



ELSEVIER

Veterinary Parasitology 111 (2003) 217–230

veterinary
parasitology

www.elsevier.com/locate/vetpar

Temporal pattern of isotype-specific antibody responses in primary and challenge infestations of sheep with *Psoroptes ovis*—the sheep scab mite

A.H.M. van den Broek^{a,*}, J.F. Huntley^b, J. Machell^b,
M.A. Taylor^c, H.R.P. Miller^a

^a Department of Veterinary Clinical Studies, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Veterinary Centre, Easter Bush, Roslin EH25 9RG, UK

^b Division of Parasitology, Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik EH26 0PZ, UK

^c Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB, UK

Received 24 May 2002; received in revised form 15 October 2002; accepted 16 October 2002

Abstract

In sheep *Psoroptes ovis* provokes an allergic dermatitis with significant *P. ovis* antigen-specific IgE responses. The kinetics of the IgE response to primary and challenge infestations of *P. ovis* were reported earlier [Parasite Immunol. 22 (2000) 407]. The present study examines IgG, IgM and IgA responses to primary and challenge infestations of *P. ovis* and the profile of antigens/allergens reacting with IgG and IgE antibodies. Antigen-specific enzyme-linked immunosorbent assays (ELISAs) demonstrate that primary infestations elicited significant increases in levels of IgG and IgM but not IgA antibodies. IgG and IgM responses to primary and challenge infestations were not significantly different. Western blots of reduced *P. ovis* proteins indicate that IgG antibodies reacted with five major antigens following primary infestation and only three of these after challenge infestation. IgE antibodies bound to three major and five minor allergens after primary infestation and two additional minor allergens after challenge infestation. Immunodominant antigens >100 and <15 kDa and allergens >100 kDa were most consistent in stimulating substantial IgG and IgE antibody responses, respectively. These antigens/allergens may be exploited in immunodiagnosis and modulation of the host immune response.

© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Sheep–arthropoda; *Psoroptes ovis*; ELISA; SDS-PAGE/Western blot; IgG; IgM; IgE

* Corresponding author. Tel.: +44-131-445-5111; fax: +44-131-445-6235.

E-mail address: adri.vandenbroek@ed.ac.uk (A.H.M. van den Broek).

1. Introduction

The astigmatid mite, *Psoroptes ovis*, is the highly contagious cause of sheep scab, a severe debilitating exudative dermatitis. This disease is accompanied by intense pruritus, extensive self-inflicted damage to fleece and skin, restlessness, loss of condition and occasionally epileptiform convulsions and death (Bygrave et al., 1993; Sargison et al., 1995; Bates, 1997). These clinical features, its rapid spread through a flock and increase in incidence since 1992 when dipping of sheep to control the disease ceased to be compulsory, make sheep scab one of major welfare and economic importance to the UK sheep industry (Kirkwood, 1986; Pearson, 1996; Anon, 1997).

A number of studies of infested sheep and cattle have suggested that IgE-mediated type 1 hypersensitivity plays a major role in the generation of cutaneous pathology (Stromberg and Fisher, 1986; Stromberg and Guillot, 1989; van den Broek et al., 2000). Host antibody responses may also contribute to the substantial protective immunity observed in sheep following primary infestations and vaccination (Bates, 2000; van den Broek et al., 2000; Smith et al., 2001). Analysis of antibody responses elicited by primary and challenge infestations and identification of antigens/allergens dominating these responses may give an indication of their role in the development of pathology and/or resistance.

P. ovis antigen-specific IgG and IgE but not IgM and IgA antibody responses to experimental infestations of naïve sheep have been reported (O'Brien, 1992; Bates, 1997; van den Broek et al., 2000). Several IgG- and IgE-reactive *P. ovis* antigens/allergens have been detected by SDS-PAGE/Western blot techniques (Boyce et al., 1991a; Matthes et al., 1996; Jayawardena et al., 1998). However, antigen binding by IgM and IgA antibodies and differences in the profile of antigen binding in primary and challenge infestations have not been reported.

In this study which extends our earlier work on the IgE antibody response (van den Broek et al., 2000), we monitored IgG, IgM and IgA antibody levels after primary and challenge infestations of sheep with *P. ovis*. In addition, SDS-PAGE/Western blot techniques were employed to identify *P. ovis* immunodominant antigens and allergens reacting with IgG and IgE antibodies, respectively, at different stages of primary and challenge infestations.

2. Materials and methods

2.1. Animals

2.1.1. Primary infestation (group 1)

Nine *P. ovis*-naïve × Suffolk sheep aged 1–2 years were infested with 25–50 ovigerous *P. ovis* mites as described previously (van den Broek et al., 2000). Blood samples were collected into vacutainer tubes, containing no anticoagulant (Becton Dickinson, Franklin Lakes, NJ, USA), by jugular venipuncture, and the serum was harvested. At 12 and 13 weeks after infestation the sheep were treated with an endectocide (Ivomec, MSB Agvet, 200 µg/kg).

2.1.2. Secondary (challenge) infestation (group 2)

Five of the treated sheep from group 1 were maintained mite-free for 11 weeks post-treatment and then were re-infested, as previously, with the 25–50 ovigerous *P. ovis* mites. Blood samples were taken from these sheep as described earlier.

2.1.3. Control (primary) infestation (group 3)

To ensure comparable pathogenicity of mites used in primary and challenge infestations, five *P. ovis*-naïve sheep (controls) were infested (group 3) with 25–50 ovigerous mites from the same batch as those used to infest the sheep in group 2. Blood samples were also taken from these sheep as described earlier.

