PERSPECTIVES ON ANIMAL BIOSCIENCES
https://doi.org/10.1071/AN21120
ANIMAL PRODUCTION SCIENCE

Fleece rot and dermatophilosis (lumpy wool) in sheep: opportunities and challenges for new vaccines

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Handling Editor:
Robin Jacob

Received: 2 March 2021
Accepted: 9 September 2021
Published: 30 November 2021

Cite this:
Denman S *et al.* (2022)
Animal Production Science, **62**(4), 301–320.
doi:10.1071/AN21120

ABSTRACT

During prolonged wetting of the fleece, proliferation of bacterial flora often dominated by *Pseudomonas aeruginosa* or *Dermatophilus congolensis* can induce dermatitis and fleece damage termed fleece rot and dermatophilosis respectively, which predispose sheep to blowfly strike. A large research effort in the 1980s and 1990s on vaccines to control fleece rot and dermatophilosis met with limited success. This review examines theoretical and technological advances in microbial ecology, pathogenesis, immunology, vaccine development and the characterisation of microbial virulence factors that create new opportunities for development of vaccines against these diseases. Genomic technologies have now created new opportunities for examining microbial dynamics and pathogen virulence in dermatitis. An effective vaccine requires the combination of appropriate antigens with an adjuvant that elicits a protective immune response that ideally provides long-lasting protection in the field. A clinical goal informed by epidemiological, economic and animal welfare values is needed as a measure of vaccine efficacy. Due to dependence of fleece rot and dermatophilosis on sporadic wet conditions for their expression, vaccine development would be expedited by *in vitro* correlates of immune protection. The efficacy of vaccines is influenced by genetic and phenotypic characteristics of the animal. Advances in understanding vaccine responsiveness, immune defence in skin and immune competence in sheep should also inform any renewed efforts to develop new fleece rot and dermatophilosis vaccines. The commercial imperatives for new vaccines are likely to continue to increase as the animal welfare expectations of society intensify and reliance on pharmacotherapeutics decrease due to chemical resistance, market pressures and societal influences. Vaccines should be considered part of an integrated disease control strategy, in combination with genetic selection for general immune competence and resistance to specific diseases, as well as management practices that minimise stress and opportunities for disease transmission. The strategy could help preserve the efficacy of pharmacotherapeutics as tactical interventions to alleviate compromised welfare when adverse environmental conditions lead to a break down in integrated strategic disease control. *P. aeruginosa* and *D. congolensis* are formidable pathogens and development of effective vaccines remains a substantial challenge.

Keywords: adjuvant, antibiotic resistance, antibody, antigenic competition, immune responsiveness, *Lucilia cuprina*, lumpy wool, microbiome, resilience, transcutaneous immunisation.

Introduction

Development of an effective vaccine requires identification of (1) relevant antigens, (2) a protective immune defence mechanism, (3) 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. Most research on vaccines for fleece rot and dermatophilosis was undertaken about three decades ago (for reviews, see Colditz *et al.* 2021; Tellam *et al.* 2021). Technological changes over the past 15 years in understanding and exploiting biology have been revolutionary and continue at a rapid pace. New

options for vaccine design to precisely elicit specific immune responses are strongly enabled by these recent technological developments (Francis 2018; Wallis *et al.* 2019). These technologies allow the identification of species and strains of bacteria causing disease using genomics tools (including identification of bacteria that cannot be grown in laboratory culture), the discovery of vaccine antigens (and complete families of antigens through genome sequencing) and the formulation of vaccines to induce specific types of immune responses.

The new technologies for interrogation of biology generate huge quantities of data that can be mined by continually improved analytical tools. The process is accelerating not only because of the technological improvements; there are now publicly available, large and expanding databases populated with tens of thousands of reference microbial genomes, huge numbers of genetic variants within a species population, small molecule reference structures, and statistical measures of units of biological complexity. Thus, biological data are being generated and processed at unprecedented rates. This information is enabling for the design of new vaccines.

Over the past two decades, there have also been many developments in design, composition and delivery of vaccines that enable improved and longer-lasting vaccine efficacy. These advances enable the tailoring of the vaccine-induced immune response to better recognise and control a threat to the host arising from an infectious microbial agent (Francis 2018; Wallis *et al.* 2019), which provide new opportunities for development of vaccines.

Two accompanying reviews examine past research on the pathogenesis, aetiology, host defence and previous vaccination trials to protect sheep from fleece rot and dermatophilosis (Colditz *et al.* 2021; Tellam *et al.* 2021). From this background, the current review examines how recent technological developments can renew prospects for effective vaccines to fleece rot and dermatophilosis through (1) more rapid and comprehensive identification of microbial populations and their abundances in complex mixtures, (2) measures of the genetic diversity of individual species of bacteria involved in initiating and sustaining fleece rot or dermatophilosis, (3) a means to monitor antibiotic resistance in bacteria, (4) more efficient identification and isolation of candidate vaccine antigens, particularly molecules secreted from bacteria to enhance their infectivity and pathogenicity in the natural state of infection (as opposed to secreted proteins identified from laboratory culture of bacteria), and novel undetected antigens discovered using bacterial genomic sequence analyses, (5) improved vaccine formulations to enhance vaccine efficacy and duration of protection, (6) more efficient means of manufacturing antigens and (7) more efficient quality control of vaccine manufacture. Recent advances in understanding immune function in sheep as the medium for host defence activated by vaccination are also examined. The review also describes some of the factors critical for commercial success of a vaccine. An electronic draft of this review was previously made available by Australian Wool Innovation as part of a larger commissioned industry report (Vuocolo *et al.* 2020).

Microbiomes

Populations of different species of bacteria at different abundances are present in fleece rot lesions and dermatophilosis (for reviews, see Colditz *et al.* 2021; Tellam *et al.* 2021). Most past investigations of the bacterial diversity and dynamics in fleece rot and dermatophilosis were technically limited by modern standards. It is likely that the full scope of numerical and functional diversity of bacterial in fleece rot samples is not accurately or comprehensively ascertained due to the following: (1) biased laboratory culture conditions for growing bacteria and the inability to grow many bacteria in laboratory culture; for example, in bacterial ecology studies, easily isolatable and cultivable bacteria may represent less than 1% of the total bacterial diversity in a sample (Hugenholtz 2002); (2) the use of molecular technologies for detection of bacterial species that did not fully capture the diversity of the bacterial population through paucity of knowledge, and the absence of quantitative analyses. The dynamics of the formation of microbial populations can now be assessed in detail by microbiome analyses.

A high priority for future research is to use modern and unbiased microbiome analyses (Qin *et al.* 2010; Denman *et al.* 2015; Forbes *et al.* 2017) to detect and quantify *all* bacterial species on normal skin and fleece, and the bacterial dynamics that lead to the expression of fleece rot and dermatophilosis. Previous research based largely on aerobic culture methods led to the conclusion that within the mixed flora of normal skin and fleece rot lesions, *Pseudomonas aeruginosa* plays a dominant role in the expression of severe fleece rot lesions and in attraction of blowflies causing body strike, as part of a disease complex (Colditz *et al.* 2021). In contrast, dermatophilosis is considered to result from a pure overgrowth of *Dermatophilus congolensis*. This organism is also the cause of strawberry foot rot, sometimes in association with scabby mouth virus (Zaria 1993). The microbiome approach may enable clarification of the role of these pathogens in fleece rot and dermatophilosis and have the added benefit of documenting strain diversity in *P. aeruginosa* and *D. congolensis*. The microbiome approach is rapid, comprehensive, quantitative and unbiased, does not require laboratory culture of bacteria, and it is now the mainstay for investigations of complex microbial communities (Qin *et al.* 2010; Denman *et al.* 2015; Forbes *et al.* 2017; Bharti and Grimm 2021). The technique relies on massively parallel sampling of DNA sequences from all bacteria in a complex population. The efficiency and utility of this process for the identification of bacteria are further advanced by the availability of whole-genome sequences for a huge variety of bacterial species.

The approach is complemented by mRNA expression analysis (transcriptomics) and proteomic approaches. In addition, the cost of these technologies per sample has dramatically decreased with time, thus enabling a more intensive investigation of the changing microbial ecology over the course of infection.

Pseudomonas aeruginosa is ubiquitous throughout nature and an opportunistic pathogen with a large genome (~6–7 Mb), allowing it to thrive in many different environments. How it achieves this is starting to be understood through 'multi-omic' approaches. *P. aeruginosa* genomes have a mosaic structure composed of a highly conserved core with inserts of flexible (found in multiple isolates) and unique genes (only in single isolates). The Pseudomonas Genome Database (ver. 20.2.1, released September 2020, https://www.pseudomonas.com/; Winsor *et al.* 2016) currently contains 9796 *Pseudomonas* spp., of which there are now 4954 *P. aeruginosa* genomes.

Studies using transposon sequencing of *P. aeruginosa* identified 321 essential genes required for growth on five different media substrates (Poulsen *et al.* 2019), while an *in silico* pan-genome comparison identified 665 core genes representing only about 1% of the entire pan-genome (Freschi *et al.* 2019). Knowledge of the core genome is essential to help define mechanisms to inhibit the proliferation of this bacterial species. On the basis of average nucleotide identity analysis, *P. aeruginosa* can be phylogenetically separated into five groups, with most isolates being assigned to Groups 1 and 2. This bias is partly based on the over-representation of clinical isolates in databases, but also reflects the low genomic diversity among isolates. *P. aeruginosa* genomes for livestock isolates are less represented, with only a single isolate from sheep mastitis infection and seven from cattle lung and faecal samples currently publicly available. Genome sequencing of isolates relevant to Australian sheep fleece rot is essential if a greater understanding of the transition of environmental strains to disease causative strains is to be achieved.

Although there is minimal correlation between bacterial genotypes and habitat, there is evidence of the emergence of dominant phenotypical *P. aeruginosa* isolates from within a community population, supporting adaption to niche specialists (Kidd *et al.* 2012; Azimi *et al.* 2020). In particular, within a population of *P. aeruginosa* strains in a cystic fibrosis antibiotic-resistant biofilm, evolutionary trajectories were measured using metagenomic methods, which identified that 60% of genomic changes to strains had evolved within 10 days (Azimi *et al.* 2020). The overall community function is determined by all the individuals in the population and not a single species. Understanding what environmental factors affect bacterial community structure and function remains critical to defining mechanisms to alter/inhibit these communities.

Pseudomonas aeruginosa and *D. congolensis* genome sequences also provide deduced protein coding sequences for all bacterial proteins, including secreted proteins that act as factors potentially involved in enhancing bacterial infectivity and pathogenesis. Thus, there is potential to rapidly identify previously unknown secreted proteins, some of which will be virulence factors. Antibody-mediated inactivation of these factors is likely to be a high-priority strategy for vaccines designed to protect sheep from fleece rot and dermatophilosis. Pan-genome sequences covering genomic strain variation within these bacterial species are currently more limited but could be rapidly generated. This information is important to ascertain the extent of strain variation in potential vaccine antigens, as this could affect vaccine efficacy.

Vaccine design and delivery

There have been significant technical developments in the design and delivery of vaccines over the past few decades, with there beign several excellent reviews of these changes (Francis 2018; Wallis *et al.* 2019). These developments have the potential to catalyse the production of new vaccines where there have been past failures in vaccine design. The recent technology improvements are aimed at producing longer-lasting, highly protective and specific cellular or humoral immune responses in the tissues affected by infection. The technology improvements have been driven by the need for a diversity of vaccine types, with each being tailored to the specific characteristics of the infective agent. Vaccines are designed for many purposes, such as, for example, killing microbes by cell-mediated and/or antibody-mediated cytotoxic pathways, decreasing microbial infectivity, decreasing microbial pathogenicity in the host, and neutralising the effects of bacterial toxins secreted by microbes (Francis 2018; Wallis *et al.* 2019). The specific purpose of a vaccine guides the type of immune response that needs to be generated by the vaccine design and the body tissue location where it needs to be effective (Francis 2018; Wallis *et al.* 2019). Other technological improvements have occurred that broaden the range of options for vaccine administration, allowing improvements in ease of use. Advances in mRNA vaccine development are providing new tools for rapid design and testing, with high levels of safety and efficacy. Lipid nanoparticle technology delivery systems combined with nucleoside modification of mRNA have increased efficacy by protecting the mRNA through suppressing innate immune responses and delivering the mRNA into the cell (Pardi *et al.* 2018). Advances in techniques to store mRNA vaccines now allow storage for up to 36 months at 5–25°C and up to 6 months at 40°C, without loss of efficacy (Alberer *et al.* 2017). Opportunities to harness immune functions in skin for protection against fleece rot and dermatophilosis through design and delivery of vaccines are considered next.

