

Reviewing Footrot in Sheep

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Abstract

Ovine footrot is the main cause of lameness in sheep around the world and is responsible for extensive economic and welfare impacts. It can be an extremely contagious disease, resulting from the invasion of the interdigital tissue by a complex mixture of bacteria, in which *Dichelobacter nodosus* is a required component. Strains of *D. nodosus* can be benign or virulent, but they are not always related with the clinical expression of footrot, complicating the diagnostic process. Several efforts have been made over recent decades to control the disease, but it remains endemic in the major sheep-raising countries of the world. The use of more efficient therapeutic procedures and better farm management practices or the development of new selective breeding tools and strategic vaccination protocols are some of the key measures that may improve footrot control in the future.

Keywords: Ovine footrot; Sheep; *Dichelobacter nodosus*

Introduction

Ovine footrot is a contagious disease affecting the feet of sheep and other ruminants and presents in two different forms. Interdigital dermatitis is the disease's mildest form and involves inflammation of the interdigital skin [1]. In contrast, severe footrot is much more aggressive, presenting as the separation of the horn from the sensitive layers of the foot, known as underrunning [2]. Footrot has been known of since the early 19th century [3]. In the mid-20th century, the virulent or progressive form and the benign or non-progressive form of footrot were identified [4]. A few decades later, Stewart *et al.* identified a more complex spectrum of virulence among different strains of *Dichelobacter nodosus*, which could be associated with different clinical expressions of the disease [5]. Nowadays, ovine footrot has been reported on the vast majority sheep-farming countries around the world [6]. The disease has a prejudicial impact on animal welfare, due to pain, discomfort and weight reduction. Consequently, it leads to productivity losses by reducing the number of lambs per ewe, reducing growth rates in lambs and adult sheep and increasing the mortality rate and fertility problems [5,7]. Beyond those losses, the costs of therapeutic, control and preventive measures can be expensive [2]. The economic impact is thus extensive, estimated at an annual cost of £24-80 M in the United Kingdom and \$18.4 M in Australia [8-10]. Most sheep farmers classify lameness as the condition of most concern in their sheep flocks and consider footrot as the main cause of ovine lameness [11,12]. It is estimated that approximately 5% of British sheep are lame at any one time [13]. That lameness is attributed to footrot in 80% of the cases [12]. Footrot has persisted for many decades in British flocks despite all the efforts made to control the disease, though the prevalence of lameness has halved over a period of 10 years, which means the results of recent research are being used to inform farmers about recommended management practices [13,14]. Even so, British farmers have been encouraged to keep footrot prevalence at a maximum of 2% [15].

Aetiology

Footrot results from the invasion of the epidermal tissue of the hooves by a complex mixture of bacteria, in which *Dichelobacter nodosus* is a necessary component [16]. This bacterium was formerly known as *Fusiformis nodosus* or *Bacteroides nodosus* and is a gram-negative, anaerobic bacterium [17,18]. Unlike other bacteria, *D. nodosus* is not usually found in sheep faeces or in soil [14]. It is possible to find the agent in normal healthy feet, but the load of bacteria present in affected feet is much higher [19]. Thus, disease progression from interdigital dermatitis to severe footrot is mainly attributed to *D. nodosus* [19].

The role of other bacteria in the pathogenesis of footrot is not fully understood, but Roberts and Egerton found that the presence of *Fusobacterium necrophorum*, a faecal organism, was also required for *D. nodosus* to start an infection in laboratory trials. Research