2.2. Antigens

P. ovis and *Psoroptes cuniculi* whole-mite extracts were prepared as follows. *P. ovis* and *P. cuniculi* mites (adults, nymphs and larvae) were collected from infestations in sheep and rabbits, respectively. The live mites were washed by vortexing for 5 min in ice-cold phosphate buffered saline (PBS, pH 7.2), followed by 1% sodium dodecyl sulphate (SDS) at room temperature (RT) and finally 10 washes in ice-cold PBS. Mites were then homogenised and sonicated in ice-cold PBS. The final homogenate was centrifuged at 10,000 g for 10 min and the supernatant (whole-mite extract (WME)) harvested, aliquoted and stored at -70°C . Protein content of the supernatant was determined, in accordance with the manufacturer's instructions, with a BCATM Protein Assay Kit (Pierce, Illinois).

Preliminary analysis of SDS-PAGE/Western blots confirmed the earlier observation of almost complete homology between in the profile of *P. ovis* and *P. cuniculi* antigens revealed by antibodies in sera from infested sheep (Boyce and Brown, 1991). These preliminary studies also indicated that ovine immunoglobulin ingested by mites harvested from sheep did not produce 'false' antigens on Western blots. In view of this and the difficulty in obtaining an adequate supply of *P. cuniculi* mites, it was decided to employ *P. ovis* antigen in the SDS-PAGE/Western blot studies. However, contamination of *P. ovis* antigen with ovine immunoglobulin (Wassall et al., 1987; Beetham, 1997; Pettit et al., 2000) did give unacceptably high background ODs in the sensitive enzyme-linked immunosorbent assay (ELISA). Therefore, antigen derived from *P. cuniculi* was used in the ELISA studies.

2.3. Enzyme-linked immunosorbent assay studies

Micro-ELISA plates (M129B, Dynex Technologies, Ashford, Middlesex) were incubated for 1 h at RT with (50 μl per well) *P. cuniculi* WME (10 $\mu\text{g}/\text{ml}$) in carbonate/bicarbonate buffer (pH 9.6) and then washed twice with PBS/0.5% (v/v) Tween 20 (PBS/T20). On the basis of preliminary titrations, sera were diluted, 1/800 for IgG/IgM and 1/5 for IgA assays, in PBS/0.5% (v/v) Tween 80/0.5 M NaCl (PBS/T80/NaCl) and 50 μl of the test serum added to the wells. After incubation for 1 h at RT, the plates were washed six times with PBS/T20. Plates were then incubated with 50 μl of the appropriately diluted mouse anti-ovine monoclonal antibody, IgG (VPM6, 1/10 in PBS/T80/NaCl), IgM (VPM13, 1/1000 in PBS/T80/NaCl) or IgA (Serotec, 1/2000 in PBS/T80/NaCl). This incubation was followed by one with 50 μl biotinylated goat anti-mouse Ig conjugate (Dako, Ely Cambs,

UK; 1/1000 dilution in PBS/T80/NaCl) and streptavidin–horse radish peroxidase (Dako, 1/2000 dilution in PBS/T80/NaCl). Each incubation was for 1 h at RT and plates were then washed six times in PBS/T20. Colour reaction was developed by incubating with 50 μ l of 3,3',5,5'-tetramethylbenzidine (TMB) substrate (Dynex Technologies) for 10 min. Incubations were at RT and stopped by the addition of 50 μ l of 0.18 M H₂SO₄ (Voller et al., 1979). The OD of the colour reaction at 450 nm was measured in a Labsystems iEMF Reader (Labsystems Life Sciences International, Hampshire, UK). In order to compare results between assays, aliquots from a pool of positive sera were stored at -20°C and serum from a fresh aliquot was included on each plate and the OD₄₅₀ of unknown samples were adjusted relative to this. In practice, between-test variability was minimal.

2.4. SDS-PAGE/Western blots

Electrophoretic profiles of *P. ovis* WME were obtained by discontinuous SDS-PAGE. Mite extracts (100 μ l) were reduced and denatured by boiling for 5 min in 100 μ l sample buffer (2.5 ml stacking buffer (30.25 g Tris in 500 ml distilled water), 3.2 ml distilled water, 2 ml glycerol, 2 ml of 20% SDS, 1 ml β -mercaptoethanol, 0.2 ml of 0.1% bromophenol blue) and then loaded (15–20 μ g per lane) onto 0.75 mm thick 12% minigels with a 4% stacking gel. Protein standards were loaded into an additional well and each gel run at 200 V for 35–45 min. The separated proteins were transferred onto nitrocellulose (NC) membrane using a semi-dry transfer apparatus at 70 A per gel for 60 min at RT. After transfer, the NC membranes were stained with 0.1% (w/v) Ponceau S in 5% (v/v) acetic acid (Sigma, Gillingham, Dorset) and cut into strips. The strip with molecular weight markers (MultiMarkTM, Novex, San Diego, USA) was dried and stain was removed from strips containing separated mite proteins by three washes in 5% acetic acid followed by a rinse in distilled water and a final wash in PBS/T80/NaCl. These were then incubated in PBS/T80/NaCl for 30 min at RT on an orbital shaker (100 cycles/min) to block non-specific protein adsorption. Subsequently, to detect reactivity with *P. ovis* antigen-specific antibodies, the blotted proteins were probed with test sera. Antigens binding IgG and IgM antibody were identified with sera prepared at a dilution of 1/100 in PBS/T80/NaCl and IgA reactivity was probed with sera prepared at a dilution of 1/10 in PBS/T80/NaCl. Reactivity with IgE antibodies was determined by incubation with pre-treated sera. Briefly, serum samples (100 μ l) were heated at 56°C for 30 min and then precipitated in 30 and 50% saturated ammonium sulphate (Kooyman et al., 1997). The final precipitate was dissolved in 200 μ l of PBS/T80/NaCl and this solution employed to probe allergen binding. After incubation for 1 h at RT with constant rocking the blots were washed three times in PBS/T80/NaCl. They were then incubated with mouse anti-ovine monoclonal antibody (IgG (VPM6, 1/10 in PBS/T80/NaCl), IgM (VPM13, 1/1000 in PBS/T80/NaCl), IgA (Serotec, 1/200 in PBS/T80/NaCl) and IgE (IE7, 1/10 in PBS/T80/NaCl)) for 1 h. This was followed by incubation with biotinylated goat anti-mouse Ig (Dako, 1/1000 in PBS/T80/NaCl) for 1 h and streptavidin–horseradish peroxidase (Dako, 1/2000 in PBS/T80/NaCl) for 30–60 min. Each incubation was at RT on an orbital shaker (100 cycles/min) and followed by three washes in PBS/T80/NaCl. Finally peroxidase activity in blots incubated with anti-ovine IgG, IgM or IgA was revealed with diaminobenzidine/urea/H₂O₂ substrate (Sigma FastTM DAB tablet, Sigma). Peroxidase activity in blots incubated with anti-ovine IgE was detected by chemiluminescence

