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Multiple resistance to macrocyclic lactones in the sheep scab mite *Psoroptes ovis*

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ABSTRACT

The astigmatid mite *Psoroptes ovis* (Acari: Proroptidae) causes the highly contagious and debilitating ovine disease, sheep scab. This ectoparasitic infection has a high economic and animal welfare impact on British sheep farming. Following recent work demonstrating resistance of *Psoroptes* mites to moxidectin, a widely used macrocyclic lactone (ML) treatment for scab, the current study compared the toxicity of three of the commonly administered macrocyclic lactone therapeutic treatments (moxidectin, ivermectin and doramectin) to *P. ovis* from outbreak populations that had appeared unresponsive to treatment. These outbreak populations were from Wales and south west England. The data presented demonstrate that there is resistance to all three available ML compounds in populations of *Psoroptes* mites. However, considerable variation in response suggested that resistance alone was not responsible for the reported lack of efficacy in all of the submitted cases; lack of response in others may be associated with inappropriate treatment application or management. These data highlight the importance of the appropriate use of these compounds to manage national scab incidence at levels that are consistent with acceptable animal welfare standards, while attempting to reduce the development and spread of resistance.

Keywords:

Acariasis; Disease; Mange; Parasite.

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1. Introduction

The non-burrowing, astigmatid mite, *Psoroptes ovis* (Hering), is an obligate ectoparasite (Sanders et al., 2000) and is the causal agent of ovine psoroptic mange, known as sheep scab (van den Broek & Huntley, 2003). Scab is one of the most important ectoparasite infections of sheep, particularly prevalent in the UK and Ireland, due to its impacts on the health and welfare of livestock and the resultant economic costs of infection on animal production (Nieuwhof and Bishop, 2005; Nixon et al., 2017). The pathogenesis of *P.ovis* infection is the result of a hypersensitivity response by the mammalian host (van den Broek et al., 2003). When the mites establish themselves on the host, guanine-rich faecal matter and antigenic salivary material is deposited on the skin, causing an immediate immune reaction. Cutaneous inflammation leads to progressive dermatitis, pruritus and self-trauma, leading to rapid weight loss (Kirkwood and Quick, 1980). Death may result from secondary infection, pneumonia and severe dehydration (Tarry 1974; Bygrave et al., 1993; Bates, 1997). The disease is highly contagious with transmission resulting from direct contact between animals, or indirectly from fomites, farm equipment or handling facilities. Due to the high rate of population growth, infection with small numbers of ovigerous female *P. ovis*, can lead to a full clinical infestation in 6 to 8 weeks (Babcock and Black, 1933; Berriatua, 2001), although progression of the clinical disease varies depending on whether it is a first or subsequent infection (Bates, 2000).

Changes in national control policy in the UK, particularly the deregulation of national dipping in 1992, led to an almost exponential increase in disease incidence (French et al., 2001), with the number of outbreaks increasing from less than 100 per year to an estimated 7,000 outbreaks per year in 2003/04 (Bisdorff et al., 2006). Scab appears to be highly localised in areas of north and western England, Wales and Scotland, with some farms in these areas reporting persistent outbreaks over several years. A questionnaire survey of 399 sheep farmers in Great Britain (Rose et al., 2009) indicated that previous exposure to sheep scab significantly affected the risk of reporting a future scab outbreak; 85% of the farms that reported at least one scab outbreak in the year of the questionnaire study had experienced at least one previous outbreak, while 27% had experienced outbreaks in more than five of the previous ten years. In contrast, 76% of farms that did not report scab had experienced no previous outbreaks. Poor fencing, the use of common grazing, neighbours with sheep, infection through markets and failure to quarantine bought in sheep have all been identified as important risk factors for scab; upland and mountain sheep remain the primary focus of infection (Rose et al., 2009). In addition to the problems associated with current and historic approaches to scab management, many aspects of the epidemiology of the disease, such as the lengthy pre-patent period, existence in cryptic sites

in a non-pathogenic form, the duration of off-host survival and strain-specific variation in pathogenicity, all contribute to making it difficult to diagnose pre-patent infection and prevent transmission, for example through markets. Given the presence of persistent foci of scab on some farms and clearly identified risk factors, it has been argued that targeted scab management programmes directed at these foci would be the most cost-effective approach to scab control (Rose et al., 2009).

Currently, only two classes of acaricidal compound are licensed for treatment of *P. ovis*: diazinon organophosphate (OP) dip and the injectable endectocide macrocyclic lactones (MLs) (Bisdorff and Wall, 2008). MLs available for scab management and prophylaxis include the avermectins, ivermectin and doramectin, and the milbemycin, moxidectin. Recently, however, the presence of resistance to moxidectin was demonstrated in outbreaks of *P. ovis* from flocks in Wales and the Welsh borders that had consistently failed to respond to ML treatment (Doherty et al., 2018). The latter study suggested that, given the pharmacological similarities between the different MLs, it was likely that the resistant populations would show cross-resistance to other compounds in this class of parasiticide. Identifying whether this is the case is important in terms of being able to offer accurate advice to farmers about appropriate product selection. The aim of this study therefore was to examine the toxicity of all three commonly used MLs against *P. ovis* from populations collected from outbreaks which appeared to be unresponsive to treatment.

