhoea and severe breech faecal soil ing were investigated over a three-year period to examine the causes of diarrhoea in sheep with low mean faecal worm egg count (WEC). All nine flocks were located in the south west of Western Australia in areas with a winter rainfall pattern (Mediterranean climate). There was no difference ($p=0.304$) in WEC of diarrhoeic sheep (loose faeces and severe breech faecal soil ing) and “normal sheep” (pelleted faeces and mild or no breech faecal soil ing). *Teladorsagia* (*Ostertagia*) *circumcincta* and *Trichostrongylus* spp were the nematodes most commonly identified by total worm counts and differentiation of larvae recovered from faeces and pasture. Larval stages of strongyle worms accounted for the largest proportion of total worm counts in both diarrhoeic and normal sheep. Adult worm burdens were small in most sheep. Diarrhoeic sheep had higher numbers of fourth stage larvae than normal sheep ($p=0.046$). There was no histopathological evidence of bacterial or viral causes of diarrhoea in any of the flocks that could be supported with bacteriology. Two flocks had marginal selenium glutathione peroxidase (selenium) levels. One flock was diagnosed with helminthosis based on rising WEC and high total worm counts. Larval hypersensitivity diarrhoea, nutritional factors or a combination of these two factors were the most likely causes of diarrhoea in the other eight flocks based on exclusion of other known causes of diarrhoea. Treatment with moxidectin and an ivermectin controlled-release capsule did not change faecal moisture content of treated sheep compared to untreated sheep three to five weeks after treatment. The findings suggest that the immune response to strongyle larvae may explain some cases of low WEC diarrhoea observed during winter-spring in immunocompetent mature sheep grazing in Mediterranean environments.

**Keywords:** diarrhoea; hypersensitivity; sheep-nematoda; faecal soil ing; controlled release technology
Introduction

Diarrhoea (also typically referred to as “scouring”) is a common and widespread problem of sheep in the south west of Western Australia. A questionnaire sent to farmers in the region in 2002 showed that moderate and severe faecal soiling of the wool around the perianal area (“breech”) and extending down the hindlimbs and tail region was more common in the winter and spring months with approximately half of the respondents reporting moderate or severe breech faecal soiling in flocks of lambs, hoggets and ewes (Jacobson, 2006). Diarrhoea is a major risk factor for breech faecal soiling (commonly referred to as “dag”) and is a production problem primarily because it increases the susceptibility of sheep to breech blowfly strike (Morley et al., 1976; Watts and Marchant, 1977). Removal of the soiled breech wool, treatment and prevention of blowfly strike is unpleasant work and represents a major economic cost for both sheep meat and wool producers (Bell, 1998).

Despite the widespread nature of the problem and the serious economic consequences for producers, very little is known about what are the common causes of diarrhoea in sheep of post-weaning age in Western Australia. A large number of infectious and non-infectious causes of diarrhoea having been identified in sheep (Bath, 2003; Farquarson, 1992; Glastonbury, 1990; Napthine, 1988), but full diagnostic investigations of cases of diarrhoea are labour intensive, expensive and often unrewarding in identifying an aetiology (or cause) of the diarrhoea.

Nematode parasites and nutritional factors associated with grazing lush green pasture are commonly implicated causes of diarrhoea in the south-west of Western Australia (Bath, 2003; Besier, 1998). Sheep that accumulate large nematode worm burdens (helminthosis) may show signs of diarrhoea associated with high faecal worm egg counts (WEC) (Besier, 2004). Alternatively, a condition has been described in which sheep that have a well-developed immunity to nematode parasites may develop “larval hypersensitivity diarrhoea” syndrome associated with intake of larvae with pasture (Larsen et al., 1999). Sheep with a well-developed immunity to nematodes generally do not develop high WEC and so larval hypersensitivity diarrhoea syndrome is typically associated
with “low WEC diarrhoea”. There are no specific diagnostic tests or post-mortem findings available for larval hypersensitivity diarrhoea syndrome and so diagnosis is generally made by exclusion of other causes. Although nutritional factors that may result in diarrhoea have not been well described in the scientific literature, “nutritional diarrhoea” is also a common diagnosis of exclusion in diarrhoeic sheep grazing lush pasture that are otherwise healthy and where no other cause of diarrhoea can be identified (Bath, 2003).