Immune responses in skin of sheep

The antibody classes IgA and IgM have strong activity in agglutinating bacteria on epithelial surfaces to prevent their invasion, whereas IgG1 and IgG2 inactivate bacterial toxins and promote phagocytosis and killing of bacteria by leukocytes (Watson *et al.* 1994*a*). All these immunoglobulin classes are present in washings from normal skin of sheep and cattle (Lloyd *et al.* 1979). In ruminant gut and mammary tissues, IgG1 is transported across epithelia by the FcRn receptor, which binds the Fc portion of the IgG1 molecule, while IgA and IgM are transported across epithelia by the poly FcR receptor (Hine *et al.* 2019*b*). From studies of antibody responses in skin washings following intradermal vaccination with *D. congolensis* in cattle, Lloyd *et al.* (1987) concluded that selective transport of IgA and IgM occurred in skin by a local secretory process, suggesting that poly FcR may be present in epithelial cells lining sebaceous glands or sweat glands to mediate transport of IgA and IgM into glandular secretions. In contrast, IgG1 and IgG2 arrived at the skin surface by a transudative process that does not involve active receptor-mediated transport. Colditz *et al.* (1992) confirmed the absence of a mechanism for active receptor-mediated transport of IgG1 across skin in sheep. Following the observation of elevated numbers of plasma cells in skin of sheep recovering from dermatophilosis (Ellis *et al.* 1987) and the presence of antibody to *D. congolensis* antigens in skin washings (Lloyd and Jenkinson 1981; Lloyd *et al.* 1987; Sutherland *et al.* 1987), methods were examined for inducing local antibody production in skin of sheep (Colditz and Watson 1993; Colditz *et al.* 2002). Using recombinant peritrophic membrane antigens from *Lucilia cuprina* larvae as experimental antigens, sheep were primed systemically then boosted by intradermal injection of antigen plus the adjuvant 'matrix immunostimulating complexes' (ISCOMs). Three weeks after boosting, interstitial fluid was harvested by the method of Watson *et al.* (1992) from the vaccination sites and from control skin sites receiving matrix ISCOMs alone without antigen. Within-animal comparison of antibody titres in interstitial fluid from the two types of site indicated that local intradermal immunisation had elevated local antibody concentrations in skin (Colditz *et al.* 2002). The isotype of the locally produced antibody was not determined.

Protein antigen incorporated into ISCOMs can induce a systemic antibody response in sheep when painted onto intact skin (Colditz and Watson 1993). In a similar fashion, bacterial ADP-ribosylating exotoxins (BARE) such as cholera toxin can induce systemic immune responses when applied to the intact surface of normal skin in a range of species including sheep (Chen *et al.* 2000; Cope and Colditz 2000; Chen *et al.* 2002) and cattle (Hammond *et al.* 2000; Morrow *et al.* 2001). This vaccine delivery method has been termed transcutaneous immunisation (TCI). BARE also act as adjuvants for the induction of immune responses to antigens co-administered by TCI (Glenn *et al.* 1998). An important feature of this vaccine delivery method is the role of skin hydration in facilitating the uptake of the epicutaneously applied antigens and BARE adjuvant (Hammond *et al.* 2000). Exotoxin A from *P. aeruginosa* is a BARE and has been found to induce systemic immune responses when applied to intact skin in mice (Glenn *et al.* 1999). Ovine isolates of *P. aeruginosa* produce exotoxin A (Burrell and MacDiarmid 1984; London and Griffith 1984); so, it is likely that the strong immunogenicity of *P. aeruginosa* reported in experimental infections and natural fleece rot may be due in part to the capacity of exotoxin A to enhance responses of the host to other pseudomonad antigens. The hydration of skin and subsequent breakdown of the epidermal barrier are features of fleece rot that would also facilitate sensitisation of the host.

While local deposition of antigen in skin, for example, by intradermal injection with a device such as the Skintraction™ injector, can induce local antibody production at the site of vaccination (Colditz and Watson 1993) as well as systemic antibody responses (Colditz and Paull 2010), local injection over the whole of the animal body by a transdermal injector is impractical. The transcutaneous immunisation route was designed for systemic vaccination via the transcutaneous route to address the risk of needle stick injuries to personnel administering vaccines and to avoid needle phobia in humans (Mitragotri 2005), rather than as a method for whole-of-skin local immunisation. The major drawback for commercial application of TCI in sheep is the requirement for skin hydration (Hammond *et al.* 2000). While natural infection with *P. aeruginosa* during fleece rot may mimic TCI by inducing a systemic immune response, failure of natural infection to induce protective immunity suggests that transcutaneous vaccination with a live attenuated *P. aeruginosa* vaccine might not be feasible, again in part because of the need for skin hydration that might itself induce fleece rot.

Despite the potential to induce local production of IgA and IgM in skin, the above considerations lead to the conclusion that systemic vaccination by a conventional route such as subcutaneous injection with a formulation designed to promote high titres of IgG1- and IgG2-class antibodies to skin pathogens is the most promising approach for development of vaccines to control fleece rot and dermatophilosis. The compatibility of this strategy with the potential inclusion of a fleece rot vaccine into existing vaccines for control of other diseases, such as the 5-in-1 vaccine, is unclear.

In summary, key points on immune responses in skin of sheep include the following: local production of IgA and IgM antibodies in skin occurs in some types of dermatitis; local production of antibodies in skin can be induced by vaccination; systemic production of IgG antibodies can be induced when antigens are applied to hydrated skin in the presence of bacterially derived adjuvants termed BAREs; exotoxin A is a BARE that is expressed by *P. aeruginosa* and its expression may augment the systemic immune response of sheep to *P. aeruginosa* antigens during fleece rot; induction of local

antibody production across the whole skin surface as a control strategy for bacterial dermatitis is impractical. The importance of systemic host immune competence to vaccination responses is considered next.

Systemic immune competence in sheep

The strength of the systemic antibody response to vaccination is influenced by several factors, including genetics of the sheep, phenotypic status of the sheep, antigens within the vaccine, adjuvant in the vaccine, and immunophysiology of antibodies.

Influence of genetics of sheep on immune responses

Individual sheep vary in the strength of their antibody response to vaccination and a portion of the variation is heritable (Nguyen 1984; Berggren-Thomas *et al.* 1987; Raadsma *et al.* 1996). Genes are considered to influence the following two aspects of the immune responsiveness: (1) specific responsiveness to individual antigens, and; (2) general responsiveness expressed broadly to many different antigens (Biozzi *et al.* 1971; Raadsma *et al.* 1996). In general terms, specific responsiveness is most strongly influenced by genes associated with the major histocompatibility complex (MHC), whereas general responsiveness is an additive genetic effect influenced by many genes (Glass 2004). These two aspects of immune responsiveness were studied in detail by Raadsma and colleagues (Raadsma *et al.* 1996, 1999) in sheep vaccinated with a commercial footrot vaccine and a 5-in-1 clostridial vaccine. The heritability of antibody titres varied from 0.22 to 0.66 for nine antigens from *Dichelobacter nodosus* (the microbial agent causing footrot), while heritability estimates for antigens from *Clostridium tetani* and *Clostridium chauvoei* were 0.12 and 0.24 respectively. Genetic correlations between antibody responses to various pairs of antigens ranged from −0.08 to +0.41. The authors concluded that there was little prospect for selective breeding of sheep with an enhanced capacity to mount strong antibody responses to *all* vaccine antigens.

The Trangie sheep selection lines for resistance and susceptibility to fleece rot and fly strike (McGuirk *et al.* 1978) support the conclusion in the previous paragraph. Whereas the resistant line expressed higher antibody responses to injection of *Pseudomonas* antigens than did the susceptible line (Chin and Watts 1991), antibody responses to *L. cuprina* antigens did not differ between lines following artificial infection with larvae. Similarly, antibody responses to the *C. tetani* component of the 5-in-1 vaccine did not differ between selection lines in the CSIRO *Haemonchus* or *Trichostrongylus* selection flocks (Colditz *et al.* 1996). Thus, immunological differences between selection lines (not discussed here) that are known to contribute to resistance to specific disease did not confer differential immune responsiveness to vaccines unrelated to the specific disease resistance under selection.

Notwithstanding these results and similar findings in other species, suggesting that selection for simultaneous antibody production to all antigens present in vaccines may not be a realistic goal, several studies have examined the potential to selectively breed dairy cattle (Hernandez *et al.* 2006), beef cattle (Hine *et al.* 2019*a*), pigs (Mallard *et al.* 1992; Wilkie and Mallard 1999) and sheep (Hine *et al.* 2017) for a trait termed general immune responsiveness (or general immune competence). In these studies, general immune responsiveness has been based on a combination of antibody- and cell-mediated immune response measures. High immune responsiveness is favourably associated with temperament and growth rate during weaning in beef cattle, growth rate in pigs, and a reduction of several diseases in dairy cattle. Estimated breeding values for general immune competence are available for beef cattle and dairy cattle for use by Australian producers (Angus Select 2020).

A recent study explored associations between general immune competence, stress-responsiveness and temperament and important health and production traits in 2613 lambs and 945 adult ewes in the Meat & Livestock Australia (MLA) resource flock (Hine *et al.* 2017). General immune competence was moderately positively correlated with haptoglobin responses to the combined effects of vaccination and management-induced stress. Favourable genetic correlations among general immune competence, internal parasite resistance and several carcase characteristic traits including tenderness and intramuscular fat were observed in lambs. Although unfavourable genetic correlations were observed between general immune competence and certain fleece traits such as fibre diameter and yield, favourable correlations were observed for other fleece traits such as staple strength. In addition, sheep with a calm temperament as assessed by flight time had moderately lower immune competence. The study demonstrated that there is potential to select sheep for *general* immune competence.

An important conceptual difference lies between studies such as Raadsma *et al.* (1996, 1999) on antibody responsiveness to vaccination and studies on general immune competence as a trait balanced for antibody- and cell-mediated activities of the immune system (Hine *et al.* 2017, 2019*a*; Reverter *et al.* 2021). Antibody- and cell-mediated responses are often antagonistic and selection simultaneously for both is thought to select for a general reactivity of the immune system that overrides this antagonism. Furthermore, phenotyping for general immune competence is undertaken in the context of stressors such as weaning and repeated handling over a short period of time. Stressors are well recognised to compromise immune function (Dantzer and Kelley 1989). Hence, general immune competence measured in the face of stressors is thought to provide a measure of resilience to the day-to-day fluctuations experienced within the production environment that compromise immune

function, health, production and welfare (Colditz and Hine 2016; Berghof *et al.* 2019*b*; Iung *et al.* 2019). In this respect, resilience addresses a diversity of physiological, behavioural and psychological factors experienced in the production environment that modulate performance of the immune system. Refinements to methods for measuring resilience are currently under investigation (Elgersma *et al.* 2018; Berghof *et al.* 2019*a*; Poppe *et al.* 2020). Immune competence as a key component of resilience may have implications for the efficacy of vaccines to help control disease. Further studies are needed on the contribution of innate immune responses to general immune competence and on the influence of selection for general immune competence on efficacy of vaccines to protect against disease.

Selection of flivestock over many decades for increased productivity has led to a decreased capacity to cope with day-to-day fluctuations in the production environment and to an increased susceptibility to disease (Rauw *et al.* 1998). For example, in sheep, selection for clean fleece weight is associated with a reduced immune function and increased susceptibility to internal parasite infections (Masters and Ferguson 2019). Selection for production modifies metabolic activities in target tissues such as muscle or the wool follicle, which leads to a change in partitioning of metabolic resources (e.g. protein and energy) between tissues. For the immune system, the penalty of reduced priority for use of resources may be especially apparent during the heightened demand associated with strong immune activation by pathogens (Colditz 2008). The inclusion of immune competence as a quantitative trait in the breeding objective provides the opportunity to improve the priority of the immune system for access to metabolic resources.