as described by the manufacturer (ECLTM Western blotting, Amersham). Pooled sera collected from sheep before primary infestation and after 10 weeks after challenge infestation were used, respectively, as negative and positive controls. In additional control blots, serum or monoclonal antibody were replaced by wash diluent.

2.5. Statistical analysis

Differences between the groups in the isotype-specific antibody responses were evaluated by analysis of co-variance as described previously (van den Broek et al., 2000). The two-tailed student's *t*-test was employed to determine the significance of differences in antibody levels within each group.

3. Results

3.1. ELISA studies

Sera were collected weekly from 0 to 14 weeks and at week 16 of the primary infestation. *P. ovis* antigen-specific IgG, IgM and IgA antibody levels were estimated in samples collected from five sheep (group 1). Later IgG, IgM and IgA antibody levels to *P. ovis* were determined in sera collected weekly from 0 to 8 weeks after challenge re-infestation of these five sheep (group 2) and the accompanying control (primary) infestations (group 3).

3.1.1. IgG antibody

A significant ($P = 0.046$) increase in mean IgG antibody level (Fig. 1) was first seen 2 weeks after primary infestation (group 1). Antibody levels of group 1 sheep rose more rapidly than those of group 3 and were significantly greater ($P < 0.003$) than those of group 3 at 7 weeks. Peak antibody levels (group 1) were achieved from 8 to 12 weeks after infestation. In challenge infestations (group 2) there was a prompt but transient IgG antibody response (Fig. 1) though statistically it was not significantly different from the baseline value. No significant differences were detected in antibody levels of sheep with primary (groups 1 and 3) and challenge (group 2) infestations.

3.1.2. IgM antibody

A significant ($P = 0.04$) rise in mean IgM antibody level was first detected 7 weeks after primary infestation (group 1). There was no statistical difference between IgM antibody levels of sheep with primary and challenge infestations (Fig. 2).

3.1.3. IgA antibody

No increase in *P. ovis* antigen-specific IgA level was detected following primary or challenge infestation.

3.2. SDS-PAGE/Western blots

As Western blots probed with anti-IgM and -IgA antibody revealed only faint labelling of antigens, which was difficult to interpret, these results are omitted.

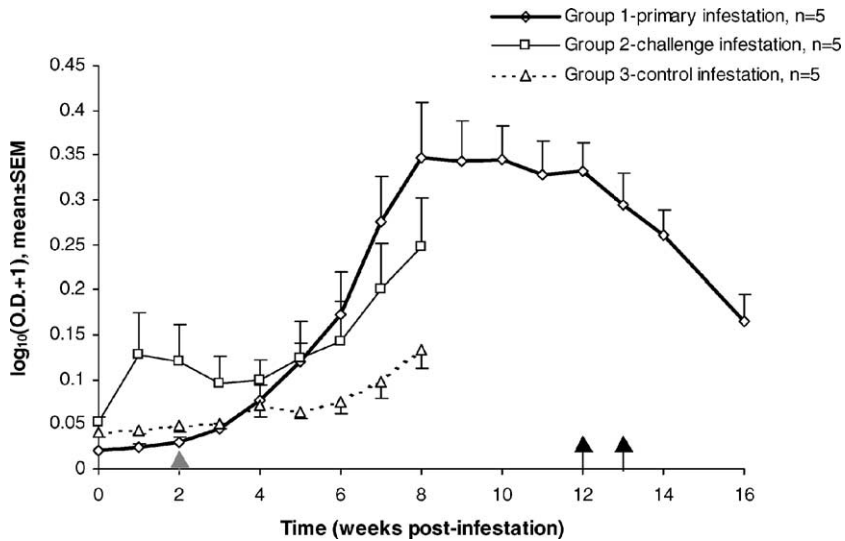


Fig. 1. *P. ovis* antigen-specific IgG levels in the course of primary and challenge infestations. The grey arrow indicates first occurrence of a significant increase in antibody level during primary infestations and the black arrows indicate treatment with endectocide. At 7 weeks the IgG level in group 1 was significantly greater ($P < 0.003$) than that in group 3 but no significant differences were detected between IgG levels of sheep with primary (groups 1 and 3) and challenge (group 2) infestations.