This study aimed to investigate the causes of “low WEC diarrhoea” in flocks of sheep in the south west of Western Australia.

Materials and methods
Selection of flocks

Nine flocks of sheep from eight properties were investigated over a three-year period. Farmers, veterinarians and advisors reported flocks with suspected “low worm egg count diarrhoea” for consideration for inclusion in the study. Requirements for inclusion in the investigation were that sheep were at least 14 months old when diarrhoea or fresh breech faecal soiling was initially observed (ie sheep were expected to have developed a functional immunity to nematode infection), sheep were actively diarrhoeic and had a mean flock WEC of less than 150 eggs per gram of faeces (epg) performed within two weeks of the visit. Only the ewes from the Bindoon and Tenterden properties were grazing with unweaned lambs and the other flocks would not have been subject to the periparturient relaxation of resistance (Leathwick et al., 1999; McAnulty et al., 2001).

All flocks were Merino sheep, except on the Bindoon property (Damara and Merino-Damara crosses). All nine flocks were investigated in the months of August to October (initial visit). Annual rainfall for the year in which sheep were sampled varied from 315 to 780 mm/annum on the nine farms. All nine properties had a Mediterranean environment characterised by cool wet winter and hot dry summer. The initial visits were conducted in August (Kendenup and Boyup...
Brook), September (Bokerup, Bindoon, Dudinin, Tenterden and Broomehill) and October (Kojonup).

Procedures for sample collection

On the day of sampling, sheep were yarded and a sample of at least 100 sheep were “dag scored” using a scale of 0 (no breech faecal soiling) to 5 (severe breech faecal soiling) (Jacobson, 2006; Larsen et al., 1994). Three flocks had been recently crutched and these were scored using a modified scale of 0 (no evidence of faecal soiling since crutching) to 3 (extensive evidence of faecal soiling since crutching) (Jacobson, 2006).

Faecal samples were collected from “diarrhoeic sheep” (active diarrhoea and severe breech faecal soiling) and “normal sheep” (pelleted faeces and mild or no breech faecal soiling) for a modified McMaster WEC and larval differentiation using methods described in Australian Standard Diagnostic Techniques for Animal Diseases Manual (Lyndal-Murphy, 1993). Faecal samples from the diarrhoeic and normal sheep were pooled separately for larval differentiation. Pasture samples were collected for pasture larval counts (Jacobson, 2006).

Two or three diarrhoeic sheep and two or three normal sheep from each flock were euthanased for post-mortem examination. In total, 22 diarrhoeic sheep and 21 normal sheep underwent post-mortem examination from the 9 flocks. Samples of the gastrointestinal tract were taken from the rumen, abomasum (fundus and pylorus), jejunum (1m and 4m from common bile duct), ileum, caecum, proximal, spiral and distal colon, fixed in 10% neutral buffered formalin and used for histopathology (Jacobson, 2006). Blood samples were collected for measurement of glutathione peroxidase (GSHPx). Sterile swabs were taken from the jejunum and large intestine for bacteriology. Mucosal scrapings were taken from the caecum and colon. Total worm counts (TWC) and differentiation of nematodes from the abomasum and small intestine were conducted, including abomasal digest (Jacobson, 2006; Lyndal-Murphy, 1993). Specifically, fourth stage larvae (L4) were described as early L4 (undifferentiated larvae immediately after third stage moult and approximately
1.6mm in length) or developing L₄ (approximately twice the size of the early L₄, exhibiting some
differentiation such as a bursa in males, but no features enabling species differentiation).

A sample of at least 20 actively diarrhoeic sheep with severe breech faecal soiling were
treated with a moxidectin drench (Cydectin®, Fort Dodge Australia Pty Ltd) and an ivermectin
controlled-release capsule (Ivomec Maximizer Controlled-release Capsule for Adult Sheep®, Merial
Australia Pty Ltd). Faecal samples were collected for WEC from the treated sheep and a sample of
20 untreated control sheep that were also actively diarrhoeic with severe breech faecal soiling. The
treated and control sheep were identified and faeces were collected from these sheep between 21
and 49 days after treatment for measurement of WEC, faecal dry matter and faecal consistency
from 8 and 3 of the flocks respectively (Jacobson, 2006; Larsen, 2000).

