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Fleece rot in sheep: a review of pathogenesis, aetiology, resistance and vaccines

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ABSTRACT

Fleece rot develops following prolonged wetting of sheep when bacterial proliferation in wool and on skin induces exudation of serum proteins onto the skin surface and causes damage to wool follicles and fibres. These processes create an attractive environment for blowflies to lay eggs, leading to body strike. Current reliance on insecticides for prevention and treatment of fly strike is being increasingly challenged by development of insecticide resistance. This review examines the large body of past research on the bacterial causes of fleece rot, the genetics of sheep susceptibility and resistance, the characteristics of the resulting immune defence reactions, and attempts to control fleece rot by vaccination. The high dependence on weather conditions for expression of fleece rot hampers studies on the disease. Normal skin and wool are populated by a dynamic microbial community. During adverse environmental conditions, natural resistance to fleece rot associated with physical characteristics of wool and skin can be overwhelmed and a complex mix of bacteria flourishes. Prolonged hydration alone, and in combination with bacterial exoproducts, induces dermatitis and exudation of immunoglobulins and other serum proteins onto the skin surface. Pathogens do not usually penetrate the epidermis. Nonetheless, during prolonged skin hydration, sheep can become sensitised to fleece rot pathogens and produce antibodies. Antibody titres rise late within a typical (3 week) case of fleece rot. High naturally acquired antibody titres may contribute to resistance to fleece rot. In contrast to some evidence for a protective role of antibody, there is little evidence for innate or adaptive cellular immune responses contributing to protection against fleece rot pathogens. Previous attempts to develop vaccines have met with mixed success. Nonetheless, there remain prospects for development of a new vaccine to control fleece rot. Further knowledge on the microbial ecology of normal and wet skin would assist this endeavour and may help identify other control strategies.

Keywords: dermatitis, disease complex, holobiont, *Lucilia cuprina*, microbial ecology, *Pseudomonas aeruginosa*, *Pseudomonas maltophilia*, vaccines.

Introduction

Fleece rot is the result of bacterial infection on the skin and in the fleece of sheep that induces exudative dermatitis and wool damage, and predisposes sheep to fly strike on the body. A large quantity of research has examined causes, the inheritance of sheep susceptibility and resistance, characteristics of the resulting innate and adaptive immune responses, and the potential of vaccines for controlling the disease. This review assesses prior research on the causes of infection, including environmental, bacterial and host factors, and successes and failures of past vaccine research that can help identify new opportunities for the development of immunological strategies to control fleece rot, with potential beneficial consequences for reducing body strike. An initial report on this topic has appeared previously (Vuocolo *et al.* 2020).

Skin provides a defensive barrier against microbial invasion. Like other body surfaces, it is populated by microbiota that co-exist in a symbiotic host–microbe ecology that can be

disrupted by adverse environmental or host-related conditions (Jackson *et al.* 2002). Disruption of the skin barrier function, typically by sustained wetting due to rain, is characterised by a sequential cascade of events involving inflammation, bacterial proliferation and serum exudation (Goodrich and Lipson 1978; James *et al.* 1984*a*; Chin and Watts 1988; Burrell 1990). During the inflammatory dermatitis, odorant attractants for gravid female blowflies are produced directly by metabolic activities of bacteria and following degradation of wool and skin by bacterial enzymes, which lead to an increased risk of blowfly strike (Merritt and Watts 1978*a*; Watts and Merritt 1981*a*). While body strike has been considered of a lesser significance than is breech strike in recent decades, ongoing improvements in genetic and non-genetic strategies for control of breech strike may lead to the re-emergence of body strike, and predisposing fleece rot as a prominent health and welfare risk for the sheep and wool industry (James 2006; Phillips 2009; James *et al.* 2019). Regardless of its association with body strike, fleece rot is a disease of importance to the economics of sheep production (Burrell *et al.* 1992; Cottle 1996); however, these costs do not appear to have been estimated in recent years and a new analysis of the importance of the fleece rot–body strike disease complex is warranted.

Background

The first description of fleece rot in Australia appeared in the second half of the 19th century when pastoralists reported a green discolouration as a fault in wool. The condition, termed water stain or weather stain, was reproduced by Stuart (1894) by transferring bacteria isolated from affected wool to the skin of healthy sheep. Seddon and McGrath (1929) recognised that sheep varied in susceptibility to this condition, and that moisture in the fleece was important for disease development, which was accompanied by an inflammatory reaction in skin (dermatitis) (Bull 1931). Following the emergence of fly strike caused by *Lucilia cuprina* as an important disease of sheep in the first few decades of the 20th century, Seddon and his colleagues undertook intensive research and established a link between wool characteristics and body conformations that favoured prolonged wetting of the fleece in some sheep, resulting in a bacterial dermatitis (fleece rot) and susceptibility to infestation by blowfly larvae (Gilruth *et al.* 1933).

Prevalence

Field studies on the microbial aetiology, progression of infection, host defence reactions and genetics of resistance to fleece rot under field conditions have been hampered by the substantial variation in prevalence and severity of the disease from year to year, due to variation in rainfall patterns. Seasonal variation in prevalence has been studied at New South Wales (NSW) Department of Primary Industries (DPI) Research Station, Trangie, NSW (32°01′S, 147°59′E), in fine- and medium-woolled Merinos. In 1976, 1977 and 1978, prevalence was 37%, 23% and 8% respectively (Atkins and McGuirk 1979), and over the subsequent 17-year period, the average prevalence for fleece rot based on 3339 records in yearling sheep was 23.5% (Mortimer *et al.* 1998). Similar variability was recorded at CSIRO 'Longford' Research Station, Armidale, NSW (30°20′S, 151°27′E) where average prevalence of fleece rot in 6213 fine- and medium-woolled Merino lambs at 10 months of age in the years 1991–1996 was 26.7%, and ranged from 0.46% in 1991 to 75.2% in 1996 (Li *et al.* 1999). Prevalence of fleece rot in one Merino flock and two Corriedale flocks in the South Island of New Zealand ranged from 65% to 100% in a 2-year interval (Keown and Reid 1997).

Young sheep are more susceptible than are older animals to fleece rot (Hayman 1955; Atkins and McGuirk 1979; Burrell *et al.* 1992). This might be due to effects of age on physical characteristics of wool and skin, such as staple structure and the durability or thickness of the lipid barrier (Hayman 1955), or could reflect the requirement of young sheep to be exposed to the infectious agents before they can express a degree of immunity as older animals (Chin and Watts 1991). Acquisition of immunity may require exposure of sheep to a succession of different bacterial strains. Immaturity of the immune system of young sheep and their sensitivity to stressors following weaning are also likely to contribute to the greater susceptibility of young animals (Colditz *et al.* 1996*a*).

