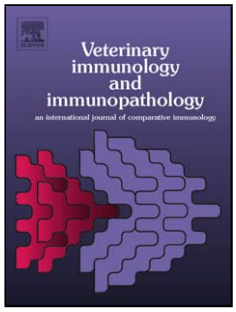

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PII: S0165-2427(09)00236-0
DOI: doi:10.1016/j.vetimm.2009.07.004
Reference: VETIMM 8108

To appear in: *VETIMM*

Received date: 16-9-2008
Revised date: 16-4-2009
Accepted date: 1-7-2009

Please cite this article as: Rodríguez-Cadenas, F., Carbajal-González, M.T., Fregeneda-Grandes, J.M., Aller-Gancedo, J.M., Rojo-Vázquez, F.A., Clinical evaluation and antibody responses in sheep after primary and secondary experimental challenges with the mange mite *Sarcoptes scabiei* var. *ovis*, *Veterinary Immunology and Immunopathology* (2008), doi:10.1016/j.vetimm.2009.07.004

Clinical evaluation and antibody responses in sheep after primary and secondary experimental challenges with the mange mite *Sarcoptes scabiei* var. *ovis*

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Abstract

In this work the clinical evolution and the specific serum IgG and IgE antibody responses in sheep after primary (n = 10) and secondary (n = 4) experimental challenges with the mange mite *Sarcoptes scabiei* var. *ovis* were studied. The primary infection was characterized by the development of mange lesions in all sheep, a detection of live *S. scabiei* mites in 70% skin scrapings taken in week 10 post-challenge (PC), strongly raised and sustained specific IgG levels and a more moderate but continuous rise in specific IgE levels. Seroconversion was detected for IgG and IgE by ELISA in 90% and 60% of the sheep in week 8 PC, respectively. By Western-blotting (WB), ten IgG-reactive bands (36-120 KDa) and four IgE-reactive bands

(90-180 KDa) were observed in week 8 PC. Following the secondary challenge the ewes developed a smaller area of mange lesion than that seen following primary challenge and live *S. scabiei* mites were not detected in skin scrapings collected in week 8 PC, suggesting that sheep had developed immunity to re-infection. Compared to primary infection, the specific IgG secondary antibody levels were transient, but in contrast there was an anamnestic IgE response, in which the specific serum IgE levels in week 2 PC were significantly higher than those demonstrated after primary infection. WB analysis revealed one additional IgG-reactive band (180KDa) and no additional IgE-reactive bands. Determining the immunodiagnostic or vaccination value of the IgG-reactive antigens and IgE-reactive allergens detected requires further studies.

Keywords: Sheep; *Sarcoptes scabiei*; ELISA: Western-blotting; Clinical-aspects; Resistance.

Abbreviations: OD: optical density; PC: post-challenge; RT: room temperature.

1. Introduction

Sarcoptic mange is a parasitic skin disease due to infection by the mite *Sarcoptes scabiei*. The lesions produced in sheep, characterized by formation of crust of up to 1 cm in thickness, are mainly located on the head (Cordero del Campillo and Rojo-Vázquez, 1999). The main clinical signs are rubbing and scratching. It also causes important financial losses due to decreases in milk production, reproductive performance and the growth of lambs born from affected ewes (Fthenakis et al., 2000, 2001).

Currently, diagnosis of sarcoptic mange in sheep is performed by visual observation of the mites in skin scrapings. The detection of specific serum antibodies against *S. scabiei* by ELISA is routinely used to diagnose sarcoptic mange in dogs (Curtis, 2001; Lower et al.,

2001) and has also been successfully used to monitor the effectiveness of eradication programmes for sarcoptic mange in pigs (Jacobson et al., 1999; Rueda-López, 2006). Recently, the authors have developed and validated an ELISA to diagnose sarcoptic mange in sheep using a crude saline extract from *S. scabiei* var. *ovis*, and demonstrated that it is highly accurate (Rodríguez-Cadenas et al., submitted to journal). However, this test is unsuitable for large scale use, owing to limitations on the amount of mites which can be collected, as there is currently no *in vitro* culture technique for breeding *S. scabiei*. Identification of the major antigens and allergens of *S. scabiei* would assist in the development of an immunodiagnostic test based on recombinant proteins (Kuhn et al., 2008). The antibody response against *S. scabiei* has already been characterized in dogs, foxes and goats (Bornstein et al. 1995; Arlian and Morgan, 2000; Tarigan, 2004), showing differences between species. These inter-species antibody profile differences are in agreement with the findings of Arlian et al. (1996a), who reported that each variety of *S. scabiei* may produce a range of proteins comprised of both those which are variety-specific, and those which are immunologically identical and shared by the different mite sub-types.

Currently, control of sarcoptic mange is mainly based on the administration of acaricides. The use of these chemical compounds has serious drawbacks; i.e. the development of drug resistance (Curie et al., 2004), adverse environmental effects (Sanderson et al., 2007), residues in animal products (Imperiale et al., 2004) and health hazards to humans (Bradberry et al., 2005). As a consequence, the development of 'non-chemical' methods, such as vaccination, is desirable. The achievement of a vaccine against sarcoptic mange is thought to be feasible as animals having recovered from a previous infection show resistance to re-infection (Arlian et al, 1994; Arlian et al., 1996b; Tarigan, 2002). Furthermore, it has been suggested that the mechanism involved in the development of acquired resistance against *S. scabiei* is related to IgE responses (Tarigan, 2003; Tarigan and Huntley, 2005).

The aim of the present study was to monitor sheep clinically after primary and secondary experimental challenges with the mange mite *S. scabiei* var. *ovis,* and to characterise the specific serum IgG and IgE antibody responses.

2. Materials and methods

2.1. Experimental animals

First, ten *S. scabiei*-naïve adult sheep (> 1 year) were challenged (primary infection group). During week 10 post challenge (PC), five randomly selected sheep were treated with ivermectin (Ivomec®, Merial, two injections one week apart s.c., at 200 µg kg^{-1} of body weight) and the other five removed from the study. One of the ivermectin-treated sheep died two weeks after treatment from causes not related to the experiment. The remaining four treated sheep were kept until eight weeks after treatment, by which time the mange lesions had disappeared. After recovering from the primary infection, the four sheep were challenged again with the same *S. scabiei* var. *ovis* strain (secondary infection group) and monitored for another eight weeks. As a positive control for this infection three *S. scabiei*-naïve adult sheep were also challenged at the same time (secondary infection control group).

