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

Kim Hamer[a,1] MA VetMB CertAVP(SHEEP) MRCVS

Stewart TG Burgess[b] BSc PhD

Valentina Busin[c] DVM, DipECSRHM, PhD, MRCVS

Neil D Sargison[a, d¥] BA VetMB PhD DSHP DipECSRHM FRCVS

a. University of Edinburgh, Royal (Dick) School of Veterinary Studies and Roslin Institute, Easter Bush Veterinary Centre, Roslin, Midlothian, Scotland EH25 9RG

b. Moredun Research Institute (MRI), Pentlands Science Park, Edinburgh, Scotland EH26 0PZ, UK

c. University of Glasgow, School of Veterinary Medicine, Garscube Estate, Glasgow, Scotland, G61 1QH

d. The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, Scotland, EH25 9RG

¥ Corresponding author: Email neil.sargison@ed.ac.uk; Tel +44 (0)131 445 4468

Word count: 2420

[1] Present address: University of Glasgow, School of Veterinary Medicine, Garscube Estate, Glasgow, Scotland, G61 1QH

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

ABSTRACT

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

KEYWORDS

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

INTRODUCTION

Psoroptes ovis is a non-burrowing parasitic mite of sheep, the causative agent of sheep scab, which has a significant detrimental effect on the welfare of clinically affected animals.[1] It is estimated to cost the UK sheep industry £8 million per year in lost production and preventative measures[2] largely due to weight loss and lamb mortality.[3] The mites spend their whole lifecycle on the host and the propagation of disease requires the transfer of at least one viable ovigerous female mite to a new animal.[4] This transfer can occur either via direct contact, or through fomites, such as pieces of wool on fence posts or handling facilities, where mites can remain viable for up to 16 days.[5 6]

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

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

a specificity of 96.5% (S. Burgess, unpublished observations). The test indicates exposure to infection, but cannot currently discriminate between an active infestation and a recently resolved infestation, as such animals can remain positive after effective treatment.[13] Therefore, it is best employed alongside treatment history and at a group, or flock level, to assess for the presence or absence of disease in a flock, rather than diagnosis in individual animals.

The Pso o 2 ELISA has been assessed in a flock outbreak of sheep scab[13] but not in a field situation without obvious clinical disease, where mite numbers may be low. The purpose of this report was to demonstrate the performance of the ELISA in circumstances where *P. ovis* mite numbers were extremely low. This included the testing of asymptomatic sheep after purchase by a lowland farm, and testing of animals on an extensive hill farm from which some of the purchased animals had come.

MATERIALS AND METHODS

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

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

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

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

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

Superficial skin scrapes and clear adhesive tape were used to collect samples from multiple locations on a mildly pruritic lamb on Farm 1 and the lambs with positive serology samples on Farm 2.[14] These samples were collected from areas of wool with yellow discolouration and skin with slight hyperkeratosis, found on the neck and flank. Both ears of the lambs on Farm 2 were flushed, as previously described.[15] These samples were examined microscopically for identification of ectoparasites.[16]

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

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

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

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

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

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%).

DISCUSSION

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

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

To prevent the excessive use of acaricides, it is important to minimise false positive results in the detection of *P. ovis*. The ELISA used here detects antibody to a single recombinant protein, Pso o 2, which is highly specific for sheep scab[12] compared with a previously developed crude *Psoroptes* mite extract based ELISA.[9 18] However, due to the longevity of the circulating IgG response, the test can give false positive results in sheep that have recently received effective acaricide treatment[13] or self-resolved.[7] Test results should therefore always be interpreted in conjunction with treatment history. Also, biological tests rarely achieve 100% specificity, so when low numbers of positive results are seen, as in this case, where one or two out of 12 samples were positive, they should be interpreted with caution, as they may not represent a current active infection. Hence repeat samples and additional testing are recommended in these circumstances, as were undertaken here.

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

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

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

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

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

seroprevalence of sheep scab was found on the extensive hill farm (Farm 1), compared with a seroprevalence of 78% found during a clinical outbreak on a lowland farm[13] this may reflect specific management characteristics of extensive hill flocks with common grazing. On extensive farms the spread of infection is prevented by low stocking densities.[20] Farm 1 had an average stocking density of approximately one breeding ewe to five acres. The breed of sheep farmed[8] and flock immunity, can also suppress clinical signs and mite numbers.[21] Flock immunity builds as a result of repeated exposure, possibly from untreated sheep that remain on the hill after a gather[20] or co-grazing with other flocks.[19]

Given the endemic nature of sheep scab in the UK[10] the low mite numbers in extensive and subclinical situations and the poor sensitivity of traditional mite identification methods[9] the use of this new serological test with high specificity for *P. ovis*[12] is necessary to improve control. Formal ways to use the test could potentially include accreditation schemes, which would allow flocks to provide evidence of freedom from *P. ovis* infection. Work would need to be done to establish whether purchasers would seek *P. ovis*-free flocks for replacements, and so encourage participation in such a scheme. Regional or national eradication strategies may also be considered, as was attempted in one Swiss region, where a crude *P. cuniculi* antigen antibody ELISA was used to target treatments.[18]

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

ABBREVIATIONS

ELISA – enzyme linked immunosorbent assay

OD – optical density

MRI – Moredun Research Institute

ETHICAL APPROVAL

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

ACKNOWLEDGEMENTS

Rebecca Mearns for advice and support with the diagnostic testing. The farm staff on Farms 1 and 2 for their willing involvement in this study.