Statistical Analysis
Categorical data were analysed using a Chi-square test for two-sided significance,
specifically Pearson Chi-square or Fisher’s exact test. Variances were tested using Levene’s test of
equality of error variance. Blood glutathione peroxidase and faecal consistency measurements were
normally distributed and the variances were not significantly different (p>0.05) and so these
parameters were analysed using ANOVA. The distribution of log-transformed WEC and TWC were
non-normal and the variance of the WEC in diarrhoeic and normal sheep were significantly
different (p<0.05), therefore WEC and TWC were compared using a two-tailed non-parametric
Mann-Whitney U test.

All statistical analyses were performed using SPSS 11.0.2 for Mac OS X.

Results
Breech faecal soiling distribution
The estimated proportion of sheep with moderate or severe breech faecal soiling (dag
score 3.0 or higher for the six flocks of uncrutched sheep, modified dag score 2.0 or higher for the
three flocks of recently crutched sheep) ranged from 30 to 85% with a mean of 48% in the nine flocks.

WEC and larval differentiation

The WEC in the diarrhoeic and normal sheep are shown in Table 1. There was no significant difference in the mean strongyle WEC of the diarrhoeic or normal sheep in any flock or for all sheep combined ($p=0.304$).

The strongyle species cultured from faeces were predominantly *Teladorsagia (Ostertagia) circumcincta* and *Trichostrongylus* spp. *Haemonchus contortus* was identified in one flock (Bokerup orange tags), but the mean flock WEC was low (100epg) and *H. contortus* accounted for only 8% of the larval differentiation.

Total Worm Counts

The mean TWC are shown in Table 2. Larval stages accounted for the largest proportion of the TWC. There was a trend towards a higher TWC ($p=0.089$) in the diarrhoeic sheep compared with the normal sheep. Diarrhoeic sheep had more fourth stage larvae (L₄) than normal sheep ($p=0.046$). There was no significant difference in the total number of adult *Teladorsagia* ($p=0.217$), *Trichostrongylus* ($p=0.710$), *Nematodirus* ($p=0.265$) or *Haemonchus* ($p=1.000$) recovered from the diarrhoeic sheep compared with the normal sheep. *T. colubriformis* and *T. vitrinus* were the *Trichostrongylus* species most commonly isolated. *T. axei* was identified in sheep from four flocks (Broomehill, Bindoon, Bokerup orange and Bokerup mixed tag). *T. rugatus* was identified in sheep from the Dudinin property. There was no significant difference in either abomasal *T. axei* ($p=0.648$) or intestinal *Trichostrongylus* spp. ($p=0.868$) between diarrhoeic and normal sheep.
Pasture larval counts

The pasture larval count results are shown in Table 3. The pasture larval counts fell into three distinctive categories: “low” (less than 500 larvae/kg pasture dry matter), “medium” (1000-2000 larvae/kg pasture dry matter) and “high” (more than 6000 larvae/kg pasture dry matter).

Tel. circumcincta and Trichostrongylus spp. were the nematode species most commonly isolated and differentiated from pasture grazed by the diarrhoeic flocks on the nine farms. H. contortus was cultured from pasture from only one paddock (Bokerup orange tag) from which H. contortus represented 5% of larvae recovered from the pasture sampled.

Treatment with controlled-release capsules

Treatment of sheep with a moxidectin drench and an ivermectin CRC was effective at reducing the WEC of treated sheep 21 to 49 days after treatment on all of the properties tested with a positive egg count identified in only a single sheep (50 epg at the Boyup Brook property).

Mean faecal dry matter of faeces post-treatment are shown in Table 4. Post-treatment faecal consistency scores of treated and control sheep were also measured on the Dudinin, Tenterden and Broomehill properties and the results are shown in Table 4. There was no significant difference in the faecal dry matter content of the faeces (eight flocks, p=0.244) or the faecal consistency score (three flocks, p=1.000) of the treated sheep compared with the untreated sheep post-treatment.

Histopathology

Of the 43 sheep that underwent post-mortem examination, one normal sheep from the Broomehill property had histopathological evidence of a recognised non-parasitic cause of diarrhoea, specifically microabscessation of the duodenum consistent with yersiniosis. One diarrhoeic sheep (Bindoon) and one normal sheep (Dudinin) had histological changes consistent
with coccidiosis, however the same diarrhoeic sheep from Bindoon also had a very high TWC (31000 worms).

