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Outbreaks of diarrhoea ('winter scours') in weaned Merino sheep in south-eastern Australia

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Objective Identify the cause of outbreaks of diarrhoea during winter that are not attributable to gastrointestinal nematodes in weaned Merino sheep in the high rainfall regions of south-eastern Australia and determine the efficacy of antimicrobials used to treat this syndrome.

Methods We investigated 45 outbreaks on 24 farms. Faecal samples from affected animals were cultured for *Yersinia*, *Campylobacter* and *Salmonella*

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spp. Risk factors, including rainfall, temperature and worm egg count (WEC), were assessed. *Yersinia* spp. were identified with molecular tests and susceptibility to four antimicrobials was determined.

Results *Yersinia pseudotuberculosis* serotype III and virulent *Y. enterocolitica* were most frequently isolated. The frequency and severity of disease varied between region, farm and year. *Yersinia pseudotuberculosis* was detected only during winter, but *Y. enterocolitica* was present in all seasons. Pathogenic *Yersinia* species were more often isolated when WECs exceeded 500 eggs/g. A high proportion of *Y. enterocolitica* and *Y. pseudotuberculosis* were resistant to sulfafurazole (64% and 86.9%, respectively).

Conclusions A bacterial enteritis caused by pathogenic *Yersinia* was the cause of the winter scours syndrome in the 24 flocks investigated. The use of molecular testing increased the sensitivity of detection and identification of *Yersinia* spp. No clear association between weather, WEC and disease was established, suggesting complex interactions between risk factors are more important than any single factor. Sulfonamides should not be routinely used to treat this syndrome. Rather, during an outbreak the targeted use of an effective antimicrobial, such as oxytetracycline, should be integrated with grazing management strategies, including moving affected mobs onto lower risk pastures and decreasing the stocking rate.

Keywords antibiotic resistance; bacterial enteritis; diarrhoea; sheep; *Yersinia* spp.

Abbreviations bp, base pairs; DM, dry matter; DSE, dry sheep equivalents; epg, eggs per gram; NSW, New South Wales; WEC, worm egg count

Diarrhoea during winter ('winter scours') is a common problem of sheep in the high rainfall regions of south-eastern Australia and has been described in young Merino sheep (< 16 months old). Outbreaks typically occur between July and October, when temperatures are low, rainfall is high (> 500 mm/year) and there is often limited pasture availability (1–2 cm or 400–600 kg dry matter/hectare [DM/ha]).^{1,2} It has been hypothesised that a complex interaction between environmental, management and animal risk factors precipitates an outbreak, but studies examining these interactions are lacking. Sheep veterinary consultants in this region note that the frequency and severity of outbreaks are increasing, especially on farms with high stocking rates (J. Webb-Ware, pers. comm.).

During an outbreak, up to 60% of sheep can be affected and the mortality rate can exceed 10%.³ Affected animals present with acute or subacute enteritis characterised by profuse, watery diarrhoea, teeth grinding and signs of abdominal discomfort ('winter scours syndrome'). A chronic scouring syndrome associated with weight loss and ill thrift has also been reported, but is less common.

Clinical experience and previously published reports^{2,4} have noted an association between elevated worm egg counts (WECs) and the winter scours syndrome, but the acute syndrome often occurs when WECs are low and persists despite treatment with an effective anthelmintic. Affected animals frequently respond to treatment with antibiotics, including sulfonamides or tetracycline, suggesting that bacterial enteritis is an important component of this syndrome.

The overall costs of the winter scours syndrome have not been estimated, but costs associated with bacterial enteritis, including deaths, lost productivity and treatment, have been estimated at A\$10–29 million annually.^{5,6} However, these estimates may be conservative because there are limited epidemiological data to support the assumptions used in those reports. In addition, gastrointestinal parasitism is often a presumptive diagnosis for scouring weaners and so winter scouring associated with bacterial enteritis may be more common than realised. *Campylobacter*, *Salmonella* and *Yersinia* spp. are the bacterial agents associated with this syndrome. Virulent *Y. enterocolitica* and *Y. pseudotuberculosis* are most commonly isolated from sheep with acute gastroenteritis, but sporadic outbreaks associated with *Campylobacter* or *Salmonella* spp. have been reported. However, outbreaks of salmonellosis are typically associated with periods of nutritional stress, confinement feeding, transportation or overcrowding and there are usually more deaths.^{1,7} Outbreaks associated with *C. jejuni* or *C. coli* occur most frequently during summer and typically have fewer deaths than outbreaks of yersiniosis.¹ A syndrome of diarrhoea associated with colitis and infection with a *Campylobacter*-like organism has also been described in East Gippsland in summer.⁸