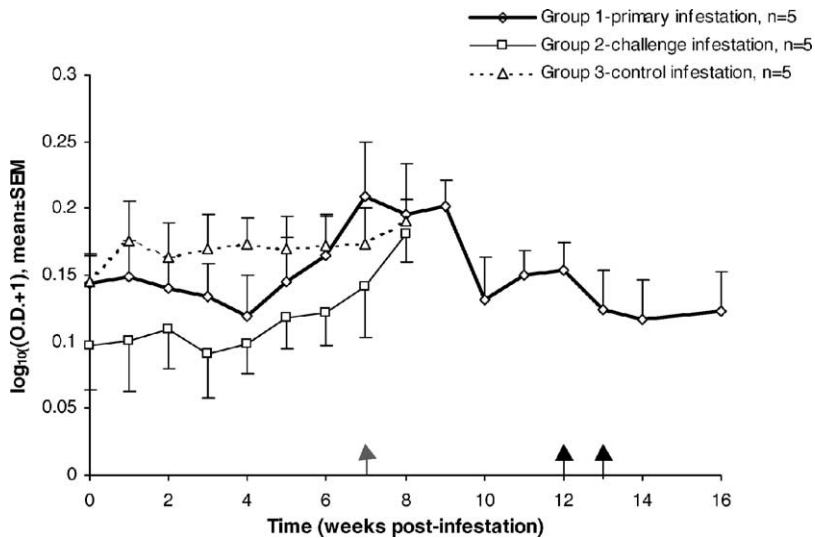


Fig. 2. *P. ovis* antigen-specific IgM levels in the course of primary and challenge infestations. No significant differences were detected between IgM levels of sheep with primary (groups 1 and 3) and challenge (group 2) infestations.

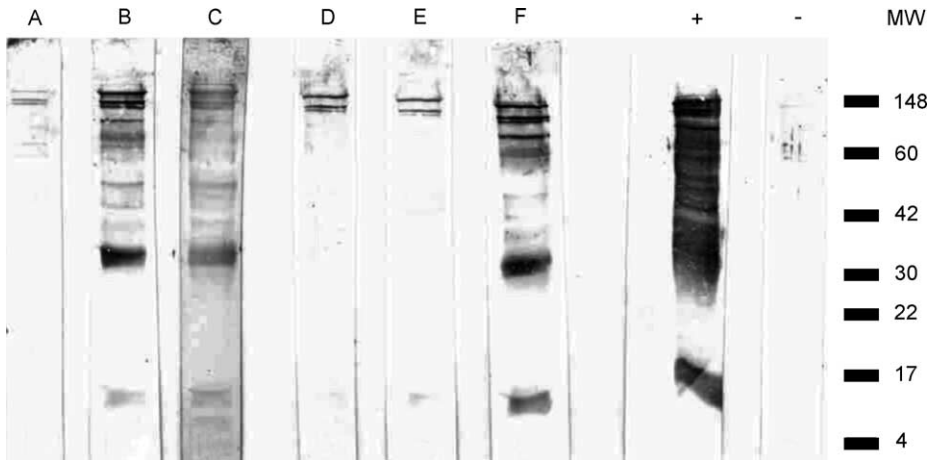


Fig. 3. Sheep 3: development of *P. ovis* antigen recognition by IgG antibodies in the course of primary and secondary infestations. Primary infestation: lane A, pre-infestation; lane B, mid-infestation; lane C, end-infestation. Challenge infestation: lane D, pre-challenge; lane E, 2 weeks post-challenge; lane F, 6 weeks post-challenge. (+) Positive control (pooled sera from terminal bleed of challenged sheep); (–) negative control (pooled sera collected from sheep before primary infestation).

Major IgG-reactive antigens and major IgE-reactive allergens were defined as those binding IgG and IgE antibodies, respectively, in at least 50% of the sera tested and demonstrating strong reactivity with at least 50% of these (Lowenstein, 1978). Blots demonstrating the development of antigen and allergen labelling by IgG and IgE antibodies, respectively, in the course of primary and challenge infestations are shown in Figs. 3 and 4, respectively.

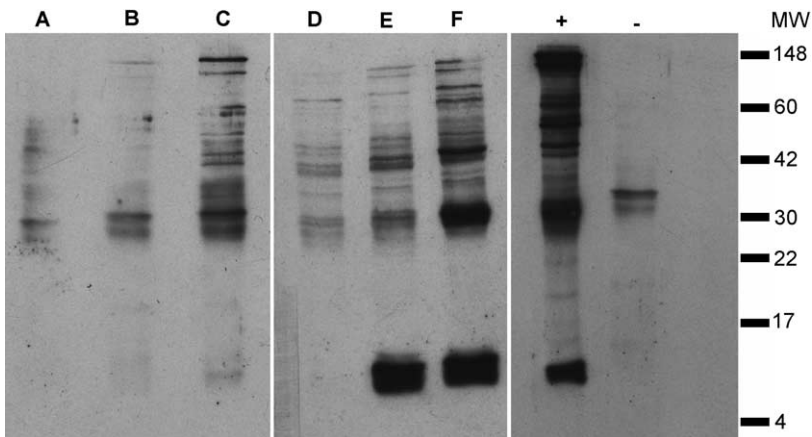


Fig. 4. Sheep 3: development of *P. ovis* allergen recognition by IgE antibodies in the course of primary and secondary infestations. The evident differences in the intensity of IgG and IgE labelling seen in Fig. 3 and this figure are partly due to the use of chemiluminescence to detect peroxidase activity in blots incubated with anti-ovine IgE, this greatly enhanced detection of IgE binding.

Table 1

Major IgG-reactive antigens detected in the course of primary infestations

Molecular weight (kDa)	Control (pre-infestation)						Weeks 4–6						Weeks 11–13						Pooled sera ^a
	1 ^b	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	
>100							s	s	s			s	m	vs	m	m	s	s	vs
90																			
80																			
70																			
65																			
54–57		m						m	w			w	s	s	w			w	m
50																			
45–47		m						m	w			w	m	s	w			s	m
40																			
28–34			w				m	m	s			m	m	s	m			s	s
<15							m	m	m			m	s	s	w	vs	vs	m	s

Intensity of antibody labelling: w, weak; m, medium; s, strong; vs, very strong.

^a Sera collected from terminal bleed at conclusion of challenge infestation.^b Sheep number.