There were no consistent differences in the nature or severity of morphological changes in the diarrhoeic sheep compared with the normal sheep. The most common histological finding was “globule leukocyte hyperplasia” in the small intestine and/or large intestine and caecum. There was no significant difference in the number of diarrhoeic sheep (16/22) with evidence of enteritis compared with the normal sheep (12/21, Pearson Chi-square p=0.284). The cellular infiltrate was described as “eosinophilic” in 20/28 sheep with evidence of enteritis, but there was no significant association between a diagnosis of “eosinophilic enteritis” and diarrhoea (p=0.280).

There was a trend towards histological changes consistent with gastrointestinal parasitism (including atrophy, clubbing, blunting and/or fusion of the small intestinal villi) being more commonly observed in the diarrhoeic sheep (14/22) compared with the normal sheep (8/21, Pearson Chi-square p=0.090).

*Bacteriology*

None of the post-mortem examinations identified known pathogenic bacteria in conjunction with histological changes indicative of bacterial disease. *Salmonella* spp. were not isolated from any sheep in the study.

One normal sheep from the Broomehill property had histological evidence of yersiniosis but no *Yersinia* spp. was cultured to support this diagnosis. *Yersinia enterocolitica* was cultured from three normal sheep from the Kojonup property, but there were no histopathological changes consistent with yersiniosis in any of the sheep from this property.
Campylobacter-like organisms were observed in mucosal scrapings taken from sheep from the Bokerup (mixed age) and Tenterden properties, but there was no histological evidence of campylobacteriosis in any of the post-mortem examinations of sheep from these properties.

Selenium

Serum GSHPx was above the reference range (>50U/g Hb GSHPx) in all of the sheep from all of the properties except for Kojonup and Broomehill. The flock mean GSHPx for the Broomehill property was 49.8 U/g Hb and for the Kojonup property was 47.2U/g Hb. There was no histopathological evidence or history of clinical signs consistent with nutritional myopathy in either of these flocks. There was no significant difference in GSHPx in the diarrhoeic sheep (178U/g Hb) compared with the normal sheep (204U/g Hb, ANOVA p>0.609).

Discussion

The finding that WEC were similar in the diarrhoeic and normal sheep was consistent with other studies demonstrating that susceptibility to diarrhoea and breech faecal soiling is not necessarily associated with the size of WEC or adult worm burdens, particularly in adult sheep (Larsen et al., 1999; Larsen et al., 1994). WEC were not corrected for faecal moisture and although increased faecal moisture may have served to dilute the WEC in the diarrhoeic sheep (Le Jambre et al., 2007) it seems unlikely that this would have a significant effect due to the minor differences in WEC between the 9 flocks or between the diarrhoeic and normal sheep within flocks on most properties. It therefore appears that in eight of the nine flocks, the likely cause of diarrhoea was consistent with that described for the larval hypersensitivity syndrome (Larsen et al., 1999). Correcting WEC for faecal dry matter in future studies may provide further useful information on the relative magnitude of WEC in diarrhoeic and normal sheep.

As the pathogenesis of this larval hypersensitivity diarrhoea syndrome has not been fully described and there are no diagnostic tests or specific clinical findings currently available to
confirm the diagnosis (Larsen et al., 1999), a presumptive diagnosis of larval hypersensitivity diarrhoea is usually made where other known causes of diarrhoea have been ruled out. There was no confirmed evidence of bacterial causes of diarrhoea in the nine flocks studied. This suggested that known bacterial agents were unlikely to be the predominant cause of diarrhoea in the nine flocks investigated. One normal sheep from the Broomehill property with no evidence of active diarrhoea or breech faecal soiling had histological changes consistent with yersiniosis. Yersinia spp. were cultured on the Kojonup property, but there was no histological changes to support a diagnosis of yersiniosis in this flock. Interestingly, these were the same two flocks with marginal selenium status.