by Bennett *et al.* found evidence of a synergetic relationship between *F. necrophorum* and *D. nodosus*. More recently, Atia *et al.* proposed an opportunistic role for this pathogen, as high loads of *F. necrophorum* were only observed once severe footrot had developed. Thus, instead of attributing to *F. necrophorum* the role of precursor in footrot pathogenesis, it is now believed that this bacterium contributes to both the disease's duration and severity [1,19-22]. *F. necrophorum* promotes inflammation and damage of the *stratum corneum*. This bacterium also produces toxins that cause necrosis of the superficial layer of the interdigital skin, enabling the establishment of other bacteria, such as spirochetes [14]. *F. necrophorum* is an anaerobic gram-negative bacterium divided in two sub-species, *F. necrophorum* sub-species *necrophorum* and *F. necrophorum* sub-species *funduliforme*. The first one is commonly found in animals, while the second is usually present in humans [23]. However, Zhou *et al.* observed that the variant more frequently found in sheep and goats was different from the two sub-species previously reported, suggesting that much has yet to be learned about this issue [24]. Other unexpected bacteria such as *Fusobacterium equinum* and *Bacteroides ureolyticus* have been isolated in footrot lesions [25,26]. The presence of *B. ureolyticus*-like organisms in footrot lesions may present a problem for diagnosis as this bacterium is phenotypically similar to *D. nodosus* and it is possible that some older researches have mis-identified the agent present in footrot lesions [26]. It is possible to conclude that bacteria other than *D. nodosus* represent an important role in the pathogenesis of the disease, but there is much more to be studied to understand the exact mechanism [1]. Some decades ago it was believed that *Treponema spp.* was also involved in the disease pathogenicity, but one recent study concluded that there was no significant connection between footrot and *Treponema spp* [17,27].

Pathogenesis

Transmission of footrot starts with naturally footrot-infected sheep acting as a source of infection to the feet of healthy animals [17]. However, the infection of healthy interdigital skin of sheep with *D. nodosus* alone is not enough for the development of footrot. The activity of normal environmental microflora present on the interdigital skin, the presence of favorable environment temperatures and the water maceration of the *stratum corneum* of the hoof reflect some of the necessary prerequisites for disease development [14,17]. Disease transmission is enhanced by temperatures above 10 °C and with consistent rainfall over several weeks, rather than a single short-lived episode of rainfall [3]. Wet weather increases vulnerability to footrot either by inducing physical changes in the hoof, making it more susceptible, or by changing the biology of the pathogens that cause footrot [1]. Therefore, transmission of footrot is higher during winter when sheep are housed in high animal densities, particularly in clay soil types [14,28].

D. nodosus can be transmitted between sheep via soil contact and is capable of surviving outside the host for long periods [1]. Myers' investigations revealed that *D. nodosus* could tolerate up to 10 days exposed to air, though more recently Muzafar has shown that it could survive longer than 30 days, despite being an anaerobic bacterium [28,29]. The duration of infectiousness of the agent outside the hoof depends on climatic conditions and Muzafar concluded that agent survival was higher at 5 °C than at 25 °C. *D. nodosus* feeds on collagen present in living dermis, digesting it, and this represents one of the main barriers for the eradication of footrot, since the causative agent has the capability of surviving in the inter-digital skin or in cryptic lesions within the hoof for several months [1,14,28].

Risk Factors Associated with Footrot

Expression of footrot in the field is determined by three key factors: the virulence of the bacteria involved, environmental conditions and host resistance [17]. Many environmental factors, such as temperature, rain fall or soil type, can affect the disease's progression by leading to injuries to the feet of sheep, thus increasing susceptibility to infection [22]. Some management practices are directly associated with an increased likelihood of flocks developing footrot and the probability of sheep developing footrot increases with flock size [15]. Stocking animals in high densities can create an extremely contaminated environment. The implementation of preventative measures such as quarantining or isolation of diseased animals can drastically reduce environmental contamination for the rest of the flock, decreasing disease prevalence [14]. All factors that can lead to the maceration of the interdigital skin will facilitate colonization of *D. nodosus* [17]. As such, the presence of moist ground with rough pasture in the areas where sheep graze is another risk factor [15]. Some non-genetic factors can have a significant influence on susceptibility to footrot. By sex, it has been reported that ewes are more resistant to the disease than rams, while by age, it has been concluded that yearling sheep are much less likely to have footrot than lambs [15,17,30]. Some ovine breeds, like Merino, have been shown to be more susceptible to footrot [31].