In summary, there are several key points on genetics of immune responses in sheep. (1) There is little prospect for selection of sheep with an enhanced genetic capacity to mount strong antibody responses to *all* vaccine antigens. (2) Within an animal, the genetic influence on antibody responses varies among the different antigenic components of a vaccine. Therefore, the relative titres of antibodies to various antigens within a vaccine will differ among individuals, and animals that respond poorly to some of the antigens in a vaccine are likely to be found in every population. Vaccines are therefore unlikely to protect all animals in a flock. (3) Resilience, which can be identified in part by the strength of immune responses to vaccination, is a measure of the capacity of animals to maintain health and production in the face of day-to-day challenges such as disease, routine husbandry practices, and fluctuations in weather. (4) Resilience highlights the fact that adaptive immune responses are not the sole means by which vaccines can improve disease resistance (Netea *et al.* 2020). (5) Resilience may enhance the capacity of commercial vaccines to protect sheep against disease.

Effect of the phenotypic status of the sheep on antibody responses

Physiological stressors such as lactation, and environmental stressors such as transport and social isolation affect immune functions in sheep (Dwyer and Bornett 2004). In comparison with mature non-pregnant, non-lactating adult sheep, antibody responses to vaccination are weaker in lambs, rams, ewes during late pregnancy, during the course of a number of diseases, when animals are in poor body condition and when animals are stressed (Watson and Gill 1991; Watson *et al.* 1994*b*). While fleece rot is usually associated with periods of wet weather lasting a week or more, it is unknown whether weather events of this nature affect antibody responses in a manner that contributes to the disease process (Dwyer and Bornett 2004).

Effect of the antigenic composition of a vaccine on antibody responses

In addition to genetic factors of the host influencing the strength of the antibody response to vaccination, the antigens present within a vaccine can interact in a process termed antigenic competition that modifies relative antibody responses to each antigen. This issue has been particularly important in the development of ovine footrot vaccines comprising multiple serotypes of closely related fimbrial antigens. Importantly, Raadsma *et al.* (1996) noted that heritability of the antibody responsiveness to footrot vaccine antigens was not correlated to mean antibody titre. Thus, just because an antigen can induce a strong antibody response does not mean that antibody responses to that antigen will have a high heritability. This suggests that non-genetic factors such as competition between antigens for recognition by the immune system can influence antibody responses (Finney *et al.* 2018). The phenomenon has importance for development of multivalent vaccines against bacterial strains with similar (but not cross-reactive) immunological identity such as footrot vaccines (Hunt *et al.* 1995) and multivalent clostridial vaccines (Rossi *et al.* 2018). Antigenic competition occurs when different antigens are mixed and administered together. Each antigen can induce an immunologic response when administered alone; however, when administered with other antigens, there is a suppressed immunological response to one or more of the antigens. This response can confound vaccine efficacy that aims to protect a host from all bacterial strains represented in the vaccine.

Immunophysiology of antibodies

The generation and decay of antibody titres following vaccination are influenced by the vaccine formulation, the half-life of the immunoglobulin isotypes and, possibly, by loss of antibody into sinks such as colostrum during lactogenesis in late pregnancy, the gut lumen during some

parasite infections, and due to enhanced catabolism in animals in poor body condition. In a typical adaptive immune response, antibody titres rise for 4–8 weeks following vaccination and, subsequently, decay in accord with the half-life of each immunoglobulin isotype that contributes to the titre. The half-life of IgG1 in sheep, measured by the decay of antibody reactivity in serum following passive immunisation, is between 12 and 24 days (Watson 1992). Similar values for IgG have been reported for other species (Hedegaard and Heegaard 2016). However, IgA and IgM may have half-lives as short as several days (Pearson and Brandon 1976). Following vaccination, plasma cells can continue to produce antibody for extended periods of time; thus, the decline of antibody titres following vaccination is slower than the decline following passive immunisation and the titre can be increased again by boosting injections.

Importantly, antigenic stimulation of the host by pathogens during the course of a disease can have a substantial impact on the kinetics of antibody titres. (This is the usual way by which antibody responses to pathogens are induced). Vaccines that rely on antibody to protect animals against pathogens that contain antigens that are normally 'hidden' from the host immune surveillance system during natural infections pose substantial difficulties for vaccine development (Willadsen *et al.* 1993). Examples are provided by the TickGard™ vaccine for cattle and the BarberVax™ vaccine for sheep. In animals primed and boosted with BarberVax™, egg counts remained suppressed at 6 weeks but not at 8 weeks following boosting (D. Smith, pers. comm.). Protection provided by this vaccine is thought to be mediated by IgG antibodies to gut antigens of *Haemonchus*, which are hidden from the sheep's immune system. Due to the absence of natural boosting by infection, efficacy of this vaccine requires frequent revaccination. Intervals up to 6 weeks between boosts are recommended in a vaccination program that is tailored to the age of the sheep and the epidemiology of the parasite (http://barbervax.com.au/how-to). The titre of antibody required to provide protection against disease can differ by orders of magnitude among pathogens (Rossi *et al.* 2018). In contrast to the rapid decline over several weeks in protection provided by BarberVax™, protection can continue for more than a year following tetanus vaccination in the absence of (known) boosting by exposure to the pathogen in the interim (Rossi *et al.* 2018; Tizard 2021). However, it is noteworthy that doubts exist as to whether plasma antibody titres are the sole determinant of protracted protection induced by clostridial vaccines in sheep (Rossi *et al.* 2018).

Studies on fleece rot indicate that animals can become sensitised to antigens from several skin pathogens in the absence of vaccination (Chin and Watts 1991). This suggests that natural boosting to vaccine antigens might occur in the field and thus reduce the frequency at which boosting is required.

Vaccine adjuvants

Antigens, adjuvants, other formulation components (termed excipients) and the route of vaccine administration all influence the immune response to vaccination. Adjuvants can influence the strength of the immune response, the bias between cell-mediated and antibody-mediated responses, and the immunoglobulin isotypes of antibodies. The effect of an adjuvant is not always consistent across different antigens. Consequently, a very common requirement in vaccine development is the need to screen a range of adjuvants for their effect on the immune responses that are targeted by the vaccine. Thus, adjuvants have been screened for their capacity to stimulate IgG antibody to experimental fly strike vaccine antigens (East *et al.* 1992), TickGard™ and BarberVax™ (David Smith, pers. comm.). In all three instances, Quil-A or the water-in-oil adjuvant Montanide ISA50V2 proved to be the most effective candidate adjuvants examined that were also commercially available, and these would be strong candidates for inclusion in a new vaccine against disease-causing skin microbiota in sheep. There have also been many technological developments in vaccine adjuvants since Tickgard™ and BarberVax™ trials were performed that may provide further options for generating strong and relevant immune responses (Reed *et al.* 2013; Young 2019). Notably, a new generation water-in-oil emulsion adjuvant Montanide ISA 61VG has been developed and found effective for sheep vaccination promoting cellular immune and IgG responses (Begg *et al.* 2019). While adjuvants have the capacity to enhance immune responses, they do not overcome the limitations imposed by age, or physiological or genetic factors on immune responsiveness in sheep (Watson *et al.* 1994*b*).

In summary, several key points on harnessing antibody responses to vaccination to control skin microbiota are evident. (1) A portion of the flock may respond poorly to some antigens in any vaccine. (2) Genetic selection for general immune competence may be more effective than genetic selection for antibody responsiveness as a strategy to improve protection from disease in vaccinated sheep. (3) Impact of weather stress on natural and acquired antibody responses to skin pathogens is unknown. (4) Natural boosting by exposure to antigens from skin pathogens could stimulate a longer duration of protection for a putative skin pathogen vaccine than is seen for vaccines such as BarberVax™ and TickGard™ that contain 'hidden' antigens. (5) During development of a new vaccine to control skin microbiota, it is likely that a panel of adjuvants would need to be screened. (6) Antigenic competition is a potential challenge to development of a vaccine that contains multiple antigens of close immunological identity. (7) Within the current knowledge base for sheep vaccinology, molecular modelling is likely to have little power to predict antigenic competition in sheep. Animal studies will be needed to establish the importance of antigenic competition for future vaccines.

Justification for the development of a fleece rot vaccine

Multiple drivers for development of a fleece rot vaccine

A number of potential benefits from development of a fleece rot vaccine can be identified, including the following: increasing wool yield and quality, and reduction in the incidence of fleece rot and flystrike; maintaining the effectiveness of insecticides for control of flystrike (not reviewed here); improving animal welfare; and enabling enhanced surveillance for bacterial resistance to antibiotics.

Reduction in the incidence of fleece rot and fly strike

The bacterium *P. aeruginosa* is thought to be the primary species that initiates and sustains fleece rot, although contributions from other pseudomonad species and other bacterial genera may also be possible (reviewed by Colditz *et al.* (2021)). At present, the incidence and severity of fleece rot caused by these bacterial species are minimised by managing environmental risk factors, such as, for example, exposure to warm and wet weather, and the timing of shearing, and by matching sheep genotype to the production environment. There is no effective antibiotic treatment. The extent of direct loss in industry productivity due to fleece rot is likely to be small and highly variable year-on-year, on the basis of incidence and existing management practices. A contemporary estimation of costs would be valuable.

The development of fleece rot in sheep is a strong predisposing factor for body strike caused by larval infestation of *L. cuprina* or *Lucilia sericata* (Watts and Merritt 1981*a*, 1981*b*; Eisemann 1988; Raadsma *et al.* 1988; Raadsma 1991*a*, 1991*b*; Dai 1997; Norris *et al.* 2008; James *et al.* 2019). Thus, the major driver for development of a fleece rot vaccine is a reduction in the incidence of blowfly strike, which has considerable impact in the industry (Tellam and Bowles 1997; James 2006; Phillips 2009). Moreover, vaccination as part of an overall integrated pest management strategy has the potential to extend the effective lifetime of existing insecticides used to control flystrike.

Maintaining the effectiveness of insecticides for control of flystrike

Insecticides have been widely used in Australia to control blowfly strike in sheep for over seven decades and, before that, various non-specific chemical agents were used (Shanahan and Roxburgh 1974; Levot 1995; Sandeman *et al.* 2014). Insecticides have worked well and the sheep wool and meat industries are currently reliant on them for improving industry productivity and maintaining good animal welfare. However, there is a long history of development of blowfly resistance to various classes of insecticides (Levot 1995; Sandeman *et al* 2014; Heath and Levot 2015). The risk of widespread resistance developing to currently used insecticides (Sales *et al* 2020) emphasises the need for constant vigilance and for use of integrated pest management practices to prolong the utility of insecticides.

Integrated pest management for flystrike control emphasises the need for rotation of different insecticide classes, consideration of the insecticides used for lice control and careful application to slow the development and spread of insecticide resistance in *L. cuprina* (Sandeman *et al.* 2014). Appropriate application minimises the presence of a sublethal dose on sheep, which can increase the risk of resistance development and spread. The ability to maintain control and rotate insecticide use to minimise resistance development may become more constrained with the recent development of resistance to cyromazine and dicyclanil (Levot 2012, 2013; Levot *et al.* 2014; Sales *et al.* 2020). Vaccines that indirectly or directly reduce the likelihood of flystrike may become an important adjunct in the integrated pest management strategy.

Improved animal welfare

Blowfly strike itself and some current blowfly control practices used on sheep are looming threats to market access in the wool and sheep meat industries due to animal welfare concerns held by markets and the public (Tellam and Bowles 1997; Lee and Fisher 2007; Sandeman *et al.* 2014). New and more acceptable approaches for control of blowfly strike are therefore required (James 2006; Phillips 2009). Vaccination to reduce fleece rot as one of the risk factors for flystrike could be cost-effective, animal-welfare friendly, and well accepted by the industry, the wider public and markets. This approach can help diminish potential societal concerns and market access issues.

Opportunities, challenges and strategy for development of a fleece rot vaccine

As noted in the Introduction, development of a new vaccine involves at least four major stages that need to be undertaken re-iteratively. They are as follows: (1) identify appropriate antigens; (2) identify an immune mechanism for protection; (3) establish a clinical measure of vaccine efficacy; and (4) develop a strategy for vaccine use that is practical for producers while addressing epidemiological characteristics of the disease in the field. Application of the enabling technologies outlined above provides opportunities to address the many challenges in developing a new vaccine against fleece rot.