*Camplylobacter*-like organisms were observed in sheep from Bokerup (mixed age wethers) and Tenterden, but as with the *Yersinia* spp., there were no histological changes to confirm a diagnosis of bacterial diarrhoea. Both *Yersina* spp. and *Campylobacter* spp. can be isolated from healthy sheep and diagnosis of bacterial diarrhoea is based on a combination of history of “stress” (ie shearing, inclement weather), clinical signs, histopathological changes and identification of the causative organism on mucosal scrapings or culture (Glastonbury, 1990).

Trace element deficiencies also appeared unlikely causal factors for the diarrhoea observed, although all properties with the exception of Dudinin were located in areas of known selenium deficiency. The selenium status was adequate in seven of the flocks, and even on the two properties where GSHPx was marginal (Broomehill and Kojonup), it was not clear whether the selenium levels were sufficiently low to cause clinical signs. With the exception of the Kojonup property (*n*=16), the number of sheep sampled was too small to comment on the selenium status of the flock as a whole. In any case, selenium deficiency is considered to be inconsistently associated with diarrhoea (Bath, 2003). It has been suggested that selenium deficiency may result in increased susceptibility to internal parasites (Glastonbury, 1990), but several reviews have concluded that there is no evidence that selenium status has any influence on parasite establishment (Lee et al., 2002; McClure, 2003; Suttle and Jones, 1989).
Specific nutritional factors have not been clearly associated with diarrhoea in sheep in Australia, although interactions between pasture status and the rate of larval intake have been suggested as influencing the severity of diarrhoea observed (Larsen, 2000). Grazing of lush green pasture typically occurs concurrently with relatively high levels of larval intake during winter and spring in Mediterranean environments, and it is hence difficult to isolate there factors (Jacobson, 2006).

The primary purpose of the histological examinations was to rule out recognised causes of diarrhoea. The results showed that histopathological evidence of known causes of diarrhoea other than strongyle worm infections were uncommon in the adult sheep included in the study.

Histologic findings lend support to larval hypersensitivity as a causal factor. Eosinophilic enteritis and villous atrophy were common findings and may represent pathology attributable to larval hypersensitivity diarrhoea syndrome (Larsen et al., 1994), although neither finding is specific to the syndrome. As there is no published descriptive grading system for the histological evaluation of “eosinophilic enteritis” in sheep, it is difficult to determine whether eosinophilic infiltration in any given case is “normal” or indicative of “disease”. Further objective measurements such as villous:crypt ratios and differential cell counts would be required to evaluate if villous atrophy or eosinophilic infiltration of the pyloric and jejunal mucosa were associated with diarrhoea in the flocks in this study.

A further indication that nematode larval intake may be causally involved with diarrhoea is the level of larval intake. Assuming consumption of 1kg herbage dry matter per day, all flocks except at Kojonup and Kendenup had an estimated larval intake in excess of 2000 L₃ per week, which has been demonstrated as sufficient to induce diarrhoea in adult sheep susceptible to severe breech faecal soiling (Larsen et al 2000).

It is of interest that although flocks were chosen on the basis of low flock worm egg counts, a number of sheep were found to have high total worm counts and larval stages accounted for the largest proportion of the nematode populations. It is well recognised that WEC have a poorer
correlation with TWC in mature sheep (McKenna, 1981), and naturally do not reflect the magnitude
of infection with larval stages of nematodes. The increased numbers of L₄ in the diarrhoeic sheep
was a novel finding and cannot be easily explained with the existing level of knowledge. The larval
stages recovered on TWC were not identified by genus, but the largest proportion of L₄ were
recovered from the abomasal contents and digest suggesting that the majority were Tel.
circumcincta as no adult H. contortus were identified in any of the TWC.

A proportion of the early L₄ recovered may have been arrested Tel. circumcincta larvae,
but it was not possible to differentiate arrested larvae from developing larval stages. There was no
reason to suspect different levels of larval intake in diarrhoeic and normal sheep grazing the same
pasture and there was no difference in the number of L₃ recovered from the diarrhoeic and normal
sheep. The pattern of hypobiosis of Tel. circumcincta in Western Australia is not well understood.
L₄ are commonly observed that may well be inhibited, and parasitologists have not recognised
hypobiosis as a routine phenomenon in Tel. circumcincta or T. colubriformis in this region. This
may be in part because the widespread practice of summer drenching would remove arrested L₃ (T.
colubriformis) and L₄ (Tel. circumcincta) before the resumption of development. If the level of
arrested development is mediated mainly by host immunity (Dobson et al., 1990; Eysker, 1978;
Seaton et al., 1989), then presumably genetic variation in the immune response could be expected.
Several authors have suggested a genetic relationship determining susceptibility to diarrhoea and
severe breech faecal soiling that is independent of WEC (Bisset et al., 1994; Greeff and Karlsson,
1998; Larsen et al., 1995; Pollott et al., 2004; Shaw et al., 1999). If higher numbers of L₄ are found
to be consistently associated with diarrhoea, then this may provide a basis for further investigation
of potential pathophysiological mechanism(s) behind “low WEC diarrhoea” in mature sheep.