2.2. Preparation of the *S. scabiei* var. *ovis* challenge-inocula and experimental challenge

Experimental challenges were performed using crusts with *S. scabiei* var. *ovis* mites collected from a severely affected ewe. Briefly, the approximate total number of *S. scabiei* mites on the donor sheep was determined on the day before challenge from the total lesion area and the mean number of mites per 1 cm^2 of lesion. The latter measure was estimated by scraping the donor sheep at several 1 cm^2 points in the lesion, and counting the *S. scabiei* larvae, nymphs and adults under the microscope after 10% KOH digestion of the crusts and concentration of

the mites by floating with saturated sucrose solution. Thereafter, on the day of challenge the donor sheep were euthanized (T-61®, Intervet International B.V.), all the crusts from the lesion removed and chopped to produce particles of around 5 mm diameter, and aliquots with approximately 2,000 mites were prepared. The sheep were challenged with an aliquot each, by maintaining the crusts in contact with the convex surface of one ear for 48 h by means of a dressing.

Blood samples were taken by jugular puncture at weekly intervals and after centrifugation at 900 g for 15 min the sera were removed and stored at −20º C.

2.3. Clinical monitoring

The mange lesions which developed after challenge in the convex surface of the ear were recorded in a diagram and subsequently graded by comparing with reference lesion pictures which had been scored in accordance with the affected area. The scale scores were as follows: score 0 if no lesion was visible on the ear; score 1 when < 10% of the area was affected; score 2 when 10-25% of the area was affected; score 3 when 25-50% of the area was affected; score 4, when 50-75% of the area was affected; and score 5 when > 75% of the area was affected.

Skin scrapings were collected from the lesion and examined for live *S. scabiei* mites after incubation at 30º C for 30 min, and in addition they were also examined using the digestion-concentration technique (see section 2.2.).

2.4. Mite extract

The mite extract used in both ELISA and Western-blotting (WB) techniques was a crude saline extract kindly supplied by Dr. John F. Huntley (MRI, Penicuik, Scotland). This extract had been prepared from *S. scabiei* var. *caprae* mites collected from a mangy goat as described by Tarigan and Huntley (2005) and stored frozen at −20º C until being processed as described

below. After defrosting, they had been washed once in ice-cold PBS, followed by another wash in PBS-1% (w/v) SDS at room temperature (RT) and 10 further washes in ice-cold PBS to remove the SDS. The mites had been then transferred to a ribolyser tube (Lysing Matrix C, Q-biogene) and homogenised in PBS with a shaker machine (FastPrep® FP120, Q-biogene) for four 30 s cycles with cooling between each. After centrifugation at 5,500 g for 5 min the supernatant (mite extract) had been removed, its protein concentration measured by the Bradford method (Bradford Reagent, Sigma) with BSA as standard and stored frozen at −20º C until use.

2.5. ELISA

High-binding microtiter plates (Costar®, Corning Incorporated, USA) were coated overnight at 4º C with 50 µl per well of a solution of the mite extract adjusted to 5 µg ml^{-1} of protein with carbonate-bicarbonate buffer (70 mM $NaHCO_3$, 30 mM Na_2CO_3, 0.2 g l^{-1} NaN_3, pH 9.6). After washing three times with PBS-T20 (PBS, 0.5 ml l^{-1} Tween 20) the plates were incubated for 1 h at 37º C with 50 µl per well of sheep sera appropriately diluted (1/200 for IgG or 1/10 for IgE) in PBS-T80-NaCl (PBS, 5ml l^{-1} Tween 80, 0.5M NaCl) and added in duplicate. The plates were washed again, then incubated for 1 h at 37º C with 50 µl per well of an appropriate mAb diluted in PBS-T80-NaCl: clone VPM6 (Bird et al., 1995) diluted 1/20 for IgG detection or clone 2F1 (Bendixen et al., 2004) diluted 1/200 for IgE detection. After a further washing, the plates were incubated for 1 h at 37º C with 50 µl per well of biotin-labelled goat anti-mouse IgG antibodies (Sigma®, USA) diluted 1/5,000 in PBS-T80-NaCl. The plates were washed once more, then incubated for 30 min at 37º C with 50 µl per well of streptavidin-HRP (GE Healthcare, UK) diluted 1/40,000 in PBS-T80-NaCl and washed again. Peroxidase activity was then visualized using the chromogen substrate OPD (OPD Tablets, Dako, Denmark) in accordance with the manufacturer's instructions. The reaction was

stopped with 50 µl per well of 0.5M H_2SO_4, then the Optical Density (OD) was measured at 492 nm using a spectrophotometer and the OD for each serum calculated (mean OD of the duplicate test wells). ELISA studies were carried out at two-week intervals throughout the study. Positive and negative control sera were tested on each plate and the ODs of the samples were adjusted relative to them to obtain comparable results between plates.

2.6. SDS-PAGE/Western-blotting

Aliquots of the mite extract with 100 µg of protein were mixed 1:1 with Laemmli reducing sample buffer (Bio-Rad Laboratories) and boiled for 5 min. Each mixture was then loaded into Tris/HCl 12% acrylamide/bisacrylamide gel, together with 10 µl of broad-range molecular weigh markers (prestained SDS-PAGE Standards, Bio-Rad Laboratories). The gels were subjected to electrophoresis at 200V for 45 min using Tris/Glycine/SDS running buffer (25mM Tris, 192 mM glycine, 5 g l^{-1} SDS). After this, the separated proteins from the gels were transferred onto nitrocellulose membranes (Trans-Blot® Transfer Medium, Bio-Rad Laboratories) at 100V for 1.5 h using Tris/Glycine transfer buffer (25mM Tris, 0.192 mM glycine, 200 ml l^{-1} methanol). Protein transfer was checked by staining the membrane with Ponceau S solution (0.1% Ponceau S pure, 50 ml l^{-1} acetic acid). Electrophoresis and transfers were done using the Mini Polyacrilamide Gel System (Bio-Rad Laboratories) and the PowerPac™ Basic Power Supply (Bio-Rad Laboratories).

