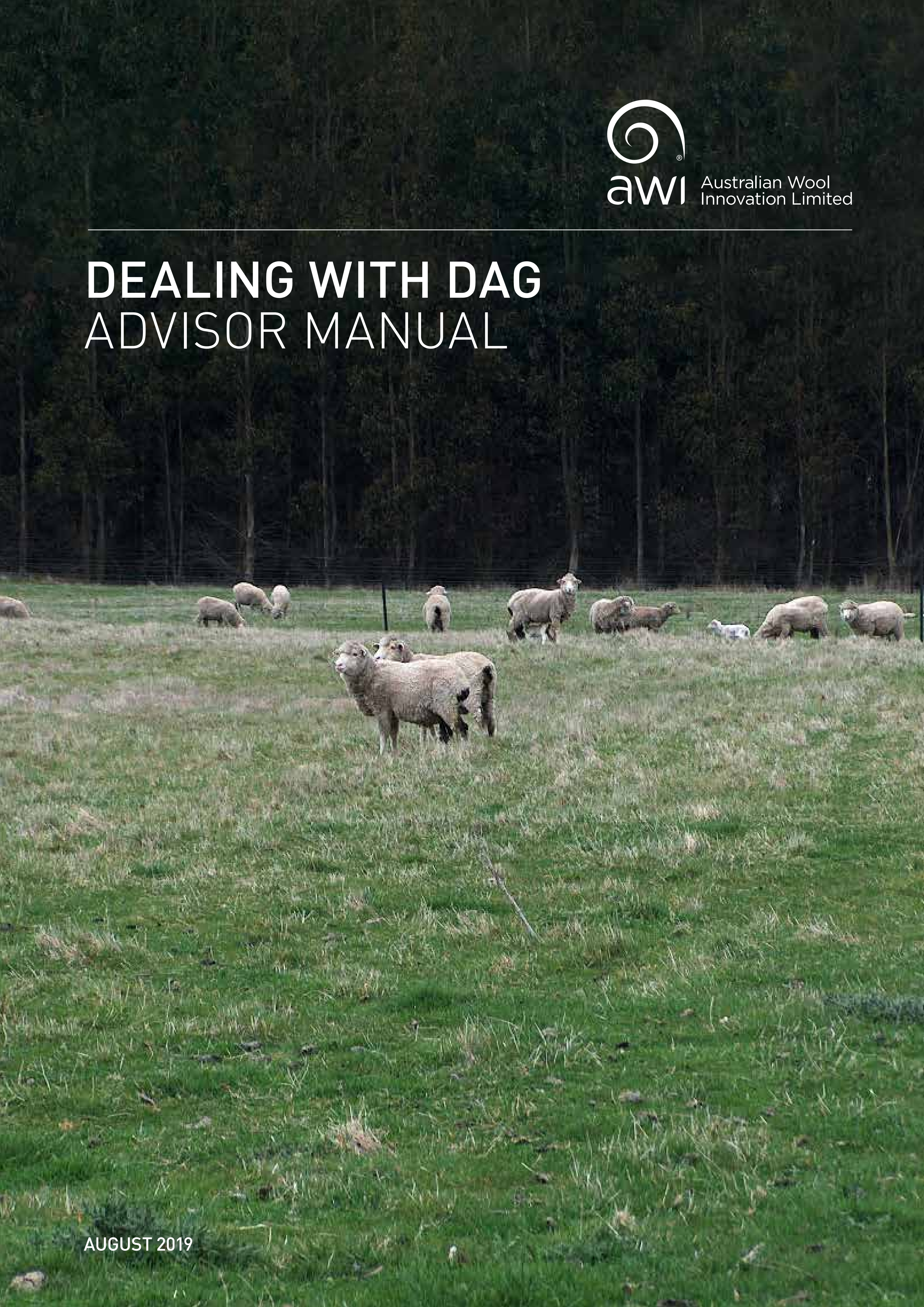

Australian Wool Innovation Limited

DEALING WITH DAG
ADVISOR MANUAL

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Cover image: Scouring ewes
in south western Western
Australia (Source: B Besier).

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DEALING WITH DAG: A REVIEW OF THE CAUSES, DIAGNOSIS, MANAGEMENT AND TREATMENT OF SCOURING IN SHEEP

01

INTRODUCTION

Scouring (diarrhoea) is a common and frustrating reality on Australian sheep properties, and a significant impost due to direct costs and the increased risk of blowfly strike. Scouring due to nematode (worm) infections in a proportion of lambs is common for the majority of flocks especially in higher rainfall areas (Sweeny *et al.*, 2012b). Anthelmintic treatment is commonly needed for remediation and prevention of scouring in lambs. There are a number of causes that may lead to scouring in young sheep, but in most cases the underlying cause is relatively easily diagnosed and effective treatments are available.

Scouring is less predictable or easily diagnosed in mature sheep. On many farms in the high winter and uniform rainfall areas of south-eastern Australia (Central Tablelands of NSW, southern Victoria, Tasmania and south eastern South Australia) up to 40% of ewes have persistent scouring and severe dag from July to October, and over 60% have a substantial amount of soiled breech wool removed at crutching (Larsen *et al.*, 1994; Larsen *et al.*, 1999). The prevalence of scouring and dag in mature sheep in areas with a pronounced Mediterranean climate tends to be less predictable. Typically, 5% of ewes are affected in the medium to high rainfall areas in Western Australia, but in some situations 20% or more (and occasionally up to 85%) can have diarrhoea and dag (Jacobson, 2006; Jacobson *et al.*, 2009).

In many cases, the cause of scouring will be relatively simple and directly related to poor on-farm worm control programs leading to excessively high burdens of *Trichostrongylus* spp. (*T. colubriformis* and *T. vitrinus,* and to a lesser extent *T. axei* and *T. rugatus*), *Teladorsagia circumcincta* and *Nematodirus* spp. (largely restricted to lambs) (Larsen *et al.*, 1994; Besier, 2004). In other cases, it will be more complex and involve interactions between the animal and its immunocompetence, diet and climatic conditions, compounded by protozoal and bacterial infections.

This review aims to discuss the various causes of diarrhoea in sheep in southern Australia and provide a guide for people helping farmers to manage and prevent scouring and dag formation in their animals.

The link between scouring and dag

The major risk factor for dag accumulation is faecal consistency rather than composition of faeces (Waghorn *et al.*, 1998). The consistency of sheep faeces varies from faecal pellets through to pasty or liquid diarrhoea (scouring). Pelleted faeces do not adhere to wool, and dags will only accumulate when faeces are not in pelleted form (Waghorn *et al.*, 1998).

Duration of scouring impacts dag accumulation. Chronic (ongoing) scouring increases the risk of dag accumulation (Watts *et al.*, 1978; Watts and Luff, 1978; James, 2006). Once some faecal material is attached to wool, drier faecal material is able to accumulate (Waghorn *et al.*, 1998). Consequently, faecal matter continues to accumulate (sometimes rapidly) and this further increases the extent of dag. Dag accumulation is further exacerbated in sheep spending time lying in faeces (Waghorn *et al.*, 1999).

Factors determining faecal consistency are complex. The moisture (water) content of faeces is highly variable and not well correlated with faecal consistency. For example, formed faecal pellets with very low risk of dag accumulation can have dry matter content as low as 23%, whereas unformed faeces (with higher risk for dag accumulation) may have dry matter content of 32% (Waghorn *et al.*, 1999). Faecal pellets are formed by contractions of the spiral colon that compress digesta into pellets (Ruckebusch and Fioramonti, 1980; Bedrich and Ehrlein, 2001). Water absorption in the large intestine is largely dependent on the osmotic gradient (Wesselink *et al.*, 1995). Faster rate of passage through the large intestine or reduced capacity to reabsorb water from digesta by the large intestine may result in failure of faecal pellet formation in the spiral colon, resulting in the production of loose faeces with higher risk for dag accumulation. Notably, sheep predisposed to dag have persistently higher faecal moisture and looser faecal consistency compared to sheep not predisposed to dag, even at times of year when ewes are not scouring (Larsen, 1997; Larsen *et al.*, 1999) . The mechanism by which this occurs is not understood.

Scouring sheep with severe dag (Source: B Besier).

02

COST OF DAG

Direct and indirect on-farm costs of dag

The direct financial penalties of dag accrue from the need for crutching and associated wool value loss, treatment costs, and importantly, an increased susceptibility to breech strike. Post-farmgate costs of dagged sheep at slaughter are an additional impost.

Costs associated with dag shown in Table 1 included variable cost of crutching, plus reduced income from selling potentially high-value wool at a discounted price. In addition, producers with flocks that are consistently 'daggy' also tend to undertake a larger crutch on all sheep which removes more clean wool than would otherwise occur, which at current wool prices can add about $0.70 to the estimated costs shown in Table 1.

Using January 2019 wool prices, 2018/2019 recommended wages and results from a 1995 study by the Mackinnon Project (Larsen *et al.*, 1995a), the increased costs from crutching and decreased income from soiled wool in sheep with severe dag (a score of 3 or more on a scale of 0-5) are estimated to be at least $1.39-2.46 per head (Table 1). These costs may increase by at least 30-50% if producers stop mulesing in the absence of genetic selection for less breech wrinkle and decreased scouring because unmulesed sheep can have up to twice as much dag and take from 30-100% longer to crutch (Larsen *et al.*, 2012).

In 1995, the direct cost of dag due to hypersensitivity scouring (see below, 'Scouring associated with low worm burdens') in Victoria was estimated to be $10 million, which adds around 15-20% to costs and production losses from roundworms (Larsen *et al.*, 1995a). This estimate didn't include costs from increased breech strike in affected sheep (estimated to be at least another $20m) or the additional treatment costs, plus other indirect and intangible costs associated with daggy sheep. Examples of indirect costs include additional insecticide treatments and labour to manage flystrike in daggy sheep. Intangible costs include concern by producers about the health and welfare of sheep. The use of additional insecticide and anthelmintic treatments to manage flystrike risk for scouring sheep may also contribute to flies and worms developing resistance to treatments (Pritchard *et al.*, 1980; Leathwick and Besier, 2014). These indirect costs, which have not been estimated, are probably at least equal to the direct costs of hypersensitivity scouring. A further intangible effect is the likely contribution to resistance by sheep worms and blowflies to control chemicals, due to the increased requirement for treatments.

Table 1: The total cost of dag for ewes in each dag score category. Adapted from Larsen *et al.* (1995a) using 2019 wool prices and 2018/19 recommended wages.

ITEM	DAG SCORE					
	0	1	2	3	4	5
A. Cost of crutching (c/head) #	10	20	30	72	90	90
B. Dag-weight (g)	68	115	204	380	737	1225
C. Wool yield (%)	36.4	28.5	22.9	19.8	13.6	14.4
D. Clean wool in crutchings (g) (B x C)	25	33	47	75	100	176
E. Foregone value of crutched wool* (c/head)	14	19	42	67	89	156
Total cost of dag (c/head) (A + E)	24	39	72	139	179	246

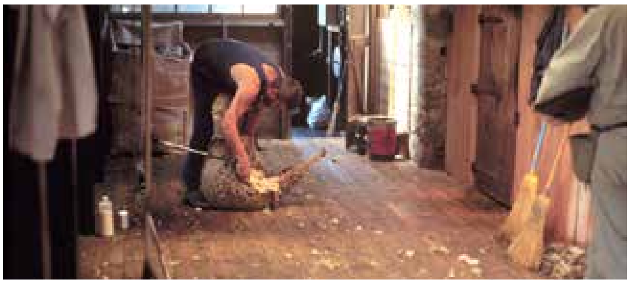
Assumes sheep with dag score of 0-2 can be dagged in the race by owner or farm labour, dag scores of 3 are crutched at the 2019 rate for 'Other crutching' and 'dag scores of 4 & 5 at the 2019 rate for 'Full crutching'.