3.2.1. Primary infestation

In primary infestations, binding of IgG antibodies to reduced antigens and of IgE antibodies to allergens was evaluated in blots of *P. ovis* proteins probed with sera collected from six sheep in group 1 before infestation, at 4–6 and 11–13 weeks after infestation. Clinically, the 4–6-week period represented the mid-stage of lesional development and by 11–13 weeks lesions were resolving.

Individual animals exhibited considerable variation in the profile of antigens and allergens labelled by IgG and IgE antibodies, respectively. As the infestations progressed, IgG and IgE antibodies reacted with a greater number of antigens and allergens, respectively, and the intensity of labelling increased. At 11–13 weeks after primary infestation five major IgG-reactive antigens (Table 1) and three major and four minor IgE-reactive allergens (Table 2) were detected on Western blots. Several other antigens were labelled by immunoglobulin IgG (MW: 80, 65, 40 and 20 kDa) and further allergens were labelled by immunoglobulin IgE (MW: 90 and 80 kDa) but these were weakly reactive and occurred less consistently. Antigens >100 and <15 kDa and allergens >100 kDa were more consistent than others in stimulating substantial IgG and IgE antibody responses, respectively.

3.2.2. Challenge infestation

In challenge infestations, binding of IgG and IgE antibody was determined in blots probed with sera collected from three sheep in group 2 immediately before challenge and 2 and 6 weeks after challenge.

IgG antibodies in pre-challenge sera reacted weakly with up to three of the major antigens identified during primary infestation (Table 3) while IgE antibodies labelled one major and up to four minor allergens (Table 4). Two of these minor allergens (MW: 80 and 90 kDa) were detected only inconsistently at 11–13 weeks after primary infestation. There was a

Table 2
Major IgE-reactive allergens detected in the course of primary infestations

Molecular weight (kDa)	Control (pre-infestation)						Weeks 4–6						Weeks 11–13						Pooled sera ^a
	1 ^b	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	
>100							m	w	w	w	m	m	vs	vs	s	s	vs	vs	vs
90																			
80																			
70					w						w		m	m	m	w	w	m	w
65					w						w		w	w	m	w	vs		w
54–57											w	w	w	s	m	s	vs	m	s
50				w	w		w	w	w	w			m	w	w	w	m		w
45–47				w	w				w	m			m	s	m	m	m		m
40																			
28–34	w	w	w	m	m		m	m	m	m	s	w	s	m	s	m	s	w	m
<15															w				s

Intensity of antibody labelling: w, weak; m, medium; s, strong; vs, very strong.

^a Sera collected from terminal bleed at conclusion of challenge infestation.

^b Sheep number.

more rapid and marked increase in the intensity of allergen labelling and in the number of allergens identified after challenge than primary infestation (Table 4). This is illustrated by comparison of the intensity of allergen labelling 4–6 weeks after primary infestation with that seen 2 weeks after challenge (Fig. 4, lanes B and E, respectively). Enhancement IgG antibody binding was also seen but developed more gradually than the increase in IgE antibody binding. This is seen by comparison of the intensity with which IgG-reactive antigens were labelled 4–6 weeks after primary infestation and 2 weeks after challenge (Fig. 3, lanes B and E, respectively).

Table 3
Major IgG-reactive antigens detected in the course of challenge infestations

Molecular weight (kDa)	Control (before re-infestation)			Week 2			Week 6		
	3 ^a	5	6	3	5	6	3	5	6
>100	m	w	w	m	w	m	s		m
90									
80									
70									
65									
54–57							w		w
50									
45–47			w	w		w	w		w
40									
28–34		w			m		s	w	
<15			w	w		w	m		w

Intensity of antibody labelling: w, weak; m, medium; s, strong.

^a Sheep number.

Table 4

Major IgE-reactive allergens detected in the course of challenge infestations

Molecular weight (kDa)	Control (before re-infestation)			Week 2			Week 6		
	3 ^a	5	7	3	5	7	3	5	7
>100				w	s	m	m	s	m
90			m	w	s	s	m	s	s
80			m				w		s
70							m		m
65									
54–57							m		m
50	w		m	w	s	s	m	s	s
45–47	w	m	m	m	s	m	s	s	s
40	w			w	m		m	m	
28–34	w	m	m	m	s	m	vs	vs	m
<15				vs			vs		vs

Intensity of antibody labelling: w, weak; m, medium; s, strong; vs, very strong.

^a Sheep number.

4. Discussion

The present ELISA studies demonstrate that experimental infestations with *P. ovis* elicit IgG and IgM antigen-specific isotypes. We also identified a defined number of major IgG-reactive antigens and IgE-reactive allergens that may be important in eliciting the immune response to this mite. The kinetics of the IgG antibody response to primary infestation was comparable to that observed in earlier studies of infestations in sheep (Bates, 1997), cattle (Fisher, 1983; Pruett et al., 1986; Lonneux et al., 1996) and rabbits (Uhlir, 1991). The reason for the divergence in serum IgG responses of group 1 and 3 sheep (Fig. 1) to primary infestations is uncertain. As reported earlier (van den Broek et al., 2000), leisional growth and by implication growth of the mite population in the two groups was similar. This suggests a that the difference may reflect a disparity in the immune responsiveness of the two groups. In cattle (Fisher, 1983), challenge infestations did not elicit an anamnestic IgG antibody response. In the present study, an anamnestic antigen-specific IgG antibody response was detected but because individual responses varied widely this response was not statistically significant. This contrasts with the report of a significant, rapid and sustained increase of antigen-specific IgE levels in the serum of sheep after challenge infestations of *P. ovis* (van den Broek et al., 2000). The observation of a gradual increase in intensity of IgG binding and rapid increase in that of IgE binding on Western blots labelled, respectively, by IgG and IgE antibodies in sera collected from sheep after challenge infestations is consistent these ELISA findings. The present study failed to detect evidence of a *P. ovis* antigen-specific IgA antibody response, though detection of faint staining on SDS-PAGE/Western blots suggested that a weak response did occur. The IgA antibody response to ectoparasites other than *Sarcoptes scabiei* does not appear to have been reported. Several studies of human patients and one of dogs with sarcoptic mange recorded a fall in total serum IgA antibody levels that was reversed after successful treatment (Falk, 1980; Morsy et al., 1993; Nassef et al., 1991; Thoday, 1993).