Clinical Signs

The disease is characterized by acute lameness, anorexia, reduced production, reduced wool quality and, in the worst-case scenario, can result in death [1]. In the beginning of the infection it is possible to observe an inflammatory process, characterized by erythema and diffuse superficial necrosis of the interdigital skin. If the disease evolves to a more severe phase, a break at the skin-horn junction will be seen. Underrunning, which starts at the heel and the posterior region of the sole, can progress along the sole to the toe. The separation can extend to the abaxial wall of the hoof in the most severe cases [17]. The separation process will create a cavity between the sensitive tissue of the claw and the hoof horn, which will be filled with a grey pasty scum which has a foul smell, characteristic of anaerobic bacterial activity [14]. Sheep with chronic footrot infection tend to present feet with overgrown and misshapen horns and with extensive necrotizing damage through the surrounding soft tissues [17]. Over recent decades many

scoring systems have been developed to establish criteria to differentiate the levels of progression of footrot infection. The first scoring system for footrot lesions was created by Egerton and Roberts and remains one of most widely implemented classifications to this day [32]:

Score 1: limited, mild interdigital dermatitis;

Score 2: more extensive interdigital dermatitis;

Score 3: severe interdigital dermatitis and underrunning of the horn of the heel and sole;

Score 4: similar to score 3, but with the underrunning extended to the walls of the hoof.

Diagnosis

The clinical diagnosis of footrot starts by performing a careful visual inspection of the hooves. The most frequent lesions identified visually range from a mild interdigital dermatitis to the underrunning of the sole, according to the disease's stage of development. The differentiation between benign and virulent forms of footrot can be a challenging process in early stages of the disease or if adverse environmental conditions are present [33]. The most significant differential diagnosis of footrot involves ovine contagious interdigital dermatitis, which is a necrotizing infection caused by *F. necrophorum* in the absence of *D. nodosus* [34]. The differentiation of this disease from footrot may be difficult since the appearance of both is similar. Other diseases often mistaken with footrot include white line disease, contagious ovine digital dermatitis and foot/toe abscesses, although experienced technicians can easily distinguish these from footrot [2]. According to Kaler and Green, terminology to describe foot diseases is often used incorrectly, mainly by farmers who tend to identify any hoof horn lesion as footrot. Definitive diagnosis can only be achieved by the demonstration of *D. nodosus* in gram stain smears collected directly from suspect lesions, as some features displayed by these colonies allow for their differentiation from others [10,12,17]. These bacteria are observed as large gram-negative rods [0,6-0,8 µm wide and 3-10 µm in length] and can be straight or slightly curved, presenting a characteristic enlargement at the ends [10]. Microbiological culture of *D. nodosus* is an expensive and laborious process that requires specialized equipment and can be particularly difficult when the sample site is contaminated with other pathogens [35]. As the bacterium is an obligate anaerobe, it requires a specially enriched medium for its isolation, such as hoof agar, trypticase arginine serine agar or Eugon agar. Prior to culture, these media need to be kept in anaerobiosis jars [36]. However, as the culture takes approximately four weeks to yield results – and thus cannot be practically used to segregate infected animals from the rest of the flock – it is rarely used as a diagnostic tool [35]. In the recent years, the use of PCR-based methods has grown because of the reliability of results [37]. A multiplex PCR is currently used for serogrouping, as it is a simple and rapid technique that may be very useful for vaccination-based footrot control [38]. Stäuble *et al.* developed a real-time PCR that detects the presence of alleles *aprV2/aprB2* directly from clinical samples, constituting a rapid and sensitive diagnostic technique to differentiate between benign and virulent footrot [39]. This technique is capable of detecting high loads of virulent strains of *D. nodosus* in clinically healthy sheep. This evidence indicates that the lesion scoring system does not always correlate with bacterial loads and the virulence of *D. nodosus* strains. Nevertheless, McPherson *et al.* have identified low rates of agreement between clinical diagnosis and PCR test results [33]. Swabs are used to collect samples directly from the interdigital skin, which can be used both for culturing and PCR analysis [27]. This technique is currently considered a better diagnostic approach than biopsy punching [39].