Pathogenesis

Early studies on the pathogenesis of fleece rot established that wetting for several days without an opportunity to dry out leads to a loss of waxes and a decrease in the protective hydrophobic properties of the waxes in wool as the free sterol content (including cholesterol, lanosterol and dihydrolanosterol) of the waxes increases (Hay and Mills 1982). Bacterial enzymes, especially protease, elastase, DNAase and phospholipase C from *Pseudomonas aeruginosa* isolates were thought to contribute to these changes (Goodrich and Lipson 1978; James *et al.* 1984*a*; Chin and Watts 1988; Burrell 1990). Burrell (1990) suggested that the role in dermatitis of Gram-positive bacteria, with the capacity to produce lipases and phospholipases, deserves further investigation. Breakdown of the skin wax layer following artificial wetting is associated with an increase in the abundance of *P. aeruginosa* in some cases (Merritt and Watts 1978*a*, 1978*b*), and chemical disruption of the sebaceous layer with petroleum ether can greatly increase the incidence of fleece rot (James and Warren 1979). The detergent action of potassium salts in suint may contribute to the disruption of the wax layer (Freney 1940) and is in accord with the association between suint content

of wool present before wetting and susceptibility to fleece rot (James *et al*. 1984*a*).

Dermatitis and development of a serous exudation commence within 6 h of wetting of healthy skin (Chapman *et al*. 1984), as summarised in Fig. 1, and with prolonged wetting, there can be disruption of normal follicular structures (Nay and Watts 1977). Human and mouse studies have shown that keratinocytes contain the pro-inflammatory cytokine interleukin-1α (Kupper *et al*. 1986) and produce other inflammatory cytokines, including interleukin-8, during skin trauma (Larsen *et al*. 1989), which may contribute to cutaneous inflammation seen following prolonged hydration of skin in sheep. Exudation of serum (Watts and Merritt 1981*b*) and infiltration of the dermis and epidermis by neutrophils and discolouration of the fleece usually occur without invasion of the epidermis by bacteria (Burrell *et al*. 1982; Burrell 1990; Chin *et al*. 1995).

Microbial ecology of skin and fleece rot lesions

Bacterial profiles on healthy skin and in fleece rot lesions have been investigated using a variety of approaches, including (1) determining infectivity and pathogenicity of bacteria applied to the skin of sheep (Stuart 1894), (2) laboratory culture and examination of colony morphology, growth characteristics and exoproducts (Watts and Merritt 1981*b*; Burrell and MacDiarmid 1984; London and Griffith 1984; MacDiarmid and Burrell 1986; Burrell 1990), (3) DNA profiling (Kingsford and Raadsma 1997; Dixon *et al*. 2007; Norris *et al*. 2008), (4) serology (Burrell and MacDiarmid 1984; MacDiarmid and Burrell 1986; Chin and Watts 1992; Dai 1997) and (5) the ability of specific vaccines to protect sheep from some fleece rot bacterial species or strains, but not others (MacDiarmid and Burrell 1986; Burrell 1990; Burrell *et al*. 1992; Dai 1997).

Bacterial culture and microbial DNA analyses have indicated that healthy fleece contains a diversity of bacterial species (Watts and Merritt 1981*a*, 1981*b*; Burrell 1990; Chin and Watts 1992; Lyness *et al*. 1994; Kingsford and Raadsma 1995, 1997; Dixon *et al*. 2007; Norris *et al*. 2008). These populations can undergo sudden and enormous growth in numbers under conditions of high moisture content of fleece and warm temperatures (Watts and Merritt 1981*b*; Burrell *et al*. 1982; Burrell 1990; Lyness *et al*. 1994; Norris *et al*. 2008). Watts and Merritt (1981*a*, 1981*b*) calculated that washings from healthy fleece contained about 3000 bacteria/mL and this number showed a remarkable increase to 700 million/mL in washings from areas affected by fleece rot.

Studies employing conventional aerobic culture methods identified a number of bacterial species that could initiate and/or sustain fleece rot (Stuart 1894; Seddon and McGrath 1929; Belschner 1937; Burrell and MacDiarmid 1984; London and Griffith 1984; Burrell 1990; MacDiarmid and Burrell 1992; Chin and Watts 1992; Lyness *et al*. 1994). Many pseudomonads were implicated, including *P. aeruginosa*, *P. maltophilia*, *P. alcaligenes*, *P. flourescens*, *P. cepacia* and *P. mendocina* (Burrell and MacDiarmid 1984; London and Griffith 1984; MacDiarmid and Burrell 1986; Burrell 1990; Chin and Watts 1992; Chin *et al*. 1995). The green colouration of wool seen in many cases of fleece rot is consistent with secretion of a green pigment (pyocyanin) by *P. aeruginosa* and its absence in other pseudomonads (Fraser and Mulcock 1956; Watts and Merritt 1981*a*, 1981*b*; MacDiarmid and Burrell 1986;

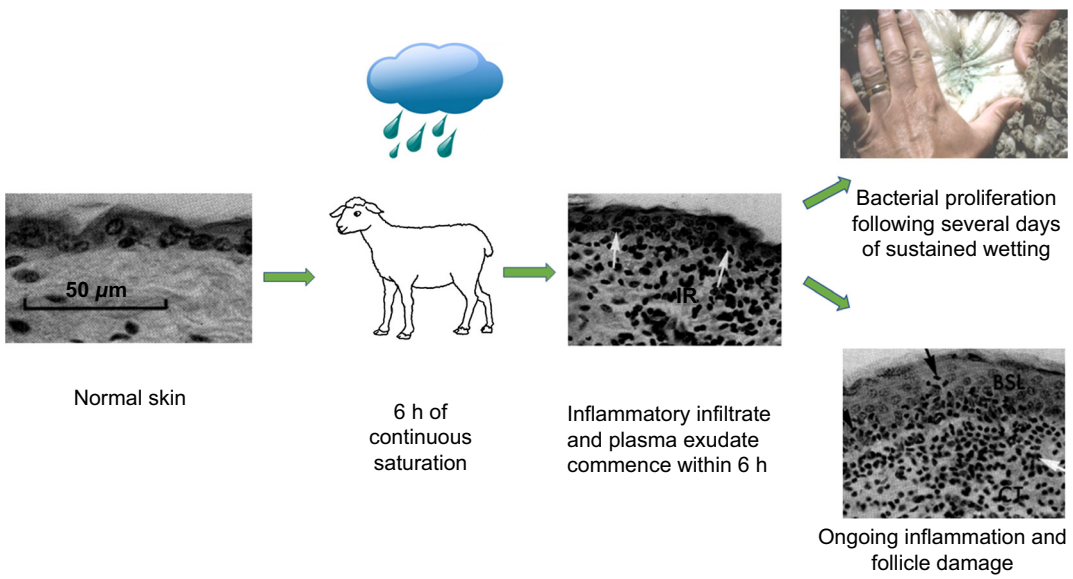

Fig. 1. Inflammation in sheep skin following prolonged wetting. Histology adapted from Hollis *et al*. (1982), with permission from CSIRO Publishing.

Burrell 1990; Chin and Watts 1992; Dai 1997; Norris *et al.* 2008). *P. maltophilia* is considered to be primarily responsible for a yellowish-brown fleece discolouration associated with fleece rot (MacDiarmid and Burrell 1986; Burrell 1990; Chin and Watts 1992). Other colourations can also occur (Mulcock *et al.* 1965; Mortimer 2009). Discolouration of wool samples *in vitro* was induced in fleece samples in the laboratory by application of *P. aeruginosa* (Merritt and Watts 1978*b*; Watts and Merritt 1981*b*).