Identification of appropriate antigens

There is an opportunity to use modern microbiome analyses to investigate the dynamics and the diversity of bacteria

involved in the microbial ecology of normal skin and in the transition to fleece rot and dermatophilosis. The goal is to identify critical stages in the development of the disease, and the diversity of bacteria and their exoproducts involved in those stages, so that relevant antigens for a vaccine can be identified. In this stage, bacterial serotype or strain diversity analyses are important because they allow identification of the comparable variations in the structures of antigens. A priority question for antigen identification is the contribution of secreted toxins, exoproducts and communication molecules to initiation and progression of fleece rot and the creation of a niche for fly strike.

Antigen identification can also be enhanced by transcriptomics and targeted proteomics. The former identifies only genes that are actively transcribing mRNA under a particular environmental condition, while the latter can directly confirm the presence of candidate proteins derived from genome bioinformatic analyses. These approaches are complementary and can assess strain-specific variation in candidate antigens.

A challenge accompanies the potential need to include several antigens in a vaccine that represent different serotypes or strains. Increased complexity of a vaccine has impacts on the manufacturing process, vaccine formulation and cost. A further concern is that competition among antigens for recognition by the immune system could reduce vaccine efficacy compared with a vaccine formulated with antigens from a single bacterial serotype and challenged with the same bacterial serotype. One strategy may be to identify essential exoproducts that contribute to infectivity or pathogenicity, but which show minimal structural variation across different serotypes or strains.

Identify an immune mechanism for protection

A number of experiments point to IgG antibody in serous exudate on the skin surface being able to provide protection against fleece rot disease progression; however, this remains to be conclusively demonstrated (Colditz *et al.* 2021). Salient supportive findings include the following: (1) there is no bacterial invasion of the epidermis, so there is little opportunity for leukocytes to control bacteria by phagocytosis or release of bactericidal products; (2) there is some IgM and IgA in natural skin secretions (probably sweat (suint)) but these immunoglobulins are better at agglutinating bacteria than neutralising toxins; (3) IgG1 and IgG2 are the immunoglobulins of choice for neutralising toxins and exoproducts from bacteria; (4) while there is evidence of potential to induce the local production of antibodies in the skin, achieving this across the whole of the skin surface would be very problematic, and there is no mechanism for active transport of IgGs into skin secretions; blood supply to the skin is excellent (especially during dermatitis), so delivery of IgGs to dermal vasculature is not limiting the availability of IgGs in the serous exudate; (5) high titres of IgGs can be achieved by a conventional systemic vaccination route with a potent adjuvant for antibody production; (6) natural boosting of the immune system by bacteria on skin may help lengthen the duration of protection following vaccination, but whether this happens is unknown.

Establishing a mechanism of immune protection can facilitate the design of an adjuvant formulation and route of administration to activate this immune defence pathway. Should IgG antibody in serous exudate prove to be protective, there is an excellent choice of commercial vaccine adjuvants registered for use in livestock that can generate high titre antibodies of this immunoglobulin isotype. There is also substantial knowledge regarding antigen formulation, suitable administration sites on the animal, injection regime (number of injections and timing), and longevity of effective IgG antibody responses for this immune defence mechanism, and therefore duration of protection.

It is not yet known whether antibody present in skin secretions prior to protracted wetting of skin can prevent the development of fleece rot. Is it necessary for a serous exudate to develop before sufficient antibody is present to control bacterial activity? This leads to the third challenge of vaccine development.

Establish a clinical measure of vaccine efficacy

Clarity over the goal of vaccination is a prerequisite for developing a clinical measure of vaccine efficacy. For fleece rot, this question can be framed in the following terms: is reduction in wool pigmentation, crusting and fibre damage (i.e. fleece rot) a sufficient goal to justify development of a new vaccine, or is reduction in body strike and potentially also breech strike a necessary criterion of vaccine efficacy?

Several issues are important. First, is it necessary for the vaccine to prevent bacterial proliferation or is it adequate to inactivate the exoproducts that confer virulence to the bacteria? As fleece rot typically resolves spontaneously when weather conditions permit the fleece to dry, it may be sufficient to inactivate exoproducts without killing or inactivating bacterial growth.

Second, if the goal is reduction in body strike, the relationship between the severity of fleece rot and susceptibility to body strike may create a threshold for vaccine efficacy. This relationship has been studied in most detail by Raadsma *et al.* (1988). In a study of 176 10-month-old wethers with 4 months wool undergoing artificial wetting, these authors observed that susceptibility to body strike was linearly related to severity of fleece rot. Body strike occurred only in sheep with exudative fleece rot (fleece rot scores 3–6; see fig. 5 in Raadsma *et al.* 1988) or with intense staining of wool in bands greater than 10 mm wide in the absence of exudate (fleece rot score 2). None of the 48 wethers with no fleece rot or with mild wool discolouration in bands less than 10 mm wide developed body strike (fleece rot scores 0 and 1 respectively; see fig. 5

in Raadsma *et al.* 1988). If this relationship holds in larger numbers of sheep with various wool types and wool lengths, then it suggests that the prevention of exudation is an important clinical measure of vaccine efficacy for control of body strike, while prevention of strong wool discolouration greater than 10 mm would provide a more stringent clinical measure. It is noteworthy that previous research on experimental blowfly vaccines indicates that antibody provides the mechanism of immune defence against larvae (Casu *et al.* 1997; Tellam *et al.* 2001). The strong association between the presence of exudate and susceptibility to body strike suggests that in sheep vaccinated against blowfly larvae, antibody would be present in exudate on the skin surface at the time larvae begin to feed on the host.

A third question is the role of odours in initiation of body strike and whether prevention of odour development during fleece rot is an important goal of vaccination. A recent review by James *et al.* (2019) examined the role of odours in predisposition to blowfly strike, and the reader is referred to that document for technical details. These authors concluded that blocking odours, for example, by vaccination, may play a role in the control of susceptibility to fly strike. They noted the following (James *et al.* 2019, p. 4):

> In sheep, odours associated with bacterial growth, particularly when in association with urine staining, scouring and diseases such as fleece rot and dermatophilosis, are critical in determining susceptibility to strike. However, there is little evidence to suggest that differences in attraction of flies to sheep, or innate odour differences between sheep, are key factors in breech strike susceptibility, other than when associated with differences in known predisposing conditions. In addition, any innate differences in sheep odour are likely to be overwhelmed by the effects of bacterial odours during strike waves.

Assessment of vaccine efficacy requires a disease challenge model. Models have been developed for inducing fleece rot by artificial wetting of sheep or by application of live bacterial cultures to the skin. Studies employing natural field challenge would be feasible but would provide a slower means of assessing efficacy. *In vitro* correlates of efficacy, such as, for instance, models of antibody effects on bacterial function or on inhibition of attraction of gravid female blowflies to bacteria-generated odorants, could play an important role in fast-tracking vaccine development. However, it would need to be kept in mind that *in vitro* correlates, on account of their specificity, may not cover the full spectrum of activities through which a vaccine confers protection. For instance, a vaccine might not only affect production of odours that attract female flies but also influence the generation of cues for oviposition and the exudation of serum used by larvae as a food source.

Develop a strategy for vaccine use in the field

For efficient use by sheep producers, the timing of vaccination needs to be integrated with routine husbandry practices if possible, while accommodating the epidemiological characteristics of the disease in the field. Some of these logistical details can be solved only when earlier stages of vaccine development are completed. For instance, sheep age can influence disease susceptibility and strength of the immune responses, and longevity of protection will influence frequency and time of year (or occurrence of weather conditions) when vaccination is necessary.

Strategies required for successful use and adoption of a new fleece rot vaccine could include (1) a stand-alone fleece rot vaccine with a prime-boost administration regimen in lambs, followed by annual boost at the beginning of seasonal period of risk in environments with a seasonal risk profile (e.g. Mediterranean), (2) a fleece rot vaccine combined with a multivalent clostridial vaccine administered in the typical clostridial vaccine regimen, (3) a fleece rot vaccine combined with a potential flystrike vaccine administered prior to onset of peak flystrike season (early spring), coinciding with husbandry practices such as crutching and drenching, and (4) a combined fleece rot and dermatophilosis vaccine administered as per the first point.

Commercialisation challenges and opportunities for a fleece rot vaccine

Currently, there are commercialisation challenges and significant opportunities associated with the development of a vaccine to protect sheep from fleece rot. Ultimately, the impact of the vaccine would be measured by a reduction in body strike in sheep, maintenance of insecticide efficacy, reduction in insecticide use and the removal of external market threats. The extent of impact of the vaccine in these areas would interact with new and independent efforts to directly control body strike.

Ideally, an efficacious vaccine would be integrated with existing management practices to ensure good market penetration and minimisation of labour. The optimum process would incorporate effective fleece rot antigens into an existing vaccine, protecting sheep from multiple unrelated diseases (e.g. a multivalent clostridial vaccine). This outcome would increase the value of the existing vaccine. The efficacies of all antigenic components in this multi-disease vaccine would need to be unaffected by the inclusion of the fleece rot antigens. Importantly, there is the implicit assumption that the protective immune responses generated in sheep for each antigen in the multi-disease vaccine would involve a similar type of immune response. Before considering this process, the efficacy of a fleece rot vaccine would need to be independently demonstrated under a range of circumstances. The inclusion of a fleece rot vaccine into existing unrelated vaccines should be considered but may not be practical for a variety of reasons.

Market size

The potential market size for a fleece rot vaccine is commercially important but difficult to calculate. There are considerable data available on the prevalence of fleece rot in Australian sheep; however, these data primarily show that prevalence is highly variable, particularly in relation to weather and location (Norris *et al.* 2008). Perhaps the best data obtained over a 17-year period at Trangie (New South Wales (NSW); Mortimer *et al.* 1998) and over 6 years at CSIRO 'Longford' Research Station (NSW; Li *et al.* 1999) are prevalence values of 23.5% and 26.7% respectively, but with a very large year-to-year variation. Market up-take could be geographically variable, being influenced by climatic conditions and management practices during at-risk periods. Uptake could also be influenced by fleece and sheep type. A vaccine could also increase the flexibility of producers to run sheep types in environments that expose animals to a heightened risk of fleece rot. The primary driver for vaccine development is not the direct value loss associated with fleece rot in sheep, rather the associated increased risk of flystrike and the need to maintain long-term efficacy of insecticides in the face of a long history of development of insecticide resistance. The cost of flystrike control in Australia has been estimated at A$173 million per year, but some control practices are also major external marketing threats to the Australian wool and sheep meat industries (Sandeman *et al.* 2014). There is some quantitative information about the relationship between the incidence and severity of fleece rot and the incidence of bodystrike (Raadsma *et al.* 1988), which may be valuable for estimation of market size.

History of past patents and commercialisation efforts

A large research effort was undertaken in the 1980s and early 1990s to develop vaccines against *P. aeruginosa* for fleece rot control. Details describing these experimental vaccines are listed in the accompanying review (Colditz *et al.* 2021). The section below highlights possible reasons for past commercial failures of a fleece rot vaccine.

Australian and New Zealand patents entitled *Improved Vaccine* were granted in 1988 but have now expired (Burrell and MacDiarmid 1988; application date, 17 January 1986; publication date, 29 February 1988; patent reference numbers, AU PG8941 19850118; NZ 214858A; inventors, D. H. Burrell and J. A. MacDiarmid; applicants, CSIRO and Biotech Australia Pty Ltd). A related patent submitted by the same inventors and applicants was filed in 1986 (Burrell and MacDiarmid 1986; granted 1986; patent reference numbers, AU 8652530-A, ZA 8600374-A; inventors, D. H. Burrell and J. A. MacDiarmid; applicants, CSIRO and Biotech Australia Pty Ltd). The major patent claim was for a vaccine that reduces predisposition to flystrike by reducing microbial activity in skin, i.e. '*The vaccine is useful for reducing the predisposition of sheep to fly strike (claimed). Action is by protecting the sheep against microbial activity in the fleece (fleece rot) and skin, preventing dermatitis lesions which attract the blowfly to oviposit*'. The vaccine could include soluble antigens or outer membrane antigens from *P. aeruginosa* alone or in combination with similar antigens from *Pseudomonas putida*, *Pseudomonas stutzeri* or *Pseudomonas maltophilia* (Burrell and MacDiarmid 1988, p. 1). It was considered at the time that a vaccine would be unable to supplant the use of insecticides used for control of blowfly strike (the major (indirect) product competition in the market). Additional factors contributing to commercial failure at the time are likely to have included *P. aeruginosa* strain variation (affects vaccine efficacy and manufacture) and a limited market size.