It should be noted that on the Bindoon property helminthosis was more likely that this
flock was diarrhoeic due to high worm burdens than larval hypersensitivity diarrhoea, as WEC had
risen sharply in the seven days between the “screening WEC” and the investigation and the sheep
had very high TWC. This indicates the importance of repeated monitoring of WEC and conducting TWC in cases of apparent “low WEC diarrhoea”.

Two or three scouring and normal sheep were used for TWC from each of the nine flocks. The decision was made to perform post-mortem examination on sheep from a number of different properties and over a three-year period to gain a “representative sample” of sheep with low WEC scouring across the south-west of Western Australia. The finding of higher numbers of L₄ in scouring sheep was previously unreported and warrants further investigation, particularly the effect of “flock”, “farm” and “year” on the number of L₄ in scouring and normal sheep. It was not feasible to examine these effects in the present study, but these should be addressed in further investigations.

There was no appreciable difference in faecal dry matter (eight farms) or faecal consistency (three farms) of the treated sheep compared with the control sheep 21 to 49 days post-treatment. This was surprising given that other studies have found that CRC are effective at reducing dag scores in treated sheep (Allerton et al., 1998; Gogolewski et al., 1997a; Gogolewski et al., 1997b; Larsen et al., 1994; Macchi et al., 2001; Rehbein et al., 1999), although some studies have shown CRC may not reduce breech faecal soiling in all instances (Allerton et al., 1998; Webb Ware et al., 2000). Differences in the response to treatment with CRC may be due to a number of factors, including timing of treatment, faecal parameters measured, interval between treatment and measurement of response and the active ingredient used. The majority of studies measured the differences in dag weight and/or dag score in the treated and untreated sheep. In these studies it appears that CRC were given before severe breech faecal soiling was evident in a large proportion of sheep, presumably prior to or soon after the onset of diarrhoea (Allerton et al., 1998; Gogolewski et al., 1997a; Gogolewski et al., 1997b; Macchi et al., 2001; Rehbein et al., 1999; Webb Ware et al., 2000) rather than after sheep were actively diarrhoeic and with a high proportion with severe breech faecal soiling as was the case in this experiment. It is therefore possible that CRC may be more effective at preventing breech faecal soiling if given to sheep prior to or soon after the onset of diarrhoea. It is also possible that the three-week interval following treatment with CRC and
moxidectin was not sufficient to allow for recovery of the gastrointestinal tissues and to observe any resulting effect of treatment on faecal consistency (Angus et al., 1979). It is possible that ivermectin CRC had no effect on faecal dry matter or consistency due to the ongoing antigenic stimulation in the treated sheep or due to ivermectin resistance. Whilst the ivermectin CRC may have been effective at killing incoming larvae, the larvae may not have been killed quickly enough to eliminate antigenic triggering of a hypersensitivity response. Ivermectin resistance is common in Western Australia can result in reduced efficacy of the drug to prevent establishment of incoming larvae (Barnes et al., 2001; Leathwick et al., 2002; Sutherland et al., 1997; Sutherland et al., 1999).

There was evidence of ivermectin resistance on the Bindoon property (86% efficacy) but not on the most recent WEC reduction test on the Kendenup, Boyup Brook, Bokerup and Broomehill properties. A recent WEC reduction test had not been performed on the other properties.