* Price of fleece wool 1200 c/kg clean, crutchings from ewes with a dag score ≤ 1 and ≥ 2 are 52% and 26% of this price, respectively.

Treatment costs

In many instances, the appearance of scouring in a mob of sheep reflects a failure of control measures, in particular, a failure of worm control. Scouring due to inadequate worm control commonly occurs where appropriate preventative programs have not been followed, or where unusual weather conditions allow worms to develop outside the usual seasonal pattern. Control is also less effective where drench resistance reduces the efficiency of strategic programs, as the continued pasture contamination with worm eggs often leads to large worm populations earlier in the season than expected. Remedial drenching treatments impose an additional cost and work effort in the short term, and further add to the selection pressure for drench resistance. In severe cases, where pastures are massively contaminated with worm larvae, multiple treatments may be necessary, especially to lambs which have not developed an effective natural immunity.

Scouring due to bacterial or protozoal causes may also require veterinary treatments, or changes to management routines and plans for paddock use.



Crutching costs and reduced fleece value impact farm profitability (Source: J Larsen).

The link between dag and flystrike

Flystrike, initiated principally by the Australian sheep blowfly, *Lucilia cuprina*, is a major animal health and welfare problem for sheep production in Australia, estimated to cost from $171-280m annually (Sackett *et al.*, 2006; Lane *et al.*, 2015). Breech strike is the main form of flystrike in high winter rainfall areas in most years, although a high incidence of body strike can occur with above average rainfall during spring, summer or autumn.

In south-eastern and south-western Australia, persistent diarrhoea ('scouring') and dag is a major risk factor for breech strike (Watts and Perry, 1975; Morley *et al.*, 1976; James, 2006; Anonymous, 2011b; Larsen *et al.*, 2012; Greeff *et al.*, 2014; Greeff *et al.*, 2018b). This is in agreement with a large number of studies worldwide confirming that scouring is the major risk factor for development of dag and/or subsequent flystrike (Morley *et al.*, 1976; Watts and Marchant, 1977; Watts *et al.*, 1978; Watts and Luff, 1978; Pownall *et al.*, 1993; French and Morgan, 1996; Leathwick and Atkinson, 1998; Pickering *et al.*, 2015).

Sheep are predisposed to breech strike by prolonged wetting of the wool on the tail or breech from faeces or urine (Miller, 1939; Morley *et al.*, 1976; Watts and Marchant, 1977; Leathwick and Atkinson, 1995). The risk of flystrike is increased as the extent of dag increases (Morley *et al.*, 1976; Watts *et al.*, 1979; Wardhaugh *et al.*, 1989; Leathwick and Atkinson, 1995; Leathwick and Atkinson, 1996). Breech strike risk is also increased by skin wrinkles around the breech and tail, which trap moisture and provide ideal egg-laying sites for *L. cuprina*.

Oviposition of blowfly eggs associated with dag (Source: J Larsen).

Accumulation of dag is the single major risk factor for breech strike (Greeff *et al.*, 2018a). Relatively minor accumulation of dag will significantly increase the risk of breech strike, and even a small amount of dag tends to override the effect of wrinkle and bare area for flystrike risk. After dag, wrinkle is the next most important risk factor for breech strike (Anonymous, 2011b; Greeff *et al.*, 2018a). This means that in low dag environments, breech wrinkle represents an important risk for breech strike, with urine stain and wool colour of relatively minor importance (Anonymous, 2011a; Smith, 2016).

Post-farmgate consequences of scouring

Post farm-gate costs associated with scouring and dag are poorly quantified, but are likely to be considerable along the entire sheep meat and wool supply chains. Both the fleece and viscera are reservoirs for microbes that include potential human pathogens and microorganisms that cause meat spoilage and product reduce shelf life (Newton *et al.*, 1978; Greer *et al.*, 1983). Faecal soiling of fleeces is an important source of microbial carcass contamination associated with reduced productivity for sheep meat processors, reduced product shelf life, potential public health risks and risks to valuable export markets. Many of Australia's international sheep meat markets have a zero tolerance for faecal contamination of meat products. In addition, chemical residues associated with endo- and ecto-parasitic treatments used for managing scouring and blowfly strike risk impact the meat and wool supply chain.

Washing sheep prior to slaughter may reduce visible contamination, but actually increases the risk of microbial contamination of the carcass irrespective of wool length (Biss and Hathaway, 1996b). Microbial transfer to the carcass can occur through rupture or leakage from the gut, direct contact between the fleece and the carcass tissues, or indirectly via aerosols, water droplets, contact with knives or equipment and the hands, arms or clothing of abattoir workers (Newton *et al.*, 1978; Smeltzer *et al.*, 1980; Sheridan *et al.*, 1992; Biss and Hathaway, 1996c, a, b; Hadley *et al.*, 1997). The degree of soiling of the fleece affects the carcass microbial load with increasingly soiled fleeces associated with greater microorganism load and a higher proportion of contaminated carcasses (Biss and Hathaway, 1995; Hadley *et al.*, 1997). Abattoir productivity is impacted because faecal contamination necessitates trimming of the affected carcass tissue. Trimming results in reduced saleable product and slows the speed of the chain, resulting in sub-optimal processing efficiency.

03

IMPACT OF BREECH (AND TAIL) CONFORMATION ON DAG

Breech conformation, specifically tail length, breech wool cover and breech wrinkle, impact the risk of dag accumulation.

Tail docking at the fourth coccygeal joint reduces severity of dag (French *et al.*, 1994; Scobie *et al.*, 1999; Webb Ware *et al.*, 2000), and reduces the risk of breech strike (Gill and Graham, 1939; Riches, 1941, 1942; Graham *et al.*, 1947; Watts and Marchant, 1977; Watts and Luff, 1978). Shorter docked tail length (less than three coccygeal joints) predisposes sheep to soiling (wet) wool extending to the skin of tail and crutch area (Watts and Marchant, 1977; Watts and Luff, 1978), plus increased risk of perineal neoplasia (Vandengraff, 1976; Hawkins *et al.*, 1981; Swan *et al.*, 1984), bacterial joint infections (Lloyd *et al.*, 2016) and rectal prolapse (Thomas *et al.*, 2003). Longer tail length has been associated with increased risk of dag formation in sheep in high dag environments (Graham *et al.*, 1947; Watts and Luff, 1978; Scobie *et al.*, 1999). Some studies have observed no impact of tail length on dag accumulation, although these studies were conducted using flocks in which most lambs did not have severe dag (Pomroy *et al.*, 1997; Scobie *et al.*, 1997; Smith *et al.*, 2012). Poor docking technique at any tail length is associated with poor health and welfare outcomes, such as delayed wound healing, shattered vertebrae and deviated tails.

The relationship between wrinkle and dag (as opposed to flystrike) remains uncertain. Breech strike selection demonstration flocks were in low dag (Armidale, NSW) and high dag (Katanning and Mount Barker, WA) environments. However, sheep with the highest wrinkle scores were concentrated in the low dag (summer rainfall) environment, which means that the relationship between high wrinkle score and dag risk in high risk (Mediterranean and winter rainfall) environments has not been fully described (Anonymous, 2011a; Smith, 2016).

Breech modification using the Mules operation, combined with docking tails at the correct length, has been very successful in reducing the risk of breech strike in Merino sheep (Watts and Marchant, 1977; Beveridge, 1984; James, 2006). Welfare issues associated with the Mules operation, including reduced growth rate and survival between marking and weaning, have recently questioned the continued use of the procedure (Shutt *et al.*, 1987; Fell and Shutt, 1989; Chapman *et al.*, 1994; Jongman *et al.*, 2000; Paton *et al.*, 2003; Paull *et al.*, 2007; Paull *et al.*, 2008a; Paull *et al.*, 2008b; Colditz *et al.*, 2009b, a; Hemsworth *et al.*, 2009; Lepherd *et al.*, 2011a; Evans *et al.*, 2012a; Playford *et al.*, 2012). Non-surgical methods have been investigated for modifying the breech and tail, including needle-less injection of compounds that destroy wool follicles, the application of plastic clips, and liquid nitrogen, with varying success (Pratt and Hopkins, 1976; Colditz *et al.*, 2009a; Levot *et al.*, 2009; Colditz *et al.*, 2010; Lepherd *et al.*, 2011c, b; Evans *et al.*, 2012b, a; Playford *et al.*, 2012; Rabiee *et al.*, 2012; Colditz *et al.*, 2015). However, persistent scouring significantly increases the risk of breech strike even in mulesed sheep (Watts and Perry, 1975; Morley *et al.*, 1976; Watts and Marchant, 1977; James, 2006), therefore control of scouring and appropriate insecticide application remains part of an integrated approach to the prevention and control of breech strike.

In the longer term, genetic selection of Merino sheep with increased breech bare area and less breech wrinkle (James 2006), decreased scouring (Larsen *et al.*, 1995b; Larsen *et al.*, 1999) and decreased dag (Greeff *et al.*, 2014; Greeff *et al.*, 2018a) are strategies that will reduce the risk of breech strike and reliance on the Mules operation. However, it will take some time for this to be implemented, first in ram-breeding flocks, then in wool-growing flocks using rams purchased from ram breeders. Consequently, in the short to medium term management strategies that might effectively control scouring and dag accumulation, plus procedures such as additional strategic crutching and appropriate application of insecticides will continue to have an important role in managing breech strike risk (James *et al.*, 2009).

FACTORS TO CONSIDER IN DETERMINING THE CAUSE OF SCOURING IN SHEEP

- Region of Australia
- Age of the sheep
- Time of year
- Type of pasture
- Proportion of the flock affected
- Rainfall

04

DIRECT IMPACT OF WORMS ON SCOURING

Infection with gastrointestinal nematodes (worms) is a major risk factor for scouring and is mostly associated with infections of *Trichostrongylus* spp. (*T. colubriformis* and *T. vitrinus* and to a lesser extent *T. axei* and *T. rugatus*) and *Teladorsagia circumcincta* and *Nematodirus* spp. (largely restricted to lambs) (Larsen *et al.*, 1994; Besier, 2004). The abomasal parasite, *Haemonchus contortus* is not associated with scouring and infections from this blood-sucking worm generally result in lower faecal water content.

Pathogenesis of scouring

The relationship between worm infection and scouring is complex. Scouring arises from a combination of both direct effects of infection and the immune response of the host (Williams and Palmer, 2012), and is not well correlated with adult worm burden (Williams *et al.*, 2010c). As scouring sheep do not necessarily carry large burdens of established adult worms, the third and fourth stage larvae and the immune response to various worm stages are presumed to be significant in the aetiology of scouring. Nevertheless, adult worm burdens are associated with scouring in some situations, most commonly in the more susceptible classes of sheep (e.g., lambs, weaners and lactating ewes, especially maiden ewes).

The intestinal-dwelling *T. colubriformis* and *T. vitrinus* cause direct and indirect damage to the upper small intestine (Beveridge *et al.*, 1989). Damage includes villus atrophy (decreased local absorptive capacity) and crypt cell hyperplasia (increased water and electrolyte secretion) (Barker and Titchen, 1982), as a result of physical changes to the gut mucosa and local immune responses (McClure *et al.*, 1992; Williams and Palmer, 2012). Immune responses to parasites increase gut permeability resulting in increased loss of blood, plasma and mucins. The abomasal-dwelling *T. circumcincta* causes stretching and damage to the abomasal gastric pits, leading to a rise in pH and a decline in acid digestion (Stear *et al.*, 2003). Infection from both genera also results in changes to gut motility, resulting in a reduced transit time of digesta (Williams *et al.*, 2010c).