The transferred membranes were then blocked for 1 h at RT with PBS-T80-NaCl, and after drying were cut into strips 3 mm wide. Each strip was incubated for 1 h at RT with 1 ml of sheep sera diluted 1/200 for IgG detection or 1/10 for IgE detection, in PBS-T80-NaCl. The strips were then washed three times, for 5 min on each occasion, with TBS (20 mM Tris, 0.5 mM NaCl, pH 7.6) and incubated for 1 h at RT with 1 ml of the appropriate mAb diluted in PBS-T80-NaCl: clone VPM6 diluted 1/20 for IgG detection or clone 2F1 diluted 1/1,000 for

IgE detection. After another washing step, the strips were incubated for 1 h at RT with 1 ml of biotin-labelled goat anti-mouse IgG antibodies (Sigma) diluted 1/5,000 in PBS-T80-NaCl. The strips were washed again, then incubated for 30 min at RT with 1 ml of streptavidin-HRP (GE Healthcare) diluted 1/20,000 in PBS-T80-NaCl. After another washing the peroxidase activity was visualized using a chemiluminescent substrate (ECL™, GE Healthcare, UK) in accordance with the manufacturer's instructions. WB studies were performed on sera collected prior to challenge (week 0), and at weeks 4 and 8 PC. Positive and a negative control sera were tested during each run of the assay. Further controls were ensured by replacing the serum or the mAb with dilution buffer.

The software package Quantity One® 4.5.0 (Bio-Rad Laboratories) was used to capture the images from the scanner (GS 800 Calibrated Densitometer, Bio-Rad Laboratories) and to determine the molecular weight of the reactive bands, using as reference the strip with the molecular weight markers. The antibody binding intensity of each serum to bands of the mite extract was scored (separately for IgG and IgE) as 0, 1, 2, 3, 4 or 5. The score was 0 when there was no binding, 1 for the weakest binding and so on up to 5 for the strongest. Thereafter, the mean binding intensity of the positive sera (score ≥ 1) to the band was graded as follows: weak (mean score 1-1.9), medium (2-2.9), strong (3-3.9) or very strong (4-5).

2.7. Statistical analysis

The one-sample Kolmogorov-Smirnov test was used to determine whether the paired-difference variables of ODs were normally distributed, and because this was always the case, the paired-samples t-test was used to compare antibody levels at different time points within each group, and to compare significantly-elevated antibody levels at the same time point between groups. When the intention was to do the latter, in order to prevent specific antibody levels being carried over from the primary infection to the secondary infection, the pre-

198 challenge antibody level was previously subtracted for each sheep and thus only the antibody
199 level elicited by the relevant challenge was used for comparison. All statistical tests were
200 performed with SPSS 15.0 for Windows®, and the alpha value was set at 0.05.
201

202 **3. Results**

203 **3.1. Clinical monitoring**

204 The results of clinical-lesion examination in weeks 1, 4 and 8 PC are shown in Table 1. After
205 the primary challenge all sheep exhibited mange lesions at the site of challenge, these
206 consisting of abundant exudates which later developed to form alopoecia and crust formations,
207 and there was a progressive growth in the mange lesion area as indicated by an increase in the
208 mean mange lesion score. Live *S. scabiei* mites were detected in skin scrapings from seven
209 sheep (70%) in week 10 PC, while a large number of dead mites (using the digestion-
210 concentration technique) were detected in all of them.
211 Following ivermectin treatment in week 10 PC, the mange lesions progressively disappeared
212 and there were no visible lesions of mange in any sheep by eight weeks later, when the
213 secondary challenge was performed.
214 After the secondary challenge, the four sheep exhibited mange lesions at the site of challenge
215 which were visually similar to those observed in the primary infection group. However, after
216 one week PC the lesions progressively disappeared, as indicated by a decrease in the mean
217 mange lesion score, to form alopoecic areas. Live *S. scabiei* mites were not detected in any of
218 the four skin scrapings collected after 8 weeks PC, while a few dead mites were detected in
219 one (25%). The mange lesion scores of the secondary infection control group approximated to
220 those of the primary infection group.
221

222 **3.2. ELISA**

The results of ELISA are shown in Figure 1. Before primary challenge the ELISA OD values were low (< 0.15 for IgG and < 0.1 for IgE) in all sheep. After the primary challenge, most of the sheep developed specific serum IgG and IgE antibodies. A significant increase in the IgG levels were first detected two weeks PC (one-tail $p < 0.05$), which were strongly increased at four weeks PC, after which the OD values levelled to a plateau. IgG seroconversion (cut-off value 0.197; defined as the mean plus 3 SD of ODs of sera at week 0) was demonstrated in nine sheep (90%) in week 4 PC, and they remained seropositive in week 8 PC. A significant increase in the IgE level was also first detected in week 2 PC (one-tail $p < 0.01$) and then progressively increased. IgE seroconversion (cut-off value 0.093) was found in five animals (50%) after 4 weeks PC and in six (60%) after 8 weeks PC.

After ivermectin treatment a decrease was detected in the specific IgG and IgE levels. Prior to the secondary challenge the IgG antibody level was still significantly higher when compared to pre-primary-challenge (one-tail $p < 0.05$), but the IgE antibody level was similar (one-tail $p > 0.05$).

After the secondary challenge, a significant increase in the specific IgG level was detected in week 4 PC (one-tail $p < 0.05$), but in week 6 and 8 PC the IgG level was similar to pre-secondary-challenge (one-tail $p > 0.05$). However, the IgG level in week 4 PC was similar to that of the same sheep in the primary-infection group (two-tail $p > 0.05$). The specific IgE level showed a rapid and strong increase in the first two weeks (one-tail $p < 0.05$) and reached the highest value at week 4 PC, while after that the level started to decrease but still remained significantly elevated at week 8 PC (one-tail $p < 0.05$). The IgE level in week 2 PC was significantly higher than that of the same sheep in the primary infection group (two-tail $p < 0.05$), but later was similar (two-tail $p > 0.05$). The IgG and IgE antibody levels in the secondary infection control group were similar to those of the primary infection group.

