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Performance of the psoroptes ovis - antibody enzyme-linked immunosorbent assay in the face of low-level mite infestation

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1 **PERFORMANCE OF THE *PSOROPTES OVIS* ANTIBODY ELISA IN THE FACE**
2 **OF LOW LEVEL MITE INFESTATION**

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18 **PERFORMANCE OF THE *PSOROPTES OVIS* ANTIBODY ELISA IN THE FACE**
19 **OF LOW LEVEL MITE INFESTATION**

20 **ABSTRACT**

21 *Psoroptes ovis* mites, the causative agent of sheep scab, can severely compromise sheep
22 welfare and production. However, in subclinical infections, mite detection is difficult,
23 increasing the risk of spread. A recent serodiagnostic test, based on detecting host antibodies
24 to the *P. ovis* allergen, Pso o 2, has made the detection of subclinical infection possible. The
25 use of this test was demonstrated in subclinical situations, through an opportunistic
26 observational study on an extensive hill farm and a lowland flock with recently introduced,
27 quarantined livestock. Twelve animals were tested from each group. Breeding ewes and
28 lambs on the hill farm had seroprevalences of 16% (12.5 – 17.8%) and 8.3% (4.8 – 10.1%),
29 respectively. Quarantined store lambs had a seroprevalence of 16.7% (13.2 – 18.5%); no
30 evidence of *P. ovis* was found in quarantined replacement ewes. By detecting subclinical
31 infection, this serological test could be a powerful tool in sheep scab control, for quarantine
32 procedures, accreditation programs and possibly regional or national eradication protocols.

33 **KEYWORDS**

34 Sheep scab, *Psoroptes ovis*, ELISA, serology, control, quarantine

35

36 INTRODUCTION

37 *Psoroptes ovis* is a non-burrowing parasitic mite of sheep, the causative agent of sheep scab,
38 which has a significant detrimental effect on the welfare of clinically affected animals.¹ It is
39 estimated to cost the UK sheep industry £8 million per year in lost production and
40 preventative measures² largely due to weight loss and lamb mortality.³ The mites spend their
41 whole lifecycle on the host and the propagation of disease requires the transfer of at least one
42 viable ovigerous female mite to a new animal.⁴ This transfer can occur either via direct
43 contact, or through fomites, such as pieces of wool on fence posts or handling facilities,
44 where mites can remain viable for up to 16 days.^{5 6}

45 Individual animals on which mite numbers are low may show mild or inapparent clinical
46 signs, so that infection can easily go undetected. This is a high-risk situation for the spread of
47 infection. Low mite numbers can occur during the ‘lag’ and ‘decline’ phases of infection,⁷
48 when the fleece is short⁸ and in some breeds without dense fleeces.⁸ Babcock and Black,
49 1933,⁵ found mites could remain hidden on sheep for up to two years. Traditional diagnostic
50 methods, using microscopic mite identification have low sensitivity, especially in these
51 subclinical infestations.⁹

52 In response to these diagnostic difficulties and the ongoing, endemic nature of sheep scab in
53 the UK,¹⁰ new immunological methods have been employed to produce an indirect antibody
54 enzyme linked immunosorbent assay (ELISA) to detect immune responses to *P. ovis*
55 infection.¹¹ A recombinant form of the *P. ovis* allergen, Pso o 2, is used to detect anti-Pso o 2
56 antibodies in sheep serum and can be used to diagnose sheep scab as early as two weeks post-
57 infestation.¹² When trialled in a variety of circumstances, the Pso o 2 ELISA has been shown
58 to be highly effective in detecting infection,¹³ with a sensitivity of 93% and specificity of
59 90%.¹² Further optimisation has resulted in an improved assay with a sensitivity of 98.2% and

60 a specificity of 96.5% (S. Burgess, unpublished observations). The test indicates exposure to
61 infection, but cannot currently discriminate between an active infestation and a recently
62 resolved infestation, as such animals can remain positive after effective treatment.¹³

63 Therefore, it is best employed alongside treatment history and at a group, or flock level, to
64 assess for the presence or absence of disease in a flock, rather than diagnosis in individual
65 animals.

66 The Pso o 2 ELISA has been assessed in a flock outbreak of sheep scab¹³ but not in a field
67 situation without obvious clinical disease, where mite numbers may be low. The purpose of
68 this report was to demonstrate the performance of the ELISA in circumstances where *P. ovis*
69 mite numbers were extremely low. This included the testing of asymptomatic sheep after
70 purchase by a lowland farm, and testing of animals on an extensive hill farm from which
71 some of the purchased animals had come.