Classifications of Footrot

Strains of *D. nodosus* differ in virulence and in their ability to induce clinical disease, being classified as benign, intermediate or virulent strains. For descriptive purposes, these three terms are also used to describe the different clinical forms of footrot [5,10]. While benign footrot refers to the mildest state of the disease, with the lesions of dermatitis almost exclusively limited to the interdigital skin, virulent footrot is related with a much more severe disease, which frequently progresses to the separation of the soft and hard horn from the underlying hoof matrix, a phenomenon known as underrunning. The expression intermediate footrot refers to all the forms located between benign and virulent footrot [1]. Virulent strains of *D. nodosus* commonly lead to a clinical expression of virulent footrot, but it must be recognized that clinical expression of the disease is also dependent on environmental conditions and host factors [16]. Likewise, if favorable climatic conditions are present, benign strains of *D. nodosus* induce severe footrot lesions in a limited proportion of susceptible animals [33]. It is important to clearly determine which form of footrot is present in each flock, since only flocks with virulent footrot may benefit from a control or eradication program. Time and resources should not be expended on cases of intermediate and benign footrot, as they will regress without treatment if environmental conditions become less propitious for the development of footrot. Moreover, the mildest forms of footrot usually have limited economic impacts [17]. The most precise method for identifying the form of footrot requires examination of a representative number of animals from the whole flock to determine the proportion of sheep presenting score 4 lesions, rather than the presence of footrot lesions of any grade [33]. If there is any doubt as to the effect of the environment or about previous treatments, it is recommended to repeat the inspection after a minimum period of two weeks. When virulent footrot is present, it is expected that more than 10% of the animals will have severe lesions. The disease develops very fast and there is little evidence of self-curing. The presence of clinical signs, such as lameness, are associated with huge production losses. In the other hand, in case of benign footrot, less than 1% of the animals will have severe lesions, mostly confined to the interdigital skin [scores 1 or 2]. Most such lesions resolve spontaneously with improved environmental conditions [17].

In contrast to Australian researches, in the UK *D. nodosus* is not typically classified according to virulence but to the presence of key signs of clinical diagnosis, such as lameness or the severity of lesions [14].

Classifications of *D. nodosus*

There are several different criteria by which the causal agent of footrot can be classified and the presence of specific virulence factors is one relevant factor used to categorize different strains of *D. nodosus*. Some of the more important virulence factors include its proteases, fimbriae and outer-membrane proteins [1,40]. The fimbriae of *D. nodosus* play an important role in virulence expression since they are required for binding to host epithelial cells, it being generally accepted that virulence is directly proportional to the number of fimbriae present [29,41]. The outer membrane proteins of *D. nodosus* interfere with the host's immune response, rather than attacking the host [29]. The secretion of extra-cellular proteases by *D. nodosus* plays a vital role in the biology of the agent due to its inability to create any amino acid. Thus, the bacterium obtains amino acids by importing them from digested proteins, due to the proteolytic ability of extra-cellular proteases [29]. The thermostability of these proteases is also deeply related to the expected virulence of the microorganisms [16]. Virulent strains of *D. nodosus* produce heat stable enzymes with caseinolytic activity, while benign strains produce heat labile enzymes [17]. The gelatine gel test is used to identify heat stable proteases, but correlation between the results of this test and the clinical expression of disease can be unreliable [6,14]. Alternatively, the elastase test measures the temporal and quantitative activity of extracellular proteases, and is probably a better option for distinguishing virulent and benign strains of *D. nodosus*, since it has a good correlation with clinical diagnosis [6,33]. In the past, some segments of the *D. nodosus* genome, such as *intA* or *vrl*, were thought to be significant virulence factors, while the genetic segment *intD* was strongly associated with a benign phenotype of *D. nodosus* [42,43]. However, more recent research has not been able to confirm the hypothesis of the *intA* gene as a virulence factor [44]. More recently, other genetic segments have been considered important virulence factors, such as *aprV2*, which encodes the acidific protease 2 (*aprV2*), a thermostable protease. Meanwhile, the orthologous *aprB2* gene, responsible for encoding a thermolabile protease, is present in the genome of *D. nodosus* benign strains [45]. The genes *aprV2* and *aprB2* have a single nucleotidic difference (TA/CG) at position 661/662 [46]. It was believed that this difference was responsible for the definition of the elastase activity, but McPherson *et al.* have identified many strains of *D. nodosus* containing the gene *aprV2* in clinically healthy herds, proving the *aprV2* is no longer a reliable virulence marker [33,45]. Another classification of *D. nodosus* refers to serogroup, which is related to fimbrial antigenicity and which is important in the development of specific vaccines [47]. The serogroup classification is denoted by letters and there are 10 different known serogroups (A-I and M) [48]. These serogroups can further be subdivided into 21 serotypes (A1, A2, B1, B2, B3, B4, B5, B6, C1, C2, D, E1, E2, F1, F2, G1, G2, H1, H2, I and M) according to cross-absorption tests [49]. Each region and country has a specific profile of isolation frequencies for the serogroups. Serogroup B is undoubtedly the most frequent serogroup in all the countries. Other frequent *D. nodosus* serogroups are A (Australia), D (Australia, New Zealand and the United Kingdom), G (Australia) and H (Australia and the United Kingdom) [50]. The median number of *D. nodosus* serogroups found in affected hooves is one, although cases of up to seven different *D. nodosus* strains on a single hoof have been reported [51,52].