However, increased numbers of IgA-positive cells have been detected in lesional skin of pigs with sarcoptic mange (Morsy and Gaafar, 1989). Studies in cattle, vaccinated intradermally with *Dermatophilus congolensis*, have provided evidence of local production of antigen-specific IgA and its secretion onto the surface of the skin in the absence of a rise in serum antigen-specific IgA (Lloyd and McEwan Jenkinson, 1981). It is possible, therefore, that IgA antibody may be involved in host defence even though it is not detected serologically.

The role, if any, of the different isotypes responses in host defence is not clear. Ovine immunoglobulin has been detected in *P. ovis* mites harvested from infested sheep (Pettit et al., 2000). Host immunoglobulins could, therefore, have a direct, adverse effect on parasite survival. In a recent report, vaccination of calves with crude preparations of *P. ovis* allergens induced immediate and 24–48 h hypersensitivity responses (Pruett et al., 1998). Although after challenge infestation no statistically significant difference was detected in mite numbers and lesional areas on vaccinated and control calves, there was a trend that suggested vaccination increased resistance to infestation. This was attributed to an augmented IH response that resulted in pruritus and increased self-grooming. In our earlier investigation of IgE antibody responses in the sheep used in the present study, we reported that the challenged sheep (group 2) were resistant to re-infestation (van den Broek et al., 2000). Although speculative, it is possible that the early IgE response rather than the IgG response was critical in the development of this resistance. This would be consistent with the observations of Pruett et al. (1998) and provides supportive evidence of the involvement of IgE-mediated mechanisms in host resistance to *P. ovis*.

The profile of *P. ovis* antigens/allergens on SDS-PAGE/Western blots labelled by sera collected during primary infestation was similar to those reported previously in sheep and confirms the diversity of individual responses to *P. ovis* antigens and allergens (Boyce et al., 1991a; Matthes et al., 1996; Jayawardena et al., 1998). Although the present study suggests that IgE antibodies react with a greater number proteins than IgG antibodies it is possible that this difference is simply attributable to the greater sensitivity of the technique (chemiluminescence) employed to detect IgE binding. A number of proteins appeared to bind both IgG and IgE antibodies. These could be proteins possessing epitopes that bind IgG or IgE or both or different proteins of the same or similar molecular weights that possess either IgG- or IgE-binding epitopes. The Western blots also indicated that as the infestations progressed, IgG and IgE antibodies reacted with a greater number of antigens and allergens, respectively, and that the intensity of antigen and allergen labelling increased. This is consistent with the observation of a progressive increase mite numbers and therefore antigenic challenge in the course of primary infestations (Bates, 1997). In addition, simulation analysis of population dynamics suggests that if the immune response reduces adult female fecundity the relative numbers of eggs, larvae, nymphs and adults challenge will alter in the later stages of primary infestations (Wall et al., 1999). As a consequence there may be a corresponding change in the profile of antigens and allergens detected on Western blots. We have previously reported the failure of challenge infestations in group 2 sheep to become established (van den Broek et al., 2000), this would result in a weaker antigenic challenge and probably accounts for the observation that fewer IgG-reactive proteins were labelled with IgG in challenge than primary infestations. The observation that some immunodominant antigens, particularly those >100 kDa, were

recognised more consistently than others, may have implications for the serodiagnosis of sheep scab. At present only two of *P. ovis* antigens/allergens have been characterised. One, povDerp1, displayed a sequence homology with the group 1 mite allergen Derp1 derived from *Dermatophagoides pteronyssinus* and migrated as a 36 kDa band (Lee et al., 2002). The other, a 16 kDa polypeptide, elicited an immediate cutaneous hypersensitivity reaction in cattle and possessed a sequence homology with group 2 mite allergens Lepd2 and Derf2, from *Lepidoglyphus destructor* and *Dermatophagoides farinae*, respectively (Pruett, 1999).

At present diagnosis of sheep scab is suggested by history and clinical signs and is confirmed by identification of *P. ovis* mites in scrapings from lesional skin. Inevitably, animals with sub-clinical disease or minimal lesions may escape detection. Although studies of experimentally infested cattle have indicated that some cattle with minimal lesions remain seronegative on ELISA (Fisher et al., 1986; Lonneux et al., 1998) and the present study indicates that sheep with similar lesion areas may have considerably different IgG responses, employment of an immunodiagnostic test may provide a more reliable method for screening a flock/herd than clinical examination. Recently, Ochs et al. (2001) described a sensitive sandwich ELISA for the diagnosis of *P. ovis* infestation in sheep. Although this assay employed crude *P. cuniculi* extract its diagnostic sensitivity was cited as 93.7% and its specificity as 96.5%. However, it is notable that in this evaluation of specificity, four out of four sheep with chorioptic mange and three out of five sheep with forage mites gave ELISA readings above the cut off value. Earlier studies employing ELISA and/or SDS-PAGE/Western blot techniques have revealed cross-reactivity in the antigen repertoire of *Psoroptes* spp. mites and related mites such as *Chorioptes bovis*, *Sarcoptes suis* and *Notedres cati* (Matthes et al., 1996) and *D. pteronyssinus* (Stewart and Fisher, 1986). Cross-reactivity with ixodid (*Dermacentor variabilis*) and argasid (*Ornithodoros* spp.) ticks has also been reported (den Hollander and Allen, 1986; Wozniak et al., 1996). This cross-reactivity may explain the observation in the present study of immunoglobulin IgE-reactive bands in a significant number of pre-infestation sera. More importantly, cross-reactivity of this nature will compromise specificity and reduce the reliability of serodiagnostic tests. However, the present study and earlier investigations suggested that a specific profile of antigen recognition may characterise *P. ovis* infestations (Boyce et al., 1991a; Matthes et al., 1996). The present study also indicated that some immunodominant antigens stimulate seroconversion early in the course of infestation. Identification and isolation of these *Psoroptes* spp.-specific antigens and their expression as recombinant proteins might improve sensitivity and specificity of immunodiagnostic tests and importantly guarantee their reproducibility. These may provide a sensitive method for screening flocks and individual animals and facilitate the implementation of control programmes. They could also be used for epidemiological surveys (Lonneux et al., 1996; Boyce et al., 1991b).