2. Materials and methods

Samples from a ML naïve population of *P. ovis* mites were obtained from the Moredun Research Institute (MRI), Edinburgh. These mites had been in continuous culture on untreated sheep, with no exposure to acaricide for at least ten years. Samples from farm scab outbreaks that were reported not to have responded to standard ML acaricidal treatment were collected by farmers or veterinary surgeons from sites across the UK and sent to the University of Bristol by post. Outbreak samples were obtained through a network of veterinary and pharmaceutical company contacts. Field samples were collected by skin scraping and were used in bioassays within 24/48 h of collection. In total, samples were supplied from 12 apparently non-responding outbreak populations. All the outbreak populations for which large numbers of mites could be obtained originated from Wales or south west England.

In the laboratory, samples of infected skin and wool were inspected using a stereomicroscope (Leica S6E) and mites were picked out of wool and skin-debris using a paintbrush. Prior to use, mites were tested for a locomotory response; only mites capable of a

full limb contraction within 5 seconds of stimulation using three paintbrush strokes across legs I and II and the gnathosoma were used. This ensured all mites used were fully mobile before treatment exposure.

The bioassay used to test for resistance was developed from the methodology of Brimer et al. (1995) and modified by Doherty et al. (2018). Sterile Petri-dishes (90 mm diameter) were partially filled with 18 ml of potato dextrose agar (agar 15g/L; dextrose 20g/L, potato extract 4g/L, Sigma-Aldrich Ltd). As it solidified, a surface layer of 1ml of 5% sterile-filtered sheep serum (Sigma-Aldrich Ltd) and 1ml of treatment solution was added. Negative control plates were made in an identical manner for each trial with 1ml absolute ethanol in place of the ML. The three ML treatments used in the bioassays were ivermectin (Oramec Sheep Drench, 0.08% w/v), moxidectin (Cydectin 20mg/ml Long Acting, Zoetis Animal Health) and doramectin (Dectomax 10mg/ml, Elanco Animal Health). These were diluted in 100% ethanol to achieve concentrations of 2000ng/ml or 4000ng/ml.

Up to 10 mites were placed in the centre of each Petri-dish. After the mites had been added, the inner rim of the Petri-dishes and lids were smeared with petroleum jelly and sealed with parafilm ensure that the mites were contained. The Petri-dishes were incubated at 20°C and 80% R.H. Replicates for each ML compound plus an ethanol control were set up; priority was given first to bioassay plates at 4,000 ng/ml since this was considered to be a discriminating dose, followed by plates at 2,000 ng/ml, where sufficient numbers of mites were available. The number of mites alive were recorded in all Petri-dishes at 24 h intervals after initial setup for 72 h. A mite was categorised as alive based on tactile stimulation and locomotory response, as described previously.

The percentage mortality in each plate and the median percentage mortality for each treatment were calculated at 72 h after Petri-dish set-up, for both the outbreak and the control populations. Mann-Whitney U-tests were used to compare the percentage mortality between treatments as the data were not normally distributed (Shapiro-Wilk W test, $P = 0.008$).

3. Results

When exposed to ethanol-treated Petri-dish plates for 72 h, the MRI-derived mites showed mortalities that varied between 10 and 40%, with a median mortality of 32% (Fig. 1). When exposed to MLs at either 2000 ng/ml or 4000ng/ml, mortalities of 80% and above were observed consistently and the median mortality, including all samples, was 100% (Fig. 1), demonstrating the high susceptibility of naive mites to all MLs at these concentrations in the bioassay conditions.

In total, twelve field outbreak samples were collected, but only six contained sufficiently large numbers of mites to allow at least one test assay plus a control; from these six populations sufficient numbers of mites were obtained for only 17 ML assays plus their respective ethanol controls. When exposed to ethanol only, mites from outbreak populations showed a low percentage mortality, with a median of 23%, which was not significantly different to that seen in the MRI populations (Mann-Whitney U test, $W = 26.0$ $P = 0.42$; Fig. 2). However, when exposed to MLs, wide ranging variation in mortality was seen, with an almost bimodal distribution, (Fig. 2): at 72h, 8 of the 17 ML assays gave mortality that was above 80% (median=100%) and were as high as seen with the MRI mites, while the other 9 had a mortality of between 0 and 66% (median=40%). The latter group had mortality that was significantly lower than that seen in the MRI mites exposed to the same treatment (Mann-Whitney $W = 153.0$, $n = 26$, $P < 0.001$).