These organisms have also been isolated from the faeces of healthy animals, suggesting that a complex interaction between environmental, management and animal factors, such as weather and weaning or shearing, are necessary for the development of disease.^{3,9} Results from case series investigations have identified several risk factors, including inclement weather and an increased burden of gastrointestinal nematodes, but these are not always associated with disease.^{2,4}

To date, no studies have specifically investigated the epidemiology of risk factors for the winter scours syndrome in south-eastern Australia and so our understanding is limited to clinical reports and case series investigations. The aims of this study were to identify the primary agents responsible for outbreaks of the winter scours syndrome of Merino weaners, to identify potential risk factors for this disease that require further investigation and evaluate the efficacy of antibiotics used for treatment.

Materials and methods

Study design

A case series investigation was conducted between June 2012 and October 2013 using targeted sampling during outbreaks of winter scours identified using a network of veterinary consultants, Government veterinarians and existing Mackinnon Project clients. Only outbreaks in 3–16-month-old Merino weaners in the high rainfall regions of south-eastern Australia (> 500 mm annual rainfall) were investigated. An outbreak was defined as acute onset of diarrhoea, of less than 1-week duration, with a minimum morbidity of 25% within an affected mob and evidence of systemic disease, including inappetence, teeth grinding, lethargy and dehydration.

Faecal samples were collected from 10–40 scouring sheep from each affected mob, before the administration of antibiotics, for parasitology and microbiology. A full postmortem examination was conducted on 1–3 representative animals from a subset of animals that had either died within 2 h of a farm visit or were euthanased after showing signs of severe disease.

Postmortem examinations

Necropsies were conducted on farm and faecal samples and mucosal scrapings were submitted for bacterial culture. Faecal samples were also examined for WECs and fresh and formalin-fixed tissues from the duodenum, ileum, jejunum, large intestine and caecum were cultured and examined by histopathology. Samples were collected within 4 h of death or euthanasia of the animal.

Parasitology

WECs were performed using a modified McMaster technique with a minimum detectable limit of 15 eggs/g (epg).¹⁰ Burdens of coccidia and *Moniezia* spp. were each estimated using a scale of 0 (none) to 3 (heavy).

Microbiology

Faecal and postmortem samples were screened for the presence of *Yersinia*, *Campylobacter* and *Salmonella* spp. by inoculation onto selective media. *Yersinia* spp. were isolated using cefsulodin-irgasan-novobiocin agar (Edwards Pty Ltd, Aust) incubated aerobically for 24–48 h at 30°C. All dark-purple colonies 0.5–5 mm in diameter were subcultured onto sheep blood and MacConkey agar plates (Edwards Pty Ltd, Aust) for further investigation. *Campylobacter* spp. were isolated using Skirrows agar (Media Preparation Unit, University of Melbourne, Aust). Samples were incubated under micro-aerophilic conditions (5% O₂, 10% CO₂ and 85% N₂; CampyGen™, Oxoid Ltd, UK), at both 37°C and 42°C for 48 h. Suspect colonies were stained with dilute carbol fuchsin and morphologically positive samples were subcultured for further analysis. Isolates that showed enhanced growth at 42°C and were positive for sodium hippurate hydrolysis were identified as *C. jejuni*. Those showing enhanced growth at 42°C, but were negative for hippurate hydrolysis, were identified as *C. coli*.

Salmonella spp. were isolated using selenite mannitol enrichment broth incubated at 37°C for 24 h. A subculture of the broth was made onto xylose lysine deoxycholate agar (Media Preparation Unit, University of Melbourne, Aust) and incubated at 37°C for 24 h. No *Salmonella* isolates were detected during this study.

Identification and serotyping of Yersinia spp. Approximately 10% of suspect *Yersinia* spp. were biochemically typed using commercial kits (API 20E®, Biomerieux, USA). However, because of problems with repeatability and interpretation of those biochemical reactions, a multiplex PCR to identify *Y. enterocolitica* and *Y. pseudotuberculosis*, and a simplex PCR to identify members of the *Yersinia* genus, were developed. Briefly, genomic DNA was extracted from pure culture by heat lysis.¹¹ All suspect samples were screened using a multiplex PCR to identify if they were either *Y. enterocolitica* (270-bp product) or *Y. pseudotuberculosis* (756-bp product).¹² A hot start, 25-μL reaction containing 2.5 mmol/L MgCl₂, 0.2 mmol/L dNTPs, 0.2 μmol/L of each primer (227F, YER, YPS2R, YE2R), 1 U of GoTaq® DNA polymerase and 2 μL of genomic DNA was performed. The PCR mixture was amplified in a Bio-Rad TM100™ (Richmond, C, USA) thermal cycler using the following cycle: 5 min at 95°C, 30 cycles of 94°C for 20 s, 66°C for 20 s and 72°C for 45 s, then a final 5-min cycle at 72°C.