AUTHOR CONTRIBUTIONS

KH collected and analysed the data and drafted the manuscript

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

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

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

All authors read and approved the final manuscript

FUNDING

Stewart Burgess is funded by the Scottish Government, Rural and Environment Science and Analytical Services (RESAS). Neil Sargison is funded by Biotechnology and Biological Sciences Research Council (BBSRC). Work undertaken by Kim Hamer was as part of a Royal (Dick) School of Veterinary Studies Senior Clinical Training Scholarship. Valentina

Busin was employed by the University of Glasgow School of Veterinary Medicine during her contribution.

COMPETING INTERESTS

The authors declare that they have no competing interests

255 REFERENCES

256 1. Kirkwood AC. History, biology and control of sheep scab. *Parasitol Today*.
257 1986;**2**:302–7.

258 2. Nieuwhof GJ, Bishop SC. Costs of the major endemic diseases of sheep in Great
259 Britain and the potential benefits of reduction in disease impact. *Anim Sci*.
260 2005;**81**:23–9.

261 3. Sargison N, Scott P, Penny C, Pirie R. The effect of an outbreak of sheep scab
262 (*Psoroptes ovis* infestation) during mid-pregnancy on ewe body condition and lamb
263 birth weights. *Vet Rec*. 1995;**136**:287–9.

264 4. van den Broek AH, Huntley JF. Sheep Scab: the Disease, Pathogenesis and Control. *J*
265 *Comp Pathol*. 2003;**128**:79–91.

266 5. Babcock OG, Black WL. The common sheep-scab mite and its control. *Texas Agric*
267 *Exp Stn Bull*. 1933;**479**:1–34.

268 6. O'Brien DJ, Gray JS, O'Reilly PF. Survival and retention of infectivity of the mite
269 *Psoroptes ovis* off the host. *Vet Res Commun*. 1994;**18**:27–36.

270 7. Bates P. The pathogenesis and ageing of sheep scab lesions - part 1. *State Vet J*
271 (United Kingdom). 1997;**7**:11–5.

272 8. Bates P. The pathogenesis and ageing of sheep scab lesions - part 2. *State Vet J*
273 (United Kingdom). 1997;**7**:13–6.

274 9. Ochs H, Lonneux J-F, Losson B., Deplazes P. Diagnosis of psoroptic sheep scab with
275 an improved enzyme-linked immunosorbent assay. *Vet Parasitol*. 2001;**96**:233–42.

276 10. Bisdorff B, Wall R, Milnes A. Prevalence and regional distribution of scab, lice and

277 blowfly strike in Great Britain. *Vet Rec*. 2006;**158**:749–52.

278 11. Lee A, Machell J, Van Den Broek A, et al. Identification of an antigen from the sheep
279 scab mite, *Psoroptes ovis*, homologous with house dust mite group I allergens.
280 *Parasite Immunol*. 2002;**24**:413–22.

281 12. Nunn FG, Burgess STG, Innocent G, Nisbet AJ, Bates P, Huntley JF. Development of
282 a serodiagnostic test for sheep scab using recombinant protein Pso o 2. *Mol Cell*
283 *Probes*. 2011;**25**:212–8.

284 13. Burgess ST, Innocent G, Nunn F, et al. The use of a *Psoroptes ovis* serodiagnostic test
285 for the analysis of a natural outbreak of sheep scab. *Parasit Vectors*. 2012;**5**:7-17.

286 14. Sargison ND. Differential diagnosis and treatment of sheep scab. *In Pract*. 1995;**17**:3–
287 9.

288 15. Crilly JP, Nunn F, Busin V, et al. Investigation and treatment of ovine psoroptic
289 otoacariasis. *Vet Dermatol*. 2016;**27**:206-252.

290 16. Ministry of Agriculture, Fish & Food. Manual of veterinary parasitological laboratory
291 techniques. H.M. Stationery Office, 1986

292 17. Doherty E, Burgess S, Mitchell S, Wall R. First evidence of resistance to macrocyclic
293 lactones in *Psoroptes ovis* sheep scab mites in the UK. *Vet Rec*. 2018;**182**:106.

294 18. Jacober P, Ochs H, Torgerson PR, Schnyder M, Deplazes P. A method for sheep scab
295 control by applying selective treatment based on flock serology. *Vet Parasitol*.
296 2006;**136**:373–8.

297 19. Rose H, Wall R. Endemic sheep scab: Risk factors and the behaviour of upland sheep
298 flocks. *Prev Vet Med.* 2012;**104**:101–6.

20. Spence T. Control of sheep scab in Britain. *Aust Vet J*. 1951;**27**:136–46.

21. van den Broek AHM, Huntley JF, Machell J, et al. Cutaneous and systemic responses during primary and challenge infestations of sheep with the sheep scab mite, *Psoroptes ovis*. *Parasite Immunol*. 2000;**22**:407–14.