Clinical presentation

Scouring associated with adult worm burdens is most common in sheep less than one-year-old and the periparturient ewe (Williams and Palmer, 2012). Young sheep have had less time (and exposure to worms) to acquire an effective immune response and this predisposes to higher worm burdens (Balic *et al.*, 2000). With reproductive ewes, a transient loss or diminution of immunity occurs in the period from prior (3-4 weeks) to after (6-9 weeks) lambing allowing for greater rates of establishment of infective larvae resulting in high worm burdens (Beasley *et al* 2010). Increased worm burdens, during a period of poor immunity exacerbate the contribution of trichostrongylids to scouring.

Scouring as a result of infection with *Trichostrongylus* and *Teladorsagia* spp. is most prevalent in winter rainfall and Mediterranean regions of southern Australia, where it is most commonly observed in sheep grazing improved pastures during winter and early spring (Larsen *et al.*, 1999). This period also coincides with high numbers of infective larvae on pasture (Anderson, 1972; Anderson, 1983; Besier, 2004). Scouring is less common in summer rainfall regions even though infection from both *Trichostrongylus* and *Teladorsagia* spp. is common (Bailey *et al.*, 2009). When scouring does occur in the summer rainfall regions it is most frequently observed in early spring when the relative proportion of these species to total worm infection is greatest (i.e. because *H. contortus* is at a low seasonal level).

Scouring and dag in lambs (Source: J Larsen).

Diagnosis and monitoring

CONFIRMATION OF PARASITISM AS A CAUSE OF SCOURING ALMOST ALWAYS REQUIRES FAECAL WORM EGG COUNTS

In some situations, parasitism may reasonably be suspected as the cause of scouring based on the clinical presentation, such as in lambs grazing green pastures in winter and spring. An immediate response to drenching generally provides confirmation, provided that an effective product is used.

However, confirmation of parasitism as a cause of scouring almost always requires faecal worm egg counts. In Australia, worm egg counting is usually according to long-established protocols based on the McMaster technique (Hutchinson, 2009), though more sensitive approaches are available, e.g. Flotac (Rinaldi *et al.*, 2011). Regardless of the laboratory technique employed, worm egg counts provide a relatively insensitive estimate of worm burdens in a group, due to inherent variations between individual animals and within laboratory processes, and to operator error (van Burgel *et al.*, 2014). Unless adequate sheep numbers are sampled or sufficient eggs counted, confidence intervals around mean counts are high (Dobson *et al.*, 2012). Egg counts must therefore be "interpreted" in the light of epidemiological factors, such as the time of year, class of sheep, paddock movements, and the nature and timing of previous treatments.

Worm egg counts are often used to assess the role of worms in flocks with scouring and plan parasite control programs (Source: B Besier).

With these caveats, worm egg counts often provide an immediate answer to the role of parasites. Low counts (typically less than 100 eggs per gram) usually indicates that primary parasitism (due to large worm burdens or heavy larval challenge) is not involved, although "hypersensitivity scouring" remains a possibility in relatively worm-immune sheep. Moderate counts (200 – 300 eggs per gram) or higher, in association with scouring, also usually indicate parasitism, though other causal factors should be considered if not consistent with the epidemiological picture. In these cases, or where counts are high (over 500 eggs per gram) without scouring, it is essential to conduct specialised laboratory tests to identify the worms involved, to exclude the possibility that significant counts are due to *H. contortus* (not associated with scouring).

A variety of procedures exist to differentiate between worm species (or more commonly, only to genus level), as only the eggs of *Nematodirus* spp. can be identified by egg morphology alone. At present, identification relies mainly on larval culture and differentiation of the third-stage larvae (van Wyk and Mayhew, 2013), despite the significant disadvantage of a lengthy time requirement (7 days), and poor differentiation between *Teladorsagia* and *Trichostrongylus* species (Besier, 2013; Roeber and Kahn, 2014). In most cases it is sufficient to distinguish only between *H. contortus* and the scour worms, and the lectin binding assay provides a result within hours of the worm egg count (Palmer and McCombe, 1996), although this has not been widely adopted by diagnostic laboratories. More recently, PCR tests based on nematode DNA have been developed (Gasser *et al.*, 2008; Roeber *et al.*, 2015). The potential for quantitative PCR tests, offering simultaneous indication of worm burden sizes and identity, has also been demonstrated (Bisset *et al.*, 2014; Melville *et al.*, 2014), although technical barriers remain to general implementation. At present, PCR testing is feasible for worm identity (Sweeny *et al.*, 2011a), but is not yet widely used in Australia, largely due to the relatively high cost and limited availability (Hunt and Lello, 2012).

However, more complex investigations, requiring additional resources than a simple worm egg count and worm identification, are indicated where egg counts are low, and hypersensitivity scouring or non-parasitic causes should be considered. If necessary, sheep may be sacrificed and total worm counts performed to indicate the size and composition of worm burdens. For obvious reasons, this approach is usually restricted to cases where sheep mortalities have occurred, and severe consequences are likely if the condition continues.

The widely recommended IPM-based worm control programs include a structured approach to monitoring worm burdens, aimed at preventing the development of excessive worm burdens, so that clinical signs, including scouring, do not occur. Guides to worm egg count schedules, appropriate for different environments and classes of sheep, are detailed at the WormBoss website (www.wormboss.com.au). In general, these aim to detect the development of large worm burdens and the requirement for drenching. Professional advice is warranted where egg counts are not easily interpreted.

Control and management

Regional worm control programs play an important role in the management of worm-related scouring (www.wormboss.com.au). These programs rely on a combination of chemical and non-chemical control options for integrated parasite management (Waller, 1999).

The centrepiece of most regional programs is the combination of strategic worm egg count monitoring and a mix of tactical and strategic anthelmintic treatments, aiming to keep worm egg counts below treatment thresholds (Besier, 2004), to minimise production loss and prevent scouring. A key aim of integrated parasite management programs is to ensure sustainability by minimising the development of resistance to anthelmintic drugs. Strategies for managing anthelmintic resistance are based on recommendations to reduce exposure to worms to anthelmintics (Pritchard *et al.*, 1980), and the concept of ensuring that part of the worm population remains *in refugia* from drenches (Besier, 2012; Leathwick and Besier, 2014).

The two most practical non-chemical worm control options are genetic selection for increased host (sheep) resistance to worms and grazing management to reduce the number of infective larvae on pasture (e.g. cropping rotations, smart grazing, cross-grazing with cattle).

Unfortunately, selecting sheep for increased resistance to worms using WEC Australian Sheep Breeding Value (ASBV) will not reduce dag formation (Williams and Palmer, 2012), and dag formation must be included as a separate trait in breeding programs. While high worm egg counts are commonly associated with scouring and dags in lambs, the relationship is not straightforward for sheep that have attained immunity to worm infection. This relationship varies between environment and sheep genetics, and described in more detail in the section 'Impact of breeding for low worm egg count on scouring'.

Good worm control is likely to be most effective at reducing worm-related scouring in worm-susceptible classes of sheep, including lambs. However, best practice worm control is unlikely to be of major benefit for controlling low-worm egg count scouring in adult sheep, as has been demonstrated on a number of occasions (Larsen, 2000; Williams and Palmer, 2012).

Controlled release anthelmintic capsules (e.g. Ivomec Maximiser™, Bionic™, Dynamax™) and long-acting moxidectin injectable products may prevent the accumulation of dag relating to high worm burdens, but are not always a cost-effective strategy. The routine use of long-acting anthelmintics may also be undesirable because these potentially select more strongly for drench resistance than short-acting drenches (Leathwick *et al.*, 2006). (See below, Control and management of hypersensitivity scouring).

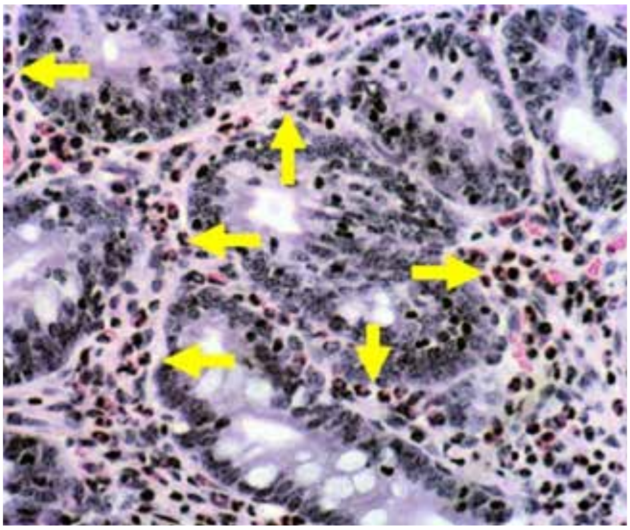
05

SCOURING ASSOCIATED WITH LOW WORM BURDENS

Best practice worm control programs will reduce the proportion of sheep with diarrhoea in each age group, but will not eliminate all diarrhoea (scouring). Even on farms with good worm control programs, scouring and dag can be observed in 10-40% of sheep, depending upon the area, seasonal conditions and availability of pasture. In south-eastern Australia, higher pasture availability (at least 800-1000 kg DM/ha) and a predominance of perennial pasture species tend to be associated with more dag. In south-western Australia, higher pasture availability (annual grasses and legumes) in late winter or early spring tends to be associated with more dag.

In many cases, scouring in sheep with low WEC will be due to 'hypersensitivity scouring', the second major cause of worm related diarrhoea (also called 'low worm egg count scouring'). The term 'hypersensitivity scouring' is used to describe a heightened inflammatory response seen in the gut of daggy sheep (Larsen *et al.*, 1994). This response is repeatable and heritable, meaning that the same sheep scour and have high dag score each year (Larsen *et al.*, 1995a). A similar predisposition to scouring has been confirmed in Merino and Romney sheep selected for resistance to internal parasites on the basis of low WEC (Bisset *et al.*, 1996; Williams *et al.*, 2010c).

Typical appearance of hypersensitivity scouring of mature Merino ewes grazing improved pastures during spring in western Victoria. Ewes given a controlled release capsule containing a drench had little or no dag (Source: J Larsen).



Section of jejunum from a ewe with a high dag score showing increased infiltration of eosinophils into the lamina propria (arrows). H & E stain, x 400 magnification (Source: J Larsen).

Pathogenesis of hypersensitivity scouring

The major inflammatory change observed in the gut of affected sheep is an increased number of eosinophils (Larsen *et al.*, 1994). Eosinophils are a type of white blood cell, typically associated with parasite infection and hypersensitivity ('allergic') responses, such as allergic dermatitis and asthma in humans (Rosenberg *et al.*, 2013; Lambrecht and Hammad, 2014; George and Brightling, 2016). In addition, changes in the populations of T-lymphocytes have been described. These included a reduced ratio of cells positive to the CD4 marker ('T-helper cells') compared to those positive to CD8 (now FoxP3, 'T-regulatory cells'), and reduced populations of cells positive to an important inflammatory mediator, gamma-interferon (γ-IFN) (Larsen *et al.*, 1999). T-lymphocytes have many sub-types. Importantly, the 'Th2' sub-population controls and directs responses that are involved in both protective immunity to worms and hypersensitivity responses.

Why some sheep express an effective immune response without scouring, whilst others have both an effective immunity (low worm egg counts) and a scouring response is not understood. Consequently, a better understanding of the inflammatory response of sheep susceptible to scouring is needed and should be a major target of any new research.