Several experimental vaccines for fleece rot protection have been investigated in the past, but few were tested in field trials and none was commercialised, although one was patented but not tested for efficacy against fleece rot (Chin 1995). Clinical trials using antigens derived from *P. aeruginosa* to protect humans from several infectious diseases caused by this bacterium have been disappointing and no commercial vaccine for human use has been developed (Priebe and Goldberg 2014; Merakou *et al.* 2018; Hoggarth *et al.* 2019). Notably, several specific potential antigens have been identified.

Progress with vaccine against human diseases caused by *P. aeruginosa*

Pseudomonas aeruginosa normally does not infect healthy people. It is a pathogen that exploits opportunities arising in individuals with injuries, chronic health conditions or deficient immune systems. Typically, *P. aeruginosa* infects broken human skin, particularly resulting from burns, and most epithelial tissue surfaces such as the lower respiratory tract (especially people with cystic fibrosis), urinary tract, gastrointestinal tract, ear and cornea (Merakou *et al.* 2018). The bacterium is one of the most prevalent causes of infectious diseases in humans and responsible for considerable morbidity and mortality (Merakou *et al.* 2018). The metabolic and genetic versatility of *P. aeruginosa* is the reason why it can successfully proliferate in diverse tissues, each with different local environmental characteristics (Vasil 1986). The feature common to all infections is the breaking down of the barrier function of epithelial tissue layers and the promotion of a moist environment in which *P. aeruginosa* proliferates, using cellular debris as a nutrient source. Treatment of infected people is difficult as infections are often resistant to multiple classes of antibiotics and the bacterium readily avoids immune defence responses in the host by using a formidable array of strategies (Döring and Pier 2008; Sharma *et al.* 2011; Merakou *et al.* 2018). Hence, antibiotic treatments are usually aggressive and often do not fully eradicate the bacterium, thereby leading to long-term chronic infection.

Since the 1970s, there have been numerous and continuing efforts to develop vaccines to protect at-risk individuals from *P. aeruginosa* infections. No vaccine has become commercially available, although promising results have been obtained

(Döring and Pier 2008; Sharma *et al.* 2011; Merakou *et al.* 2018). One reason for the lack of success is that *P. aeruginosa* chronic infections often occur in people whose immune system is compromised by other health factors and, hence, they cannot mount an adequate immune response to an administered vaccine. Moreover, for some triggers of infection, such as burns and injuries, there may be insufficient time for a strategically administered vaccine to induce an effective immune response. In the latter instance, passive immunisation involving the transfer of effective antibodies from another source to the injured person may be a quicker and more effective strategy.

The ability to test a new vaccine in a relevant human cohort is difficult due to limited patient numbers who often receive variable, and therefore confounding, additional treatments, particularly antibiotic treatments. These factors in clinical trials often lead to statistically inconclusive results or non-confirmation in subsequent trials. There may also be ethical and clinical issues associated with the use of human control groups that remain unvaccinated. Further, the results for vaccines tested in animal models of human diseases caused by *P. aeruginosa* often do not translate well when tested in humans (Döring and Pier 2008; Sharma *et al.* 2011; Merakou *et al.* 2018).

The search for effective human vaccines has focussed on identifying protective antigens from *P. aeruginosa*, generating specific immune responses by optimising vaccine formulations and administration routes, designing vaccines to manage *P. aeruginosa* strain variation, and producing different vaccines for each type of medical condition (Döring and Pier 2008; Sharma *et al.* 2011; Merakou *et al.* 2018). Effective vaccines against *P. aeruginosa* should be characterised by several features. First, the vaccine should elicit an antibody response that mediates opsonophagocytic killing by neutrophils and macrophages, and neutralises virulence factors. Second, the vaccine antigen should be present in the *P. aeruginosa* strain causing disease or the antigen should be conserved in structure and thereby generate cross-reactive protection against multiple *P. aeruginosa* strains. Alternatively, the vaccine antigen may contain the spectrum of structural forms of an antigen that are present in multiple strains causing disease. Third, the target antigens in *P. aeruginosa* need to be expressed in sufficient quantities in the bacterium and be available for recognition by antibodies. Fourth, it is considered that the vaccine should also generate a T cell response (T_H17), although there is some debate as to whether this is of primary importance (Merakou *et al.* 2018).

Numerous antigens from *P. aeruginosa* have been tested in experimental and clinical vaccines designed to protect humans from *P. aeruginosa* infections. Table 1 summarises the tested antigens (adapted from reviews by Döring and Pier 2008; Sharma *et al.* 2011; Merakou *et al.* 2018). For simplicity, the table lists classes of antigens and does not contain antigens tested by passive immunisations. The latter would not be practical for use in livestock animals. The antigens with some extent of protection include lipopolysaccharide, polysaccharide repeating unit of LPS (O-antigen), outer membrane proteins, mucoid exopolysaccharides (alginate), polysaccharide-protein conjugates, flagella proteins, pili proteins, whole dead *P. aeruginosa*, live-attenuated *P. aeruginosa*, attenuated *Salmonella* and attenuated adenovirus engineered to express various *P. aeruginosa* antigens, surface-expressed T3SS proteins involved in Type 3 secretion of bacterial virulence proteins directly into epithelial cells, exotoxin A and secreted proteases (Döring and Pier 2008; Sharma *et al.* 2011; Merakou *et al.* 2018). Some of the tested vaccines included combinations of antigens. In the list of antigens, there is an emphasis on virulence factors that help *P. aeruginosa* adhere to cell surfaces, damage tissues for the dissemination of nutrients or increase the bacterial survival rate.

Justification for development of a dermatophilosis vaccine

Many of the comments regarding the justification for the development of a fleece rot vaccine outlined in the previous section also pertain to the development of a vaccine for control of dermatophilosis, although there are some important differences.

Dermatophilosis causes wool production losses and an increased risk of blowfly strike, especially on the sheep body, back and sides (Wilkinson 1979; Gherardi *et al.* 1981, 1983; Edwards 1985; Edwards *et al.* 1985; Bateup and Edwards 1990). However, the extent of its contribution to blowfly strike risk is unclear and may be less than fleece rot. Dermatophilosis is currently managed by culling affected sheep, by monitoring the natural self-healing of sheep and by using antibiotics in severe cases. Moreover, good management practices can limit the spread of dermatophilosis within a flock. The implication is that the market size for a vaccine protecting sheep from dermatophilosis is small and can be quantified from knowledge of antibiotics use and cost of labour for this control purpose. A much larger market is possible if vaccine efficacy extended to other animals, particularly livestock (cattle) and pets. The bacterial species causing dermatophilosis in sheep, *D. congolensis*, causes dermatophilosis in a wide range of animals but it is not an important pathogen in humans (Amor *et al.* 2011). Strain variation changes in sheep could be occurring as a result of antibiotic use for dermatophilosis control and thereby influence vaccine antigen design. In addition, livestock industries may be required in the future to monitor antibiotic resistance development in bacterial populations relevant to the control of livestock diseases, to offset future infection risks in livestock and the risk to human health through indirect transfer of antibiotic resistance genes to other bacterial species.

Table 1. Summary of *P. aeruginosa* antigens tested in human clinical trials and animal models of human infections.

Antigen	Function in *P. aeruginosa*	Advantage	Limitation
Live-attenuated *P. aeruginosa*	Live *P. aeruginosa* missing aroA gene	Presentation of multiple Ag to immune system; opsonophagocytic Ab (i.e. pathogen marked by Ab for ingestion by phagocytes)	Residual virulence
Killed *P. aeruginosa*	–	Presentation of multiple Ag to immune system	Toxic
Lipopolysaccharide (LPS)	Potent toxin causing tissue inflammation	Generation of high levels of opsonic Ab	Toxic; pyrogenic; high heterogeneity; low immunogenicity
O-polysaccharides (O-Ag)	Polysaccharide repeating unit of LPS	Generation of high levels of opsonic Ab	Toxic; pyrogenic; high heterogeneity; low immunogenicity
Alginate (mucoid polysaccharide that is primary determinant of serogroup)	Secreted exopolysaccharide; confers mucoid phenotype; immobilises diffusion of antibiotics, Ab and phagocytes; responsible for biofilms (retain hydration)	Generation of opsonic Ab; low structural heterogeneity; Ab-binding enables phagocytosis by neutrophils; Ab prevents *P. aeruginosa* attachment to epithelial surface	Ab show variable opsonic ability
Flagella proteins	Motility; attaches to mucin for mucin colonisation; chemotaxis; invasiveness	Adjuvant effect; moderate heterogeneity	*P. aeruginosa* can shed flagella
Pilin proteins	Adhesion to epithelial cells; biofilm formation; twitching motility	High immunogenicity; multiple proteins	High heterogeneity; cell receptor binding site hidden
T3SS translocator proteins	Translocates bacterial proteins into host cells to invade tissue; virulence factor	Induces T_H17 immune cells; blockage by Ab binding enhances phagocytosis	Not widely tested yet
Outer membrane proteins	Structural and functional components of bacterial cell wall; biofilm formation; virulence factor by binding innate defence components (C3b adhesion factor; IFN gamma)	Highly conserved and immunogenic; quorum-sensing through IgF-gamma binding; surface exposed	Nil
Exotoxin A (mono-ADP-ribosyltransferase) and proteases (elastase and alkaline protease)	Virulence factors; initiate tissue invasion, cell death, availability of nutrients for bacterial proliferation	Ab binding neutralises cytotoxic effects and pathology	Less effective in bacterial clearance; Ag requires inactivation to be used in vaccine
Attenuated *Salmonella* and attenuated adenoviral vectors engineered to express various *P. aeruginosa* Ag	–	High immunogenicity and adjuvant properties	Pre-existing anti-adenovirus immunity; *Salmonella* vector restricted to mucosal immunity

Note: the table contains merged and abbreviated information from reviews by Döring and Pier 2008; Sharma *et al.* 2011; Merakou *et al.* 2018.
Ab, antibodies; Ag, antigen.

Opportunities and challenges

The scientific challenges associated with the development of a vaccine to protect sheep from dermatophilosis are similar to those listed for a fleece rot vaccine. One common aspect includes the need for a sheep immune response to bacterial antigens that results in the production of antibodies that can be released onto the skin. However, one difference from fleece rot is that the invasive hyphae of *D. congolensis* penetrate the upper skin layer and are therefore more exposed to the sheep's immune surveillance system than is *P. aeruginosa*. Consequently, phagocytic activity and cell-mediated toxicity could contribute to vaccine-induced immune defence against *D. congolensis*. A vaccine against *D. congolensis* may also require consideration of the diversity of *D. congolensis* strains in the vaccine formulation. The identification of specific protective antigens from *D. congolensis* will require consideration of life stage and potential antigen function. The priority strategy for antigen identification for inclusion in a vaccine should focus on secreted virulence and pathogenicity factors and the inactivation of their normal activities by specific IgG antibodies induced in skin or present in serous exudate on the skin surface.

Commercialisation challenges and opportunities

Market size

The commercialisation issues for a vaccine protecting sheep from dermatophilosis will need to consider market size and penetration, as most affected sheep self-heal. The cost of administering antibiotics to chronically affected sheep or culling sheep is the direct market competition for a vaccine.

The average yearly incidence of dermatophilosis in the national flock is unclear due to considerable year-to-year and geographical variation. Berry and Watt (2017) reported incident rates in Merino lambs from a number of mobs on two properties in the central tablelands of NSW. The incident rates in lambs from all mobs ranged between 10% and 80%, with most mobs averaging 10–30%. Most affected sheep self-healed and remained untreated with antibiotics except for one mob where 80% of lambs (2600 lambs) were severely affected and treated. However, lamb treatment rates in most mobs ranged between 0% and 13%, and are likely to have averaged approximately 5–10%. The variation among mobs may relate to local weather differences and differences in sheep genetics, as some mobs may be better suited to the local environment through use of historical sheep bloodlines. If these numbers translate across the industry, then the total treatment cost is likely to be, in aggregate, small, as most affected lambs self-heal. Moreover, older sheep are more resistant to dermatophilosis due to maturation of the immune system and skin structure, possible immune acquisition of resistance and, indirectly, through the culling of chronically infected sheep before they become adults. Thus, there is unlikely to be a large market for a stand-alone dermatophilosis vaccine unless its utility extends to control of strawberry footrot in sheep and dermatophilosis in cattle and pets. Most other commercialisation factors are similar to those listed above for a fleece rot vaccine. It is also noteworthy that the efficiency of the treatment of choice for sheep severely affected by dermatophilosis in a small study undertaken by Berry and Watt (2017) was 64%. However, at shearing, 40% of the untreated sheep were incompletely shorn due to active lesions, compared with 10% for those

Box 1. A strategy for development of new vaccines for control of fleece rot and dermatophilosis in sheep.