The SDS-PAGE/Western blots also identified allergens dominating IgE antibody responses to primary and challenge infestations. Histopathology suggests that IgE-mediated responses contribute to cutaneous pathology while the IgE antibody response in challenge infestations indicates that they may also be involved in host defence (van den Broek et al., 2000). These allergens may, therefore, be suitable candidates for inclusion in vaccines designed to modulate the host response to infestation. They would also be of value in more detailed studies of the immunopathogenesis of sheep scab.

Acknowledgements

The funding of this work by MAFF (Contract CSA 3503) and advice of I. McKendrick of BioSS in statistical analysis are gratefully acknowledged. Dr. J.F. Huntley received funding from the Scottish Executive, Environment and Rural Affairs Department.

References

- Anon, 1997. Vet. Rec. 140, 163.
- Bates, P., 1997. The pathogenesis and aging of sheep scab lesions. Part 1. State Vet. J. 7 (3), 13–16.
- Bates, P., 2000. Differences between primary and secondary infestations with the sheep scab mite, *Psoroptes ovis*. Vet. Rec. 146, 528–529.
- Beetham, P.K., 1997. Sheep scab mite (Acari: Psoroptidae): general morphology and immunocytochemistry using serum from infested animals. Ann. Entomol. Soc. Am. 90, 202–207.
- Boyce, W.M., Brown, R.N., 1991. Antigenic characterization of *Psoroptes* spp. (Acari: Psoroptidae) mites from different hosts. J. Parasitol. 77, 675–679.
- Boyce, W.M., Jessup, D.A., Clark, R.K., 1991a. Serodiagnostic antibody responses to *Psoroptes* sp. infestations in bighorn sheep. J. Wildl. Dis. 27, 10–15.
- Boyce, W.M., Mazet, J.A., Mellies, J., Gardner, I., Clark, R.K., Jessup, D.A., 1991b. Kinetic ELISA for detection of antibodies to *Psoroptes* sp. (Acari: Psoroptidae) in bighorn sheep (*Ovis canadensis*). J. Parasitol. 77, 692–696.
- Bygrave, A.C., Bates, P.G., Daniel, N.J., 1993. Epileptiform seizure in ewes associated with sheep scab mite infestation. Vet. Rec. 132, 394–395.
- den Hollander, N., Allen, J.R., 1986. Cross-reactive antigens between a tick, *Dermacentor variabilis* (Acari: Ixodidae), and a mite, *Psoroptes cuniculi* (Acari: Psoroptidae). J. Med. Entomol. 23, 44–50.
- Falk, E.S., 1980. Serum immunoglobulin values in patients with scabies. Br. J. Dermatol. 102, 57–61.
- Fisher, W.F., 1983. Development of serum antibody activity as determined by enzyme-linked immunosorbent assay to *Psoroptes ovis* (Acarina: Psoroptidae) antigens in cattle infested with *P. ovis*. Vet. Parasitol. 13, 363–373.
- Fisher, W.F., Guillot, F.S., Cole, N.A., 1986. Development, experimental and decline of serum antibody activity to *Psoroptes ovis* antigens in infested cattle in an endemic and non-endemic scabies area of Texas. Exp. Appl. Acarol. 2, 239–248.
- Jayawardena, K.G., Heller-Haupt, A., Woodland, R.M., Varma, M.G., 1998. Antigens of the sheep scab mite *Psoroptes ovis*. Folia Parasitol. 45, 239–244.
- Kirkwood, A.C., 1986. History, biology and control of sheep scab. Parasitol. Today 2, 302–307.
- Kooyman, F.N., Van Kooten, P.J., Huntley, J.F., MacKellar, A., Cornelissen, A.W., Schallig, H.D., 1997. Production of a monoclonal antibody specific for ovine immunoglobulin E and its application to monitor serum IgE responses to *Haemonchus contortus* infection. Parasitology 114 (Part 4), 395–406.
- Lee, A.J., Machell, J., van den Broek, A.H.M., Miller, H.R.P., Isaac, R.E., Huntley, J.F., 2002. Identification of an antigen from the sheep scab mite, *Psoroptes ovis*, homologous with house dust mite group I allergens. Parasite Immunol. 24, 413–422.
- Lloyd, D.H., McEwan Jenkinson, D.M., 1981. Serum and skin surface antibody responses to intradermal vaccination of cattle with *Dermatophilus congolensis*. Br. Vet. J. 137, 601–607.
- Lonneux, J.F., Nguyen, T.Q., Hollanders, W., Losson, B.J., 1996. A sandwich enzyme-linked immunosorbent assay to study the antibody response against *Psoroptes ovis* in cattle. Vet. Parasitol. 64, 299–314.
- Lonneux, J.F., Nguyen, T.Q., Hollanders, W., Denis, M., Thiry, E., Pastoret, P.P., Losson, B., 1998. Humoral and cell-mediated immune responses of beef and dairy cattle experimentally infested with *Psoroptes ovis*. Am. J. Vet. Res. 59, 583–587.
- Lowenstein, H., 1978. Quantitative immunoelectrophoretic methods as a tool for the analysis and isolation of allergens. Prog. Allergy 25, 1–62.
- Matthes, H.F., Harrison, G.B., Shaw, R.J., Heath, A.C., Pfeffer, A., Hiepe, T.H., 1996. Cross-reacting antibodies to *Sarcoptes suis*, *Chorioptes bovis* and *Notoedres cati* and anti-*P. ovis* IgE in sera from sheep infested naturally with *Psoroptes ovis*. Int. J. Parasitol. 26, 437–444.