Clinical presentation

There appear to be key differences in the nature of this syndrome between the high rainfall areas of south-west Western Australia, which has shorter growing seasons and a marked Mediterranean climate, and the high rainfall areas of south-eastern Australia in which scouring is a problem in virtually every year (e.g. Central Tablelands of NSW, most of Victoria and Tasmania, and the southeast of South Australia). The Mackinnon Group (Faculty of Veterinary Science, University of Melbourne, Werribee) conducted detailed field investigations into scouring and dag of adult Merino ewes in western Victoria between 1992 and 2000 (Larsen *et al.*, 1995a; Larsen *et al.*, 1995b; Larsen *et al.*, 1999; Larsen, 2000). In this area up to 30-40% of older sheep develop persistent diarrhoea and severe dag. Scouring typically begins in late June or July and persists until mid- to late-spring (October or November). On farms which were studied over 3-4 consecutive years, sheep identified as being susceptible to dag started to scour at around the same time each year.

The presentation in south eastern Australia is quite different to the more transient (6-8 week) scouring response in sheep in Western Australia during late winter, often attributed to hypersensitivity scouring associated with exposure to increasing populations of infective larvae after the seasonal break (Jacobson *et al.*, 2009). In Western Australia, scouring consistent with 'hypersensitivity scouring' (Larsen *et al.*, 1994) is considered to occur, but the prevalence is variable between farms, flocks and sheep classes, and it has not proved possible to predict the extent of apparent hypersensitivity scouring from year to year. There is a distinct seasonal pattern in producer observations of scouring (across all sheep classes) in the major sheep zones of Western Australia (Besier and Bell, 1999; Jacobson, 2006; Sweeny *et al.*, 2012b). Scouring was reported to be virtually absent in summer and autumn, but present to a variable degree on up to 90% of farms in winter and spring in most sheep classes. Moderate to severe scouring was reported on half of the farms surveyed (Jacobson, 2006). Although the survey findings cannot be related to flock worm egg counts, a conceptual model of hypersensitivity scouring in a Mediterranean climate has been proposed in association with field observations and data from Department of Agriculture and Food WA laboratory submissions.

Explanation for different presentations in south-eastern and south-western Australia

Interactions between seasonal worm larval exposure and the relative degree of acquired worm immunity are believed to largely explain the different patterns of low worm egg count scouring seen in different age classes of sheep. In a Mediterranean climate, worm larval intake ceases abruptly with the onset of hot and dry conditions in mid-to late spring (Besier, 2004). After the autumn seasonal break, cooler temperatures and new pasture growth allow larval development and migration onto the herbage. The size of the peak in larval population is then determined by the level of pasture contamination with worm eggs from grazing animals, and in more temperate areas, survival through summer in faecal pellets, as occurs in south-eastern Australia (Besier and Lyon, 1990).

This model of hypersensitivity scouring proposed for Mediterranean areas suggests that, with sufficient larval intake, the immunological status of an animal determines the nature of the scouring. Worm-naive lambs, and possibly immunocompromised mature sheep, are likely to develop 'classical' helminthosis, indicated by relatively high worm egg counts and response to anthelmintic treatment. However, sheep that have developed a functional immune response, through exposure to sufficient larval challenge earlier in life, are likely to reject most infective larvae (Dobson *et al.*, 1990b; Dobson *et al.*, 1990a; Vlassoff *et al.*, 2001). This occurs after the larvae exsheath, but before they develop into adult worms. It appears that this does not invariably cause 'hypersensitivity scouring', characterised by gut inflammation (hyper-eosinophilia) and diarrhoea, suggesting that other factors may apply where this form of scouring occurs.

In addition to the acknowledged genetic variation in the immune response (referred to as 'worm resistance'; see below), it has been suggested that the period during which there is little or no larval challenge can affect the character of the immunological response in areas with a Mediterranean climate. During this period, which is far longer in a Mediterranean compared to a temperate environment, there may be a decrease ('waning') of immune competency. Other factors, yet to be fully defined, are presumed to explain the wide variation in the prevalence of scouring between flocks which have experienced a similar challenge with worm larvae. These factors could include the size and nature of worm burdens retained during the period without larval challenge, and the timing and rate of larval challenge during winter. Similar to temperate environments, the degree of scouring is also influenced by the type, amount and quality of pasture. In this context, it is of note that hypersensitivity scouring has rarely been reported from overseas environments where continual larval exposure is usual, such as in the UK or New Zealand, although whether this indicates that the syndrome is not commonly found in these situations, or a failure to report it in the literature, is not known.

Mediterranean low WEC scouring: hoggets/yearlings

In some hogget mobs (10-15 months-old), scouring associated with high worm egg counts (> 500 eggs per gram [epg]), typical of sheep that are susceptible to worms, often occurs 6-10 weeks after the autumn break. Drenching these sheep with an anthelmintic usually leads to rapid resolution of the scouring, and these sheep show resistance to further worm infection from then onward (shown by low WECs). In other hogget flocks, outbreaks of scouring can occur during the winter and spring in association with low worm egg counts (< 200 epg), which is more typical of the hypersensitivity scouring syndrome. Other scouring hogget flocks may have moderate counts (200-400 epg), with the distribution of individual worm egg counts indicating that these sheep are at varying stages in the process of developing and expressing immunity to larval challenge. There appears to be no consistent response to a single short-acting anthelmintic drench for these flocks in low or moderate worm egg count categories, with both good and poor responses reported. It is likely that these differences reflect varying opportunities to develop an acquired immunity prior to the onset of summer conditions in a Mediterranean climate. For example, it has been postulated that the longer grazing period of lambs born earlier in the year (April to June) would allow enough exposure to larval challenge for immune development, hence an increased risk of hypersensitivity scouring following the autumn break the next year. In contrast, lambs born in late winter would have decreased exposure, hence an incomplete immunity to worms. These lambs would be susceptible to helminthosis in the following winter but would not be predisposed to hypersensitivity scouring. However, the role of birth date and other management factors that influence worm exposure, and whether different degrees of immune development can affect the response to treatment with short-acting anthelmintics, have not yet been investigated in detail. In addition, no reliable diagnostic tests are available to differentiate the likely response to treatment of diarrhoea in these weaned lambs.

Low WEC scouring in Merino hoggets in south west Western Australia, a region with a Mediterranean environment (Source: C Jacobson).

Mediterranean low WEC scouring: adults

In mature sheep in Mediterranean environments, scouring in association with high worm egg counts is not common, except occasionally in ewes during lactation. In this circumstance, drenches are usually effective when given in the post-partum period, which is typical practice in this region (Cornelius *et al.*, 2015). In the south-west of Western Australia (especially the Great Southern region, south-east of Perth), two main forms of scouring associated with low worm egg counts and a poor response to drenching have been observed. Most commonly, a small proportion of the flock (< 5%) scour severely while other sheep are largely unaffected. This would be consistent with a genetic predisposition to scouring, and is likely to respond to the culling of affected individuals. Less commonly, but unpredictably, a large proportion of a flock (> 20%, and sometimes > 50%) scour relatively severely (Jacobson *et al.*, 2009). Although this may also reflect a genetic predisposition to hypersensitivity scouring in response to larval challenge, it is difficult to explain the sporadic nature of sporadic outbreaks of high-prevalence scouring when worm egg counts remain low. This typically involves just one of several flocks of mature sheep on a property, and only a minority of farms in a district are affected in any one year. The time of onset for both low worm egg count scouring syndromes varies but is always after the autumn break, when sheep are grazing green annual pastures.

Studies of flocks in Western Australia in which a large proportion of animals were scouring but mean worm egg counts were low provide good evidence that this is a hypersensitivity response, and also emphasise the difficulty of confirming this diagnosis other than by excluding other possible causes (Jacobson *et al.*, 2009). Intensive investigations in eight scouring flocks of ewes with low WEC (mean egg count, 104 epg) found histopathological intestinal changes and immunological indices indicative of a hypersensitivity response to worm larvae, although this was not definitive as there was no consistent difference between the diarrhoeic and normal sheep. However, larval hypersensitivity scouring was the presumptive diagnosis on the basis that other diseases (infectious and metabolic) were excluded as causes, and the epidemiological factors were consistent with the syndrome. Of note, is the lack of any clear association between the occurrence of low worm egg count scouring and the effectiveness of strategic worm control measures in a flock.

Low WEC scouring in mixed age Merino wethers in south west Western Australia (Mediterranean environment) with 60% sheep classed as moderate or severe dag (Source: C Jacobson).

Diagnosis of hypersensitivity scouring

DIAGNOSIS OF HYPERSENSITIVITY SCOURING IS USUALLY MADE WHEN OTHER CAUSES OF SCOURING HAVE BEEN EXCLUDED (E.G. HIGH WORM BURDENS, BACTERIAL ENTERITIS, PROTOZOAL DISEASE, ACUTE ACIDOSIS) IN SCOURING SHEEP THAT HAVE LOW WEC.

There are no diagnostic tests or clinical findings that allow a definitive diagnosis of larval hypersensitivity scouring syndrome, and no specific treatment. The longer-term strategy of selection for both worm resistance and lower dag scores provides the best approach to its management.

Increased eosinophils are observed in the mucosal cellular infiltrate on gut histopathology at post mortem (Larsen *et al.*, 1994), and a number of studies have noted correlations between faecal consistency and gut tissue eosinophils or IgE (Bisset *et al.*, 1996; Shaw *et al.*, 1998; Shaw *et al.*, 1999; Williams *et al.*, 2010a; Williams *et al.*, 2010b). However, observed differences described to date were relative to unaffected sheep. There are no published descriptions or grading systems for the histological evaluation of 'eosinophilic enteritis' in sheep, and associations between variable eosinophilic infiltration and clinical signs (diarrhoea) have not been determined. This makes it challenging to determine whether eosinophilic infiltration in any given case is indicative of disease, and relative severity of any such changes.

Pasture larval counts can be used to demonstrate exposure to strongyle larvae. However, pasture larval counts are labour intensive. In addition, interpretation is difficult given the small numbers of larvae required to induce scouring in susceptible sheep (Larsen, 2000), so pasture larval counts are of limited value in diagnosis of larval hypersensitivity scouring.

Control and management of hypersensitivity scouring

An important feature of hypersensitivity scouring in sheep is that it is triggered by exposure to only a relatively low number of worm larvae (Larsen, 2000). Unfortunately, this means that best-practice worm control programs will often not eliminate dag caused by hypersensitivity scouring in susceptible sheep.

Hypersensitivity scouring is primarily associated with the response of the sheep to infective larvae. The scouring response to nematode challenge is heritable and repeatable (Larsen *et al.*, 1995b). Heritability for faecal consistency has been estimated around 0.22, although this varies between studies (McEwan *et al.*, 1992; Karlsson and Greeff, 1996; Greeff and Karlsson, 1999; Pollott *et al.*, 2004). Consequently, genetic selection is the best long-term option currently available for control of hypersensitivity scouring. As a visual trait, dag is readily observed by farmers and stud-breeders and is cheap to measure, and thus may readily be incorporated into a breeding objective. Importantly, hypersensitivity scouring is not associated with poor protective immunity to worms as dag and WEC are separate genetic traits (Pollott *et al.*, 2004). This has important implications for managing hypersensitivity scouring as breeding sheep for enhanced parasite resistance using WEC ASBV will not decrease dag. This is counter-intuitive, and contrary to the expectations of most farmers and advisers. As these are independent traits, genetic progress on both dag and WEC ASBV can be made simultaneously where both traits are included in selection index (Karlsson and Greeff, 2006).

As hypersensitivity scouring is repeatable, phenotypic culling of ewes with repeated dag will reduce the burden of dag on farm (Larsen *et al.*, 1995b; Larsen *et al.*, 1999).