DNA from negative species-specific PRCs was analysed using a *Yersinia* genus, simplex PCR.¹² Reaction conditions were: 2.5 mmol/L MgCl₂, 0.2 mmol/L dNTPs, 0.3 μmol/L of each primer (227F, 669R), 0.5 U GoTaq® DNA polymerase and 2 μL of genomic DNA. Amplification conditions were: 95°C for 5 min, 30 cycles of 94°C for 20 s, 68°C for 20 s and 72°C for 15 s, followed

by 72°C for 5 min. Positive samples were classified as ‘other *Yersinia* spp.’, which were non-pathogenic for sheep (i.e. without virulence genes).

Virulent *Y. enterocolitica* were identified using a two-step simplex PCR that identified plasmid (*YadA*) and chromosomal (*YstA*) virulence genes.¹³

Optimised PCR conditions for *YadA* detection were: 2 mmol/L MgCl₂, 0.1 mmol/L dNTPs, 1 µmol/L of each primer (YadA F, YadA R), 0.5 U GoTaq® DNA polymerase and 2 µL of genomic DNA. Optimised PCR conditions for *YstA* detection were: 2 mmol/L MgCl₂, 0.1 mmol/L dNTPs, 0.5 µmol/L of each primer (YstA F, YstA R), 0.5 U GoTaq® DNA polymerase and 2 µL of genomic DNA. Amplification conditions for both virulence PCRs were: 95°C for 5 min, 30 cycles of 95°C for 15 s, 61°C for 30 s and 72°C for 1 min, followed by 72°C for 5 min. The *Y. enterocolitica* isolates lacking both virulence genes were classified as ‘avirulent’.

The *Y. pseudotuberculosis* isolates were serotyped by slide agglutination using commercial antisera against ‘O’ antigens I–VI (Denka Seiken Co. Ltd, Tokyo, Japan).

Antimicrobial susceptibility testing

The antimicrobial susceptibility of at least half of the *Yersinia* isolates from each farm was determined. Susceptibility to amoxycillin (25 µg), tetracycline (10 µg), trimethoprim (5 µg) and sulfafurazole (300 µg) (Oxoid Ltd, UK) was assessed using a disc diffusion technique.¹⁴

The disc diffusion results for sulfafurazole were ambiguous and were verified using a two-fold agar dilution technique with Muller Hinton agar plates containing a sulfafurazole concentration of 1, 2, 4, 8, 16, 32, 64, 128, 256, 512 or 1024 µg/mL.¹⁴ The resistance breakpoint was 64 µg/mL.

Outbreak investigations

A total of 45 outbreaks were investigated on 24 farms between June 2012 and October 2013 (2 from north-central Victoria, 2 from north-east Victoria, 10 from south-west Victoria, 4 from Gippsland, 6 from central New South Wales (NSW)). The average farm was 1700 ha (range 240–3500 ha), with an average annual rainfall of 675 mm (range 540–960 mm), an average stocking rate of 14 dry sheep equivalents per hectare (DSE/ha, range 7–22 DSE/ha) and had 1000–9000 breeding Merino ewes. Farm visits were conducted by a veterinarian for 7/45 outbreaks; on all other occasions, faecal samples were collected by the producers.

Results

Identification of bacterial species

A total of 1020 faecal samples were collected from the 24 farms. Overall, *Y. pseudotuberculosis* was the most frequently isolated pathogen (n = 189; 18.5%), followed by virulent *Y. enterocolitica* (n = 139; 13.6%, Table 1). One outbreak of *C. jejuni* was recorded on a farm in north-central Victoria in October 2012, but no *Salmonella* spp. were detected.

Serotyping was performed on 135/189 isolates of *Y. pseudotuberculosis*; 97.7% (n = 132) were serotype III, a single isolate was serotype II and the remaining two isolates were inconclusive. Virulence genes were detected in 107/139 isolates of *Y. enterocolitica*. Of these, 91.6% (n = 98) were virulent, having either the plasmid (n = 91; 85.1%) or chromosomal (n = 7; 6.5%) gene sequence. All avirulent samples (n = 9; 8.4%) originated from an outbreak investigation on a single farm in October 2013, but no alternative cause of disease was identified in this case.