72

73 MATERIALS AND METHODS

74 To demonstrate the application of the Pso o 2 ELISA in situations where *P. ovis* mite
75 numbers may be low, the test was applied in a quarantine situation on a lowland farm, where
76 the purchased sheep had no clinical signs of sheep scab, and on an extensive hill farm, where
77 subclinical infection may have been present. In early September 2017, 50 Scottish Blackface
78 store lambs were sold from an extensive hill farm (Farm 1) in the west of Scotland to a
79 lowland commercial sheep flock situated in the south east of Scotland (Farm 2). A possibility
80 of subclinical infection with *P. ovis* existed owing to the common grazing and unfenced
81 boundaries on the hill farm. The consequences of introducing *P. ovis* to a naïve flock can be
82 severe.³

83 The flock on Farm 1 consisted of 900 Scottish Blackface breeding ewes. Scottish Blackface
84 sheep are not densely fleeced and can maintain *P. ovis* mite numbers at low levels without
85 clinical signs.⁸ They were grazed at low stocking densities on 1677 hectares of common hill
86 grazing at 170 to 1025 metres above sea level. The area of this farm that the purchased store
87 lambs had come from was separated into two ‘hefts’ (groups of sheep accustomed to grazing
88 in a certain area of the hill). Staff on Farm 1 had not observed signs of sheep scab for at least
89 three years; nevertheless, all breeding sheep were treated with 1ml per 20kg bodyweight of
90 2% long-acting injectable moxidectin (20mg/ml, Cydectin LA, Zoetis) in October every year
91 (including 2017) as a precautionary measure.

92 The flock on Farm 2 was free from clinical signs of sheep scab and consisted of 300 breeding
93 ewes and ten terminal sire rams. The sheep were intensively grazed on enclosed, improved
94 pasture and rough common pasture, unused by other flocks. Sixty replacement Scottish mule
95 ewes had also been bought into Farm 2 from another source in early September and placed in
96 the field next to the store lambs, with only a single wire fence separating them. Due to a
97 failure of quarantine procedures the new stock (store lambs and replacement ewes) had had
98 contact with other sheep on the farm, without the use of precautionary acaricide treatments.
99 Therefore the risk of the introduction of infection was high and the incoming animals were
100 screened for sheep scab to provide evidence for the justification of whole flock treatment.

101 Blood samples were analysed from 12 Scottish Blackface store lambs (originating from Farm
102 1) and 12 replacement ewes (from other sources) on Farm 2, six weeks after purchase. The
103 seropositive lambs from Farm 2 were re-tested, plus a further 12 store lambs from the same
104 group. In addition, blood samples were analysed from 25 ewes (a minimum of 12 from each
105 heft) and 12 lambs from Farm 1, in November 2017. All blood samples were collected as
106 whole blood into vacutainers without anticoagulant and allowed to clot, then refrigerated

107 until testing was undertaken. The samples were tested using the Pso o 2 sheep scab ELISA,
108 using reagents and conditions developed by MRI.¹² Testing was undertaken by MRI; except
109 for the samples from the first 12 store lambs on Farm 2 and repeat samples from the positive
110 animals in this group, which were carried out by Biobest Laboratories Ltd.

111 Superficial skin scrapes and clear adhesive tape were used to collect samples from multiple
112 locations on a mildly pruritic lamb on Farm 1 and the lambs with positive serology samples
113 on Farm 2.¹⁴ These samples were collected from areas of wool with yellow discolouration
114 and skin with slight hyperkeratosis, found on the neck and flank. Both ears of the lambs on
115 Farm 2 were flushed, as previously described.¹⁵ These samples were examined
116 microscopically for identification of ectoparasites.¹⁶

117 The indication to test twelve animals per management group is based on an estimated within
118 flock prevalence of 20%, providing a minimum test accuracy of 95%, and test sensitivity of
119 98.2% and specificity of 96.5% at the selected optical density (OD) cut-off (S. Burgess,
120 unpublished observations). The test sensitivity and specificity were used to calculate the
121 minimum (*Min*) and maximum (*Max*) potential seroprevalence in each group tested, using the
122 following formula:

$$123 \quad Min = 100 * (P - ((1 - (SP / 100)) * N)) / N$$

$$124 \quad Max = 100 * (P + ((1 - (S / 100)) * N)) / N$$

125 Where *P* is the number of positive test results, *S* is the test sensitivity, *SP* is the test
126 specificity and *N* is number of animals tested. The arithmetic mean of the OD was calculated
127 for each test group by adding together all the OD results for that group and dividing by the
128 number tested.

129 RESULTS

130 On Farm 1 (Table 1), one of 12 lambs (8.3%) was found to be seropositive, giving a potential
 131 group seroprevalence between 4.8-10.1%. Four of 25 ewes (16%) were found to be
 132 seropositive, hence the potential group seroprevalence in the ewe flock was estimated to be
 133 between 12.5-17.8%.