Treatment

Different treatment approaches and management methods are used worldwide according to the specific production system in question. The choice of management method depends on the size of the flock, the stocking rate, the availability of medication and other resources and the acceptance of different management and treatment policies within each market [1]. Furthermore, some farmers choose not to treat affected animals owing to a belief that treatment is expensive and may not be ultimately profitable, though Wassink *et al.* have concluded that economic losses will occur if animals are left untreated for seven days or more [7,9]. Winter and Green have produced a cost-benefit analysis of different approaches of controlling footrot in 116 sheep flocks and concluded that farms with higher prevalence of lameness had a much higher overall cost per animal per year than farms with lower prevalence rates (£6.53 versus £3.90) [9]. Some of the methods used routinely some years ago to control footrot, such as foot-trimming, are currently associated with higher levels of prevalence and incidence, even when done properly [53,54]. Foot-trimming has been used for decades and its goal was to remove diseased tissue and to promote a good hoof conformation [1]. This procedure reduces bacterial load, diminishing environment contamination, and exposes deeper tissue to oxygen, which is toxic to anaerobic bacteria. However, the use of foot-trimming needs to be done carefully, as the overuse of foot-trimming may damage the sensitive tissues and induce foot bleeding, leading to lameness [13,55]. This situation gives infectious agents an opportunity to penetrate the hooves [56]. Green *et al.* reported that the prevalence of footrot increased after routine foot-trimming sessions, which suggests that excessive use of trimming may represent a risk factor for disease transmission [53]. In addition, as foot-trimming is a time-consuming practice that requires much physical effort, its cessation could potentially save farmers significant amounts of time and money [9]. The use of foot-bathing can have a beneficial effect in lameness prevention in the initial stages of footrot, but the recommended procedures cannot be easily implemented in the vast majority of commercial farms as each sheep should remain inside the footbath for 10 minutes once a week [13,57]. Moreover, following treatment, all animals need to be moved to an area that has been free of other animals for a minimum period of two weeks [56]. Another disadvantage of footbathing is that the vast majority of chemical solutions used are toxic both to the environment and to the people applying them, with such solutions also often contain copper salts, zinc sulphate and formalin, which can be painful for animals, though it is believed there are possible alternatives to these chemical solutions [58]. Winter *et al.* performed a study based on 1260 postal questionnaires received