Detection of Yersinia spp. The *Yersinia* spp. isolated in faecal samples from sheep in 2012 and 2013 are summarised in Table 1. In both years, and across all farms and regions, the detection of pathogenic *Yersinia* spp. was highest during winter, with the exception of *Y. enterocolitica* in spring 2012 (Table 1). The proportion of animals shedding each species varied markedly between region and farm, ranging from 0% to 75% and from 0% to 55%, for *Y. enterocolitica* and *Y. pseudotuberculosis*, respectively.

In 2013, when the number of investigations from Victoria (16 outbreaks, 300 samples) and NSW (10 outbreaks, 226 samples) were comparable, isolation of *Y. pseudotuberculosis* was significantly higher in Victoria (26% vs 0.5% in NSW, $\chi^2 = 69.7$, $P < 0.001$). No difference for isolation of *Y. enterocolitica* was detected in 2013 (20% vs 14%; $\chi^2 = 3.1$, $P = 0.08$).

To further describe the winter scours syndrome, data collected during the winter and spring were collated and the average WEC per farm included (Table 2). The proportion of animals shedding pathogenic *Yersinia* spp. and the primary species detected varied between farms within the same region. For example, farms B and C were located within a 15-km radius, but the proportion of each *Yersinia* spp. detected differed markedly. In 2012, very few *Yersinia* spp. were detected on farm B, while on farm C *Y. pseudotuberculosis* was the primary species detected (30/43, 61%). By comparison, in 2013 *Y. pseudotuberculosis* (11/20, 55%) was the primary species detected on farm B, while *Y. enterocolitica* (26/54, 48%) was the primary species detected on farm C.

Weather conditions

The north-central, north-east and south-west regions of Victoria have a winter-dominant rainfall pattern, typically recording more than one-third of their

annual rainfall between June and August each year. Average winter rainfall and winter temperatures recorded at Bureau of Meteorology sites closest to each of the farms with outbreaks within these regions, were comparable between study years, with the exception of Lake Eildon (farms B and C), which recorded a higher than average rainfall in 2013.

In contrast, Gippsland and central NSW have a more uniform rainfall distribution, with significant rainfall occurring in both summer and winter. In these regions, average winter temperatures, as well as average winter rainfall, were higher in 2013 than in 2012.

When comparing averages across all regions, winter rainfall (+44 mm) and minimum (+0.7°C) and maximum (+0.7°C) winter temperature ranges were higher in 2013, indicating that it was a wetter but warmer year than 2012. The largest differences between rainfall (+12 to 188 mm) and temperatures (min. +0.3°C to +1.9°C; max. -0.1°C to +1.8°C) in 2013 were recorded in Gippsland and NSW.

Parasitology

Primary gastrointestinal parasitism principally associated with *Teladorsagia* and *Trichostrongylus* spp. was excluded as a differential diagnosis, with sheep recently treated with an effective anthelmintic or having a low WEC. WECs were available for 28 outbreaks monitored in 17 flocks (Table 2). Of these, 9 flocks had counts that exceeded the accepted threshold for anthelmintic treatment in Merino weaners (> 300 epg).¹⁵ Where WECs were > 550 epg, the combined proportion of pathogenic *Yersinia* spp. detected was > 50%. When WECs were between 400 and 550 epg, the detection of pathogenic *Yersinia* spp. was more variable, ranging from 3% to 61%. In cases with an unknown history

of anthelmintic use, it was not possible to assess the association between the presence of pathogenic *Yersinia* spp. and WECs < 400 epg, because in those cases the low WECs may have been associated with either a low parasite burden or recent anthelmintic treatment. Burdens of coccidia and *Moniezia* spp. were low on all farms.

Postmortem investigations

Necropsies were performed on between 1 and 3 affected weaners from 7 farms (14 in all). Because of advanced autolysis, the samples from the large intestines of two animals were not examined by histology.

The primary abnormalities reported were moderate to severe lymphocytic–histiocytic enteritis and colitis. Inflammatory cell infiltrate typically contained lymphocytes, macrophages, plasma cells, neutrophils and eosinophils.

Ulceration or abscessation was observed in 5 of 14 small intestinal samples and in 6 of 12 large intestinal sections. Microabscessation with bacterial aggregates was reported on 2 of 14 occasions.

Antimicrobial sensitivity testing of Yersinia spp.

Most *Y. enterocolitica* isolates were resistant to ampicillin (89%), with resistant isolates detected on all farms (Table 3). The disc diffusion test indicated that only 8% of *Y. enterocolitica* isolates were resistant to sulfafurazole, but the proportion assessed as resistant was higher when using the agar dilution test (64%). The small proportion of *Y. enterocolitica* isolates (2%) resistant to tetracycline originated from one farm. No resistance of *Y. enterocolitica* to trimethoprim was detected.