134 Of the Scottish Blackface store lambs that were tested on Farm 2, two of 24 lambs (8.3%)
 135 were found to be seropositive (Table 1) giving a potential group seroprevalence of between
 136 4.8 and 10.1%. One of these lambs was found to be seropositive when first tested and then
 137 found to be seronegative on re-test. There was no evidence of exposure to sheep scab in the
 138 replacement ewes (Table 1). All ELISA results are available in Appendix 1.

139 No mites were found in any of the superficial skin scrape or ear flush samples.

Table 1: Distribution of animals, from a Scottish hill farm (Farm 1) and bought in sheep on a
 Scottish lowland farm (Farm 2), classified as positive by anti-Pso o 2 ELISA.

Animals sampled	Farm	Number sampled	Number of positive results	Mean OD ⁴⁵⁰ (range)	Group seroprevalence (%)
Lambs	1	12	1 positive 1 inconclusive	0.205 (0.093 – 0.519)	8.3 (4.8 – 10.1)
Breeding ewes	1	25	4	0.355 (0.07 – 1.97)	16 (12.5 – 17.8)
Store lambs (First set tested)	2	12	2	0.337 (0.08 – 2.04)	16.7 (13.2 – 18.5)
Store lambs (repeats)	2	2	1	1.125 (0.22 – 0.203)	-
Store lambs (Second set tested)	2	12	2 inconclusive	0.215 (0.13 – 0.4)	-

Replacement ewes	2	12	0	0.185 (0.1 – 0.3)	0
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Numbers of animals classified as seropositive for sheep scab based on the ELISA results.

For samples tested at Biobest, $OD^{450nm} > 0.4$ = suspicion of infestation, $OD^{450nm} > 0.5$ = positive. For samples tested at MRI, $OD^{450nm} > 0.4$ = suspicion of infestation, $OD^{450nm} > 0.432$ = positive. Also shown, an estimate of the group seroprevalence and the prevalence range, based on test sensitivity (98.2%) and specificity (96.5%).

140

141

142 DISCUSSION

143 Here we have demonstrated the use of a serological diagnostic assay, using a single recombinant
 144 protein, for the detection of *Psoroptes ovis* infestation in sheep,¹² in a subclinical situation
 145 where traditional diagnostic methods of mite identification failed. It has also been
 146 demonstrated here that this new test has the potential to be an effective tool in preventing
 147 disease incursion during the introduction of new or returning stock. As such, the detection of
 148 *P. ovis* in subclinical situations represents a step forward for the control of sheep scab, with
 149 potential for the detection and removal, by appropriate treatment, of infection in
 150 asymptomatic flocks and the prevention of *P. ovis* propagation to uninfected farms.

151 Recently bought-in animals were assessed using the ELISA, the results of which were used to
 152 justify treatment with macrocyclic lactones; for the whole flock on Farm 2 and previously
 153 untreated lambs on Farm 1. The judicious use of these products is important to maintain their
 154 efficacy against ectoparasites and endoparasites, as well as reduce their environmental
 155 impact. This is especially pertinent given the recent UK report of *Psoroptes ovis* mite
 156 resistance to moxidectin.¹⁷

157 To prevent the excessive use of acaricides, it is important to minimise false positive results in
158 the detection of *P. ovis*. The ELISA used here detects antibody to a single recombinant
159 protein, Pso o 2, which is highly specific for sheep scab¹² compared with a previously
160 developed crude *Psoroptes* mite extract based ELISA.^{9 18} However, due to the longevity of
161 the circulating IgG response, the test can give false positive results in sheep that have recently
162 received effective acaricide treatment¹³ or self-resolved.⁷ Test results should therefore
163 always be interpreted in conjunction with treatment history. Also, biological tests rarely
164 achieve 100% specificity, so when low numbers of positive results are seen, as in this case,
165 where one or two out of 12 samples were positive, they should be interpreted with caution, as
166 they may not represent a current active infection. Hence repeat samples and additional
167 testing are recommended in these circumstances, as were undertaken here.

168 To obtain meaningful results, additional testing needs to be undertaken in a risk-based
169 manner. The analysis of risk should incorporate the number of positive results from initial
170 testing, the degree of positivity of these results and the on-farm situation. The farm
171 assessment should include whether animals are displaying clinical signs consistent with *P.*
172 *ovis* infection, movement of animals, use of common grazing, quarantine and biosecurity
173 measures, proximity of neighbouring flocks and history of sheep scab in those flocks. If very
174 few (1 or 2) of the original samples had low positive results, and the on-farm risk was
175 considered to be low, monitoring without further testing may be appropriate, or additional
176 testing could be delayed to increase the likelihood of finding positive animals if infection is
177 present or recent. Where low numbers of highly positive samples or potential biosecurity
178 breaches exist, additional testing would be recommended. Ideally the same positive animals
179 should be re-tested, alongside additional animals from the same group, making it pertinent to
180 record animal identity at the time of sampling. Where testing of the same animals is not

181 possible a representative proportion of the group should be re-tested and further work is
182 required to determine what proportion this would be.