A picture of the complex microbiology of fleece rot lesions has been provided by Burrell (1990). In a survey of over 1000 samples from healthy skin, fleece rot lesions and fleece rot lesions in the early stages of fly strike, 32 genera of Gram-negative bacteria and 22 species of Gram-positive bacteria were isolated. Following fly strike, there was a rapid change in the bacterial flora, with appearance of *Proteus mirabilis*, *Providencia stuartii*, *P. rettgeri*, and a large increase in *Alcaligenes faecalis*. Flies and larvae were identified as carriers of these bacteria, as well as carrying *Bacillus* spp. and *Micrococcus* spp. It was suggested that bacteria seeded by flies and larvae may contribute to a change in microflora following strike. Bacterial flora were found to differ among different sites on the sheep, among sheep within a flock, among flocks and among geographic regions. Over the 72 h following wetting, if there was the emergence of *P. aeruginosa* in a fleece rot lesion, it was observed to displace *Bacillus cereus*, *Staphylococcus* spp. and *Micrococcus* spp. but not *Corynebacterium* spp. Exudation was most serious in lesions populated by *P. aeruginosa* and *P. maltophilia*.

A study by Chin and Watts (1992) provided similar findings on the progression of fleece microbiology following wetting. Swab samples from wool and skin taken before and after natural exposure of 23 sheep to heavy rain were examined by aerobic culture on sheep blood agar plates, and colonies were identified by morphology, commercial diagnostic kits and gas chromatography. At 96 h after the commencement of rain, four sheep showed no signs of fleece rot, seven developed lesions dominated by green pigmentation and 12 developed lesions dominated by yellow and orange pigmentation. Of the 12 most abundant bacterial genera present before exposure to rain (*Acinetobacter* spp., *Alcaligenes* spp., *Bacillus* spp., *Corynebacterium* spp., *Enterobacter* spp., *Flavimonas* spp., *Micrococcus* spp., *Moraxella* spp., *Proteus* spp., *Pseudomonas* spp., *Serratia* spp., *Staphylococcus* spp.), only *Pseudomonas* spp. and *Micrococcus* spp. were detected in fleece rot lesions at 96 h. *P. aeruginosa* was present in the greatest abundance. In sheep with green lesions, *P. aeruginosa* and *P. maltophilia* were detected, and in sheep with yellow/orange lesions, *P. aeruginosa*, *P. maltophilia*, *P. fluorescens*, *P. alcaligenes* and *P. cepacia* were isolated. Bacteria detected after the rain event on the four sheep with no signs of fleece rot were not reported. Serological responses to skin flora following development of fleece rot in this study are described below.

Several other studies have also reported findings on resident bacteria in healthy wool and skin. In a survey of wool samples from flocks in South Australia and Western Australia, Lyness *et al.* (1994) detected *Bacillus cereus* on 100%, *Bacillus thuringiensis* on 92% and *Pseudomonas* spp. on 88% of samples. The number of samples examined in the survey was not reported. Jackson *et al.* (2002) examined bacterial populations on normal wool and skin of Coopworth and Romney sheep in New Zealand by culture methods. The most abundant species were Gram-positive cocci, especially *Micrococcus* spp., Gram-negative rods, especially *Pseudomonas* spp., and Gram-positive rods such as *Corynebacterium* spp. More than 80% of colonies were pigmented and more than 95% of bacteria were isolated from the outer zone of the wool staple, while relatively few were isolated from skin. The distribution of species was similar among different zones of the staple and skin, while abundance was greater on the upper parts of the body than on the belly and legs. These several features led the authors to conclude that bacteria on wool and skin were not adventitious environmental contaminants. Rather, they suggested that 'the fleece contains a specialised microflora which poses no threat to wool quality but that disturbance of the microflora may allow proliferation of detrimental bacteria leading to fleece problems. ... The role of the endogenous microflora in suppressing fleece disorders such as fleece rot and fly strike should be investigated' (Jackson *et al.* 2002, p. 54).

Only limited molecular genetic analyses have been conducted on the bacteria present on normal skin and during fleece rot. Kingsford and Raadsma (1995), using primers designed to amplify a *P. aeruginosa*-specific region of the *16S* rRNA gene, examined fleece washings collected from a flock of 100 sheep. In an examination of diagnostic specificity, polymerase chain reaction (PCR) results agreed with bacteriological isolation of *P. aeruginosa* in 89% of fleece samples tested, 2% of samples contained organic PCR inhibitors in the fleece washings, and 3% were below the sensitivity of detection. Use of nested PCR did not increase the sensitivity of detection compared with using a single pair of primers in PCR. Other bacterial species present in the samples were not identified. The PCR test measured only the presence or absence of the bacterium, i.e. the test was not quantitative and therefore did not provide data on the abundance of *P. aeruginosa* in the samples. The PCR methods were subsequently used to confirm *P. aeruginosa* identity in a bacterial culture study (Kingsford and Raadsma 1997). The authors surveyed 1568 sheep and identified fleece rot in 646 sheep. Of the affected sheep, 14% were positive for *P. aeruginosa*, thereby implying the involvement of other bacterial species in the generation of fleece rot. However, there was a strong association of the presence of *P. aeruginosa* with the severity of fleece rot. In a subsample of animals, *P. aeruginosa* was isolated from 60% of fleece rot lesions that were also fly struck, whereas it was present in 6% of lesions that were not struck. The authors concluded that 'the surveys

consistently showed that the presence of *P. aeruginosa* was associated with increased severity of fleece rot and subsequent flystrike. Although it might only contribute in part to the disease complex, the importance of *P. aeruginosa* cannot be underestimated and warrants consideration for inclusion in future fleece rot vaccines' (Kingsford and Raadsma 1997, p. 275).

More recently, Dixon *et al.* (2007) used *16S* rRNA sequence profiles to identify predominant bacterial populations representing eight major bacterial orders in samples from skin and wool in two resistant and two susceptible sheep from the NSW DPI selection lines before and following 3 days of artificial wetting. Sheep were free of fleece rot prior to artificial wetting. At the time of sampling following wetting, the two resistant sheep had fleece rot scores of zero and the two susceptible sheep had scores of five. Considerable bacterial diversity was found. Bacteria were classified to 183 taxonomic groups, most of which had not previously been associated with the skin or fleece of sheep. Four taxonomic groups were strongly differentially represented in the sheep from the resistant and susceptible lines. The samples acquired from the susceptible sheep after wetting contained abundant populations of eight *Pseudomonas* species; however, *P. aeruginosa* was not detected. On the basis of these findings and other reports cited above, the authors concluded that *P. aeruginosa* was 'not the causal agent [of fleece rot], but merely an opportunistic bacterium taking advantage of a situation caused by other bacteria' (Dixon *et al.* 2007, p. 745).