from British farmers and concluded that the use of footbathing in sheep presenting interdigital dermatitis has a beneficial effect preventing disease progression, as disinfectants can inactivate surface pathogens [13,17]. However, footbathing was not found to be effective against cases of severe footrot, as footbath solutions cannot penetrate deep into affected tissues. According to Winter and Green, it is extremely important to persuade both farmers and veterinarians to discontinue outdated procedures that are inefficient, physically difficult and time-consuming in favor of currently recommended methods [9]. Even though there are many farms that have altered their routine procedures, a significant number of farmers continue to regularly practice hoof-paring on their sheep, against the advice of recent research [59]. Farmers that follow the most up-to-date recommendations for reducing the prevalence of lameness in sheep have significantly lower expenses per animal than those who do not use such management methods [9]. These recommendations discourage whole-flock interventions, which are expensive and considered ineffective in reducing lameness, and focus on the early treatment of affected animals with antibiotics, which is associated with lower costs and higher treatment effectiveness in footrot [7,13]. Winter *et al.* investigated the cost-benefit of different strategies to treat ovine footrot in 116 English flocks and concluded that prompt treatment of lame ewes with parenteral antibiotics is the most efficient and cost-effective strategy [9]. However, straight extrapolations for different farming scenarios are inadvisable, as treatment effectiveness depends on flock size, footrot prevalence and the pathogenicity of *D. nodosus* strains involved. There is little evidence for antibacterial resistance in *D. nodosus*, with the microorganism showing in-vitro sensitivity to different antibiotic classes such as tetracyclines, macrolides, penicillin, cephalosporins and fluoroquinolones [54,60,61]. Oxytetracycline has been used effectively in the treatment of footrot for decades [62]. Even so, other antibiotic options have been discovered more recently. Strobel *et al.* compared the efficacy of the use of gamythromycin with oxytetracycline in the treatment of sheep presenting footrot lesions [60]. The difference between treatments was significant, with sheep injected with a single dose of gamythromycin revealing a better clinical cure rate. The use of systemic antibiotics targets anaerobic bacteria located deep within the feet, reducing inflammation. The effect of medication is rapid and lame sheep can become sound within a period of 3 to 4 days, though poor blood supply to the hoof can reduce the effectiveness of systemic therapy. Thus, the use of topical antibacterial sprays may be a useful option. Local antibiotics have another advantage as they inactivate surface *D. nodosus*, promptly reducing environmental contamination [14]. Kaler *et al.* concluded that replacing foot-paring with the use of parental antibiotics would accelerate the recovery of over a million British footrot affected lame sheep per year, with subsequent gains in production [55]. However, the extensive use of parenteral antibiotics has some limitations, since the maximum effect of antibiotics occurs when sheep are held in dry conditions for 24 hours after the injection, which may not be possible for most sheep flocks [63]. Another disadvantage is the inability to sell sheep for human consumption until after the withdrawal period, which can last several weeks for some antibiotics [64]. The overuse of antibiotics also leads to the development of drug resistance and the European commission has made limiting these medications in both human and veterinary medicine a priority [65]. Additionally, growing numbers of organic herds, in which the use of antibiotics is prohibited, has led to demand for new environmentally friendly therapies to be found [58].

Szponder *et al.* performed a study on sheep suffering from footrot and implemented an alternative therapy consisting of ozone therapy and the application of autologous platelet-rich plasma [58]. Ozone is a strong antioxidant which promotes oxidative stress and restricts some inflammatory cell factors and is successfully used to heal many kinds of wounds [66,67]. Platelet-rich plasma has been extensively used to promote healing in lesions since it locally introduces increased concentrations of growth factors and other bioactive molecules in injured tissues [68]. The use of this therapy was successful in the totality of the animals and no side-effects were observed in treated sheep. As such, the local application of ozone and platelet-rich plasma may be considered an effective treatment for footrot as a replacement for the conventional use of antibiotics and disinfectants demands to be replaced. However, this protocol is expensive and time-consuming, which probably precludes its large-scale use [58].