Long-acting treatments

The use of long-acting anthelmintics, given as slow release capsules or depot injections of moxidectin, have been considered as potential tools for reducing the risk of hypersensitivity scouring, presuming an effect against the infective larval stage after ingestion from pasture. These treatments are widely used in Australia, with production benefits due to both the immediate reduction in parasitism and the longer-term epidemiology effect of reducing pasture larval contamination (Gogolewski *et al.*, 1997; Allerton *et al.*, 1998; Larsen *et al.*, 2009). However, these studies were not aimed at investigating (and did not report in depth) whether these treatments were associated with a reduction in worm-induced scouring. Despite some anecdotal indications by sheep producers of positive effects, objective information is conflicting, with observations from a marked reduction in scouring through to no evidence of improvement (Jacobson *et al.*, 2009).

The sustained suppression of worms in sheep that results in production benefits would also be expected to reduce or prevent scouring related to adult worm burdens, and long-acting products may therefore be of significant benefit in animals subjected to primary parasitism. However, where scouring results from an excessive immunological response, as occurs with hypersensitivity scouring, a positive effect may not necessarily result. Although long-acting macrocyclic lactone anthelmintics prevent the development of infective larvae to adult worms, an immunological response is apparently still incited (Dever *et al.*, 2015). Given that relatively small numbers of larvae are required to initiate hypersensitivity scouring, the syndrome may still occur despite long-acting anthelmintic treatment.

06

IMPACT OF BREEDING FOR LOW WORM EGG COUNT ON SCOURING

Breeding sheep for "worm resistance" (low worm egg counts) has been advocated for some decades as a strategy to permanently reduce pasture contamination with worm eggs, and hence the risks of sheep acquiring excessive worm burdens (Woolaston and Windon, 2001; Karlsson and Greeff, 2006). Although the worm burdens of worm resistant sheep are not necessarily reduced to the same degree as their worm egg counts, the lower rates of larval challenge from pasture (due to lower egg output) are expected to reduce the pathogenic effects of worms over time.

However, an association between worm resistance and an increased propensity for dag has been observed in sheep old enough to have developed immunity to worms from lines selected for low worm egg count (Douch *et al.*, 1995; Karlsson *et al.*, 2004) This poses the question of whether scouring is an unintended (and paradoxical) side-effect that partially offsets benefits of reduced worm egg excretion, and whether this occurs only in specific and defined situations. It has been postulated that the basis of worm resistance is a more intensive immunological response to worm larvae, and that in some individuals, gut inflammation resulting from this immunological response is sufficient to lead to scouring (Larsen *et al.*, 1995b; Shaw *et al.*, 1999; Williams *et al.*, 2008; Williams *et al.*, 2010c).

While dag score and the related faecal consistency score are low-to-moderately heritable traits (Pollott *et al.*, 2004), the reported correlation between dags and worm resistance appears to vary considerably between studies (Colditz *et al.*, 1996; Larsen *et al.*, 1999). Several studies in New Zealand have indicated a negative genetic relationship between dag scores in Romney lines selected for low worm egg count (Bisset *et al.*, 2001). This was clearly shown in a trial comparing lambs originating from ewes in worm resistant or worm susceptible selection lines, in which mean worm egg counts were some 50 times lower in the resistant groups, but the incidence of scouring was significantly greater (Bisset *et al.*, 1997). Of interest, no epidemiological benefit of the reduction in worm eggs contaminating pastures was evident; this is consistent with the demonstration by Larsen and Anderson (2000) that hypersensitivity scouring could be incited by relatively low numbers of infective larvae intake. It is also of interest that Romney lines selected for tolerance to the effects of worms (resilience) showed significantly lower dag scores, but no trend towards lower worm egg counts (Bisset and Morris, 1996; Wheeler *et al.*, 2008).

In Merino sheep, negative correlations between worm resistance and dag scores have been observed in the Rylington Merino flock in Western Australia at hogget age where mean worm egg counts were markedly lower in the resistant Rylington line compared to unselected sheep, and had a negative genetic correlation with dag score of -0.67 in winter (Karlsson and Greeff, 1996). However, the correlation between worm egg count and dag score varied with season and was less unfavourable in seasons when worm challenge was lower. Pollott *et al.* (2004) reviewed Rylington Merino results in the light of earlier studies and concluded that "The trend of a positive correlation post-weaning and a negative correlation at yearling to hogget age fits with the theory of an age-related change from high to low FEC scouring". This is consistent with the conceptual basis of hypersensitivity scouring in worm-immune sheep. On the basis of these results, Greeff and Karlsson (1999) noted that the unfavourable relationship cannot be ignored, therefore worm egg count and dag should be treated as two separate traits for ram selection. They later reported that since including faecal consistency score and dag score in Rylington breeding indexes, the mean dag score had decreased significantly (Karlsson and Greeff, 2007).

IN WINTER RAINFALL REGIONS, RAM BREEDERS SHOULD INCLUDE BOTH WORM RESISTANCE AND DAG SCORE IN SELECTION INDEXES.

As an indirect indication of the complexity of the worm resistance-dag relationship, several investigations have confirmed that worm resistant sheep are not necessarily associated with low dag scores. Observations on three farms in Victoria (Larsen *et al.*, 1994) showed that sheep with low worm egg counts were just as likely to develop severe dag as those with high counts. A later study (Larsen, 2000) included a line of Merinos with consistently high dag scores associated with low worm egg counts, although a similarly worm resistant line had consistently low-dag scores suggesting both high and low WEC sheep can accumulate severe dag. More recently, pen studies in Western Australia showed that worm resistant Merino rams challenged with worm larvae for 6 weeks maintained low worm egg counts and had low worm burdens at slaughter, although this was associated with looser faecal consistency in those rams that were genetically predisposed to dag (Williams *et al.*, 2010c). Both Victorian and Western Australian studies included groups of highly worm resistant sheep that were not genetically predisposed towards higher dag scores, indicating that selection for worm resistance does not necessarily carry a penalty of increased dag development. This confirms that dag and WEC are separate genetic traits, and both of these traits should be included in selection indexes where the goal is to reduce dag.

Of note, all of the studies reported above were from winter rainfall regions, where hypersensitivity scouring is a significant factor. In summer rainfall regions, where *Haemonchus contortus* is the major worm species and scouring is of most importance in worm-susceptible lambs, the correlation between worm resistance and dag score is far lower (Woolaston and Ward, 1999). These authors concluded that there was little benefit of selecting against dag score, although their analyses did indicate a slight reduction in dag score in sheep selected for worm susceptibility. Further environmental effects are expected to be evident from analyses of worm egg count and dag scores data from the Information Nucleus flocks managed by the Sheep CRC. Progeny tests conducted indicate that phenotypic and genetic correlations between WEC and DAG at individual age-stages were generally low, and dag score is a poor indicator for worm resistance (Smith, 2011). Analyses by sheep age and environment may clarify these relationships.

It therefore appears that in environments predisposed to hypersensitivity scouring (i.e., winter rainfall regions), breeding for worm resistance (low worm egg count using WEC ASBV) alone is likely to entail co-selection for increased dag scores in some individuals, presumably as a result of a greater immunological response to worm challenge. However, only some worm resistant sheep have this propensity, and including selection against both dag scores and WEC in the genetic index can minimise the risk of an unfavourable side-effect. The demonstration of negative correlations with animal production in some trials (Eady *et al.*, 1998; Bisset *et al.*, 2001) underlines the necessity for a multi-trait selection index in breeding against parasitic effects (Greer, 2008; Williams, 2011).

Breeding for worm resistance (low worm egg count) has been variably adopted since its incorporation into Australian Standard Breeding Values, and is yet to be accepted by many ram breeding operations. Even where worm egg counts are routinely measured to provide a worm resistance index, it is not always appreciated that worm resistance and dag (due to hypersensitivity scouring) are separate traits, and that there is in fact a negative correlation between them (i.e. that in winter rainfall regions the most worm resistant sheep have an increased propensity to scour). It is now recommended that dag is specifically selected against, especially in highly worm resistant animals (Bisset *et al.*, 2001; Karlsson and Greeff, 2012).

This is not straightforward, as selection against dag is best conducted in hogget-age sheep, even though the correlation between worm egg counts as weaners compared to hoggets is probably sufficient to allow inferences over rankings for worm resistance (especially at high and low extremes). However, as it is necessary to delay dag observations until differences considered due to hypersensitivity scouring are evident between sire-groups, the worm challenge must be allowed to persist until scouring occurs in many sheep in progeny groups. With the obvious implications for flystrike risk and the need for crutching, this will not appeal to many ram breeders. It is fair to say that at present, there is no consistent or structured approach to breeding against the propensity for hypersensitivity scouring, despite the inadvertent implications for failing to do this where worm resistance is incorporated into ASBVs in sheep in winter rainfall regions. Agreement is needed regarding protocols for the measurement of dag and faecal consistency (scouring) score as a trait, along with the communication of its importance to ram breeders.

Concurrent selection for low WEC and low dag scores is important for managing susceptibility to dag in worm-resistant sheep (Source: B Besier).

07

PROTOZOAN PARASITES

PROTOZOAN PARASITE INFECTIONS MAY BE ASSOCIATED WITH DIARRHOEA AND OTHER SIGNS OF ILL-HEALTH IN SHEEP. INTERPRETATION OF DIAGNOSTIC TESTS AND CONFIRMATION OF PROTOZOAN DISEASE IN SCOURING SHEEP IS NOT STRAIGHTFORWARD BECAUSE INFECTIONS ARE COMMONLY ASYMPTOMATIC AND OCCUR CONCURRENTLY WITH OTHER PARASITIC AND BACTERIAL AGENTS.

Coccidiosis

Coccidiosis is caused by microscopic protozoan (single-celled) parasites of the *Eimeria* genus, of which at least 11 species have been reported in Australian sheep (O'Callaghan *et al.*, 1987; Yang *et al.*, 2016). *Eimeria* species vary in pathogenicity, with *E. ovinoidalis* and *E. crandallis* associated with more severe disease (Wright and Coop., 2008; Andrews, 2013).

Sheep are infected by oral ingestion of infective sporulated oocysts via the faecal-oral route. Sporulated oocysts can persist in the environment and may remain viable for more than a year under favourable conditions (Foreyt, 1990). Almost all sheep encounter coccidia as lambs, typically via contaminated pasture, feed, bedding or udder of ewes.