1. Undertake an investigation of microbial ecology and pathogenesis of dermatitis so as to identify appropriate vaccine antigens.
   (a) Complete a comprehensive microbiome analysis of microbial ecology in fleece rot, dermatophilosis and the breech of sheep susceptible to breech strike. A microbiome analysis would greatly assist the identification of bacterial isolates required for *in vitro* or challenge efficacy trials and antigenic targets for vaccine development.
   (b) Compile a list of possible vaccine antigens by undertaking comprehensive bioinformatics analyses of publicly available *P. aeruginosa* and *D. congolensis* genome sequences. Identify all secreted proteins, all known infectivity and pathogenicity protein factors, and known antibiotics resistance genes. Cross-reference the information obtained with strains of *P. aeruginosa* and *D. congolensis* identified by experimental microbiome analysis of fleece rot and dermatophilosis. From the list of antigens, identify strain-specific and species-specific structural variants.
   (c) Identify non-protein secreted products from *P. aeruginosa* and *D. congolensis*. The secreted microbial products will include a subset of infectivity and pathogenesis factors, which are prime candidates for inclusion in respective vaccines. Mass spectrometry can experimentally identify the secreted factors. Structural differences in non-protein antigens for different bacterial strains will need to be determined.
   (d) Finger-print *P. aeruginosa* strain-specific genetic differences in fleece rot samples. The approach will determine *P. aeruginosa* population structures (strains) in fleece rot samples, antibiotic resistance profiles and similarities of population structures with *Pseudomonas* populations that infect humans. Strain variation will be important from the perspective of fleece rot vaccine design.
2. Identify mechanism(s) of immune-mediated protection induced by vaccination.
   (a) Use candidate antigens from results of microbial analyses, and establish a primary goal of vaccination: to control fleece rot, dermatophilosis and body strike, or body strike and some breech strike.
   (b) Develop *in vivo* models to assess mode of vaccine action for use in Stage 3.
3. Develop *in vitro* correlates of immune-mediated protection.
   (a) Use *in vitro* immune function correlates of protection (e.g. antibody mediated neutralisation of bacterial exoproduct activity) to inform adjuvant choice and vaccine delivery route and regimen.
   (b) Use an *in vitro* model to examine potential duration of action in the field.
4. Undertake vaccination efficacy trials and develop field strategies for use of the vaccine.
   (a) Establish clinical efficacy in the field against primary and secondary vaccine indications: i.e. to control fleece rot, dermatophilosis and body strike, or body strike and some breech strike as per Stage 2a).
   (b) Determine duration of vaccine action.
   (c) Determine whether the vaccine(s) can be combined/formulated with other current sheep vaccines.
   (d) Develop protocols for vaccine use that accommodate the influence of management practices, sheep age, duration of action, and climatic variables on practicality and efficacy of vaccination.

that were treated with the antibiotic; that is, antibiotic treatment for control of dermatophilosis may have increased total fleece value. A dermatophilosis vaccine that indirectly decreases the incidence of flystrike would enhance its intrinsic value. Currently, the quantitative relationship between dermatophilosis incidence and severity with flystrike incidence is unknown.

Combination vaccines

One commercial possibility is that a vaccine could be developed that combines antigens based on secreted products from *D. congolensis* and *P. aeruginosa*, to produce a vaccine protecting sheep from both fleece rot and dermatophilosis. The impetus for this approach is the likely common requirement of both vaccines to produce IgG antibody on skin that represses the biological functions of secreted virulence products, the likelihood of compatibility of antigens in the vaccine and commercial efficiency. Both diseases also have similar timing in relation to weather events and season and, hence, the seasonal requirements for timing of maximum vaccine efficacy are aligned. However, individual protective vaccines would first need to be developed before the antigens were trialled together.

Conclusions

The starting point for any venture into new vaccine development is the assessment of the economic significance of the problem being addressed and the likelihood of industry adoption of a new vaccine. Despite impressive technological advances that facilitate rational design, the process of vaccine development remains an iterative exercise of trial and error. Issues to address include identification of combinations of antigens that are commercially feasible to manufacture and can be combined into a vaccine that is practical to administer in the field in a manner that provides long-lasting protection. An outline of steps for development of new fleece rot and dermatophilosis vaccines is provided in Box 1. A strong theme of disease control in recent years is the need to move beyond reliance on single modalities such as vaccines or pharmacotherapeutics to broad-based integrated management strategies. In this context, the efficacy of vaccines can be complemented by genetic selection of sheep for immune responsiveness and resistance to specific disease. Optimal expression of these traits requires good management practices that minimise exposure of animals to stressors and to environments that favour the spread of pathogens. The strategy should help preserve the efficacy of pharmacotherapeutics for use as tactical interventions to alleviate compromised welfare when adverse environmental conditions lead to a break down in integrated strategic disease control.

References

Alberer M, Gnad-Vogt U, Hong HS, Mehr KT, Backert L, Finak G, Gottardo R, Bica MA, Garofano A, Koch SD, Fotin-Mleczek M, Hoerr I, Clemens R, von Sonnenburg F (2017) Safety and immunogenicity of a mRNA rabies vaccine in healthy adults: an open-label, non-randomised, prospective, first-in-human phase 1 clinical trial. *The Lancet* **390**, 1511–1520. doi:10.1016/S0140-6736(17)31665-3

Amor A, Enriquez A, Corcuera MT, Toro C, Herrero D, Baquero M (2011) Is infection by *Dermatophilus congolensis* underdiagnosed? *Journal of Clinical Microbiology* **49**, 449–451. doi:10.1128/JCM.01117-10

Angus Select (2020) Prediction of breeding values for commerical animals now a reality. Angus Bulletin WInter 2020.

Azimi S, Roberts AEL, Peng S, Weitz JS, McNally A, Brown SP, Diggle SP (2020) Allelic polymorphism shapes community function in evolving *Pseudomonas aeruginosa* populations. *The ISME Journal* **14**(8), 1929–1942. doi:10.1038/s41396-020-0652-0

Bateup BO, Edwards JR (1990) Processing of wool contaminated with dermatophilosis scab. *Australian Veterinary Journal* **67**, 154–155. doi:10.1111/j.1751-0813.1990.tb07742.x

Begg DJ, Dhungyel O, Naddi A, Dhand NK, Plain KM, de Silva K, Purdie AC, Whittington RJ (2019) The immunogenicity and tissue reactivity of *Mycobacterium avium* subsp paratuberculosis inactivated whole cell vaccine is dependent on the adjuvant used. *Heliyon* **5**, e01911. doi:10.1016/j.heliyon.2019.e01911

Berggren-Thomas PL, Kaattari S, Hohenboken WD, Shrestha JNB, Heaney DP (1987) Inheritance of active and acquired immunity traits in sheep. *Journal of Animal Science* **64**, 1302–1312. doi:10.2527/jas1987.6451302x

Berghof TVL, Bovenhuis H, Mulder HA (2019a) Body weight deviations as indicator for resilience in layer chickens. *Frontiers in Genetics* **10**, 1216. doi:10.3389/fgene.2019.01216

Berghof TVL, Poppe M, Mulder HA (2019b) Opportunities to improve resilience in animal breeding programs. *Frontiers in Genetics* **9**, 692. doi:10.3389/fgene.2018.00692

Berry W, Watt B (2017) High prevalence mild dermatophilosis (lumpy wool) in unweaned Merino lambs following a wet spring. Available at http://www.flockandherd.net.au/sheep/ireader/dermatophilosis.html [Accessed 6 November 2020]

Bharti R, Grimm DG (2021) Current challenges and best-practice protocols for microbiome analysis. *Briefings in Bioinformatics* **22**, 178–193 doi:10.1093/bib/bbz155

Biozzi G, Stiffel C, Mouton D, Bouthillier Y, Decreusefond C (1971) Genetic regulation of the function of antibody-producing cells. In 'Progress in Immunology'. (Ed. B Amos) pp. 529–545. (Academic Press: New York, NY, USA)

Burrell DH, MacDiarmid JA (1984) Characterisation of isolates of *Pseudomonas aeruginosa* from sheep. *Australian Veterinary Journal* **61**, 277–279. doi:10.1111/j.1751-0813.1984.tb06008.x

Burrell DH, MacDiarmid JA (1986) Vaccine containing *Pseudomonas maltophilia* antigens to reduce pre-disposition of sheep to fly strike. Australian Patent Office AU8652530-A.

Burrell DH, MacDiarmid JA (1988) Vaccines against flystrike. New Zealand Patent Office NZ214858A.

Casu R, Eisemann C, Pearson R, Riding G, East I, Donaldson A, Cadogan L, Tellam R (1997) Antibody-mediated inhibition of the growth of larvae from an insect causing cutaneous myiasis in a mammalian host. *Proceedings of the National Academy of Sciences* **94**, 8939–8944. doi:10.1073/pnas.94.17.8939

Chen D, Colditz IG, Glenn GM, Tsonis CG (2000) Induction of systemic immune responses in sheep by topical application of cholera toxin to skin. *Veterinary Immunology and Immunopathology* **77**, 191–199. doi:10.1016/S0165-2427(00)00236-1

Chen D, Colditz IG, Glenn GM, Tsonis CG (2002) Effect of transcutaneous immunization with co-administered antigen and cholera toxin on systemic and mucosal antibody responses in sheep. *Veterinary Immunology and Immunopathology* **86**, 177–182. doi:10.1016/S0165-2427(02)00037-5

Chin J (1995) Composition for use in raising an immune response. Australian Patent Office AU1995022089.

Chin JC, Watts JE (1991) Dermal and serological response against *Pseudomonas aeruginosa* in sheep bred for resistance and