Control

Control programs aim to minimize disease's adverse effects in cases where complete elimination of the pathogen is not possible [14]. The control of footrot focuses on some major goals such as limiting the spread of the disease, decreasing the severity of clinical signs in affected animals and improving resistance to disease so that sheep can better withstand environmental challenges [14]. The control involves a combination of strategies such as different treatment methods, quarantine, and vaccination, culling chronically infected sheep or selective breeding for improved genetic resistance [69]. It is expected that such measures will lead to a decrease in prevalence inside the flock and reduce the severity of clinical signs in the animals that remain infected. However, it is important to note that none of the procedures used to control the disease represent a lasting approach to disease management [17]. The development of a successful control program needs to take into consideration various factors, such as the strains of *D. nodosus* present in each region and the exact seasonality of the disease in the area where the herd is located [27]. Environmental specificities can lead to adjustments in the periods in which control measures should be implemented [17]. The non-transmission period is the most effective period to implement most control strategies, however a vaccination protocol can be successfully implemented at any time, irrespective of the flock's disease status [10,70]. Factors that increase susceptibility to footrot should be avoided. Animals should not be exposed to wet conditions or to abrasive pasture, since these conditions may damage foot integrity [14]. Footbathing is an effective strategy to limit the spread of footrot within a flock, as the disinfectant can kill bacteria present on the foot surface and reducing the environmental contamination [54]. The use of parenteral antibiotics is one of the most effective procedures to control footrot, though the costs associated with this treatment are not always justified in mild cases [17,60]. Other effective

mandatory control measures are the non-acquisition of sheep from flocks of unknown footrot status and preventing animals from grazing in the same areas as neighboring flocks [17].

Eradication

Eradication programs aim to permanently eliminate all cases of footrot in a limited region. Once eradication has been achieved, it is expected that no further cases of footrot will occur, unless it is reintroduced from another source [17]. Eradication is obviously a challenging goal, but its effects are permanent and the advantages long-lasting. As the annual cost of eradicating footrot from a flock can be over \$ 10 per animal, several important factors should be taken into consideration in order to minimize financial waste before undertaking an eradication program [33]. It is important to understand the various transmission patterns occurring over the season as these determine the best period in which to implement specific preventive measures. It is also imperative that the owner of the flock is conscious that this method is costly and time-consuming. All clinical cases of footrot should be detected as soon as possible, so that operators can recognize them in the early stages of development [17]. There are several methods of eradication. The most simple and effective is whole flock disposal, though owners are often reluctant to dispose of the entire flock [70]. *Disposal of affected animals* is another effective option, but can only be applied when prevalence is low. *Identification and treatment of affected animals* is possible, but has a much lower probability of success [17]. Mills *et al.* developed a survey of 196 flocks that eradicated virulent footrot using different methods and concluded that the *whole flock disposal* was clearly the most efficient strategy [70]. Cattle are a reservoir of benign footrot strains of *D. nodosus* for sheep, but there is no evidence that virulent strains of *D. nodosus* can infect feet of cattle [17]. Goats, on the other hand, can be reservoirs of virulent footrot for sheep [71]. This indicates the improbability of eradicating footrot on those farms where sheep graze in the same area as other ruminants. All flocks to have successfully undergone an eradication program should be subjected to a regular surveillance program in following years in order to maintain footrot-free status [17]. It is important to note that an eradication programs generate flocks with animals that are highly susceptible to footrot infection if the disease is reintroduced in the herd, thus new animals should be acquired exclusively in farms free from footrot and animal contact between neighboring flocks should be avoided [14].