Resistance of *Y. pseudotuberculosis* to sulfafurazole was detected on all farms and the agar dilution test detected more resistance (87%) than the disc diffusion

test (30%). Resistance to ampicillin (10%) was less than that recorded for *Y. enterocolitica*; a small proportion of isolates was resistant to trimethoprim (3%) and no resistance to tetracycline was detected.

Discussion

This cross-sectional study investigated 45 outbreaks of diarrhoea ('scouring') in Merino weaners on 24 farms in south-eastern Australia. The aim was to determine the agent responsible for causing diarrhoea in weaner sheep during the winter that was not caused by gastrointestinal nematodes ('winter scours syndrome'). It was found that bacterial enteritis caused by *Y.*

pseudotuberculosis and *Y. enterocolitica* was a major contributor to the winter scours syndrome in the flocks in this study. This result supported previous investigations in this area of Australia that identified pathogenic *Yersinia* spp. as an important cause of diarrhoea of young sheep during the winter,^{2,16,17} but is the first study to provide such a strong association between the winter scours syndrome and *Yersinia* spp. Although yersiniosis does occur in other countries, the prevalence of winter scours in countries with large sheep populations, such as New Zealand and the United Kingdom, is currently unknown.^{7,18}

In this study, similar proportions of outbreaks of diarrhoea in Merino weaners were associated with *Y. pseudotuberculosis* and *Y. enterocolitica*. By contrast, previous studies have reported *Y. pseudotuberculosis* to be the primary organism associated with the winter scours syndrome and that enteritis associated with *Y. enterocolitica* was either uncommon or sporadic.^{2,4,16} The molecular techniques used to identify pathogenic *Yersinia* spp. in our study were more specific than the biochemical methods used previously, which probably accounts for this difference. Alternatively, a change in the

epidemiology and expression of this disease may explain the difference, but is considered less likely. Regardless, the results from this study indicated that *Y. enterocolitica* may play a far more important role in outbreaks of diarrhoea in young sheep than previously recognised.

Hypothesised risk factors for outbreaks of this syndrome include inclement weather, recent stress from shearing, poor nutrition, high challenge with gastrointestinal nematode larvae and higher stocking rates.^{3,9} Although identification of specific risk factors was beyond the scope of the present study, the timing of the outbreaks, as well as their association with season, region, climate and burdens of gastrointestinal nematodes, was assessed.

Previous research has found that faecal shedding of *Y. pseudotuberculosis* is highest during winter and spring, whereas the shedding of *Y. enterocolitica* is less seasonal, with peaks in both spring and summer.^{3,19} It was not possible to determine peak shedding periods for either organism during this study, because the investigation concentrated primarily on outbreaks of diarrhoea occurring during the winter and spring. However, the present study has provided evidence that *Y. enterocolitica* and *Y. pseudotuberculosis* are associated with outbreaks of bacterial enteritis and diarrhoea during winter and spring. In the summer and autumn, outbreaks of scouring were predominantly associated with *Y. enterocolitica*, suggesting that, under suitable conditions, *Y. enterocolitica* may cause disease throughout the year.

The observations presented in this study suggest a different epidemiology for the two *Yersinia* spp., including their maintenance within a population of sheep and circulation to initiate an annual recurrence of disease. The ability of pathogenic *Yersinia* spp. to survive in the environment or establish a latent

infection may contribute to such differences. A recent laboratory study investigating the effect of high and low temperatures and a lack of moisture on the survival of pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* in soil found the latter species was significantly more resilient.²⁰ Both species survived fixed and fluctuating temperatures ranging from -20°C to 20°C for more than 40 days, but when soil temperature was fixed at 20°C , *Y. enterocolitica* survived only 14 days. When soil temperature was fixed at 40°C , a temperature frequently exceeded in faecal masses in this region during the summer, neither spp. survived for more than 48 h.

From these observations, and the typical pattern of a progressively increasing rate of cases during an outbreak of winter scours, it is likely that pasture contamination and environmental survival during cooler months play an important role in oral–faecal cycling of *Yersinia* spp. within affected populations of sheep. Consequently, moving affected mobs onto low risk pastures (those with reduced risk of gross contamination with *Yersinia* spp.) may be a suitable adjunct to antibiotic treatment to control a disease outbreak during the winter.

During the summer and autumn, outbreaks were only associated with *Y. enterocolitica*. Because it was detected in faecal samples during this period, oral–faecal infection may contribute to circulation of this organism.⁷ However, given that *Y. enterocolitica* survives in the environment for only a short time in temperatures greater than 20°C ,²⁰ it is expected that outbreaks would most likely occur when a high proportion of animals were shedding the bacteria and when stocking rates are high. Because the detection of *Y. pseudotuberculosis* in faeces was negligible during summer and autumn, and that environmental

survival was also poor at high temperatures, circulation of this organism may rely on 'carrier' animals or a maintenance host.