3.3. WB

The results of WB analysis are shown in Table 2, Table 3 and Figure 2. Prior to the primary challenge IgG-reactive bands of 120, 52, 46, 44, 38 and 36 KDa, and IgE-reactive bands of 120 and 90 KDa were detected in some sheep, albeit weakly. Following primary infection, additional IgG and IgE-reactive bands were demonstrated. The IgG analysis at week 4 PC showed that there was reaction to four additional bands of 100, 85, 47, and 39 KDa in most of the ewes, and the binding intensities and/or frequencies to the reactive bands detected before challenge also increased. A similar profile was demonstrated in week 8 PC, with only a small increase in binding intensities and frequencies. IgE reactivity in week 4 PC was also increased, with six sheep sera showing strong labelling intensity to the 120 KDa band, although reactivity to the 90 KDa band remained unchanged. In week 8 PC, weak IgE-reactions to two additional bands of 180 and 100 KDa was detected. The binding intensities and frequencies for the 120 and 90 KDa IgE-reactive bands were also increased. The immunodominant bands were defined as bands reacting with at least 50% sheep sera and with a very strong or strong mean binding intensity. Based on this definition, the immunodominant IgG-reactive bands in weeks 4 and 8 PC were 120, 52 and 44 KDa, while in contrast there was only one immunodominant IgE-reactive band in weeks 4 and 8 PC, which had 120 KDa.

The antibody profile prior to the secondary challenge was similar to that observed prior to the primary challenge. Following the secondary challenge, one additional IgG-reactive band (180 KDa) was detected, but in general there were lower binding intensities than those demonstrated following primary challenge. The secondary IgE–reaction was also mainly confined to the 120 KDa band, with peak binding intensity observed in week 4 PC. This 120KDa band appeared to be immunodominant for IgE antibodies following secondary challenge.

4. Discussion

In this study it was shown that primary and secondary experimental challenges of sheep with *S. scabiei* var. *ovis* induce the development of mange lesions and the elicitation of serum IgG and IgE antibodies directed to proteins/peptides of a wide range of molecular weights. Interestingly, the secondary infection corresponded with reduced mange lesion area, absence of detectable live *S. scabiei* mites in skin scrapings collected in week 8 PC and a concomitant increase in specific serum IgE antibodies.

This study was based on the detection of antibodies elicited by *S. scabiei* var. *ovis* using a mite extract prepared from *S. scabiei* var. *caprae*. It is known that each variety of *S. scabiei* may produce a range of specific proteins (Arlian et al., 1996a). However, the level of cross-antigenicity between them is usually high and a heterologous variety can be successfully used to detect specific antibodies. For instance, ELISAs based on extracts from *S. scabiei* var. *vulpes* have been used for diagnosing sarcoptic mange in pigs, dogs and chamois (Bornstein et al., 1996; Bornstein and Wallgren, 1996; Lower at al., 2001; Rambozzi et al., 2004). In the present study, the specific antibody responses detected by using the *S. scabiei* var. *caprae*-based ELISA to detect antibodies in sheep after challenge were significant and quite pronounced, suggesting that this heterologous variety can be used with high performance to detect antibodies elicited by *S. scabiei* var. *ovis*. Nevertheless, it is possible that *S. scabiei* var. *ovis* has specific antibody-reactive proteins, and as a result they would not have been identified.

Prior to primary challenge and despite ELISA OD values being low, serum reactivity to several bands of the mite extract was detected by WB analysis. This may have been due to the presence of antibodies elicited by free-living mites such as *Dermatophagoides pteronyssinus* or *Acarus siro* which cross-react with *S. scabiei* proteins (Arlian et al., 1991; van der Heijden et al., 2000). In the primary infection, live *S. scabiei* mites were detected in only 70% skin

298 scrapings in week 10 PC, but all sheep developed mange lesions that spread over the ear and
299 in addition a large number of dead mites were also detected. This would indicate that the
300 technique used to detect live mites had relatively low sensitivity, a finding in agreement with
301 Gutiérrez et al., (1996) who studied sarcoptic mange in pigs. The kinetics of specific serum
302 IgG and IgE antibodies were similar to the findings of other authors studying sarcoptic mange
303 in rabbits, foxes and goats (Arlian et al., 1994; Bornstein et al., 1995; Tarigan, 2004). As the
304 infection progressed, the average IgG and IgE values tended to rise which is consistent with
305 the higher number of reactive bands, and the increase in the labelling intensities and
306 frequencies detected by WB. However, despite the development of clinical lesions in all
307 sheep, IgG and IgE seroconversion was not found in 10% and 40% of sheep, respectively, in
308 week 8 PC. The absence of detectable levels of IgE antibodies in a significant proportion of
309 sera from *S. scabiei*-infected humans, dogs and goats has previously been reported (Arlian
310 and Morgan, 2000; Morgan et al., 1997; Tarigan, 2004).
311 In the present study, the antibody profile was studied by WB and reactions were demonstrated
312 to bands of a wide range of molecular weights. However, heterogeneity of reaction within
313 each of these bands was not determined and they may represent two or more different
314 antibody-reactive proteins with the same or similar molecular weights. As a consequence, the
315 present studies cannot confirm either that bands which reacted with both IgG and IgE
316 antibodies were due to the same antigens being detected by both isotypes. The
317 immunodominant bands include those proteins/peptides more intensively and frequently
318 recognized by the immune system, which are more likely to be the best candidates for a
319 sensitive immunodiagnostic test based on the detection of induced antibodies. The
320 immunodominant IgG-reactive bands detected during the primary infection (120, 52 and 44
321 KDa) were reactive with most of the sheep sera collected in weeks 4 and 8 PC, and therefore
322 include antigens which are candidates for developing a highly sensitive immunodiagnostic

test for sarcoptic mange in sheep. In contrast, the 120 KDa allergen seems to have limited immunodiagnostic value, as it was reactive with only 70% of sheep sera taken in week 8 PC. Of note at this point was the finding that also some pre-challenge sera produced weakly reaction to the immunodominant bands. The origin of these cross-reactive antibodies is unclear, as stated before. Nevertheless, antibodies to these bands were markedly increased in a time-dependent manner following primary challenge, indicating that these proteins may still be relatively specific. Furthermore, previous studies have demonstrated little cross-antigenicity between some closely related mite proteins (Cheong et al., 2003; Kuo et al., 2003).