Vaccination

The first vaccine against footrot in sheep was developed in 1969, but only in 1974 it was possible to recognize that whole cell bacterins vaccines were not capable of protecting sheep against heterologous serogroups [72,73]. Vaccines need to induce antibody titres against fimbrial antigens from each specific serogroup of *D. nodosus*, since there is no cross-protection between them [17]. This is explained by the phenomenon of antigenic competition, in which a weak antibody production occurs against individual components if a multivalent (containing three or more antigens) vaccine is used [74]. In that way, an efficient vaccine should lead to efficient antibodies production, maintaining high antibody titres for the longest period possible [10]. Monovalent vaccines can be quite useful if used strategically in farms or regions where only one serogroup of *D. nodosus* is present [1]. The implementation of that approach was responsible for the eradication of footrot in Nepal and Bhutan [75,76]. Bivalent vaccines can also be effective, without any reduction on humoral immune response. However, multivalent vaccines are not efficient because they produce a weak and short-term antibody response [68]. While multivalent vaccines are able to protect animals for up to 10 weeks, mono or bivalent vaccines can induce an extended immunity of 16 weeks or longer [10]. Nevertheless, consecutive rounds of bivalent vaccinations, with an inter-vaccination interval of 90 days, can be successfully implemented in flocks affected by several serogroups of *D. nodosus* [77]. Vaccination against *D. nodosus* has been shown to be effective not only to prevent footrot, but to treat sheep already presenting footrot lesions [78]. The therapeutic effect can range from reduction in lameness to full healing of lesions [79]. Winter *et al.* reported an average 20% reduction in prevalence after the commencement of a vaccination program [13]. Currently, the antigenic variation is mainly attributed to fimbriae, so it is expected that specific (mono or bivalent) fimbrial vaccines will remain the best option until an antigen covering all serogroups is found [10]. Thus, future research should be focused in sequencing the *D. nodosus* genome, as it could be the key factor in developing a universal cross-protective vaccine [28].

Genetic Improvement

Some British ovine breeds such as Romney have proven to be more resistant to the development of severe footrot than the Merino breed [31]. This resistance is expressed by a quick resolution of benign lesions limited to the interdigital skin. In addition, these breeds responded more favorably to topical, parenteral and immunological therapy [10]. The natural resistance of sheep exposed to the same environmental conditions varies considerably, ranging from no clinical signs of infection to severe cases of footrot. The variation of disease resistance is expected to have an important genetic basis [80]. The estimated value of heritability of resistance to footrot could be as high as 0.31, suggesting that the use of crossbreeding with resistant animals may be very useful in producing more resilient flocks [1,80]. This will only be possible after comparing data of genetic markers with phenotypic assessment [81]. Nieuwhof *et al.* estimates that the benefits (prevalence reduction) of selecting for footrot resistance can exceed the results predicted by the existing genetic models [82]. It would be remarkable if molecular techniques could be used to identify resistant animals, which would help breeders select for footrot resistance [83]. Thanks to such techniques, it was possible to identify an association between resistance to footrot and MHC class II markers, probably because the ovine MHC class II plays an important role in modulating the response of sheep to footrot challenge [80]. This link is the basis of a commercial gene test developed in New

Zealand, which is used to select more resistant animals without the need to expose them to footrot infection [81]. However, it is expected that specific genetic tests need to be developed for each particular breed and population [1]. Mucha *et al.* attempted to identify molecular predictors of footrot resistance in Texel sheep using the genome-wide screening approach [83]. This study did not identify any potential candidate genes for footrot susceptibility, suggesting that the genetic background of footrot has a polygenic determinant. However, that study was only the first step on searching any genomic regions involved in resistance to footrot, and further researches should be employed.

Conclusion

Ovine footrot is associated with a large economic and welfare impact, remaining an important problem nowadays, despite being intensively researched for a long period. Eradication programs have been successfully implemented in limited regions, but it hasn't been possible to apply them on large-scale due to disease complexity. The diversity of *D. nodosus* strains and the environmental particularities of each region may require different control strategies. Therefore there are several areas in which researchers may gather important information that can be useful to control the disease in the future. The knowledge of the aetiology of the disease and the development of effective management practices may be key areas to control footrot. Furthermore, the production of more resilient stock can be achieved, in a short term, by implementing strategic vaccination protocols, but the protective effect will not last long if that protocols are interrupted. However, the use of genetic selection programs will be the key to produce flocks with increased resilience and resistance. In Alentejo region, Portugal, it has been developed a research in white and black Merino sheep whose goal is to identify genetic markers involved in resistance to footrot in those breeds.

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