In addition to differences in the seasonal shedding of *Yersinia* spp., this study demonstrated that the nature of outbreaks varied considerably between year, region and farm. This syndrome is most often reported in Victoria, but is endemic throughout the high rainfall regions of south-eastern Australia, including central NSW. The prevalence of shedding of both *Yersinia* spp. was markedly higher in Victoria than in NSW, although sampling bias associated with distance and access to farms during an outbreak may have influenced results. However, these results suggested that either the winter scours syndrome in NSW is different to that which occurs in Victoria or that too few 'typical' outbreaks were investigated in NSW. A prospective epidemiological study to assess and compare patterns of excretion and specific risk factors associated with *Yersinia* spp. in each region would be needed to establish this relationship. The current study provided some evidence in support of a previously observed association between high WECs and bacterial enteritis caused by *Yersinia* spp.^{2,4} The isolation of pathogenic *Yersinia* spp. was greatest when WECs exceeded 500 epg, but there were too few observations to clearly define this relationship. Consequently, further studies are needed to examine this association in more detail.

In most cases, the microscopic changes observed in tissue samples were non-specific and typical lesions associated with *Yersinia* spp. infection, including mucosal abscessation in distal small intestine and colon,^{2,21} were present in only 2 of 14 cases. In each case, pathogenic *Yersinia* spp. were isolated from the corresponding faecal material. The reasons for this are unclear, although only

1–3 animals were euthanased for a detailed postmortem examination, and this was usually done early in an outbreak. Taken together or by themselves, neither histology nor culture results without growth scores provided enough information to accurately interpret the significance of these findings.

The treatment of winter scours is often not based on a definitive diagnosis. Typically, affected sheep are treated first with an anthelmintic, because the diarrhoea is assumed to be primarily caused by gastrointestinal parasites. When animals do not respond to this treatment they are often then treated with an antibacterial, typically sulfonamides. Thus, on some farms, the severity and recurrence of outbreaks means that multiple antibiotic treatments are undertaken each year.

Until now, few studies have investigated the antimicrobial sensitivity of *Yersinia* spp. isolated from sheep. Our results were similar to those reported by Philbey et al.,² although a higher proportion of *Y. enterocolitica* were resistant to ampicillin and sulfonamide and fewer were resistant to tetracycline.

Similarly, a large proportion of *Y. pseudotuberculosis* isolates were resistant to sulfonamide, but fewer were resistant to ampicillin and none were resistant to tetracycline. Our results are probably a more accurate estimate of antimicrobial resistance because a larger number of *Yersinia* spp. isolates from a wider geographical area were tested compared with the study conducted by Philbey et al. (n = 202 vs n = 44, respectively).

In this study, the compound to which *Yersinia* spp. had developed resistance, as well as the proportion of resistant isolates, varied between farms. This was not investigated in detail, but may be associated with the frequency and severity of previous outbreaks, the strategy for antibiotic use (e.g. all animals or only those

affected were treated) and the compound most often used on each farm. Given the extremely high level of *Yersinia* spp. resistance to sulfafurazole, an innate rather than acquired resistance to this antibiotic may have contributed to this result. Our results indicated that sulfonamides are unlikely to be effective in many cases and are therefore not recommended for the routine treatment of yersiniosis. Given that resistance to tetracycline was not common, this may be a more appropriate choice for the treatment of bacterial enteritis associated with *Yersinia* spp. However, to minimise the development of resistance, it might be better to use this broad-spectrum drug strategically, treating only clinically affected animals rather than the whole mob unless a very high proportion have diarrhoea and are ill.

Whenever possible, and in all cases of non-response to treatment, bacterial culture and sensitivity testing should be undertaken to determine the most suitable treatment. Given the difficulties interpreting the disc diffusion techniques for assessing resistance to the sulfonamides, and large discrepancy between the disc and agar diffusion plating results, sensitivity testing for these compounds should be based solely on agar dilution methods.