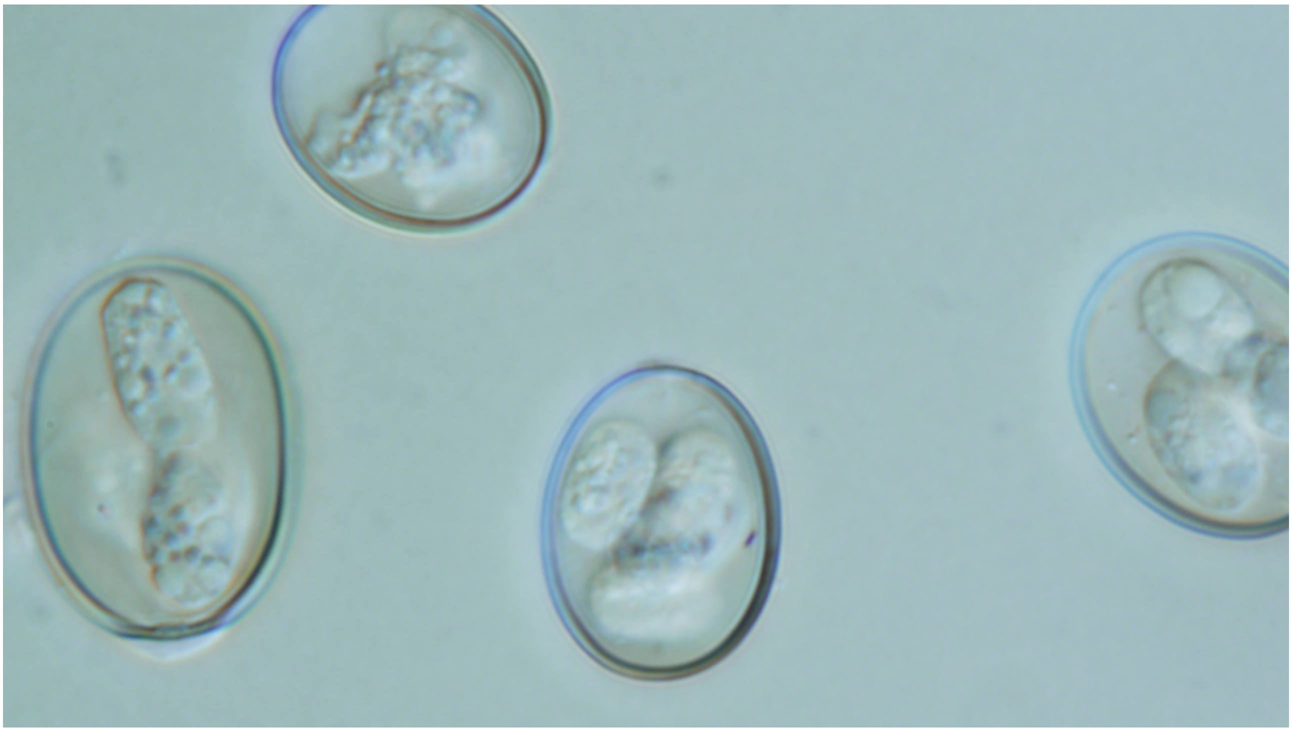
Asymptomatic infections are common in sheep. Gut damage and inflammation may cause scouring within a few weeks of birth. Lambs typically develop a strong immunity whether or not clinical signs occur, although chronic infections with shedding can occur in sheep of any age that act as carriers.

Severe coccidiosis outbreaks associated with scouring, inappetence, weight loss and deaths of weaker lambs can occur. Outbreaks are typically sporadic, usually occur in young sheep (less than 6 months old), and are associated with high stocking densities (overcrowding, particularly for sheep housed in barns or feedlots) or other stressors such as inclement weather (such as prolonged cold wet conditions), poor nutrition, stressful management procedures (such as weaning) or concurrent disease (Foreyt, 1990; Wright and Coop., 2008; Chartier and Paraud, 2012). Whilst outbreaks of clinical disease are most commonly associated with intensive husbandry, outbreaks in extensively managed unweaned lambs may occur where ewes are in poor condition and lambs are forced to graze earlier than usual. Clinical coccidiosis can occur in older sheep when there is overwhelming infection pressure (usually associated with overcrowding) and concomitant stressors.

DIAGNOSIS OF COCCIDIOSIS IS USUALLY BASED ON A COMBINATION OF HIGH FAECAL OOCYST COUNTS USING SALT OR SUGAR FLOATATION, PRESENCE OF CLINICAL SIGNS, CHARACTERISTIC GROSS OR HISTOPATHOLOGICAL CHANGES, AND HISTORY OF PREDISPOSING FACTORS.

The site of infection and gross or histopathological changes in small and large intestine varies between *Eimeria* species and specific life cycle stage. Oocysts counts should be interpreted with caution. Oocyst shedding varies between species, may be intermittent, and it is common for moderate oocyst counts to be observed in the absence of clinical disease. Diarrhoea may precede oocyst shedding in acute disease. Speciation is recommended to differentiate non-pathogenic species (Keeton and Navarre, 2018), but distinguishing *Eimeria* species in disease investigations is challenging. Methods based on oocyst morphology, pre-patent period, site of infection, or minimum sporulation time are labour intensive, time-consuming and lack specificity due to overlapping morphological characteristics between species (Tenter *et al.*, 2002; de Waal, 2012). Molecular methods based on DNA detection can be used for detection, quantitation and species identification in sheep (Yang *et al.*, 2016), but are not widely available outside of research settings.

Disease investigations where coccidiosis is suspected should consider concurrent infections that increase severity of clinical disease, and conversely *Eimeria* infection is correlated with increased susceptibility to other infections (Foreyt, 1990; Mohammed *et al.*, 2000; Wright and Coop., 2008; Andrews, 2013).



Microscopy appearance of *Eimeria* oocysts. These oocysts have been sporulated to identify the species present (Source: A Elliot, Murdoch University).

Treatment for coccidiosis

Clinical coccidiosis is usually considered a self-limiting disease (Chartier and Paraud, 2012). Treatment is rarely warranted in cases of coccidiosis in Australia. Treatments with evidence of efficacy for coccidiosis include triazones (toltrazuril, diclazuril) and amprolium (Chartier and Paraud, 2012; Andrews, 2013; Keeton and Navarre, 2018). However, these treatments are not registered for use in sheep in Australia. Sulphonamide antibiotics are sometimes used for managing outbreaks. Sulphonamides only have activity against the last stage of the lifecycle (Chartier and Paraud, 2012), and it has been suggested clinical response may be related to controlling secondary infection rather than direct impact on *Eimeria* spp. (Keeton and Navarre, 2018). Further, sulphonamide antibiotics do not have label claims for coccidiosis in Australia, and the recommendation that treatment be given over a period of 3-5 days further limits practicality for treatment of outbreaks (Chartier and Paraud, 2012). In-feed coccidiostats (monensin, lasolacid, decoquinate) may be warranted where heavy environmental contamination with infective oocysts is likely and on-going prevention is justified (Keeton and Navarre, 2018), such as occasionally occurs in feedlot situations. Of these, monensin and lasolacid sodium are currently registered for use in sheep in Australia with label claims for prevention of coccidiosis. Guidelines are available for assessing drug efficacy against *Eimeria* spp. (Joachim *et al.*, 2018).

Cryptosporidium

Cryptosporidiosis is widely recognised as an important cause of diarrhoea in lambs (de Graaf *et al.*, 1999; Paraud and Chartier, 2012; Robertson *et al.*, 2013). Whilst cryptosporidiosis has been considered a problem mainly for sheep raised under intensive conditions, *Cryptosporidium* infections have been associated with looser faecal consistency and faecal soiling in Australian lambs aged 1-8 months old raised under extensive conditions (Sweeny *et al.*, 2011b; Sweeny *et al.*, 2012a). Further, there is emerging evidence that natural infections in older sheep (beyond weaning age) under Australian conditions may be associated with reduced liveweight, reduced carcass weight and reduced processing efficiency (Jacobson *et al.*, 2016).

The impact of infection on sheep health and production are not fully understood. Asymptomatic infections are common, and clinical disease is generally considered self-limiting (Paraud and Chartier, 2012). However, recent studies have shown that naturally acquired *C. parvum* infections detected in the post-weaning period may impact carcass weight at slaughter approximately 10 weeks after shedding was detected (Jacobson *et al.*, 2016). Further challenging the notion that infections are self-limiting and impacts are restricted to very young lambs, Australian studies have shown that repeated detection of *C. parvum* shedding by sheep from weaning age on was associated with greater impacts on carcase weight at slaughter (Jacobson *et al.*, 2016).

Seven *Cryptosporidium* species have been reported in sheep worldwide, of which *C. xiaoi* (formerly *C. bovis*-like genotype), *C. parvum* and *C. ubiquitum* (formerly cervine genotype) are most commonly reported in Australian sheep (Ryan *et al.*, 2005; Yang *et al.*, 2009; Sweeny *et al.*, 2011c; Sweeny *et al.*, 2012a; Yang *et al.*, 2014c; Yang *et al.*, 2015). Of these, *C. parvum* and *C. ubiquitum* subtypes isolated from Australian sheep are recognised as zoonotic and with public health significance (Xiao, 2010; Li *et al.*, 2014). *Cryptosporidium xiaoi* infections have been recently in immunocompromised humans, and this species should be considered potentially zoonotic (Adamu *et al.*, 2014).

Cryptosporidium shedding in faeces is common and widespread in Australian sheep with mean shedding detection ranging 6-68% sheep for studies conducted in asymptomatic flocks sampled on a single occasion (Ryan *et al.*, 2005; Yang *et al.*, 2009; Sweeny *et al.*, 2011c; Yang *et al.*, 2014c; Yang *et al.*, 2015). As with *Eimeria*, *Cryptosporidium* shedding is more common in younger sheep and this has been shown to be the case under Australian conditions (Ryan *et al.*, 2005; Sweeny *et al.*, 2011c; Yang *et al.*, 2014c).

Infections are transmitted through ingestion of oocysts via contaminated feed or water, or by licking contaminated materials. A peri-parturient rise in faecal oocyst shedding has been reported in ewes (Xiao *et al.*, 1994; Ortega-Mora *et al.*, 1999). The infectious dose for lambs is as low as one oocyst (Blewett *et al.*, 1993).

Diagnosis of cryptosporidiosis is challenging for field investigations. Oocysts can be detected in faeces using microscopy with specific staining techniques, indirect immunofluorescence and enzyme immunoassays, but these methods lack sensitivity compared to molecular techniques (Casemore, 1991; Elliot *et al.*, 1999; Ryan *et al.*, 2005; Brook *et al.*, 2008). Molecular techniques are used in epidemiological studies to quantify shedding and molecular characterisation (Yang *et al.*, 2014c), but are not widely available for routine diagnostic investigations. Characteristic histopathological changes on post-mortem exam can be used to confirm diagnosis in outbreaks with high morbidity and mortality. Catarrhal enteritis, distension of caecum and colon, congestion and haemorrhagic inflammation in the last third of the ileum, and/or hypertrophy of the mesenteric lymph nodes may be observed on gross post-mortem examination, but are not pathognomonic for cryptosporidiosis (Paraud and Chartier, 2012).

Treatments for *Cryptosporidium*

There are limited treatment options for treatment or prevention of cryptosporidiosis outbreaks. European studies have assessed treatments including halofuginone lactate, paromomycin and β-cyclodextrin for management of naturally-acquired coccidiosis in young lambs (Viu *et al.*, 2000; Castro-Hermida *et al.*, 2001; Giadinis *et al.*, 2007), but these are not currently registered for use in sheep in Australia.

Microscopic appearance of (1) *Giardia* cysts and (2) *Cryptosporidium* oocysts. These oocysts are smaller than *Eimeria* and nematode oocysts, making them more challenging to identify in faecal samples (Source: A Elliott, Murdoch University).

Giardia

GIARDIA HAS BEEN ASSOCIATED WITH LOOSER FAECAL CONSISTENCY IN LAMBS UP TO 10 MONTHS OF AGE.

Giardia infections have been associated with diarrhoea in young lambs (Olson *et al.*, 1995; Aloisio *et al.*, 2006; Jafari *et al.*, 2014). In Australia, naturally-acquired infections have been associated with looser faecal consistency in lambs up to 10 months old (Sweeny *et al.*, 2011b; Sweeny *et al.*, 2012a). As with other protozoan parasites, giardiasis may occur concurrently with other infections (Taylor *et al.*, 1993). Asymptomatic infections are common, and the role of *Giardia* as a primary cause of diarrhoea in livestock remains poorly understood (Geurden *et al.*, 2010).

Giardia is generally considered as a self-limiting infection in sheep. However, experimental and natural infections have been associated with reduced growth rate and reduced carcase weight weeks or months after detection of infection (Olson *et al.*, 1995; Sweeny *et al.*, 2011b; Jacobson *et al.*, 2016).