The clinical and antibody response of sheep after a secondary challenge with *S. scabiei* var. *ovis* was also studied. A positive control for this infection was obtained by applying aliquots of challenge inocula to three *S. scabiei*-naïve ewes at the same time. The mange lesion area and the ELISA antibody levels of these positive control animals were similar to those observed in the primary infection group, which suggests that the pathogenicity of the mite doses used in the primary and secondary challenges was similar, so the two infections can validly be compared. The secondary infection group had a marked reduction in the mean mange lesion score compared with that of the same sheep in the primary infection group and no live *S. scabiei* mites were detected in any skin scraping collected in week 8 PC. These results suggest that the sheep had developed some immunity during the primary infection which was able to ameliorate the secondary infection, thus confirming previous reports concerning dogs, goats and rabbits (Arlian et al., 1994; Arlian et al., 1996b; Tarigan, 2002). This secondary infection was characterized by an anamnestic IgE response resulting in specific serum IgE levels significantly higher than those detected during the primary infection. The mechanism involved in the development of resistance to re-infection by *S. scabiei* remains unclear but it is believed that the early immune response occurring in the local skin

plays a major role and may be associated with IgE-mediated responses (Tarigan, 2003). This conclusion is also tentatively supported by the absence of specific serum IgE antibodies in a vaccination trial which failed to provide protection against infection in goats, despite the presence of high levels of specific serum IgG antibodies (Tarigan and Huntley, 2005). This lack of correlation between specific serum IgG levels and resistance agrees with the findings of Arlian et al. (1994), and the results from the present study supports the view that the development of resistance to *S. scabiei* is more closely correlated with the presence of specific serum IgE antibodies.

In summary, this study demonstrates that primary and secondary challenges of sheep with *S. scabiei* var. *ovis* results in both IgG and IgE antibody responses, and that secondary infections are ameliorated. The IgG antibody responses react to a number of antigens which would represent good targets for developing an immunodiagnostic test to detect *S. scabiei* var. *ovis* infections; conversely antigenic epitopes for IgE antibodies were mainly restricted to a 120 KDa band which would include a vaccine candidate.

5. Acknowledgements

The present study was partially financed by a grant (LE010A05) from the Education Bureau of the Castile and Leon Regional Government (Consejería de Educación de la Junta de Castilla y León). FRC holds a fellowship from the Education Bureau of the Castile and Leon Regional Government, co-financed by the European Social Fund. The authors are very grateful to Dr. J.F. Huntley (Moredun Research Institute, Penicuik, Scotland) for reading the manuscript with a critical eye and for kindly supplying the VPM6 and 2F1 mAbs, and the mite extract used in this work.

References

373 Arlian L.G., Morgan M.S., 2000. Serum antibody to *Sarcoptes scabiei* and house dust mite
374 prior to and during infestation with *S. scabiei*. Vet. Parasitol. 90, 315-326.
375 Arlian L.G., Morgan M.S., Arends L.L., 1996a. Immunologic cross-reactivity among various
376 strains of *Sarcoptes scabiei*. J. Parasitol. 82, 66-72.
377 Arlian L.G., Morgan M.S., Rapp C.M., Vyszenski-Moher D.L., 1996b. The development of
378 protective immunity in canine scabies. Vet. Parasitol. 62, 133-142.
379 Arlian L.G., Morgan M.S., Vyszenski-Moher D.L., Stemmer B.L., 1994. *Sarcoptes scabiei*:
380 the circulating antibody response and induced immunity to scabies. Exp. Parasitol. 78, 37-
381 50.
382 Arlian L.G., Vyszenski-Moher D.L., Ahmed S.G., Estes S.A., 1991. Cross-antigenicity
383 between the scabies mite, *Sarcoptes scabiei*, and the house dust mite, *Dermatophagoides*
384 *pteronyssinus*. J. Invest. Dermatol. 96, 349-354.
385 Bendixsen T., Windon R.G., Huntley J.F., Mackellar A., Davey R.J., McClure S.J., Emery
386 D.L., 2004. Development of a new monoclonal antibody to ovine chimeric IgE and its
387 detection of systemic and local IgE antibody responses to the intestinal nematode
388 *Trichostrongylus colubriformis*. Vet. Immunol. Immunopathol. 97, 11-24.
389 Bird P., Jones P., Allen D., Donachie W., Huntley J., McConnell I., Hopkins J., 1995.
390 Analysis of the expression and secretion of isotypes of sheep B cell immunoglobulins with a
391 panel of isotype-specific monoclonal antibodies. Res. Vet. Sci. 59, 189-194.
392 Bornstein S., Thebo P., Zakrisson G., 1996. Evaluation of an enzyme-linked immunosorbent
393 assay (ELISA) for the serological diagnosis of canine sarcoptic mange. Vet. Dermatol. 7,
394 21-27.
395 Bornstein S., Wallgren P., 1997. Serodiagnosis of sarcoptic mange in pigs. Vet. Rec. 141, 8-
396 12.

Bornstein S., Zakrisson G., Thebo P., 1995. Clinical picture and antibody response to experimental *Sarcoptes scabiei* var. *vulpes* infection in red foxes (*Vulpes vulpes*). Acta Vet. Scand. 36, 509-519.

Bradberry S.M., Cage S.A., Proudfoot A.T., Vale J.A., 2005. Poisoning due to pyrethroids. Toxicol. Rev. 24, 93-106.

Cheong N., Soon S.C., Ramos J.D.A., Kuo I.C., Kolortkar P.R., Lee B.W., Chua K.Y., 2003. Lack of human IgE cross-reactivity between mite allergens Blo t 1 and Der p 1. Allergy 58, 912-920.

Cordero del Campillo M., Rojo-Vázquez F.A. *Parasitología Veterinaria* (1999). McGraw-Hill Interamericana, Madrid, Spain.

Curie B.J., Harumal P., McKinnon M., Walton S.F., 2004. First documentation of *in vivo* and *in vitro* ivermectin resistance in *Sarcoptes scabiei*. Clin. Infect. Dis. 39, 8-12.

Curtis C.F., 2001. Evaluation of a commercially available of an enzyme-linked immunosorbent assay for diagnosis of canine sarcoptic mange. Vet. Rec. 148, 238-239.