Conclusions

Both *Y. pseudotuberculosis* and *Y. enterocolitica* were responsible for outbreaks of the weaner winter scours syndrome in the high rainfall regions of Victoria, but their role in the outbreaks in NSW was less clear. Molecular testing to identify pathogenic *Yersinia* spp. provided more sensitive and specific results than biochemical typing techniques. Outbreaks associated with *Y. pseudotuberculosis* occurred only in winter. However, those associated with *Y. enterocolitica* occurred throughout the year and this organism should be

included as a differential diagnosis for outbreaks of scouring in Merino weaners at any time. The continued use of sulfonamides for the treatment of bacterial enteritis associated with the winter scours syndrome is not recommended. Rather, the targeted use of tetracycline, together with management interventions such as decreasing the stocking rate and moving affected mobs onto pastures with a lower risk of bacterial contamination, is likely to be a more effective and sustainable approach. Further studies of the environmental, management and animal factors that increase the prevalence or severity of disease are needed. These will provide a more complete understanding of the epidemiology of this syndrome and enable the development of more effective control strategies.

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Conflicts of interest and sources of funding

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Table 1. Summary of *Yersinia* spp. isolated from investigations of outbreaks of diarrhoea in weaned Merino lambs on 24 farms in south-eastern Australia in 2012 and 2013

Region	Seas on	2012					2013							
		No. of investiga tions	No. of samples					Total samp les	No. of investiga tions	No. of samples				Total samp les
			No. of samples							No. of samples				
			No. of samples							No. of samples				
			No. of samples							No. of samples				
<i>Y. ente ro</i>	<i>Y. pseud o</i>	Other <i>Yersi nia spp.^a</i>	Not <i>Yersi nia</i>		<i>Y. enter o</i>	<i>Y. pseud o</i>	Other <i>Yersi nia spp.^a</i>	Not <i>Yersi nia</i>						
South-west Victoria ^b	A	0					0	2	4 (8)	0	0	46 (92)	50	
	W ^c	6	21 (15)	41 (28)	2 (1)	80 (55)	146	7	22 (18)	57 (47)	0	42 (35)	121	
	Sp	2	7 (16)	6 (13)	0	32 (71)	45	1	0	0	0	20 (100)	20	
Gippsland,	W	2	4 (4)	18	4 (4)	86	112	2	10	0	0	14	24	

Victoria ^{b,d}				(16)		(76)			(42)			(58)	
	Sp	1	6 (75)	0	0	2 (25)	8	0					0
North-east Victoria ^b	A	0					0	1	0	0	0	10 (100)	10
	W ^e	2	3 (5)	30 (51)	0	20 (34)	59	2	26 (47)	10 (18)	0	18 (33)	55
	Sp	2	1 (3)	0	1 (3)	29 (94)	31	1	0	11 (55)	0	9 (45)	20
	W	1	3 (9)	14 (42)	1 (3)	15 (46)	33	0					0
North-central Victoria ^{b,d}	Sp	2	0	0	0	30 (100)	30	0					0
New South Wales	Su	0					0	1	0	0	0	20 (100)	20

Overall	A	0				0	7	12 (8)	1 (1)	3 (2)	140 (88)	156	
	W	1	1 (3)	1 (3)	0	28 (93)	30	1	11 (37)	0	0	19 (63)	30
	Sp	0					0	1	8 (40)	0	0	12 (60)	20
	Su	0					0	1	0	0	0	20 (100)	20
	A	0					0	10	16 (7)	1 (0.5)	3 (1.5)	196 (91)	216
	W	12	32 (9)	104 (27)	7 (2)	229 (60)	380	12	69 (30)	67 (29)	0	93 (40.5)	230
	Sp	7	14 (12)	6 (5)	1 (1)	93 (82)	114	3	8 (13.5)	11 (18.5)	0	41 (68)	60

Total	19	46 (9)	110 (23)	8 (2)	322 (65)	494	26	93 (17.7)	79 (15)	3 (0.6)	350 (66.5)	526
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^aNon-pathogenic *Yersinia* spp. were identified by PCR to genus level only. ^bNo outbreak was recorded in this region during summer. ^cTwo mixed cultures of *Y. enterocolitica* and *Y. pseudotuberculosis* were detected in 2012. ^dNo outbreak was recorded in this region during autumn. ^eSix mixed cultures of *Y. enterocolitica* and *Y. pseudotuberculosis* were detected in 2013 and one mixed culture detected in 2013.

A, autumn; Sp, spring; Su, summer; W, winter.