Conclusion

Investigation of diarrhoea in nine flocks with low flock WEC found that although helminthosis was the likely cause of diarrhoea in one flock, no definitive diagnosis could be made in the other eight flocks despite extensive diagnostic work. Bacterial infections or selenium deficiencies did not appear to be a common cause of diarrhoea in the flocks investigated. It is considered that the most likely cause of diarrhoea was intake of nematode larvae in worm-immune sheep and associated larval hypersensitivity diarrhoea (Larsen et al., 1999), although diarrhoea related to dietary factors in pasture could not be ruled out and may contributed to the severity of diarrhoea observed. A striking finding was increased numbers of L₄ in diarrhoeic sheep compared to those with no evidence of diarrhoea.

Acknowledgements

We thank Mal Boyce, Robin Jacob, Kylie Blades, Alexandra Pugh, Brian Taylor, Rob Suter and Michylla Seal for their help with the field work and to the staff in the Animal Health Laboratories
in Albany and South Perth for processing the samples. Robert Dobson assisted with statistical analysis. We also gratefully acknowledge the farmers who contributed sheep and allowed us to use their facilities. Caroline Jacobson’s scholarship was funded in-part by Meat and Livestock Australia and the Australian Sheep Industry CRC.

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Table 1: Arithmetic mean faecal strongyle worm egg counts (WEC) in diarrhoeic and normal sheep

(Mann-Whitney U non-parametric test for significance)

<table>
<thead>
<tr>
<th>Property</th>
<th>WEC (eggs per gram ± standard error (range) n)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diarrhoeic sheep</td>
<td>Normal sheep</td>
</tr>
<tr>
<td>Kendenup</td>
<td>42±19 (0-550) n = 33</td>
<td>725±325 (400-1050) n = 2</td>
</tr>
<tr>
<td>Boyup Brook</td>
<td>180±58 (0-1050) n = 23</td>
<td>204±141 (0-1800) n = 13</td>
</tr>
<tr>
<td>Kojonup</td>
<td>87±72 (0-2250) n = 31</td>
<td>4±4 (0-50) n = 13</td>
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<tr>
<td>Bokerup-orange</td>
<td>104±26 (0-400) n = 27</td>
<td>100±18 (0-200) n = 12</td>
</tr>
<tr>
<td>Bokerup-mixed</td>
<td>65±28 (0-750) n = 27</td>
<td>112±81 (0-1000) n = 12</td>
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<tr>
<td>Bindoon</td>
<td>476±115 (0-2100) n = 27</td>
<td>525±168 (0-1650) n = 12</td>
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<tr>
<td>Dudinin</td>
<td>163±65 (0-1900) n = 36</td>
<td>154±98 (0-1300) n = 13</td>
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<tr>
<td>Tenterden</td>
<td>36±20 (0-700) n = 39</td>
<td>71±66 (0-800) n = 12</td>
</tr>
<tr>
<td>Broomehill</td>
<td>126±31 (0-1000) n = 43</td>
<td>66±19 (0-200) n = 14</td>
</tr>
<tr>
<td>All flocks</td>
<td>134±19 (0-2250) n = 286</td>
<td>163±35 (0-1800) n = 103</td>
</tr>
</tbody>
</table>

n/a: not applicable (WEC conducted on only two “normal” sheep)

n: number of sheep sampled
Table 2: Arithmetic mean total worm counts ± standard error (SE) in diarrhoeic and normal sheep
(Mann-Whitney U non-parametric test for significance)