susceptibility to fleece-rot. *Australian Veterinary Journal* **68**, 28–31. doi:10.1111/j.1751-0813.1991.tb09841.x
Colditz IG (2008) The costs of immune responses. In 'Resource allocation theory applied to farm animal production'. (Ed. WM Rauw) pp. 192–209. (CABI Publishing: Wallingford, UK)
Colditz IG, Hine BC (2016) Resilience in farm animals: biology, management, breeding and implications for animal welfare. *Animal Production Science* **56**, 1961–1983. doi:10.1071/AN15297
Colditz IG, Paull DR (2010) Needle-free vaccination in sheep. *Australian Veterinary Journal* **88**, 368–368. doi:10.1111/j.1751-0813.2010.00611.x
Colditz IG, Watson DL (1993) Immunisation to induce antibody production in skin of sheep. In 'Vaccines 93: Erskine House, Lorne, Vic., 28–31 March 1993'. (Organising Committee). Abstract J94.
Colditz IG, Altmann KG, Watson DL (1992) Intradermal and percutaneous transudation of IgG1 and transferrin in sheep. *Immunology and Cell Biology* **70**, 323–327. doi:10.1038/icb.1992.41
Colditz IG, Watson DL, Gray GD, Eady SJ (1996) Some relationships between age, immune responsiveness and resistance to parasites in ruminants. *International Journal for Parasitology* **26**, 869–877. doi:10.1016/S0020-7519(96)80058-0
Colditz IG, Watson DL, Eisemann CH, Tellam RL (2002) Production of antibodies to recombinant antigens from *Lucilia cuprina* following cutaneous immunisation of sheep. *Veterinary Parasitology* **104**, 345–350. doi:10.1016/S0304-4017(01)00648-3
Colditz IG, Vuocolo T, Denman S, Ingham A, Wijffels G, James P, Tellam R (2021) Fleece rot in sheep: a review of pathogenesis, aetiology, resistance and vaccines. *Animal Production Science*. doi:10.1071/AN21118
Cope RB, Colditz IG (2000) Effect on systemic antibody concentrations of topical application of choleratoxin to skin of sheep. *Australian Veterinary Journal* **78**, 121–123. doi:10.1111/j.1751-0813.2000.tb10540.x
Dai Y (1997) Potential role of *Pseudomonas aeruginosa* in sheep fleece rot and the associated serological response. PhD thesis. University of Wollongong, Wollongong, NSW, Australia.
Dantzer R, Kelley KW (1989) Stress and immunity: an integrated view of relationships between the brain and the immune system. *Life Sciences* **44**, 1995–2008. doi:10.1016/0024-3205(89)90345-7
Denman SE, Martinez Fernandez G, Shinkai T, Mitsumori M, McSweeney CS (2015) Metagenomic analysis of the rumen microbial community following inhibition of methane formation by a halogenated methane analog. *Frontiers in Microbiology* **6**, 1087. doi:10.3389/fmicb.2015.01087
Döring G, Pier GB (2008) Vaccines and immunotherapy against Pseudomonas aeruginosa. *Vaccine* **26**, 1011–1024. doi:10.1016/j.vaccine.2007.12.007
Dwyer CM, Bornett HLI (2004) Chronic stress in sheep: assessment tools and their use in different management conditions. *Animal Welfare* **13**, 293–304.
East IJ, Eisemann CH, Vuocolo T, Pearson RD, Donaldson RA, Cadogan LC (1992) Vaccines against blowfly strike: the effect of adjuvant type on vaccine effectiveness. *International Journal for Parasitology* **22**, 309–314. doi:10.1016/S0020-7519(05)80008-6
Edwards JR (1985) Sale and processing of wool affected with dermatophilosis. *Australian Veterinary Journal* **62**, 173–174. doi:10.1111/j.1751-0813.1985.tb07284.x
Edwards JR, Gardner JJ, Norris RT, Love RA, Spicer P, Bryant R, Gwynn RVR, Hawkins CD, Swan RA (1985) A survey of ovine dermatophilosis in Western Australia. *Australian Veterinary Journal* **62**, 361–365. doi:10.1111/j.1751-0813.1985.tb14208.x
Eisemann CH (1988) Upwind flight by gravid Australian sheep blowflies, *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae), in response to stimuli from sheep. *Bulletin of Entomological Research* **78**, 273–279. doi:10.1017/S0007485300013031
Elgersma GG, de Jong G, van der Linde R, Mulder HA (2018) Fluctuations in milk yield are heritable and can be used as a resilience indicator to breed healthy cows. *Journal of Dairy Science* **101**, 1240–1250. doi:10.3168/jds.2017-13270
Ellis TM, Robertson GM, Sutherland SS, Gregory AR (1987) Cellular responses in the skin of Merino sheep to repeated inoculation with *Dermatophilus congolensis*. *Veterinary Microbiology* **15**, 151–162. doi:10.1016/0378-1135(87)90140-4
Finney J, Yeh C-H, Kelsoe G, Kuraoka M (2018) Germinal center responses to complex antigens. *Immunological Reviews* **284**, 42–50. doi:10.1111/imr.12661
Forbes JD, Knox NC, Ronholm J, Pagotto F, Reimer A (2017) Metagenomics: the next culture-independent game changer. *Frontiers in Microbiology* **8**, 1069. doi:10.3389/fmicb.2017.01069
Francis MJ (2018) Recent advances in vaccine technologies. *The Veterinary Clinics of North America: Small Animal Practice* **48**, 231–241. doi:10.1016/j.cvsm.2017.10.002
Freschi L, Vincent AT, Jeukens J, Emond-Rheault JG, Kukavica-Ibrulj I, Dupont M-J, Charette SJ, Boyle B, Levesque RC (2019) The *Pseudomonas aeruginosa* pan-genome provides new insights on its population structure, horizontal gene transfer, and pathogenicity. *Genome Biology and Evolution* **11**, 109–120. doi:10.1093/gbe/evy259
Gherardi SG, Monzu N, Sutherland SS, Johnson KG, Robertson GM (1981) The association between body strike and dermatophilosis of sheep under controlled conditions. *Australian Veterinary Journal* **57**, 268–271. doi:10.1111/j.1751-0813.1981.tb05809.x
Gherardi SG, Sutherland SS, Monzu N, Johnson KG (1983) Field observations on body strike in sheep affected with dermatophilosis and fleece-rot. *Australian Veterinary Journal* **60**, 27–28. doi:10.1111/j.1751-0813.1983.tb02805.x
Glass EJ (2004) Genetic variation and responses to vaccines. *Animal Health Research Reviews* **5**, 197–208. doi:10.1079/AHR200469
Glenn GM, Rao M, Matyas GR, Alving CR (1998) Skin immunization made possible by cholera toxin. *Nature* **391**, 851–852. doi:10.1038/36014
Glenn GM, Scharton-Kersten T, Vassell R, Matyas GR, Alving CR (1999) Transcutaneous immunization with bacterial ADP-ribosylating exotoxins as antigens and adjuvants. *Infection and Immunity* **67**, 1100–1106. doi:10.1128/IAI.67.3.1100-1106.1999
Hammond SA, Tsonis C, Sellins K, Rushlow K, Scharton-Kersten T, Colditz I, Glenn GM (2000) Transcutaneous immunization of domestic animals: opportunities and challenges. *Advanced Drug Delivery Reviews* **43**, 45–55. doi:10.1016/S0169-409X(00)00076-4
Heath A, Levot GW (2015) Parasiticide resistance in flies, lice and ticks in New Zealand and Australia: mechanisms, prevalence and prevention. *New Zealand Veterinary Journal* **63**, 199–210. doi:10.1080/00480169.2014.960500
Hedegaard CJ, Heegaard PM (2016) Passive immunisation, an old idea revisited: basic principles and application to modern animal production systems. *Veterinary Immunology and Immunopathology* **174**, 50–63. doi:10.1016/j.vetimm.2016.04.007
Hernandez A, Quinton M, Miglior F, Mallard BA (2006) Genetic parameters of dairy cattle immune response traits. In 'Proceedings 8th world congress on genetics applied to livestock production, Belo Horizonte, Minas Gerais, Brazil, 13–18 August 2006'. pp. 15–18. (International Committee for World Congress on Genetics Applied to Livestock Production: Belo Horizonte, Minas Gerais, Brazil)
Hine B, Ingham A, Smith J, Colditz I (2017) Associations between immune competence, health and performance of sheep in the resource flock. Meat & Livestock Australia. Available at https://www.mla.com.au/download/finalreports?itemId=3635 [Accessed 29 September 2019]
Hine BC, Bell AM, Niemeyer DDO, Duff CJ, Butcher NM, Dominik S, Ingham AB, Colditz IG (2019a) Immune competence traits assessed during the stress of weaning are heritable and favourably genetically correlated with temperament traits in Angus cattle. *Journal of Animal Science* **97**, 4053–4065. doi:10.1093/jas/skz260
Hine BC, Hunt PW, Colditz IG (2019b) Production and active transport of immunoglobulins within the ruminant mammary gland. *Veterinary Immunology and Immunopathology* **211**, 75–84. doi:10.1016/j.vetimm.2019.04.006
Hoggarth A, Weaver A, Pu Q, Huang T, Schettler J, Chen F, Yuan X, Wu M (2019) Mechanistic research holds promise for bacterial vaccines and phage therapies for *Pseudomonas aeruginosa*. *Drug Design, Development and Therapy* **13**, 909–924. doi:10.2147/DDDT.S189847
Hugenholtz P (2002) Exploring prokaryotic diversity in the genomic era. *Genome Biology* **3**, reviews0003.1. doi:10.1186/gb-2002-3-2-reviews0003
Hunt JD, Jackson DC, Wood PR, Stewart DJ, Brown LE (1995) Immunological parameters associated with antigenic competition in a multivalent footrot vaccine. *Vaccine* **13**, 1649–1657. doi:10.1016/0264-410X(95)00145-Q

Iung LHdS, Carvalheiro R, Neves HHdR, Mulder HA (2019) Genetics and genomics of uniformity and resilience in livestock and aquaculture species: a review. *Journal of Animal Breeding and Genetics*. **137**, 263–280. doi:10.1111/jbg.12454

James PJ (2006) Genetic alternatives to mulesing and tail docking in sheep: a review. *Australian Journal of Experimental Agriculture* **46**, 1–18. doi:10.1071/EA05100

James P, Brien F, Anderson A (2019) A review of predisposing factors for breech flystrike. Australian Wool Innovation Limited. Available at https://www.wool.com/globalassets/wool/sheep/research-publications/welfare/flystrike-research-update/review-predisposing-factors-breech-flystrike-final-report.pdf [Accessed 6November 2020]

Kidd TJ, Ritchie SR, Ramsay KA, Grimwood K, Bell SC, Rainey PB (2012) *Pseudomonas aeruginosa* exhibits frequent recombination, but only a limited association between genotype and ecological setting. *PLoS One* **7**, e44199. doi:10.1371/journal.pone.0044199

Lee C, Fisher AD (2007) Welfare consequences of mulesing of sheep. *Australian Veterinary Journal* **85**, 89–93. doi:10.1111/j.1751-0813.2007.00114.x

Levot GW (1995) Resistance and the control of sheep ectoparasites. *International Journal for Parasitology* **25**, 1355–1362. doi:10.1016/0020-7519(95)00070-I

Levot GW (2012) Cyromazine resistance detected in Australian sheep blowfly. *Australian Veterinary Journal* **90**, 433–437. doi:10.1111/j.1751-0813.2012.00984.x

Levot GW (2013) Response to laboratory selection with cyromazine and susceptibility to alternative insecticides in sheep blowfly larvae from the New South Wales Monaro. *Australian Veterinary Journal* **91**, 61–64. doi:10.1111/avj.12011

Levot GW, Langfield BJ, Aiken DJ (2014) Survival advantage of cyromazine-resistant sheep blowfly larvae on dicyclanil- and cyromazine-treated Merinos. *Australian Veterinary Journal* **92**, 421–426. doi:10.1111/avj.12251

Li Y, Swan A, Purvis I (1999) Genetic variation in resistance to fleece rot in CSIRO's fine wool flock. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* **13**, 524–527.

Lloyd DH, Jenkinson DM (1981) Serum and skin surface antibody responses to intradermal vaccination of cattle with *Dermatophilus congolensis*. *British Veterinary Journal* **137**, 601–607. doi:10.1016/S0007-1935(17)31538-5

Lloyd DH, McEwan Jenkinson D, Mabon RM (1979) Location of immunoglobulins in the skin of cattle and sheep. *Research in Veterinary Science* **26**, 47–52. doi:10.1016/S0034-5288(20)30940-1

Lloyd DH, Jenkinson DM, Miller PN, Sykes TJ (1987) Sequential production of specific antibodies in serum and at the skin surface of cattle following intradermal vaccination with *Dermatophilus congolensis*. *Veterinary Immunology and Immunopathology* **16**, 251–257. doi:10.1016/0165-2427(87)90021-3

London CJ, Griffith IP (1984) Characterization of pseudomonads isolated from diseased fleece. *Applied and Environmental Microbiology* **47**, 993–997. doi:10.1128/aem.47.5.993-997.1984

Mallard BA, Wilkie BN, Kennedy BW, Quinton M (1992) Use of estimated breeding values in a selection index to breed Yorkshire pigs for high and low immune and innate resistance factors. *Animal Biotechnology* **3**, 257–280. doi:10.1080/10495399209525776

Masters DG, Ferguson MB (2019) A review of the physiological changes associated with genetic improvement in clean fleece production. *Small Ruminant Research* **170**, 62–73. doi:10.1016/j.smallrumres.2018.11.007

McGuirk BJ, Atkins KD, Kowal E, Thornberry K (1978) Breeding for resistance to fleece rot and body strike – the Trangie programme. *Wool Technology and Sheep Breeding* **26**, 17–24.