In accord with the association between *P. aeruginosa* in fleece rot lesions and fly strike noted by Burrell (1990), Kingsford and Raadsma (1997) and others, in *in vitro* studies adult *L. cuprina* exhibited a six-fold greater preference for potential ovipositing in wool samples growing *P. aeruginosa* than in those growing *Bacillus subtilis*, which is commonly found in healthy fleece (Merritt and Watts 1978*a*; Watts and Merritt 1981*a*). The potential contribution of other bacteria to production of fly-attractant odours is also recognised (Emmens and Murray 1982; Emmens and Murray 1983; James *et al.* 2019). Importantly, in these *in vitro* studies, the presence of *P. aeruginosa* in mixed cultures greatly increased attractiveness for flies, in comparison with pure cultures of *P. aeruginosa* or other species, suggesting that interactions among bacterial species contribute to odour generation (Emmens and Murray 1982, 1983).

Together, these studies on microbial ecology suggest that (1) a diversity of bacterial species constitutes an normal resident microflora on skin and wool, (2) prolonged wetting can disrupt this resident microflora and modify the abundance of species, (3) differences among individual sheep in composition of the resident flora may influence the microbial responses to prolonged wetting, (4) *Pseudomonas* spp, especially *P. aeruginosa*, are often present in fleece rot lesions that go on to be struck by flies, and (5) other microbial species may also contribute to development of fleece rot lesions and attraction of flies.

Some characteristics of *Pseudomonads* as pathogens

The attention of several research groups has been drawn to characteristics of *Pseudomonas* spp. that could contribute to the pathogenesis of fleece rot. London *et al.* (1984) detected 13 *Pseudomonas* species in cultures from fleece rot lesions that produced proteases capable of digesting unscoured wool. Sheep isolates of *P. aeruginosa* were found to produce keratinase, lipase, exotoxin A, protease, elastase, phospholipase, DNAase and lecithinase, with activities varying among isolates (Burrell and MacDiarmid 1984; Burrell 1990). Isolates lacking protease, elastase and DNAse activities were not able to induce experimental dermatitis (Burrell 1990). Sheep isolates of *P. maltophilia* were found to produce chitinase, collagenase, DNase, elastase, hyaluronidase, chondroitin sulfatase, mucinase, phospholipase and albuminase but not exotoxin A (MacDiarmid and Burrell 1986; Burrell 1990). *P. maltophilia*, but not *P. stutzeri* or *P. putida*, was able to induce experimental dermatitis when live cultures were applied to skin (Burrell 1990). Phospholipase C produced by sheep isolates of *P. aeruginosa* induced tissue necrosis when injected intradermally, and sheep that developed fleece rot following artificial wetting developed antibodies to phospholipase (Chin and Watts 1988). *P. aeruginosa* inhibited *in vitro* growth of sheep isolates of several skin bacteria, and pyocyanin prepared from *P. aeruginosa* inhibited growth of sheep isolates of *Bacillus cereus, B. coagulans* and *Staphylococcus epidermidis* (Dai 1997). Other studies have shown that pyocyanin has many potent biological actions, including inhibition of microbial competitors, activation of macrophages, production of oxygen radicals by phagocytes, degranuation of neutrophils and induction of inflammation in the lungs of sheep (Lauredo *et al.* 1998; Mavrodi *et al.* 2001).

Pyocyanin is a phenazine compound. Phenazines are a family of secondary metabolites produced by flourescent *Pseudomonas* species (Mavrodi *et al.* 2001). These compounds are brightly coloured pigments and, as noted above, are the main cause of the wool staining seen in fleece rot. *P. aeruginosa* produces a broad range of phenazines, including pyocyanin, phenazine-1-carboxylic acid, 1-hydroxyphenazine and phenazine-1-carboxamide (oxychlororaphine; Dyer *et al.* 2007). Analysis of bright yellow stain in wool (canary yellowing) identified pyocyanin and several of its derivatives bound to the fibre cuticle. The presence of pyocyanin, which is diagnostic of *P. aeruginosa*, implicates the bacterium in this non-scourable wool fault (Dyer *et al.* 2007). Pyocyanin expression acts as a quorum sensing signal in *P. aeruginosa*, promoting bacterial motility and initiating expression of virulence factors (Dietrich *et al.* 2006).

Dai (1997) noted that the predominant phenotypic form of *P. aeruginosa* isolated from fleece rot lesions was a mucoid strain, which secreted copious quantities of an extracellular glycocalyx (alginate) that prevented dehydration. Mucoid

P. aeruginosa infections in humans are particularly difficult to treat as the alginate aids biofilm formation, physically hinders normal host defence mechanisms and is a barrier to diffusion of antibiotics (Döring and Pier 2008; Sharma *et al.* 2011; Mann and Wozniak 2012; Merakou *et al.* 2018). In accord with the observation that strain variation influences development of fleece rot in sheep (Burrell 1990), phenotypic diversity also influences pathogenicity in *P. aeruginosa* in humans. In cystic fibrosis, non-mucoid *P. aeruginosa* strains often initiate infection and are then supplanted by a mucoid *P. aeruginosa* strain, which is much more difficult to eradicate and often leads to chronic infection. *P. aeruginosa* is highly motile and causes a broad group of opportunistic infections in humans, including infections of injured skin (especially burns), urinary tract, middle ear, cornea, and lung, especially in immunocompromised individuals (Priebe and Goldberg 2014; Merakou *et al.* 2018; Hoggarth *et al.* 2019). *P. aeruginosa* uses aerobic respiration for optimal metabolism, although it can also respire anaerobically on nitrate or other alternative electron acceptors. This considerable metabolic plasticity is a major reason for the ubiquitous presence of *P. aeruginosa* in humans, animals and their environments. Also underpinning the metabolic versatility of *P. aeruginosa* in causing infections at very different anatomical sites on animals is the 'extremely complex genomic diversity' in the population, which includes mobile genetic elements superimposed on genetic diversity (Lozano *et al.* 2018; Winstanley and Rumbaugh 2018; Hoggarth *et al.* 2019; Freschi *et al.* 2019). In particular, some mobile genetic elements contain the antibiotic resistance gene, beta-lactamase, which makes *P. aeruginosa* a formidable human pathogen. Human patients infected with *P. aeruginosa* usually require aggressive and prolonged antibiotic treatments.