Giardia duodenalis comprises a species complex consisting of eight genetic assemblages. Assemblages A and E have been widely reported in Australian sheep (Ryan *et al.*, 2005; Yang *et al.*, 2009; Yang *et al.*, 2014a; Yang *et al.*, 2015), and assemblage B has been sporadically reported in sheep overseas (Zhang *et al.*, 2012). *Giardia duodenalis* assemblages A and B are considered zoonotic and of public health significance (Feng and Xiao, 2011). There is emerging evidence that assemblage E may also be zoonotic (Abdel-Moein and Saeed, 2016; Zahedi *et al.*, 2017).

As with other protozoan infections, diagnosis of giardiasis is not straightforward. Cysts can be detected in faeces using microscopy (with or without concentration using sucrose, zinc sulphate or formalin) or immunofluorescence assay, but these cannot differentiate species or assemblages (Geurden *et al.*, 2010; Adeyemo *et al.*, 2018). Further, cyst-shedding by infected sheep is sporadic, and low numbers of cysts or steatorrhea can interfere with *Giardia* detection using microscopy (Geurden *et al.*, 2010; Soares and Tasca, 2016; Adeyemo *et al.*, 2018). The sensitivity and specificity of immunoassays is highly variable due to antibody cross-reactions and sensitivity can be as low as 44% (Soares and Tasca, 2016). As with *Cryptosporidium*, quantitative molecular techniques with increased sensitivity for detection of cysts in faeces and capacity to distinguish genotypes are used in epidemiological studies (Yang *et al.*, 2014a; Soares and Tasca, 2016), but are not widely available for routine diagnostic investigations in Australia. Serological tests are not considered to be reliable indicators of disease (Yanke *et al.*, 1998; O'Handley *et al.*, 2003).

Treatments for *Giardia*

There is little data available on treatment efficacy for giardiasis in sheep (Geurden *et al.*, 2010). A three-day course of fenbendazole reduced cyst shedding (but not faecal consistency or weight gain) in housed lambs aged 12 weeks old (Geurden *et al.*, 2011). However, fenbendazole is not registered for treatment of giardiasis in sheep in Australia.

08

BACTERIAL CAUSES OF SCOURING

CLINICAL BACTERIAL ENTERITIS OUTBREAKS ARE OFTEN ASSOCIATED WITH CONCURRENT STRESS OR DISEASE, INCLUDING NUTRITIONAL STRESS, WORM INFECTIONS, CROWDING, AND INCLEMENT WEATHER.

Bacterial enteritis and scouring is recognised as an important and emerging disease syndrome, particularly for weaned sheep in the high winter rainfall areas of southern Australia. Bacterial enteritis was ranked fifth in terms of costs to the Australian sheep industry behind internal parasites, blowfly strike, lice and perennial ryegrass toxicity (Sackett *et al.*, 2006). A subsequent review estimated costs for bacterial enteritis at $10.27 million per annum (Lane *et al.*, 2015). The recent experience of many specialist animal health advisers and consultants in south-eastern Australia suggests that the weaner enteritis is occurring more commonly and severely each year.

The bacterial agents most commonly associated with scouring in Australia are *Yersinia* and *Campylobacter spp*. Of these, clinical disease outbreaks are more commonly reported with *Yersinia,* although *Campylobacter* outbreaks sporadically cause significant mortality and production loss in affected flocks. Scouring may also be observed with salmonellosis (Watts and Wall, 1952; Dennis, 1965) and enterotoxaemia (*Clostridium perfringens* type D) (Lewis, 2000). Ovine Johne's disease is not a frequent cause of scouring, with severe body wasting more commonly observed.

Diagnosis of bacterial enteritis is complicated because several bacteria species that may be associated with enteritis and/or colitis are commonly isolated from asymptomatic sheep. Bacterial causes of scouring are easily distinguished from parasitic or hypersensitivity scouring by characteristic gross and histopathological changes, bacterial isolation by cultures or molecular diagnostics, response to treatment with antibacterial drugs, and association with known risk factors such as sheep age, concurrent stressors and climatic conditions. Recommendations have been established for managing risk in confined feeding scenarios (More, 2002). Some bacteria associated with scouring in Australian sheep have zoonotic potential and management options are increasingly complicated by antimicrobial resistance, therefore management of risk factors for bacterial shedding has important public health implications throughout sheep supply chains (Adams *et al.*, 1997; Humphrey *et al.*, 2007; Abraham *et al.*, 2014; Yang *et al.*, 2017).

Bacterial enteritis in a Merino weaner caused by *Yersinia pseudotuberculosis* showing enlarged mesenteric lymph nodes and diphtheritic enteritis in the jejunum (Source: J Larsen).

Yersiniosis – 'winter scours'

Yersinia pseudotuberculosis serotype III and *Y. enterocolitica* may cause enteritis in sheep (Slee and Button, 1990; Philbey *et al.*, 1991; Slee and Skilbeck, 1992). A recent study found *Y. pseudotuberculosis* and virulent *Yersinia enterocolitica* were the most frequently isolated organisms from weaned Merino lambs in south-eastern Australia affected with bacterial enteritis (Stanger *et al.*, 2018a). *Yersinia* spp. were isolated more frequently in lambs with high worm egg counts (> 500 eggs per gram of faeces). In a two-year study on four high-risk farms, shedding of *Y. pseudotuberculosis* occurred predominantly in winter, whereas *Y. enterocolitica* was isolated from faeces throughout the year. This was consistent with the timing of outbreaks of yersiniosis, which occurred only during the winter in association with *Y. pseudotuberculosis*, but also during summer and autumn when *Y. enterocolitica* was the main cause (Stanger *et al.*, 2018b). *Yersinia enterocolitica* caused a similar number of outbreaks of yersinosis to *Y. pseudotuberculosis* in this case series and so is probably a more important cause of bacterial enteritis than previously recognised.

Yersiniosis typically affects weaner sheep in winter, when pasture growth rates are low and there is stress from cold, wet and windy conditions and increased challenge with worm larvae. Yersiniosis may be associated with scouring and ill thrift, and death rates of 3-20% are reported in clinical outbreaks. Outbreaks are not uncommon in south western Victoria with 10% of leading wool growers in the South Roxby project indicating yersiniosis was a primary concern in managing Merino weaners, with 13% of respondents reporting outbreaks in which from 1-15% of weaners died (Larsen, 1999). In addition, *Yersinia* spp. in sheep include known human pathogens, and so faecal shedding represents potential public health risk (Sutherland *et al.*, 2009). The impact of sub-clinical infections are not well studied, and *Y. enterocolitica* infection may be associated with reduced liveweight in slaughter lambs under Australian conditions, even in the absence of scouring (Jacobson *et al.*, 2018).

A high proportion of *Yersinia pseudotuberculosis* serotype III (64%) and virulent *Yersinia enterocolitica* (87%) isolates from south-eastern Australia were resistant to sulfafurazole. This antibacterial is often used as a first line of treatment in cases of bacterial enteritis, but such widespread resistance indicates that other antibacterial agents should be considered as appropriate, in addition to moving affected mobs to less contaminated pasture (Stanger *et al.*, 2018a).

Campylobacteriosis - weaner colitis

Campylobacter jejuni and *C. coli* shedding is commonly reported in otherwise healthy sheep under Australian conditions (Yang *et al.*, 2014b; Yang *et al.*, 2017). As with yersiniosis, campylobacteriosis outbreaks are typically associated with high stocking density and concurrent stressors including poor weather and concurrent infections (Napthine, 1988; Glastonbury, 1990). During outbreaks, sheep with diarrhoea may be clinically unwell with ill thrift and depression, and deaths may occur.

Nine-month-old Merino weaner with bacterial enteritis caused by *Yersinia pseudotuberculosis* (Source: J Larsen).

09

ENDOPHYTE

Alkaloid toxins (lolitrem and ergovaline) produced by wild-type endophytes (fungi) associated with perennial ryegrass have been reported to increase the incidence of diarrhoea in lambs (Eerens *et al.*, 1992; Fletcher and Sutherland, 1993; Pownall *et al.*, 1993). Newer cultivars of perennial ryegrass contain the AR1 endophyte, sourced from European cultivars that do not produce lolitrem or ergovaline. The grazing of perennial ryegrass pastures with the modified endophyte is associated with increased growth rates and decreased faecal soiling in lambs (Fletcher *et al.*, 1999) and increased milk production in dairy cows (Bluett *et al.*, 2005).

While endophytes and their alkaloids have the potential to cause scouring, the highest levels of alkaloids are often detected in ryegrass in summer and early autumn, in response to the environmental stressors of high temperature and low rainfall, and increased pasture litter. Consequently, scouring associated with alkaloid toxoids is usually separated in time from other possible causes of scouring and dag formation.

Severe dag in a Merino hogget with a low worm egg count (<150 eggs per gram). The diarrhoea was attributed to hypersensitivity scouring exacerbated by exposure to an old stand of Victorian Perennial Ryegrass (Source: J Larsen).

10

NUTRITIONAL SCOURING

DIAGNOSIS OF 'NUTRITIONAL SCOURING' TENDS TO BE A NON-SPECIFIC DIAGNOSIS OF LAST RESORT.

Diet, and especially pasture, is commonly ascribed as a cause for scouring. However, the specific nutritional components of pasture that may induce scouring, and interaction between nutrition and other causes of scouring remain poorly understood. Feed components hypothesised to cause scouring include carbohydrate, crude protein, nitrate, fat, water and macro and trace minerals. Other pasture components linked to scouring include alkaloid toxins (principally lolitrem B and ergovaline) produced by wild-type endophytes most commonly associated with perennial ryegrass and Demeter fescue as discussed in the previous section.

Carbohydrates and sub-clinical ruminal acidosis

Nutritional scouring is most commonly observed when sheep are grazing green lush rapidly growing forages. Such pastures are typically rich in non-structural carbohydrates and organic acids, and low in neutral detergent fibre. This has led to the suggestion that high levels of fermentable carbohydrates may lead to altered fermentation patterns and/or an increase in the osmotic potential of digesta leading to changes in motility and greater retention of water. Acidosis is caused by rapid fermentation of carbohydrates in the rumen or the hindgut (caecum and large intestine), which leads to the accumulation of organic acids (volatile fatty acids and lactic acid), and a fall in ruminal and/or caecal pH (Plaizier *et al.*, 2008). Acute acidosis can be observed after rapid introduction to diets that are rich in starch, and acidosis is a widely recognised risk when feeding grain to sheep (Reference Advisory Group on Fermentative Acidosis of Ruminants, 2007).

There is considerable debate as to the definition of ruminal acidosis, with subacute ruminal acidosis particularly challenging to define (Plaizier *et al.*, 2018). In cattle, it is now accepted that diagnosis of ruminal acidosis based solely on rumen pH values is inaccurate. Instead, diagnosis should be based on combination of assessments of milk, blood and faeces, as well as consideration of herd management and key dietary risk factors (including NDF, physically effective NDF and non-fibre carbohydrates) (Plaizier *et al.*, 2018). A large number of studies have identified that low rumen pH does not predict clinical outcome (Plaizier *et al.*, 2018), and alternative rumen measures including combination of rumen VFA concentrations, particularly valerate and propionate and rumen ammonia are better predictors of outcome for cows fed diets high in non-fibre carbohydrates and lower NDF (Bramley *et al.*, 2013; Lean *et al.*, 2013).

Looser faecal consistency and diarrhoea is observed with sub-acute rumen acidosis in cattle, including cattle fed predominantly pasture (Bramley *et al.*, 2008; O'Grady *et al.*, 2008; Plaizier *et al.*, 2008; Bramley *et al.*, 2013). Increasing levels of pasture in the diet have been associated with an increased risk of diarrhoea in dairy cattle (Bramley *et al.*, 2013). High-risk pastures are characterised by lower neutral detergent fibre and higher non-structural carbohydrate content of pasture (Kolver and de Veth, 2002; Bramley *et al.*, 2008).