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Diarrhoeic sheep (n=22)</th>
<th>Normal sheep (n=21)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ±SE</td>
<td>range</td>
<td>mean ±SE</td>
</tr>
<tr>
<td><strong>Adult nematodes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tel. circumcincta</td>
<td>368±127</td>
<td>0-2000</td>
<td>224±91</td>
</tr>
<tr>
<td>T. axei</td>
<td>539±358</td>
<td>0-7550</td>
<td>283±134</td>
</tr>
<tr>
<td>Intestinal Trichostrongylus spp.</td>
<td>2668±1363</td>
<td>0-24 200</td>
<td>2069±1357</td>
</tr>
<tr>
<td>H. contortus</td>
<td>0±0</td>
<td>0-0</td>
<td>0±0</td>
</tr>
<tr>
<td>Nematodirus spp.</td>
<td>118±69</td>
<td>0-1200</td>
<td>52±37</td>
</tr>
<tr>
<td><strong>Immature nematodes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immature adults/L₃</td>
<td>70±30</td>
<td>0-600</td>
<td>133±61</td>
</tr>
<tr>
<td>All L₄</td>
<td>14 273±3997</td>
<td>100-72 050</td>
<td>6300±1832</td>
</tr>
<tr>
<td>Early L₄</td>
<td>13 860±3895</td>
<td>100-70 500</td>
<td>5905±1733</td>
</tr>
<tr>
<td>Developing L₄</td>
<td>414±152</td>
<td>0-2500</td>
<td>395±216</td>
</tr>
<tr>
<td>TOTAL IMMATURE/LARVAE (excluding L₃)</td>
<td>14 343±4014</td>
<td>100-72 050</td>
<td>6433±1861</td>
</tr>
<tr>
<td><strong>TOTAL NEMATODES (excluding L₃)</strong></td>
<td>18 055±4925</td>
<td>100-86 600</td>
<td>9057±2778</td>
</tr>
<tr>
<td>L₃</td>
<td>636±138</td>
<td>0-2250</td>
<td>631±178</td>
</tr>
</tbody>
</table>

8 L₅: fifth stage larvae
9 L₄: fourth stage larvae
10 L₃: third stage larvae
11 n: number of sheep sampled
Table 3: Pasture larval counts

<table>
<thead>
<tr>
<th>Property</th>
<th>Larvae/kg pasture DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kendenup</td>
<td>73</td>
</tr>
<tr>
<td>Boyup Brook</td>
<td>1052</td>
</tr>
<tr>
<td>Kojonup</td>
<td>68</td>
</tr>
<tr>
<td>Bokerup (orange tag)</td>
<td>1780</td>
</tr>
<tr>
<td>Bokerup (mixed tag)</td>
<td>1018</td>
</tr>
<tr>
<td>Bindoon</td>
<td>6424</td>
</tr>
<tr>
<td>Dudinin</td>
<td>767</td>
</tr>
<tr>
<td>Tenterden</td>
<td>6140</td>
</tr>
<tr>
<td>Broomehill</td>
<td>330</td>
</tr>
</tbody>
</table>

DM: dry matter
Table 4: Mean faecal dry matter and mean faecal consistency score (scale 1-5) for treated and untreated diarrhoeic sheep 21-49 days after treatment with moxidectin and ivermectin controlled-release capsule (ANOVA test for significance)

<table>
<thead>
<tr>
<th>Property</th>
<th>Treated</th>
<th>Untreated</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Faecal dry matter (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kendenup</td>
<td>17.7±0.9</td>
<td>18.3±0.6</td>
<td>0.603</td>
</tr>
<tr>
<td>Boyup Brook</td>
<td>19.0±1.2</td>
<td>21.2±1.5</td>
<td>0.250</td>
</tr>
<tr>
<td>Kojonup</td>
<td>21.1±1.5</td>
<td>20.5±1.3</td>
<td>0.768</td>
</tr>
<tr>
<td>Bokerup (orange)</td>
<td>21.2±0.7</td>
<td>20.8±0.9</td>
<td>0.675</td>
</tr>
<tr>
<td>Bokerup (mixed)</td>
<td>18.2±0.6</td>
<td>20.9±1.2</td>
<td>0.090</td>
</tr>
<tr>
<td>Bindoon</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dudinin</td>
<td>15.8±0.6</td>
<td>15.8±0.7</td>
<td>0.969</td>
</tr>
<tr>
<td>Tenterden</td>
<td>18.6±0.6</td>
<td>18.2±0.6</td>
<td>0.620</td>
</tr>
<tr>
<td>Broomehill</td>
<td>21.7±0.8</td>
<td>21.2±1.0</td>
<td>0.665</td>
</tr>
<tr>
<td>TOTAL</td>
<td>18.9±0.4</td>
<td>19.7±0.4</td>
<td>0.172</td>
</tr>
</tbody>
</table>

| **Faecal consistency (score 1-5)** |         |           |         |
| Dudinin           | 3.8±0.1 | 3.5±0.1   | 0.091   |
| Tenterden         | 3.8±0.1 | 4.0±0.1   | 0.181   |
| Broomehill        | 3.8±0.1 | 3.9±0.2   | 0.828   |
| TOTAL             | 3.8±0.1 | 3.8±0.1   | 1.000   |