Resolution of fleece rot lesions

At annual shearing, bands of discolouration or crusting in wool indicate the occurrence of episodes of fleece rot in the preceding year. In field cases, fleece rot usually resolves in days or weeks after weather conditions improve. Nonetheless, few studies appear to have specifically addressed the factors contributing to the resolution of fleece rot lesions. Hollis *et al.* (1982) noted in a histological study of fleece rot induced by artificial wetting, that inflammatory cells infiltrated the dermis within 6 h of wetting. During 9 days of wetting, there was substantial thickening of the epidermis. Thirteen days after the cessation of wetting, epidermal thickness had returned towards normal and a band of white wool had emerged below wool discoloured by bacteria; however, focal dermatitis was still evident in some sites. Visual scores of susceptibility to fleece rot are based on the width of the band of staining and crusting (less than or greater than 5 mm) and presence or absence of crusting within the band of staining as a measure of the severity of the case of fleece rot, as described in the AWI/MLA Visual Scoring Guide.[1] Since wool grows about 1 mm every 4 or 5 days, mild cases of fleece rot evident as colouration less than 5 mm in width are likely to have resolved within 3 weeks. Many of the weather events inducing fleece rot are likely to last less than 3 weeks, in which timeframe moisture content of wool could return to normal, thereby permitting microbial ecology to restore normal skin flora. This theoretical scenario is in accord with wetting and drying rates of the fleece being factors contributing to fleece rot susceptibility (Raadsma *et al.* 1989). However, the fleece rot bands are hydrophilic and the previous occurrence of fleece rot facilitates more rapid wetting during subsequent rain events, potentially leading to the re-occurrence of lesions (Belschner 1937; Copland 1982). Furthermore, Eisemann (1995) suggested that the activation of previously desiccated bacteria following re-wetting of damaged wool and old lesions leads to production of bacterial odours that may contribute to a rapid increase in attractiveness to sheep blowflies. As an alternative mechanism of resolution, the antibody titres to fleece rot pathogens begin to increase in serum at about 2 weeks and could be contributing to resolution of lesions within this same timeframe (Chin and Watts 1992). We are unaware of quantitative studies examining the susceptibility of resolved fleece rot lesions to fly strike in the field.

Genetic resistance of sheep to fleece rot

The early observations by Seddon *et al.* (1931*a*, 1931*b*) on phenotypic and genetic variation of sheep in susceptibility to fleece rot led to a substantial research effort to better characterise resistance traits for use in sheep breeding programs. Hayman (1953) confirmed that resistance to fleece rot was heritable. As is commonly seen with genetic parameters, estimates of heritability of resistance vary among genotypes of sheep, and among environments, with values from 0.13 to 0.41 being reported in the literature (McGuirk and Atkins 1984; James *et al.* 1984*b*; Raadsma and Rogan 1987; Raadsma *et al.* 1989; Li *et al.* 1999). Estimates of the heritability of severity of fleece rot infection range from 0 to 0.52. Differences among studies in the scoring system used to categorise lesions adds a further level of complexity to estimates of heritability of resistance and severity. The occurrence of environmental conditions conducive to expression of the disease can be sporadic; thus, there has been considerable interest in the identification of correlated (indicator) traits that would enable indirect selection of sheep for resistance.

[1]Visual Sheep Scores. Producer ver. 3 2019 https://www.wool.com/globalassets/wool/sheep/welfare/breech-flystrike/breeding-for-breech-strike-resistance/visual-sheep-scores-producer-version-2019.pdf

Most studies have examined traits measured on sheep or the fleece at or around annual shearing.

The indicator traits examined fall into the following three areas: (1) structural faults of the sheep; (2) physical characteristics of wool such as colour, structure and fibre diameter; and (3) chemical characteristics of wool including wax and suint that affect wettability of the fleece. Some conformation characteristics of the withers and breech, and skin wrinkle were recognised as undesirable in the 1920s and 1930s (Belschner 1937); however, not all studies support this conclusion (Hayman 1953, 1955; Raadsma *et al.* 1987; Raadsma 1993). White, bright greasy wool colour, a trait that is often highly heritable, is usually associated with resistance to fleece rot (Holdaway and Mulhearn 1934; Belschner 1937; Hayman 1953; Paynter 1961; James *et al.* 1984*a*, 1987; Li *et al.* 1999). In Peppin Merinos studied at Trangie, NSW, the association of fleece rot with greasy wool colour was not strong (McGuirk and Atkins 1984; Raadsma and Wilkinson 1990). Unfavourable relationships between fleece rot incidence and some wool quality characteristics including greasy wool colour and staple structure and character have also been noted (Li *et al.* 1999). Raadsma and Wilkinson (1990) found that objectively measured colour and colour development following incubation of wool samples for 5 days in a moist atmosphere at 40°C were more highly genetically correlated with resistance to fleece rot than was subjectively assessed greasy wool colour. Among other physical characteristics of wool, variability of fibre diameter (usually expressed as the coefficient of variation of fibre diameter) is highly heritable, easily measured and genetically correlated with resistance to fleece rot (James and Ponzoni 1992; Raadsma 1993). Lower fibre diameter has been associated with resistance in most studies (James and Ponzoni 1992), with some exceptions (Lipson *et al.* 1982). Staple length has a negligible to moderately negative genetic correlation with the severity of fleece rot (Raadsma 1993; Li *et al.* 1999). Potassium ion concentration in suint is positively associated with wool colour and may influence the lipid coat on fibres and skin via a detergent action following wetting (Hay and Mills 1982; Aitken *et al.* 1994). Factors reducing wettability of the fleece include higher wax content, lower suint content, lower suint insoluble nitrogen concentration and lower pH (Hayman 1953; Paynter 1961; Lipson *et al.* 1982; Pascoe 1982; James *et al.* 1984*a*; Dowling *et al.* 2006). On the basis of the associations between wool traits and fleece rot, and using typical wool values and costs of management, Cottle (1996) estimated that indirect selection for resistance to fleece rot through selection for correlated traits could result in a net financial benefit to the producer. Together, these results indicate that there is potential for indirect selection for resistance to fleece rot, although better characterisation of genetic correlations with production traits may be required (Mortimer 2001). More recently, immune competence has been identified as a potential trait for indirect selection and is discussed in more detail in an accompanying review (Denman *et al.* 2021). The interactions between these collective traits and seasonal variation may need further investigation.

In view of the limitations imposed on field studies by seasonal variation in expression of the condition, experimental conditions for inducing fleece rot in large numbers of sheep were developed by NSW DPI, by housing animals indoors and artificially wetting them *via* overhead sprinklers (McGuirk *et al.* 1978). Sheep were scored for fleece rot and blowfly strike associated with natural weather events and for these two conditions induced by artificial wetting. The phenotypic data were used to establish divergent resistant and susceptible selection lines. Following 17 years of selection, annual divergence in prevalence between the lines from the population mean of 23.5% for natural fleece rot has been 2.8%, and from the population mean of 5.8% for natural flystrike has been 0.4% (Mortimer *et al.* 1998). These changes are in accord with the high genetic correlation between the traits reported by Raadsma *et al.* (1989). Selection continued for 26 years, and during this time wool characteristics and physiological responses have been extensively studied.

Research in New South Wales and Western Australia on breech conformation traits associated with breech strike recorded little association between breech strike and body strike. In the Armidale flock, the incidence of body strike in weaners was 4%, whereas breech strike incidence was 18% (Smith 2016). Body strike heritability was estimated at 0.16 and was poorly correlated both phenotypically and genetically with breech strike (0.08 and 0.00 respectively; Smith 2016). Phenotypic correlations between body strike and fleece traits were all low to negligible (range −0.09 to 0.12). Genetic correlations between body strike and fleece traits were also negligible except for fleece rot (0.56), fibre curvature (−0.41) and assessed wool character (0.28). Fleece rot was not correlated phenotypically or genetically with breech strike (0.02 and 0.07 respectively; Smith 2016). There were no strong genetic correlations between breech strike and fleece traits. Interestingly, the fleece trait with strongest genetic correlation was coefficient of variation of fibre diameter (CVD), but the sign was reversed in the two flocks (−0.27 vs 0.31). Thus, there was no consistent effect. Greasy wool colour was not correlated with breech strike in either flock, which is in contrast to the literature cited above that describes a genetic relationship between greasy wool colour and fleece rot/body strike.