Fthenakis G.C., Karagiannidis A., Alexopoulos C., Brozos C., Papadopoulos E., 2001. Effects of sarcoptic mange on the reproductive performance of ewes and transmission of *Sarcoptes scabiei* to newborn lambs. Vet. Parasitol. 95, 63-71.

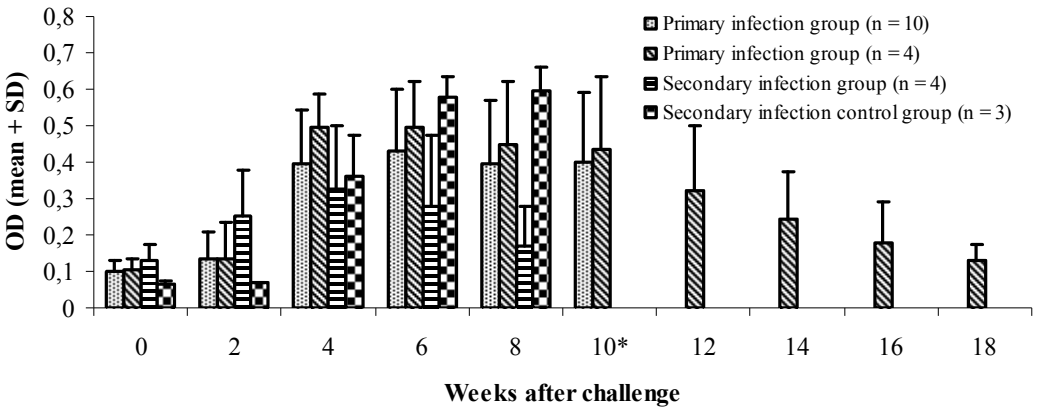
Fthenakis G.C., Papadopoulos E., Himonas C., Leontides L., Kritas S., Papatsas J., 2000. Efficacy of moxidectin against sarcoptic mange and effects on milk yield of ewes and growth of lambs. Vet. Parasitol. 87, 207-216.

Gutiérrez J.F., Méndez de Vigo J., Castellá J., Muñoz E., Ferrer D., 1996. Prevalence of sarcoptic mange in fattening pigs sacrificed in a slaughterhouse of northeastern Spain. Vet. Parasitol. 61, 145-149.

420 Imperiale F.A., Busetti M.R., Suárez V.H., Lanusse C.E., 2004. Milk excretion of ivermectin
421 and moxidectin in dairy sheep: assessment of drug residues during cheese elaboration and
422 ripening period. J. Agric. Food. Chem. 52, 6205-6211.
423 Jacobson M., Bornstein S., Wallgren P., 1999. The efficacy of simplified eradication
424 strategies against sarcoptic mange mite infections in swine herds monitored by an ELISA.
425 Vet. Parasitol. 81, 249-258.
426 Kuhn C., Lucius R., Matthes H.F., Meusel G., Reich B., Kalinna B.H., 2008. Characterisation
427 of recombinant immunoreactive antigens of the scab mite *Sarcoptes scabiei*. Vet. Parasitol.
428 153, 329-337.
429 Kuo I.C., Cheong N., Trakultivakorn M., Lee B.W., Chua K.Y., 2003. An extensive study of
430 human IgE cross-reactivity of Blo t 5 and Der p 5. J. Allergy Clic. Immunol. 111, 603-609.
431 Lower K.S., Medleau, L.M., Hnilica, K., Bigler, B., 2001. Evaluation of an enzyme-linked
432 immunosorbent assay (ELISA) for the serological diagnosis of sarcoptic mange in dogs.
433 Vet. Dermatol. 12, 315-320.
434 Morgan M.S., Arlian L.G., Estes S.A., 1997. Skin test and radioallergosorbent test
435 characteristics of scabietic patients. Am. J. Trop. Med. Hyg. 57, 190-196.
436 Rambozzi L., Menzano A., Lavin S., Rossi L., 2004. Biotin-avidin amplified ELISA for
437 detection of antibodies to *Sarcoptes scabiei* in chamois (*Rupicapra* spp.). Vet. Res. 35, 701-
438 708.
439 Rueda-López M., 2006. Elimination of mange due to *Sarcoptes scabie* var. *suis* from a 1800-
440 sow farrow-to-finish farm. Vet. Rec. 159, 595-597.
441 Sanderson H., Laird B., Pope L., Brain R., Wilson C., Johnson D., Bryning G., Peregrine A.S.,
442 Boxall A., Solomon K., 2007. Assessment of the environmental fate and effects of
443 ivermectin in aquatic mesocosms. Aquat. Toxicol. 85, 229-240.

444 Tarigan S., 2002. Dermatopathology of caprine scabies and protective immunity in sensitised
445 goats against *Sarcoptes scabiei*. Jurnal Ilmu Ternak dan Veteriner 7, 265-271.
446 Tarigan S., 2003. Histopathological changes in naïve and sensitised goats caused by
447 *Sarcoptes scabiei*. Jurnal Ilmu Ternak dan Veteriner 8, 114-121.
448 Tarigan S., 2004. Antibody responses in naïve and sensitised goats infested by *Sarcoptes*
449 *scabiei*. Jurnal Ilmu Ternak dan Veteriner 9, 258-265.
450 Tarigan S., Huntley J.F., 2005. Failure to protect goats following vaccination with soluble
451 proteins of *Sarcoptes scabiei*: evidence for a role for IgE antibody in protection. Vet.
452 Parasitol. 133, 101-109.
453 Van der Heijden H.M.J.F., Rambags P.G.M., Elbers A.R.W., van Maanen C., Hunneman
454 W.A., 2000. Validation of ELISAs for the detection of antibodies to *Sarcoptes scabiei* in
455 pigs. Vet. Parasitol. 89, 95-107.
456