4. Discussion

Previous assessment of resistance in *P. ovis*, examined only the ML moxidectin, and identified resistance in four non-responding populations derived from Wales and the Welsh border (Doherty et al., 2018). The pattern of mite mortality seen in the present study suggests that there is resistance to all 3 common ML treatments in some of the outbreak populations; consistently high levels of mortality were seen in the naïve populations obtained from MRI to the drug concentrations tested. However, lack of susceptibility to MLs was not seen universally in all the outbreak assays using mites from populations that were described as non-responding and, in some cases, reported lack of response to treatment in the outbreaks was likely to have been due to improper application of the acaricide or management of sheep after treatment, complicating the bioassay analysis. The latter is an important finding of this study, because inappropriate treatment or inadequate application may result in sub-optimal residues and further hasten selection for resistance.

It is notable that a median of 32% mortality was recorded in the ethanol-only treatment controls which, in an acaricide treatment trial, would be considered too high for the result to be meaningful (Abbot, 1925). However, such a conclusion relating to control mortality does not apply in this context because in a laboratory bioassay with *Psoroptes*, because following removal from the host mites start to die, and even in the negative control most mites only survive for 5 days or so. Hence, negative control mortality will always be high, and it is the differential rate of mortality in control and treatment groups that need to be compared with care. Hence, mortality counts were carried out 72 h after the set-up of each bioassay. To allow clear discrimination

between resistant and susceptible mites, relatively high concentrations of active ingredient were used here to provide a significant level of challenge. However, concentrations in the agar bioassay cannot be equated meaningfully to plasma concentrations. It should also be noted that the ivermectin used here was a drench formulation rather than the injectable formulation that would be routinely used in scab control. Since the active ingredient, ivermectin, is the same, regardless of formulation, and given that it was used in a bioassay not *in vivo*, it was considered that the formulation would make no difference. That this expectation was valid was demonstrated by the high mortality in the controls when exposed to this formulation of ivermectin.

Where the large-scale administration of any compound is used for parasite control, particularly where there is limited variation or rotation in the treatments available, selection for resistance would be expected to be almost inevitable (Bates, 1998). The rapid life-cycle and high rate of reproduction of *P. ovis*, combined with the limited range of available acaricidal products, would be expected to facilitate the development of resistance under strong acaricidal selection pressure. The first report of pyrethroid-resistant scab was in 1995 (Synge et al., 1995; Coles 1998). Resistance may have been facilitated by asymptomatic scab cases being exposed to low dose concentrations when pyrethroids were used to treat other ectoparasites. It was predicted that resistance to avermectins and milbemycins would develop due to the incomplete treatment and low-level exposure in subclinical scab populations (Coles, 1998). Avermectins and milbemycins are glutamate-gated chloride channel allosteric modulators and their similar mode of action would be expected to confer cross-resistance across the class (Wolstenholme, 2012). As the MLs are relied upon heavily for the control of gastrointestinal nematodes, it is of particular concern that the dependence on MLs to manage scab is also likely to hasten selection for resistance in nematode parasites, and *visé versa*.

A key problem, inherent in these analyses, was obtaining sufficient mites from an infected sheep to allow replicated bioassays to more than one drug plus control. Here only six outbreak populations yielded large enough numbers of *P. ovis* to allow tests against at least one MLs plus control at the same time, and no samples were large enough to allow replicated tests against all three MLs. As a result, the current work has been unable to definitively demonstrate cross-resistance to all three drugs in mites from the same population which, at this stage, can only be inferred. A more comprehensive, replicated assay where all MLs are tested simultaneously on the same population would be desirable, but this would require much larger mite sample sizes than can routinely be obtained at clinical inspection.

The development of resistance to insecticides and acaricides is influenced by a combination of factors, from the genetic mechanisms underlying its action, through to the frequency, dose and method of acaricide application. Studies have suggested that most resistant alleles are recessive and disadvantageous in the absence of endectocide; lower reproductive output in individuals with resistant alleles have been demonstrated *in vitro* (Georghiou and Taylor 1977). To more efficiently manage and prevent selection for resistance, rotations of acaricides/insecticides with different modes of action are recommended. In practice however, the lack of alternatives to MLs makes this difficult. The ideal long-term approach to scab management on a farm, of course, would be to eliminate all mites by appropriate treatment of all sheep, for example with OP, and then maintaining scab free stock by applying appropriate biosecurity and quarantine measures. However, if ML resistance spreads, greater reliance on OP dip alone would inevitably impose selection pressure for resistance to this compound.