Together, these results from quantitative genetic studies indicate that (1) direct and indirect genetic selection provide opportunities to reduce susceptibility to body strike, and (2) susceptibility to breech strike shows little genetic or phenotypic association with body strike, or with wool characteristics associated with susceptibility to body strike.

Molecular genetic studies of resistance to fleece rot

Genetic markers associated with resistance to fleece rot can potentially be used in breeding programs to accelerate selective breeding for resistant sheep. Variants associated with differential expression of genes in skin from six resistant and six susceptible sheep have been identified during experimentally induced fleece rot (Smith *et al.* 2010). Two markers in the gene *Fatty Acid Binding Protein 4* (*FABP4*) were identified and explained a small extent (2–6.8%) of the total phenotypic variation for the fleece rot severity trait. These markers have not been independently confirmed. *FABP4* encodes a protein typically expressed in large quantities in adipocytes and at lower levels in macrophages. The protein functions as an intracellular transporter of large fatty acids to cellular organelles and has strong roles in regulating lipid metabolism (Furuhashi *et al.* 2019; Trojnar *et al.* 2019). Some of the fatty acids transported by this protein are important intracellular signalling molecules. In a non-classical manner, the FABP4 protein is also released from adipocytes and macrophages and acts as a potent proinflammatory signalling protein, a function consistent with the inflammation observed in skin tissues affected by fleece rot. Notably, the level of FABP4 protein in blood was increased in people with the skin inflammatory condition, psoriasis (Baran *et al.* 2017). Moreover, it was recently demonstrated that *FABP4* genetic variants were potentially associated with susceptibility to flystrike in sheep (Burrows 2018). The small percentage of the genetic variation for fleece rot severity explained by the ovine *FABP4* DNA polymorphisms is insufficient for practical use in breeding programs. More extensive genome-wide genetic association studies are required to provide a practical tool for selective breeding.

Immunological mechanisms of resistance to fleece rot

The presence of a serous exudate on the skin surface during fleece rot, the tendency for fleece rot lesions to resolve over time and the variation among individuals in resistance to fleece rot have motivated studies on immune mechanisms that might influence the expression and progression of the disease. For detailed reviews of immune responses in skin of sheep and their involvement in resistance to fleece rot, see Watson *et al.* (1993, 1994), Colditz and Tellam (2000), Colditz *et al.* (2001) and Norris *et al.* (2008). Many studies have drawn on the NSW DPI selection lines to investigate these mechanisms. Leukocyte populations in blood did not differ between ewes from the two lines (Colditz *et al.* 1996*b*); however, rams from the resistant line had a higher number of CD5+ lymphocytes in blood in one of two cohorts studied (McColl *et al.* 1997). In response to intravenous injection of endotoxin from *P. aeruginosa*, higher counts of neutrophils and monocytes were observed in blood from the resistant line (Colditz *et al.* 2001). Plasma leakage in responses to intradermal injection of agents that enhance vascular permeability tended to be greater in sheep from the susceptible line than the resistance line (Colditz *et al.* 1992). Mast cells can play an important role in inducing vascular permeability by release of vasoactive compounds. Mast cells identified histologically by the binding of antibody to IgE were more prevalent in normal skin of sheep from the resistant line (Colditz *et al.* 1994; Nesa 1994). In an unrelated flock selected for clean fleece weight, IgE+ cells were more prevalent in skin of sheep with no history of fleece rot than in sheep with a history of fleece rot. Endotoxin from *P. aeruginosa* is a potent inducer of cellular inflammation. When endotoxin was injected intradermally, there was no difference between selection lines in the intensity of neutrophilic infiltration; however, greater numbers of gamma delta + T cells and eosinophils were present in normal and inflamed skin from resistant sheep.

In addition to neutrophils (Burrell *et al.* 1982; Chin *et al.* 1995), the dermatitis caused by fleece rot is heavily infiltrated by other leukocyte classes. Following the application of a staple of wool soaked in an overnight culture of *P. aeruginosa* to skin, there was a significant increase in the number of CD1+ leukocytes and CD4+ T lymphocytes in dermal biopsies taken at 6 h (Watson *et al.* 1994). Numbers of CD8+ T lymphocytes and gamma delta + T lymphocytes were significantly elevated by 24 h. The bacterial infection did not induce changes in cell populations present in efferent lymph draining the infection site, indicating that the reaction in this experimental model was very localised. When broad areas of an animal are affected by fleece rot, there is likely to be a stronger systemic effect on leukocyte dynamics. As bacteria do not penetrate the epidermis (Burrell *et al.* 1982; Chin *et al.* 1995), there would appear to be little opportunity for host defences to control bacteria by phagocytosis. It remains possible that cellular effector mechanisms such as extracellular release of lysosomal enzymes, reactive oxygen species and neutrophil extracellular nets could kill bacteria; however, scope for activity of these effector mechanisms seems limited. Thus, humoral effectors such as antibody and complement appear to hold the greatest promise as immune mechanisms for control of bacterial dermatitis.

Antibody responses following natural fleece rot and experimental induction of fleece rot with *P. aeruginosa* have also been studied. The antibody response to fleece rot induced by a natural rain event was studied in 10 sheep with a prior history of at least two episodes of natural rain exposure (Chin and Watts 1992). Six weeks following the rain exposure event that was the basis of the study, antibody was assessed by a whole cell ELISA against the nine most abundant bacterial species isolated from fleece rot lesions on the animals, namely, *Alcaligenes faecalis*, *Bacillus cereus*, *Micrococcus lylae*,

Corynebacterium ovis, *P. aeruginosa*, *Enterobacter aerogenes*, *Serratia marescens*, *Staphylococcus aureus* and *S. epidermis*. High titres were detected against *P. aeruginosa*, whereas titres against other species were low and did not differ from levels detected prior to the rain event. The time course of the development of the antibody response was examined for six of the bacterial species, including *P. aeruginosa*, *A. faecalis*, *B. cereus*, *E. agglomerans*, *M. lylae* and *S. epidermidis*. Antibody titres did not change during the 6-week period of the study against bacterial species other than *P. aeruginosa*. Titres against this bacterium had increased significantly by 2 weeks and were at a high level at 6 weeks. Further investigations showed serum of these sheep, which came from animals presenting with either green or yellow/orange fleece rot lesions, also reacted significantly to *P. fluorescens*, but not *to P. putida*, *P. cepacia*, *P. stutzeri*, *P. mendocina*, *P. maltophilia*, *P. alcaligenes, P. diminuta* or *P. vesicularis*. The authors concluded that sheep can develop an antibody response to *P. aeruginosa* during fleece rot conditions under field conditions (Chin and Watts 1992).