183 One of the store lambs from Farm 2 was initially found to be sero-positive but then displayed
184 a reduction in test OD value upon re-test, becoming sero-negative. As previously stated the
185 test is unable to distinguish between active and recently resolved infections, however
186 reductions in serological responses are observed post-treatment/resolution and a significant
187 decline in test OD value can be detected within 10 days of treatment (S. Burgess, unpublished
188 observations). As such, this observation may indicate a resolved infection in this individual.

189 In these subclinical situations, consideration should also be given to the number of animals
190 sampled, as the recommendation of sampling 12 animals per group of 2000 sheep is based on
191 an assumed within flock prevalence of 20%. The seroprevalence on Farm 1 and the store
192 lambs of Farm 2 was potentially lower than this, between 4.8 and 18.5%, which may have
193 reduced the likelihood of detecting infection. However, by testing 12 lambs from a group of
194 50, or 12 ewes from a heft of 400 to 500, a higher proportion of each group was tested than
195 the recommendations stipulate, therefore the likelihood of detecting infection may not have
196 been reduced overall. Further work will be required to determine how many animals should
197 be tested in situations with low seroprevalence. However, there is a need to balance the
198 accuracy of testing with the cost to individual farms. Also, quarantine treatment, rather than
199 testing, cannot be justified on the basis of cost alone, but an argument should be made for
200 encouraging the judicious use of acaricides.

201 Prophylactic use of acaricides is standard practice on farms with common grazing in the UK,
202 including the one described here, there is a ten-fold increase in the risk of sheep scab
203 incursion on these farms compared with farms without common grazing.¹⁹ Conversely, a low

204 seroprevalence of sheep scab was found on the extensive hill farm (Farm 1), compared with a
205 seroprevalence of 78% found during a clinical outbreak on a lowland farm¹³ this may reflect
206 specific management characteristics of extensive hill flocks with common grazing. On
207 extensive farms the spread of infection is prevented by low stocking densities.²⁰ Farm 1 had
208 an average stocking density of approximately one breeding ewe to five acres. The breed of
209 sheep farmed⁸ and flock immunity, can also suppress clinical signs and mite numbers.²¹
210 Flock immunity builds as a result of repeated exposure, possibly from untreated sheep that
211 remain on the hill after a gather²⁰ or co-grazing with other flocks.¹⁹

212 Given the endemic nature of sheep scab in the UK¹⁰ the low mite numbers in extensive and
213 subclinical situations and the poor sensitivity of traditional mite identification methods⁹ the
214 use of this new serological test with high specificity for *P. ovis*¹² is necessary to improve
215 control. Formal ways to use the test could potentially include accreditation schemes, which
216 would allow flocks to provide evidence of freedom from *P. ovis* infection. Work would need
217 to be done to establish whether purchasers would seek *P. ovis*-free flocks for replacements,
218 and so encourage participation in such a scheme. Regional or national eradication strategies
219 may also be considered, as was attempted in one Swiss region, where a crude *P. cuniculi*
220 antigen antibody ELISA was used to target treatments.¹⁸

221 The study described here is helpful as an example of how the sheep scab ELISA performs in
222 a subclinical situation and can be used as part of a quarantine protocol. We have shown that
223 it is a powerful tool for flock level surveillance of sheep scab, to target the use of whole flock
224 treatments and reduce the risks associated with introduced animals.

225

226 ABBREVIATIONS

227 ELISA – enzyme linked immunosorbent assay

228 OD – optical density

229 MRI – Moredun Research Institute

230 ETHICAL APPROVAL

231 The work was undertaken as a clinical investigation using validated and commercialised
232 diagnostics, therefore ethics approval was not sought.

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236 AUTHOR CONTRIBUTIONS

237 KH collected and analysed the data and drafted the manuscript

238 SB advised on study design, performed the testing and critiqued the manuscript

239 VB assisted with data analysis and interpretation, and contributed significantly to manuscript
240 revision and intellectual content

241 NS was responsible for study conception and design, data interpretation, manuscript revision
242 and intellectual content

243 All authors read and approved the final manuscript

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251 **COMPETING INTERESTS**

252 The authors declare that they have no competing interests

253

254

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