Sub-acute rumen acidosis is not well understood in sheep (Reference Advisory Group on Fermentative Acidosis of Ruminants, 2007; Fanning, 2016), with work required to elucidate whether this syndrome plays a role in scouring for pasture-fed sheep. Clinical acidosis has been reported in sheep fed pasture diets without access to grain (Ellem *et al.*, 2016). Provision of roughage (hay, straw) to sheep on high-risk pastures has been associated with lower incidence of moderate-severe dag in ewes, but the study didn't investigate rumen fluid characteristics with different diets, or the mechanism by which provision of roughage may have impacted faecal consistency (Davidson *et al.*, 2006).

Diet changes

The practice of moving sheep between forage sources has been implicated as a cause of scouring. It is possible that maladaptation of rumen micro-flora to a higher quality feed source could account for scouring during transition to new forage source.

Protein and nitrates

The impact of dietary protein on faecal consistency is not well studied in ruminants. Cattle fed diets higher in crude protein had looser faecal consistency, however source of protein seems to have greater impact than level of dietary protein, and considerable variation was observed between cattle fed the same diet (Ireland-Perry and Stallings, 1993).

Young rapidly growing forages are typically high in crude protein, and specifically degradable protein. This protein is degraded by microbes in the rumen, with ammonia accumulating in rumen or hindgut contents if the rate of production exceeds that of microbial utilisation. It has been proposed that accumulation of ammonia may lead to gut damage or altered motility responsible for scouring in sheep, but supporting evidence is lacking (Wesselink *et al.*, 1995).

As discussed previously, forages that have been anecdotally associated with scouring include capeweed (*Arctotheca calendula*), forage oats (*Avena sativa*) and various brassica crops. Apart from high levels of non-fibre carbohydrates and crude protein, these species may accumulate nitrate with nitrates as high as 2.0-4.4% DM in capeweed during rapid growth (Harris and Rjodes, 1969). Nitrates may be lethal at more than 1.5% dry matter due to the production of methemoglobinaemia resulting in hypoxia and death. Sub-lethal ingestion of nitrates at 0.5 and 1.5% DM may have a direct caustic effect on gut mucosa resulting in gastrointestinal irritation and diarrhoea (Everist, 1974; Radostits *et al.*, 1994), and this could explain diarrhoea in sheep grazing affected pasture. Some adaptation may occur with chronic exposure to nitrates (Galey, 1998).

Nitrate accumulation by plants varies widely and is affected by a range of factors that influence the balance between nitrogen update from soil and utilisation by plants, including plant species, soil factors, light and temperature conditions, and herbicide treatments (Galey, 1998). Nitrate levels in plants fluctuate, making it challenging to ascribe nitrates as a cause of scouring retrospectively in investigations of so-called scouring outbreaks. Nutritional scouring is challenging to replicate in experimental settings (Pethick and Chapman, 1991), and it has been suggested that variability in nitrate levels in pasture complicates replication of field conditions in pen experiments. Water contaminated via biological runoff, industrial effluent and fertilizer may also contribute to nitrate toxicity in sheep (Galey, 1998).

Minerals

Mineral imbalances have been hypothesised as a cause of nutritional scouring in sheep grazing pasture. High potassium levels (3-4% DM) are widely reported in lush pasture, particularly if there has been a recent history of application of potassium fertilizer (Pethick and Rowe, 1998). Short green pastures with high potassium relative to sodium and calcium have

Scouring and dag in crossbred lambs grazing a rape crop (Source: J Larsen).

been associated with scouring in ewes (Trengove, 1999). Dietary cation content, particularly potassium and sodium, affect the osmolality of digesta. Little potassium absorption occurs in the large intestine (Wesselink *et al.*, 1995), therefore high dietary cation levels or cation malabsorption (due to disease or rapid flow rate) may be associated with increased osmolarity for digesta and subsequent retention of water in faeces. Magnesium absorption is also likely to be inhibited on pastures with very high potassium content (Pethick and Rowe, 1998).

Trace elements

Scouring may be observed as a minor clinical sign in flocks with selenium deficiency. Other clinical signs may include nutritional degenerative myopathy (white muscle disease), reduced wool growth and ill-thrift. Scouring is described as "selenium responsive" as it is not consistently observed in flocks with confirmed deficiency, so caution should be taken when considering selenium deficiency as a primary cause of scouring. The pathogenesis of scouring with selenium deficiency is not understood, although it has been suggested that it could be related to depressed immune state resulting in increased susceptibility to internal parasites (Glastonbury, 1990). However, several studies have shown that selenium supplementation of weaner sheep fed selenium deficient diets did not impact nematode burdens (Jelinek *et al.*, 1988; McDonald *et al.*, 1989) and a number of reviews concluded there is no evidence that selenium status is associated with parasite establishment (Suttle and Jones, 1989; Lee *et al.*, 2002; McClure, 2003).

Scouring may also be a minor clinical finding with selenium toxicity following accidental overdosing. Other clinical signs include dyspnoea, bloat and abdominal pain and death due to respiratory failure.

Scouring may be observed with chronic cobalt (vitamin B_{12}) deficiency (Glastonbury, 1990). More common clinical signs include depressed appetite, ill thrift, anaemia, weepy eyes, reduced wool production and infertility (Napthine, 1988; Glastonbury, 1990).

Scouring is a minor clinical sign in sheep with copper deficiency, and is more commonly observed where copper deficiency is secondary to excessive molybdenum intake. Other signs associated with chronic copper deficiency include enzootic ataxia, loss of wool crimp, pigment and tensile strength, brittle bones, anaemia and ill thrift (Napthine, 1988; Glastonbury, 1990). The pathogenesis of scouring is not understood, but possibly associated with impaired tissue oxidation and interference with intermediary metabolism (Suttle and Jones, 1989). Scouring, gastroenteritis and abomasal ulceration may also be observed as a minor clinical finding with copper toxicity (Napthine, 1988; Farquarson, 1992).

Dietary moisture

High water intake, either in feed (through consumption of feed high in moisture) or due to polydipsia (drinking), typically increases the amount of urine produced without significantly affecting faecal dry matter or consistency. The majority of water consumed in the diet is excreted via urine, with much smaller quantities excreted via respiration, perspiration and faecal loss (Suttle and Field, 1967). Excess water ingested with pasture is likely to be excreted as urine and unlikely to be related to changes in faecal consistency in otherwise healthy sheep.

Forages commonly linked with scouring

Phalaris aquatica, in particular older stands of Australian phalaris, is often associated with severe scouring and breech soiling in south-eastern Australia. Most of these cases, particularly in sheep more than one-year-old, are most likely primarily due to hypersensitivity scouring, but are undoubtedly exacerbated by some (as yet unidentified) component of phalaris.

Similarly, sheep producers commonly consider capeweed (*Arctotheca calendula*) to be a primary cause of scouring in winter. However, it has proved challenging to replicate scouring in experimental settings where sheep were fed fresh capeweed (Pethick and Chapman, 1991), and sheep often graze capeweed-dominant pastures without scouring. This suggests that variability in specific components within these plants (e.g. nitrate, non-structural carbohydrate or mineral content) or interactions with other agents (e.g. nematode larvae) explain scouring observed in grazing sheep.

Scouring is also sometimes observed in sheep grazing standing cereal and fodder crops, but these effects have not been studied in detail.

Severe scouring and dag in recently shorn Merino ewes near Mortlake, Victoria. These ewes had a low worm egg count (<100 eggs per gram) and the diarrhoea was attributed to hypersensitivity scouring exacerbated by exposure to a pure stand of Australian phalaris (Source: J Larsen).

Condensed tannins

Grazing forages containing condensed tannins have been associated with reduced dag and moderate anthelmintic effects, hence it has been suggested that this could form part of integrated management of scouring and the magnitude of production losses from gastro-intestinal nematodes in sheep (Leathwick and Atkinson, 1995, 1996; Niezen *et al.*, 1995; Min *et al.*, 1998; Niezen *et al.*, 1998; Cole and Heath, 1999; Ramírez-Restrepo *et al.*, 2004). The mechanism by which dietary condensed tannins may impact faecal consistency has not been defined, and other factors may influence the response observed. For example a study in New Zealand showed increased dag in sheep grazing chicory (Niezen *et al.*, 1994), and it was suggested that other feed component (including soluble carbohydrates and dietary moisture) may explain the variable response. There are agronomic challenges in most sheep producing regions that limit the application of grazing condensed tannin forages as a solution to addressing scouring and dag.

Control of nutritional scouring

Control of nutritional scouring is a difficult task, simply because causative factors are seldom fully identified and control strategies uncertain. Typical on-farm responses to control scouring in sheep grazing lush and rapidly growing pastures and forages is to provide access to a source of roughage, usually in the form of hay or an area of lower quality pasture (Davidson *et al.*, 2006). This may assist by slowing digesta transit and support establishment of normal osmotic gradient in the large intestine (Waghorn *et al.*, 1999). However, there is little documented evidence that this strategy is successful in controlling scouring. Furthermore, dietary substitution of highly digestible pastures with a lower quality forage has the potential to depress production.

Loose mixtures containing bentonite (hydrated sodium calcium aluminosilicate) can also be provided to grazing animals). Bentonite is known to have a large surface area capable of adsorbing a range of nutritional and anti-nutritional compounds and has been shown to increase faecal dry matter (Waghorn *et al.*, 1994). There is anecdotal evidence that bentonite can relieve symptoms associated with endophyte alkaloids but this remains to be confirmed as a reliable management option for controlling scouring.

Other on-farm responses to control scouring include simple avoidance strategies, whereby sheep are gradually introduced to forage crops to allow adjustment of ruminal micro-organisms, and removed from suspect pastures where scouring is observed. However, this does not provide reliable protection from scouring. Further, moving sheep to alternative pasture is not always practical. Where scouring is associated with grazing cereal or forage crops, impacts of scouring may be offset with production benefits.

11

CONCLUSION

Diagnosing the cause of scouring in a mob of sheep need not be a daunting prospect. In most cases, a systematic approach, working through the potential causes and considering appropriate epidemiological factors (age of the sheep and proportion of the mob affected; region, type of pasture and rainfall; and the time of year) will elucidate the most likely cause.

The best approach begins with a thorough history and a faecal worm egg count. In south eastern and south western Australia, infection with gastrointestinal nematodes (worms) is a major risk factor for scouring in sheep. In these regions, response to treatment with anthelmintics in sheep with high faecal worm egg counts (typically >500 epg) helps confirm the diagnosis.

When faecal worm egg counts are low, sampling for protozoa and/or bacteria may be warranted depending on the age of the animals and the presence of other risk factors. Close examination of the diet of the sheep may uncover a precipitating factor, bearing in mind that nutritional scouring is often a diagnosis of last resort.

Although not well recognised in other countries, hypersensitivity to recently-ingested larval worms ("hypersensitivity scouring") is known to occur in south eastern and south-western Australia, chiefly in sheep which have developed an acquired immunity to worms. Worm hypersensitivity scouring should be considered when all other potential causes of scouring have been ruled out in sheep with low faecal worm egg counts. Over time, breeding programs that select against dag formation will help minimise the problem.

Regardless of the cause of scouring in sheep, it is important to remember that in southern Australia scouring and dag formation are the major risk factor for breech flystrike. Therefore, in southern Australia, managing sheep to prevent scouring and dag formation is vital to sheep health and well-being. By working through the various causes of scouring discussed in this review, advisors will be well equipped to help farmers prevent scouring and dag formation in their sheep, and to eliminate the problem when it does occur.

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