Similar results were obtained during experimentally induced fleece rot. Sheep developed antibody to a range of outer membrane proteins of *P. aeruginosa* following application of live bacteria to skin (Chin *et al.* 1995). Higher titres of antibody to *P. aeruginosa* were observed in sheep from the resistant line when live *P. aeruginosa* cultures were applied to the surface of wetted skin than in sheep from the susceptible line (Chin and Watts 1991). Following intradermal injection of antigens, antibody responses were generally greater in sheep from the resistant line, although the strength of responses differed among studies and among progenies of sires within each selection line (Chin and Watts 1991; Gogolewski *et al.* 1996).

The presence of antibody to *P. aeruginosa* (and *P. flourescens*) following natural infections raises the question of the role of antibody in acquired resistance to fleece rot. Some infectious diseases, especially some viral diseases, induce an adaptive immune response during infection that contributes to the clearance of infection and provides strong protection against re-infection for many years. In other instances, animals can become sensitised to pathogens during infection but the acquired immune reactivity may fail to control the infection. The results described above provide preliminary evidence that antibody to the skin pathogens present in field cases may contribute to resistance to fleece rot. Nonetheless, there is a need for additional and direct proof that a highly specific antibody can be identified in fleece rot lesions and that the antibody is not rapidly inactivated by bacterial activity. Passive immunisation of sheep with antibody of known specificity could provide important information about the availability and stability of antibodies in skin exudates as an immune mechanism for protection against fleece rot.

Vaccines against fleece rot

A large research effort was undertaken in the 1980s and 1990s to develop vaccines against *P. aeruginosa* for control of fleece rot and body strike (Schiller *et al.* 1981*a*, 1981*b*; Burrell *et al.* 1982, 1992; Burrell 1985, 1990; Burrell and MacDiarmid 1986, 1988; Chin 1995). Vaccines prepared from a suspension of killed cells or from culture filtrates of *P. aeruginosa* reduced the occurrence of fleece rot in pen and field experiments and also reduced the incidence of body strike (Burrell *et al.* 1982, 1992; Burrell 1985, 1990). In pen studies, a vaccine based on a culture filtrate protected sheep against challenge with the homologous strain. In a comparison of three vaccinated sheep with three controls, vaccination protected sheep against challenge with three heterologous strains of *P. aeruginosa*. In a field trial, the same vaccine protected all of 26 vaccinated sheep from natural fleece rot and fly strike, whereas of the 115 control sheep, 61 developed fleece rot. Twenty-one of the sheep with fleece rot also developed body strike. Mixed cultures of gram-positive and gram-negative bacteria were isolated from fleece rot lesions in control sheep in these pen and field studies (Burrell 1985). Climatic conditions during the field trial were conducive to fleece rot during a period 8–12 weeks after vaccination. Antibody titres to vaccination are likely to have been near peak levels during this period. Larger field trials were later reported (Burrell 1990; Burrell *et al.* 1992). In late 1983, a trial was conducted in 2548 sheep on 14 commercial properties in the Riverina, and Southern and Northern Tablelands of NSW. The primary outcome measure of efficacy was body strike. On four properties, there was significant reduction in body strike over the summer following vaccination, with the number of strikes in vaccinated sheep being about one-fifth the number seen in unvaccinated controls. The strain of *P. aeruginosa* isolated from fleece rot lesions in control sheep on these four properties was homologous with the vaccine strain. On the 10 properties where there was no effect of vaccination on body strike, heterologous strains of *P. aeruginosa* were identified in fleece rot lesions. Across the 14 properties, the prevalence of fleece rot in control sheep during the trial ranged from 20% to 80% (Burrell 1990). The author concluded that vaccination reduced body strike on properties where fleece rot was associated with a strain of *P. aeruginosa* homologous with the vaccine strain. In a subsequent trial, young sheep were vaccinated on two properties in late 1989. During a period of high rainfall in the subsequent winter, the prevalence of coloured fleece rot was reduced from approximately 38% to 8% and from approximately 12% to 2% in control vs vaccinated sheep on the two properties (Burrell *et al.* 1992). The authors concluded that vaccination of sheep as weaners or hoggets with a prime-boost regimen should protect young sheep for up to 2 years. The work of this group led to the issuing of a patent that provided for inclusion of bacterial culture filtrate

and/or whole bacterial cells from *P. maltophilia*, *P. aeruginosa, P. stutzeri* and *P. putida* in the vaccine (Burrell and MacDiarmid 1986, 1988). Exudation of antibody against bacterial exoproducts onto skin was claimed in the patent to be the mechanism of protection conferred by vaccination. In a review of this work, Burrell (1990) concluded that the principal objective of vaccination was to reduce body strike. Achieving this goal, he suggested, was predicated on *P. aeruginosa* being the primary cause of odours in fleece rot lesions that attract gravid *L. cuprina* (Burrell 1990). It was recognised that the vaccine would not protect all sheep from fleece rot or body strike.

Following the observation that sheep developed antibodies to surface antigens of *P. aeruginosa* during experimental infections (Chin *et al.* 1995), Chin *et al.* (1996) examined the potential of outer membrane proteins of the bacteria as vaccine candidates. The antigens, especially phospholipase C, were highly immunogenic and a patent was filed (Chin 1995); however, vaccine efficacy trials have not been reported in the scientific literature.

Schiller *et al.* (1981*a*, 1981*b*) vaccinated sheep with seven strains of *P. aeruginosa* and demonstrated that sheep immunoglobulins containing highly specific antibody titres could protect mice from experimental challenge with *P. aeruginosa*. The study was a feasibility investigation for the generation of a vaccine for protection of human burns patients from *P. aeruginosa* infection; however, the research was not progressed further. The studies provided evidence that antibodies produced by sheep following vaccination with *P. aeruginosa* can exert antibacterial activities against the organism.