To date, most of the resistant *P. ovis* populations that have been detected were located in Wales and English counties adjacent to the Welsh border, as was the case here. However, this was based solely on reports of non-responding populations received by the investigators; future comprehensive investigations in other parts of the UK may well reveal other non-responding populations where resistance may be present. Nevertheless, scab outbreaks have been shown to demonstrate local space-time clustering, with higher incidences within a 5-month time span in farms in close proximity to each other, highlighting the importance of contact between infected flocks in scab transmission (French et al., 1999). This is supported by data showing that scab risk is highest on farms that use common grazing (Rose et al., 2012; Chivers et al. 2018). It might be expected that the spread of resistant mite populations would therefore be relatively slow between contiguous farms. However, long distance sheep movements particularly in relation to markets, are also likely to be important in increasing the rate of dispersal of resistant populations, highlighting the importance of quarantine for bought in stock. It may be possible to slow the spread of resistant genes through appropriate heightened biosecurity, although this will be difficult; genetic markers for resistance would be useful to help identify resistant populations quickly and effectively.

In summary, the study demonstrates that there is resistance to all three available ML compounds in populations of *Psoroptes* mites. However, considerable variation in response suggests that resistance alone was not responsible for the reported lack of efficacy in all of the submitted cases; lack of response in others may be associated with inappropriate treatment application or management. These data highlight the importance of the appropriate use of these compounds to manage national scab incidence at levels that are consistent with acceptable

animal welfare standards, while attempting to reduce the development and spread of resistance. To counteract the spread of resistance, co-operation between farmers including co-ordination of treatment, and novel approaches to diagnosis and treatment are required (Scott et al., 2007). This is likely to require the development and implementation of integrated regional approaches to scab management.

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Figure Legends

Fig. 1. The percentage mortality of *Psoroptes ovis* obtained from the Moredun Research Institute and exposed for 72 h to agar and sheep serum plates, treated with ethanol (squares) or moxidectin, ivermectin or doramectin at 2000 ng/ml (solid circles) or 4000ng/ml (open circles).

Fig. 2. The percentage mortality of *Psoroptes ovis* obtained from non-responding outbreak populations and exposed for 72 h to agar and sheep serum plates, treated with ethanol (squares) or moxidectin, ivermectin or doramectin at 2000 ng/ml (solid circles) or 4000ng/ml (open circles).

Figure 1

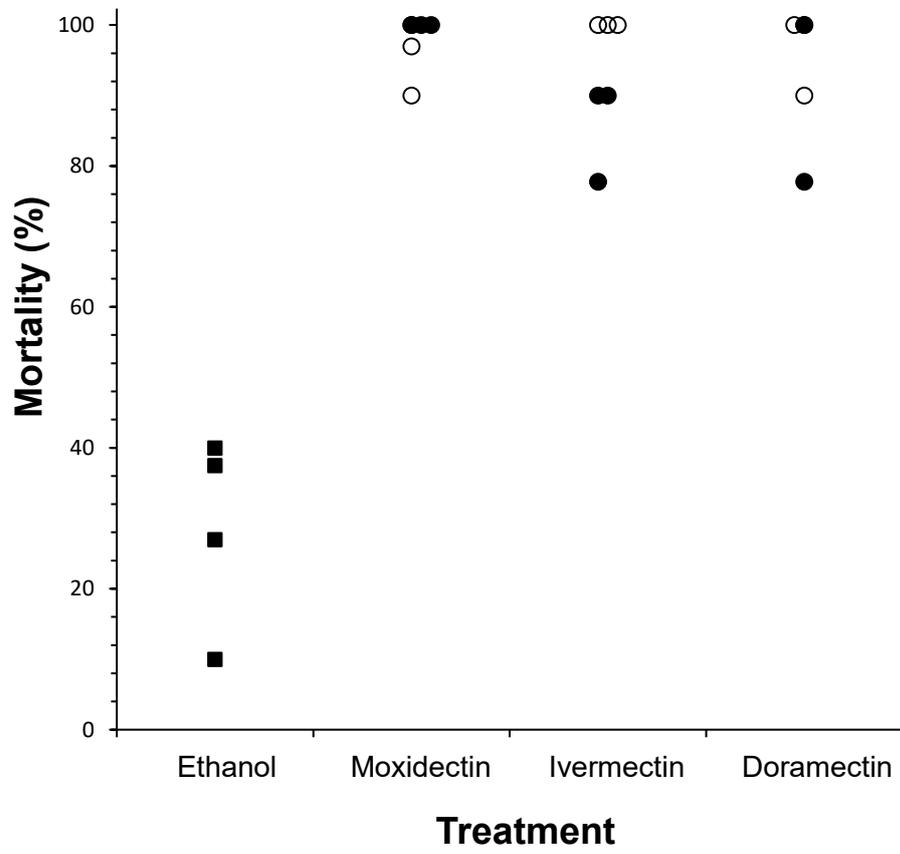


Figure 2