Table 2. Summary of the number of investigations, average worm egg count (WEC) and number (%) of samples positive for each *Yersinia* spp. isolated from outbreaks of the ‘winter scours syndrome’ during the winter and spring of 2012 and 2013 ^a

Year	Region	Farm	No. of investigations	WEC (epg) ^b	No. of samples (%)				Total samples
					<i>Y. entero</i> ^c	<i>Y. pseudo</i> ^c	Other <i>Yersinia</i>	Not <i>Yersinia</i>	
2012	South-west								
	Victoria	A	5	267	17 (12)	38 (27)	0	86 (60)	141
		D	2	211	7 (21)	7 (18)	0	20 (61)	34
		G	1		0	1 (14)	2 (29)	4 (57)	7
		I	1		4 (50)	2 (25)	0	2 (25)	8
	Gippsland,								
	Victoria	H	2	10	3 (7)	12 (27)	3 (7)	26 (59)	44
		L	1		6 (75)	0	0	2 (25)	8
		X	2	0	1 (1)	6 (9)	1 (1)	60 (88)	68
North-east	B	2	401	1 (3)	0	1 (3)	39 (94)	41	

2013	Victoria								
		C	1	790	3 (6)	30 (61)	0	10 (20)	43
	North-central								
	Victoria	F	2	597	3 (9)	14 (42)	1 (3)	15 (46)	33
		K	1	0	0	0	0	30 (100)	30
	New South								
	Wales	J	1	445	1 (3)	1 (3)	0	28 (94)	30
	Total		21		46 (9)	111 (23)	8 (2)	322 (66)	487
	<hr/>								
	South-west								
	Victoria	A	1	413	1 (2)	25 (56)	0	19 (42)	45
		E	1		0	12 (92)	0	1 (8)	13
		I	1		0	1 (10)	0	9 (90)	10
		P	1		0	0	0	10 (100)	10
		Q	1		9 (90)	0	0	1 (10)	10
		T	1	360	2 (7)	8 (29)	0	18 (64)	28
		U	2	205	10 (38)	11 (42)	0	5 (19)	26

	W	1	0	0	0	0	20 (100)	20
Gippsland,								
Victoria	L	1		0	0	0	4 (100)	4
	S	1	155	10 (50)	0	0	10 (50)	20
North-east								
Victoria	B	1	2015	0	11 (55)	0	9 (45)	20
	C	2	470	26 (48)	10 (19)	0	18 (33)	54
New South								
Wales	M	1	413	11 (37)	0	0	19 (63)	30
	N	1		0	0	1 (10)	9 (90)	10
	O	1		9 (47)	0	0	10 (52)	19
	V	1		8 (40)	0	0	12 (60)	20
Total		18		86 (25.4)	78 (23)	1 (0.3)	174 (51.3)	339

^aTo more accurately describe the winter scours syndrome, only farms with investigations of outbreaks of diarrhoea during winter or spring are reported. ^bArithmetic mean. ^cMixed infections with *Y. enterocolitica* and *Y. pseudotuberculosis* are not reported.

Table 3. Proportion () of *Yersinia enterocolitica* and *Y. pseudotuberculosis* isolates, collected during outbreak investigations of weaner winter scouring syndrome, that were resistant to ampicillin (AMP), tetracycline (TETRA), trimethoprim (TRIM) and sulfafurazole (SULFA)

Region	Farm	<i>Yersinia enterocolitica</i> ^a					<i>Yersinia pseudotuberculosis</i> ^b				
		No. tested	AMP ^c	TETRA ^d	SULFA DISC ^f	SULFA AGAR ^g	No. tested	AMP ^c	TRIM ^e	SULFA DISC ^f	SULFA AGAR ^g
South-west Victoria	A	43	30	0	0	20	16	0	0	50	75
	D	20	100	0	0	100	6	0	0	33	83
	E	0 ^g					5	0	0	20	40
	G	0 ^g					1	100	0	0	100
	I	4	100	0	50	100	3	0	0	33	100
	Q	7	100	0	0	100	0 ^f				
	T	2	100	0	50	0	4	25	0	50	100
	U	6	100	0	0	100	5	0	0	60	100

Gippsland, Victoria	H	3	100	0	0	33	8	0	13	38	100
	L	5	100	0	0	100	0 ^g				
	S	7	71	0	0	71	0 ^g				
	X	0 ^g					6	0	0	33	67
North-east Victoria	C	43	38	0	25	100	4	0	0	0	100
North-central Victoria	R	3	100	0	0	100	9	0	0	22	78
	Y	0 ^g					11	0	27	18	100
New South Wales	J	1	100	0	0	100	0 ^g				
	M	5	100	0	0	20	0 ^g				
	O	5	100	0	0	20	0 ^g				
	V	6	100	33	0	0	0 ^g				

Total	160	89	2	8	64	89	10	3	30	87
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^aNo isolates were resistant to trimethoprim. ^bNo isolates were resistant to tetracycline. ^cAmpicillin 25 µg disc. ^dTetracycline 10 µg disc. ^eTrimethoprim 5 µg disc. ^fSulfafurazole 300 µg disc. ^gSulfafurazole serial agar dilution technique: 1–1024 µg/mL, breakpoint 64 µg/mL. ^hNo isolates were available for testing.

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