An ecological perspective of fleece rot

In 1937, Seddon concluded that 'in most cases of fleece rot the wool is greyish, dirty yellow or light brown and the bacterial flora is very mixed, but where there is a frank colouration some particular chromogenic organism usually predominates' (Seddon 1937, p. 96). This picture of a complex bacterial aetiology has been borne out by many subsequent studies. The presence of a specialised adapted flora on the wool and skin (Jackson *et al.* 2002) and variation among animals in this flora (Dixon *et al.* 2007) support the concept that resident flora can provide a defence barrier against overgrowth of pathogenic species. This concept of a complex skin microbiome participating in a dynamic ecology with the host has also emerged in studies of human skin in recent years (Oh *et al.* 2016). For example, some skin bacteria can produce a range of trace amines that act as neuromodulators influencing signal transduction in peripheral nerves, immune functions, and skin wound healing (Luqman *et al.* 2020). The absence of certain coagulase-negative *Staphylococcus* species on human skin is associated with susceptibility to *Staphylococcus aureus* infection in atopic dermatitis (Nakatsuji *et al.* 2017), and resident bacteriophages, which can differ among individuals, also influence bacterial dynamics on skin (Byrd *et al.* 2018; van Zyl *et al.* 2018). The host can also actively support commensal bacteria as a defence mechanism against infection (Ayres 2020). For example, during anorexia in mice associated with activation of the innate immune system, fucose is liberated from gut epithelial cells, providing a nutrient source to support gut microbiota that are faced with a shortage of glucose due to the decreased food intake by the anorectic host (Pickard *et al.* 2014). In this ecological view of health and disease, the host organism is considered to constitute an ecological community comprised of a host and attendant populations of transmissible replicating elements (including prions, viruses, archaebacteria, eubacteria, fungi, protists, and metazoans) that are acquired from reservoirs within the host's environment at some stage of its life cycle; the organism is a holobiont (Pitlik and Koren 2017). The contribution of the transmissible replicating elements to the ecological integrity of the holobiont falls along a continuum between essential, beneficial, benign, deleterious and lethal. The influence of transmissible replicating elements on health and disease can change across the developmental trajectory of the host, or with a change in the phenotypic state of the host (Pitlik and Koren 2017). This ecological model draws attention to the dynamic status of host resistance in the face of changing abiotic (e.g. weather) and biotic (e.g. social interactions, animal management) conditions (Ayres 2020). Consistent with this model is the proposal that elevated body temperature in sheep, for example following ingestion of alkaloids produced by endophytic fungi in rye grass (Henry *et al.* 2016), can modify skin microflora and increase susceptibility to body strike (Young *et al.* 2004). In the context of the current review, the disease complex associated with fleece rot includes the influence of fleece rot on susceptibility to body strike. Within this disease complex, a prominent contribution of *P. aeruginosa* to the severity of fleece rot lesions and susceptibility to body strike at fleece rot sites is recognised. Notwithstanding the importance of *P. aeruginosa* to endstage disease, the ecological model points to a need for a more detailed knowledge of the microbiome on healthy sheep skin and microbial dynamics following protracted wetting (Jackson *et al.* 2002; Norris *et al.* 2008). Opportunities provided by such information could include genetic selection for host factors favouring a protective microbiome, probiotic interventions or phage therapies to modify resident bacterial flora that make the holobiont less susceptible to disturbance by environmental perturbations.

Conclusions

Several key findings are evident from many decades of research on aetiology, pathogenesis and host response to

fleece rot. The high dependence on weather conditions for expression of fleece rot hampers studies on the disease. Instances of high prevalence, even when they occur at a low level of severity, suggest that in adverse environmental conditions, natural resistance to fleece rot associated with physical characteristics of wool and skin may be overwhelmed in most sheep, and pathogenic bacteria flourish. Dermatitis induced by prolonged wetting of the skin can precede fleece rot and is accompanied by leakage of immunoglobulins present in plasma onto the skin surface. In this setting, the complex mix of bacterial species present do not generally penetrate the epidermis. Prolonged skin hydration is necessary for sheep to become sensitised and produce antibodies during an episode of fleece rot. Fleece rot pathogens, through a release of extracellular products, can exacerbate dermatitis caused by prolonged skin wetting. Antibody titres to fleece rot pathogens rise late within a typical (3 week) case of fleece rot. High antibody titres are associated with resistance to fleece rot. In the absence of antibody to fleece rot pathogens, greater exudation of plasma proteins onto the skin surface is likely to be associated with an increased susceptibility to fleece rot. In contrast to circumstantial evidence of a role for antibody, there is little evidence for effector activities of innate or adaptive immune cells to protect against fleece rot pathogens.

Some control points for limiting the impact of fleece rot on the health of sheep and the commercial value of wool emerge from these collective findings (Fig. 2). Direct and indirect genetic selection can play an important role in reducing the incidence of disease. Managing the microbial ecology of the skin is a second strategy. In addition to possibilities of selection for a protective microbiome and intervention with probiotics mentioned above, a common approach to controlling microbial flora and their exoproducts is vaccination. For some bacterial pathogens, vaccines can eliminate infection

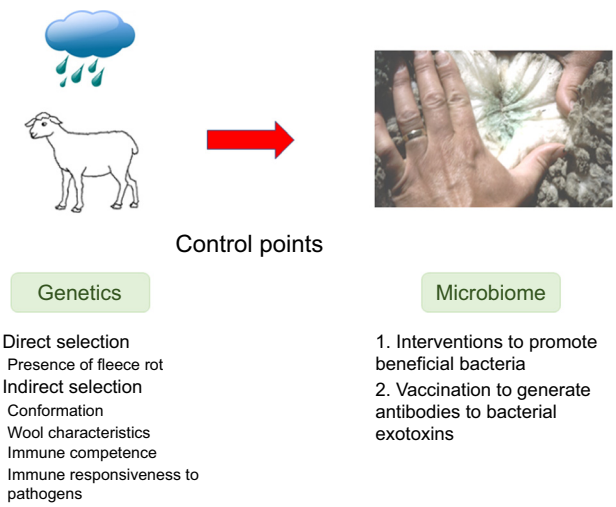

Fig. 2. Control points for fleece rot.

(e.g. by promoting phagocytosis and killing of bacteria), they can inactivate the bacterial factors that promote host pathology (e.g. exotoxin A and phospholipase C of *P. aeruginosa*), or they can reduce production of bacterial products that contribute to disease progression (e.g. quorum sensing signals released by bacteria that modify swarming behaviour and expression of virulence factors (Park *et al.* 2007). In the context of fleece rot, vaccines may be able to assist the host to limit proliferation of undesirable bacteria, limit activity of bacterial products that induce exudative dermatitis, or limit activity of bacterial factors (odorants) that attract blowflies to the sheep. Fleece rot vaccination studies have focussed on pseudomonads, especially *P. aeruginosa*, as the target. The view of fleece rot as a disease complex casts a perspective on this approach. Experience with vaccines against pathogens involved in other disease complexes in farm animals, such as bovine respiratory disease complex, has highlighted the difficulty of attaining a high level of disease control through vaccination (Theurer *et al.* 2015; Cusack and Mahony 2019). Typically, development of complex infectious diseases is not reliant on a single microbial pathogenic mechanism. Consequently, vaccination against a single pathogen is not always sufficient to control the disease complex. Notwithstanding these complexities, bacterial vaccines may yet have a role to play in controlling fleece rot and body strike. Manipulation of the resident flora by vaccination to promote abundance of protective microbial species, either separately or together with vaccination to control endstage pathogens such as *P. aeruginosa*, may also be possible.

Important questions raised by these opportunities for new vaccines include the following: (1) which bacterial species and bacterial virulence factors are needed in a vaccine; (2) can antibody titres in skin be increased prior to the development of wetting-induced dermatitis; and (3) is the functional activity of antibody on skin sufficient to control skin microbiota? Development of a vaccine requires identification of (1) relevant antigens, (2) a protective immune defence mechanism, (3) a goal of vaccination (e.g. reduction of wool staining, reduction of body strike) that can be linked to a clinical measure of vaccine efficacy, and (4) a strategy for vaccine use that is practical for producers while addressing epidemiological characteristics of the disease in the field. Advances in microbiology, immunology and vaccine technology that may help answer these questions are examined in the accompanying review (Denman *et al.* 2021).

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