457 Figure 1. Specific serum IgG (A) and IgE (B) antibodies measured by ELISA in the course of
458 primary and secondary experimental infections of sheep with *S. scabiei* var. *ovis*. * indicates
459 treatment with ivermectin of the primary infection group (n = 4), which corresponded with the
460 four sheep which took part in both primary and secondary infections.
461
462 Figure 2. WB strips showing recognition of *S. scabiei* var. *caprae* proteins by serum IgG (A)
463 and IgE (B) antibodies in the course of primary and secondary experimental infections of
464 sheep number 7 with *S. scabiei* var. *ovis*. Lane 1: prior to primary challenge; lane 2: week 4
465 after primary challenge; lane 3: week 8 after primary challenge; lane 4: prior to secondary
466 challenge; lane 5: week 4 after secondary challenge; and lane 6: week 8 after secondary
467 challenge.
468



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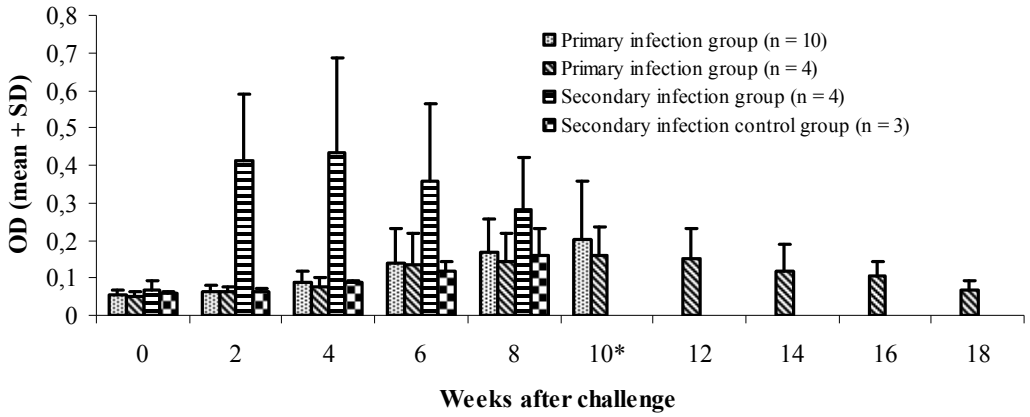

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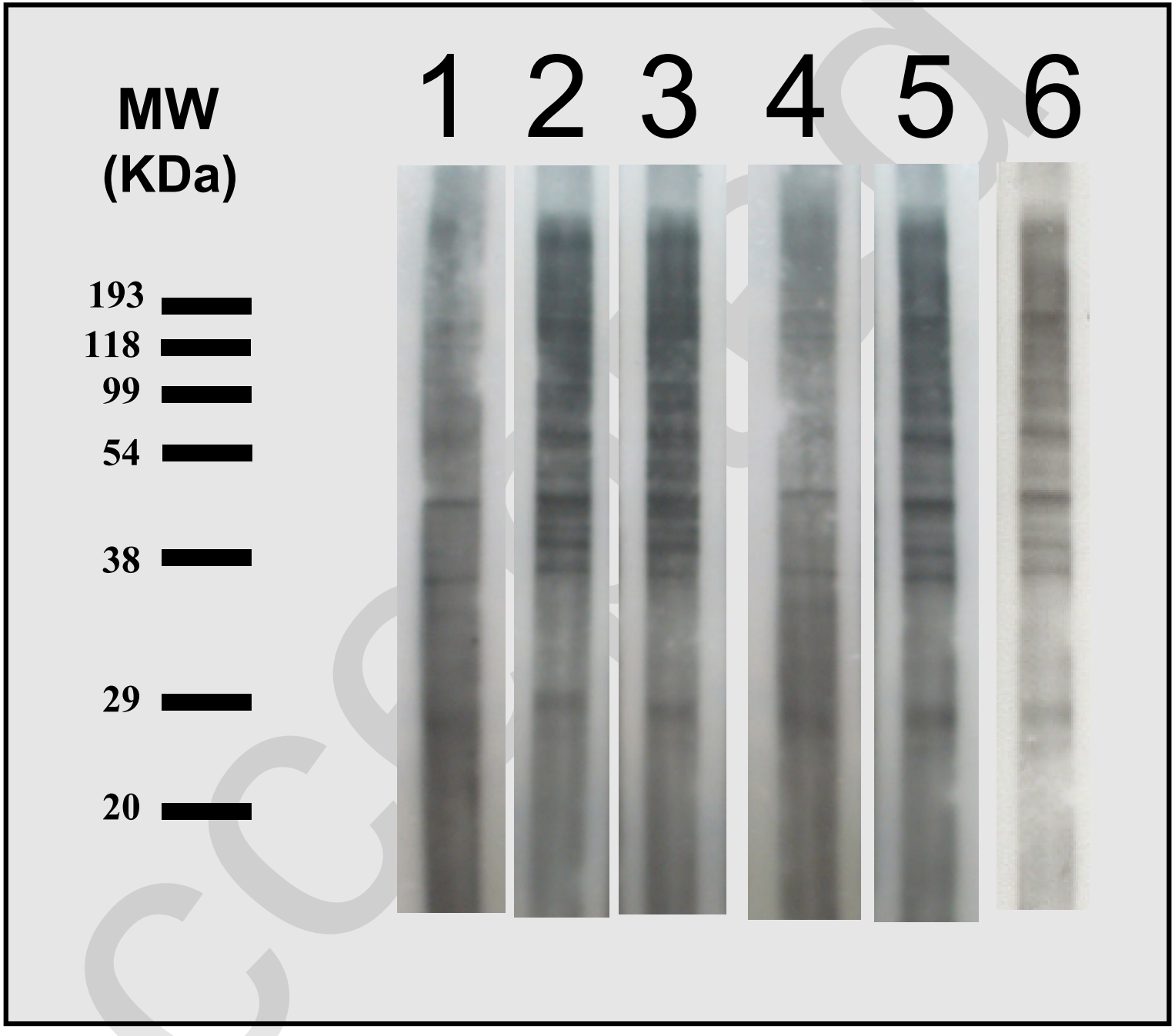
MW
(KDa)
193
118
99
54
38
29
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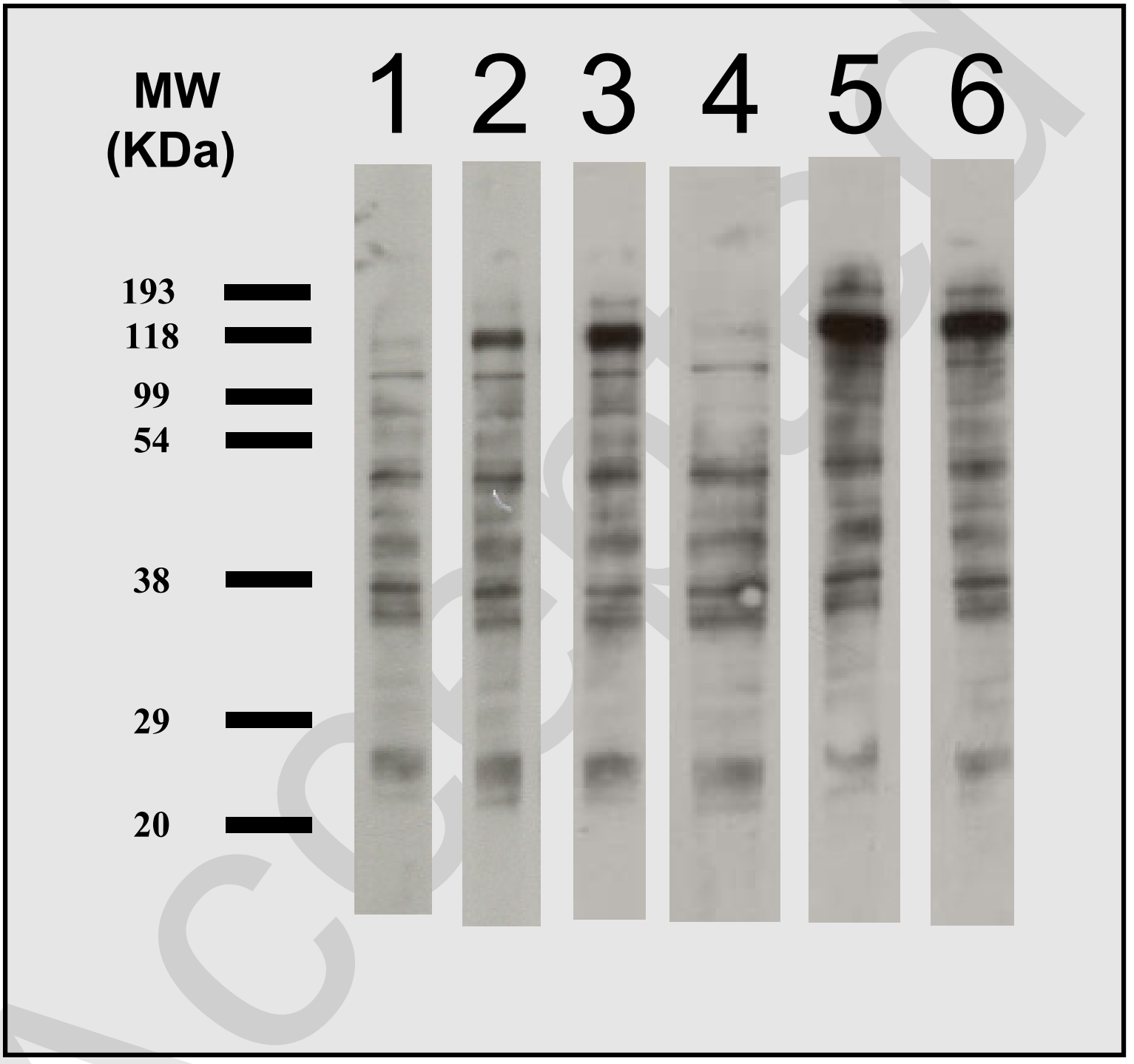
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Table 1. Mean mange lesion scores at different times in the course of primary and secondary experimental infections of sheep with *S. scabiei* var. *ovis*.