Merakou C, Schaefers MM, Priebe GP (2018) Progress toward the elusive *Pseudomonas aeruginosa* vaccine. *Surgical Infections* **19**, 757–768. doi:10.1089/sur.2018.233

Mitragotri S (2005) Immunization without needles. *Nature Reviews Immunology* **5**, 905–916. doi:10.1038/nri1728

Morrow CK, Colditz IG, Cope RB (2001) Simulated solar UVB exposure inhibits transcutaneous immunization to cholera toxin via an irradiated skin site in cattle. *Veterinary Immunology and Immunopathology* **83**, 107–114. doi:10.1016/S0165-2427(01)00377-4

Mortimer SI, Atkins KD, Raadsma HW (1998) Responses to selection for resistance and susceptibility to fleece rot and body strike in Merino sheep. In 'Proceedings of the 6th world congress on genetics applied to livestock production, Vol. 27, Armidale, NSW, Australia, 11–16 January 1998'. pp. 283–287. (Univeristy of New England: Armidale, NSW, Australia)

Netea MG, Domínguez-Andrés J, Barreiro LB, Chavakis T, Divangahi M, Fuchs E, Joosten LA, van der Meer JW, Mhlanga MM, Mulder WJ, Riksen NP, Schlitzer A, Schultze JL, Stabell Benn C, Sun JC, Xavier RJ, Latz E (2020) Defining trained immunity and its role in health and disease. *Nature Reviews Immunology* **20**, 375–388. doi:10.1038/s41577-020-0285-6

Nguyen TC (1984) The immune response in sheep: analysis of age, sex and genetic effects on the quantitative antibody response to chicken red blood cells. *Veterinary Immunology and Immunopathology* **5**, 237–245. doi:10.1016/0165-2427(84)90037-0

Norris BJ, Colditz IG, Dixon TJ (2008) Fleece rot and dermatophilosis in sheep. *Veterinary Microbiology* **128**, 217–230. doi:10.1016/j.vetmic.2007.10.024

Pardi N, Hogan MJ, Porter FW, Weissman D (2018) mRNA vaccines – a new era in vaccinology. *Nature Reviews Drug Discovery* **17**, 261–279. doi:10.1038/nrd.2017.243

Pearson LD, Brandon MR (1976) Effect of fetal thymectomy on IgG, IgM, and IgA concentrations in sheep. *American Journal of Veterinary Research* **37**, 1139–1141.

Phillips CJC (2009) A review of mulesing and other methods to control flystrike (cutaneous myiasis) in sheep. *Animal Welfare* **18**, 113–121.

Poppe M, Veerkamp RF, van Pelt ML, Mulder HA (2020) Exploration of variance, autocorrelation, and skewness of deviations from lactation curves as resilience indicators for breeding. *Journal of Dairy Science* **103**, 1667–1684. doi:10.3168/jds.2019-17290

Poulsen BE, Yang R, Clatworthy AE, White T, Osmulski SJ, Li L, Penaranda C, Lander ES, Shoresh N, Hung DT (2019) Defining the core essential genome of *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences* 116, 10072–10080. doi:10.1073/pnas.1900570116

Priebe GP, Goldberg JB (2014) Vaccines for *Pseudomonas aeruginosa*: a long and winding road. *Expert Review of Vaccines* **13**, 507–519. doi:10.1586/14760584.2014.890053

Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto J-M, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, MetaHIT Consortium, Bork P, Ehrlich SD, Wang J (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59–65. doi:10.1038/nature08821

Raadsma HW (1991*a*) Fleece rot and body strike in Merino sheep. V. Heritability of liability to body strike in weaner sheep under flywave conditions. *Australian Journal of Agricultural Research* **42**, 279–293. doi:10.1071/AR9910279

Raadsma HW (1991*b*) The susceptibility to body strike under high rainfall conditions of flocks selected for and against fleece rot. *Australian Journal of Experimental Agriculture* **31**, 757–759. doi:10.1071/EA9910757

Raadsma HW, Gilmour AR, Paxton WJ (1988) Fleece rot and body strike in Merino sheep. I. Evaluation of liability to fleece rot and body strike under experimental conditions. *Australian Journal of Agricultural Research* **39**, 917–934. doi:10.1071/AR9880917

Raadsma HW, Attard GA, Nicholas FW, Egerton JR (1996) Disease resistance in Merino sheep. V. Genetic heterogeneity in response to vaccination with *Dichelobacter nodosus* and clostridial antigens. *Journal of Animal Breeding and Genetics* **113**, 181–199. doi:10.1111/j.1439-0388.1996.tb00604.x

Raadsma HW, McEwan JC, Stear MJ, Crawford AM (1999) Genetic characterisation of protective vaccine responses in sheep using multi-valent *Dichelobacter nodosus* vaccines. *Veterinary Immunology and Immunopathology* **72**, 219–229. doi:10.1016/S0165-2427(99)00135-X

Rauw WM, Kanis E, Noordhuizen-Stassen EN, Grommers FJ (1998) Undesirable side effects of selection for high production efficiency in farm animals, a review. *Livestock Production Science* **56**, 15–33. doi:10.1016/S0301-6226(98)00147-X

Reed SG, Orr MT, Fox CB (2013) Key roles of adjuvants in modern vaccines. *Nature Medicine* **19**, 1597–1608. doi:10.1038/nm.3409

Reverter A, Hine BC, Porto-Neto L, Li Y, Duff CJ, Dominik S, Ingham AB (2021) ImmuneDEX: a strategy for the genetic improvement of immune competence in Australian Angus cattle. *Journal of Animal Science* **99**, skaa384. doi:10.1093/jas/skab007

Rossi A, Mónaco A, Guarnaschelli J, Silveira F, Iriarte A, Benecke AG, Chabalgoity JA (2018) Temporal evolution of anti-*Clostridium* antibody responses in sheep after vaccination with polyvalent clostridial vaccines. *Veterinary Immunology and Immunopathology* **202**, 46–51. doi:10.1016/j.vetimm.2018.06.010

Sales N, Suann M, Koeford K (2020) Dicyclanil resistance in the australian sheep blowfly, *Lucilia cuprina*, substantially reduces flystrike protection by dicyclanil and cyromazine based products. *International Journal for Parasitology: Drugs and Drug Resistance.* **14**, 118–125. doi:10.1016/j.ijpddr.2020.04.005

Sandeman RM, Levot GW, Heath ACG, James PJ, Greeff JC, Scott MJ, Batterham P, Bowles VM (2014) Control of the sheep blowfly in Australia and New Zealand – are we there yet? *International Journal for Parasitology* **44**, 879–891. doi:10.1016/j.ijpara.2014.08.009

Shanahan GJ, Roxburgh NA (1974) The sequential development of insecticide resistance problems in *Lucilia cuprina* Wied. in Australia. *PANS Pest Articles & News Summaries* **20**, 190–202. doi:10.1080/09670877409413633

Sharma A, Krause A, Worgall S (2011) Recent developments for *Pseudomonas* vaccines. *Human vaccines* **7**, 999–1011. doi:10.4161/hv.7.10.16369

Sutherland SS, Ellis TM, Robertson GM, Gregory AR (1987) Serum and skin surface antibody responses in Merino sheep given three successive inoculations with *Dermatophilus congolensis*. *Veterinary Microbiology* **15**, 209–218. doi:10.1016/0378-1135(87)90075-7

Tellam RL, Bowles VM (1997) Control of blowfly strike in sheep: current strategies and future prospects. *International Journal for Parasitology* **27**, 261–273. doi:10.1016/S0020-7519(96)00174-9

Tellam RL, Eisemann CH, Vuocolo T, Casu R, Jarmey J, Bowles V, Pearson R (2001) Role of oligosaccharides in the immune response of sheep vaccinated with *Lucilia cuprina* larval glycoprotein, peritrophin-95. *International Journal for Parasitology* **31**, 798–809. doi:10.1016/S0020-7519(01)00195-3

Tellam RL, Vuocolo T, Denman S, Ingham A, Wijffels G, James PJ, Colditz IG (2021) Lumpy wool in sheep: a review of pathogenesis, aetiology, resistance and vaccines. *Animal Production Science.* doi:10.1071/AN21119

Tizard IR (2021) Chapter 17 – Sheep and goat vaccines. In 'Vaccines for veterinarians'. (Ed. IR Tizard) pp. 215–224.e1. (Elsevier: St Louis, MO, USA) doi:10.1016/B978-0-323-68299-2.00026-5

Vasil ML (1986) *Pseudomonas aeruginosa*: biology, mechanisms of virulence, epidemiology. *Journal of Pediatrics* **108**, 800–805. doi:10.1016/S0022-3476(86)80748-X

Vuocolo T, Denman S, Ingham A, Wijffels G, Colditz I, Tellam R, James P (2020) Control of fleece rot and lumpy wool by vaccination: feasibility review and options. Australian Wool Innovation Limited. Available at https://www.wool.com/globalassets/wool/sheep/research-publications/welfare/flystrike-control/200702-on-00723-fleece-rot-final-report.pdf [Accessed 3 November 2020]

Wallis J, Shenton DP, Carlisle RC (2019) Novel approaches for the design, delivery and administration of vaccine technologies. *Clinical and Experimental Immunology* **196**, 189–204. doi:10.1111/cei.13287

Watson DL (1992) Biological half-life of ovine antibody in neonatal lambs and adult sheep following passive immunization. *Veterinary Immunology and Immunopathology* **30**, 221–232. doi:10.1016/0165-2427(92)90140-L

Watson DL, Gill HS (1991) Post natal ontogeny of immunological responsiveness in Merino sheep. *Research in Veterinary Science* **51**, 88–93. doi:10.1016/0034-5288(91)90037-O

Watson DL, Lea J, Burke GL (1992) A method for collecting interstitial fluid from skin of sheep. *Australian Veterinary Journal* **69**, 14–15. doi:10.1111/j.1751-0813.1992.tb09856.x

Watson DL, Colditz IG, Gill HS (1994*a*) Immunological effector mechanisms in ruminants. In 'Vaccines in agriculture: immunological applications to animal health and production'. (Eds PR Wood, P Willadsen, JE Vercoe, RM Hoskinson, D Demeryer) pp. 21–36. (CSIRO Information Services: Melbourne, Vic., Australia)

Watson DL, Colditz IG, Andrew M, Gill HS, Altmann KG (1994*b*) Age-dependent immune response in Merino sheep. *Research in Veterinary Science* **57**, 152–158. doi:10.1016/0034-5288(94)90051-5

Watts JE, Merritt GC (1981*a*) Body strike in sheep. In 'Proceedings of the University of Sydney Postgraduate Society for Veterinary Science, Vol. 58, Sydney, 10–14 August 1981'. pp. 177–191.

Watts JE, Merritt GC (1981b) Leakage of plasma proteins onto the skin surface of sheep during the development of fleece-rot and body strike. *Australian Veterinary Journal* **57**, 98–99. doi:10.1111/j.1751-0813.1981.tb00461.x

Wilkie B, Mallard B (1999) Selection for high immune response: an alternative approach to animal health maintenance? *Veterinary Immunology and Immunopathology* **72**, 231–235. doi:10.1016/S0165-2427(99)00136-1

Wilkinson FC (1979) Dermatophilosis of sheep association with dipping and effects on production. *Australian Veterinary Journal* **55**, 74–76. doi:10.1111/j.1751-0813.1979.tb15169.x

Willadsen P, Eisemann CH, Tellam RL (1993) 'Concealed' antigens: expanding the range of immunological targets. *Parasitology Today* **9**, 132–135. doi:10.1016/0169-4758(93)90177-H

Winsor GL, Griffiths EJ, Lo R, Dhillon BK, Shay JA, Brinkman FS (2016) Enhanced annotations and features for comparing thousands of *Pseudomonas* genomes in the *Pseudomonas* genome database. *Nucleic Acids Research* **44**, D646–D653. doi:10.1093/nar/gkv1227

Young AJ (2019) Adjuvants: What a Difference 15 Years Makes! *Veterinary Clinics: Food Animal Practice* **35**, 391–403. doi:10.1016/j.cvfa.2019.08.005

Zaria L (1993) Dermatophilus congolensis infection (dermatophilosis) in animals and man! An update. *Comparative Immunology, Microbiology and Infectious Diseases* **16**, 179–222. doi:10.1016/0147-9571(93)90148-X

Data availability. Data sharing is not applicable as no new data were generated or analysed during this study.

Conflicts of interest. All authors were involved in writing a related commissioned industry report that was submitted to Australian Wool Innovation but have no other conflicts of interest.

Declaration of funding. This research was funded by Australian Wool Innovation (AWI) Project ON-00723. AWI is funded by Australian woolgrowers through a wool levy and by the Australian Government which provides a matching contribution for eligible R&D activities.

Acknowledgements. We thank three anonymous reviewers for constructive comments on the paper.

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