	Prior to challenge	Week 1 after challenge	Week 4 after challenge	Week 8 after challenge
Primary infection group (n = 10/n = 4) *	0/0	1.9/2.25	2.7/2.75	3.5/3.75
Secondary infection group (n = 4)	0	2	1.75	1.25
Secondary infection control group (n = 3)	0	2	2.67	4

* n = 10 includes the ten sheep, while n = 4 includes only the four sheep which took part in both primary and secondary infection groups.

Table 2. Frequencies of labelling (%) and mean binding intensities* of IgG in sheep sera collected at different stages in the course of primary and secondary experimental infections to *S. scabiei* var. *caprae* proteins.

Molecular weight (KDa)	Primary infection (n = 10)			Secondary infection (n = 4)		
	Prior to challenge	Week 4 after challenge	Week 8 after challenge	Prior to challenge	Week 4 after challenge	Week 8 after challenge
180	0%	0%	0%	0%	0%	25% / s
120	30% / w	80% / s	90% / s	0%	100% / m	50% / m
100	0%	80% / m	90% / m	0%	75% / w	50% / w
85	0%	80% / m	80% / m	0%	75% / w	50% / w
52	90% / w	100% / s	100% / s	100% / m	100% / m	75% / w
47	0%	80% / w	80% / w	0%	50% / w	75% / w
46	30% / w	100% / m	100% / m	0%	50% / m	75% / w
44	70% / w	100% / s	100% / s	75% / w	100% / m	100% / m
39	0%	90% / w	100% / w	0%	25% / w	25% / w
38	90% / w	100% / m	100% / m	50% / w	75% / w	75% / w
36	100% / w	100% / m	100% / m	50% / w	75% / w	75% / w

* It was estimated from only the sera positive to the band: w: weak; m: medium; s: strong; vs: very strong.

Table 3. Frequencies of labelling (%) and mean binding intensities* of IgE in sheep sera collected at different stages in the course of primary and secondary experimental infections to *S. scabiei* var. *caprae* proteins.

Molecular weight (KDa)	Primary infection (n = 10)			Secondary infection (n = 4)		
	Prior to challenge	Week 4 after challenge	Week 8 after challenge	Prior to challenge	Week 4 after challenge	Week 8 after challenge
180	0%	0%	40% / m	0%	75% / m	75% / w
120	10% / w	60% / s	70% / vs	50% / m	100 / vs	75% / s
100	0%	0%	10% / w	0%	25% /w	25% / w
90	10% / w	10% / w	50% / w	0%	100% / w	75% / w

* It was estimated from only the sera positive to the band: w: weak; m: medium; s: strong; vs: very strong.