- Morsy, G.H., Gaafar, S.M., 1989. Responses of immunoglobulin-secreting cells in the skin of pigs during *Sarcoptes scabiei* infestation. Vet. Parasitol. 33, 165–175.
- Morsy, T.A., Kenawi, M.Z., Zohdy, H.A., Abdalla, K.F., el Fakahany, A.F., 1993. Serum immunoglobulin and complement values in scabietic patients. J. Egypt. Soc. Parasitol. 23, 221–229.
- Nassef, N.E., Makled, K.M., Elzayat, E.A., Sanad, M.M., 1991. Humoral and cell mediated immune responses in scabietic patients. J. Egypt. Soc. Parasitol. 21, 765–770.
- O'Brien, D.J., 1992. Studies on the epidemiology of psoroptic mange in Ireland. Ph.D. thesis, National University of Ireland, Dublin.
- Ochs, H., Lonneux, J., Losson, B.J., Deplazes, P., 2001. Diagnosis of psoroptic sheep scab with an improved enzyme-linked immunosorbent assay. Vet. Parasitol. 96, 233–242.
- Pearson, P., 1996. Parasitic damage to sheepskins. In: Proceedings of the Conference on Sheep Scab, Tralee, Republic of Ireland, pp. 22–25.
- Pettit, D., Smith, W.D., Richardson, J., Munn, E.A., 2000. Localisation and characterisation of ovine immunoglobulin within the sheep scab mite, *Psoroptes ovis*. Vet. Parasitol. 89, 231–239.
- Pruett, J.H., 1999. Identification and purification of a 16 kDa allergen from *Psoroptes ovis* (Acarina: Psoroptidae). J. Med. Entomol. 36, 544–550.
- Pruett, J.H., Guillot, F.S., Fisher, W.F., 1986. Humoral and cellular immunoresponsiveness of stanchioned cattle infested with *Psoroptes ovis*. Vet. Parasitol. 22, 121–133.
- Pruett, J.H., Temeyer, K.B., Fisher, W.F., Beetham, P.K., Kunz, S.E., 1998. Evaluation of natural *Psoroptes ovis* (Acarina: Psoroptidae) soluble proteins as candidate vaccine immunogens. J. Med. Entomol. 35, 861–871.
- Sargison, N.D., Scott, P.R., Penny, C.D., Pirie, R.S., 1995. Effect of an outbreak of sheep scab (*Psoroptes ovis* infestation) during mid-pregnancy on ewe body condition and lamb birthweight. Vet. Rec. 136, 287–289.
- Smith, W.D., van Den, B.A., Huntley, J., Pettit, D., Machell, J., Miller, H.R., Bates, P., Taylor, M., 2001. Approaches to vaccines for *Psoroptes ovis* (sheep scab). Res. Vet. Sci. 70, 87–91.
- Stewart, G.A., Fisher, W.F., 1986. Cross-reactivity between the house dust mite *Dermatophagoides pteronyssinus* and the mange mites *Psoroptes cuniculi* and *P. ovis*. I. Demonstration of antibodies to the house dust mite allergen Dpt12 in sera from *P. cuniculi*-infested rabbits. J. Allergy Clin. Immunol. 78, 293–299.
- Stromberg, P.C., Fisher, W.F., 1986. Dermatopathology and immunity in experimental *Psoroptes ovis* (Acari: Psoroptidae) infestation of naïve and previously exposed Hereford cattle. Am. J. Vet. Res. 47, 1551–1560.
- Stromberg, P.C., Guillot, F.S., 1989. Pathogenesis of psoroptic scabies in Hereford heifer calves. Am. J. Vet. Res. 50, 594–601.
- Thoday, K.L., 1993. Serum immunoglobulin concentrations in canine scabies. In: Ihrke, P.J., Mason, I.S., White, S.D. (Eds.), Advances in Veterinary Dermatology, vol. 2. Pergamon Press, Oxford, pp. 211–227.
- Uhler, J., 1991. Humoral and cellular immune-response of rabbits to *Psoroptes cuniculi*, the rabbit scab mite. Vet. Parasitol. 40, 325–334.
- van den Broek, A.H., Huntley, J.F., Machell, J., Taylor, M., Bates, P., Groves, B., Miller, H.R., 2000. Cutaneous and systemic responses during primary and challenge infestations of sheep with the sheep scab mite, *Psoroptes ovis*. Parasite Immunol. 22, 407–414.
- Voller, A., Bidwell, D.F., Bartlett, A., 1979. The Enzyme-Linked Immunosorbent Assay (ELISA). A Guide with Abstracts of Microplate Applications. Nuffield Laboratories of Comparative Medicine, The Zoological Society of London, Regents Park, London.
- Wall, R., Smith, K.E., Berriatua, E., French, N.P., 1999. Simulation analysis of the population dynamics of the mite, *Psoroptes ovis*, infesting sheep. Vet. Parasitol. 83, 253–264.
- Wassall, D.A., Kirkwood, A.C., Bates, P.G., Sinclair, I.J., 1987. Enzyme-linked immunosorbent assay for the detection of antibodies to the sheep scab mite *Psoroptes ovis*. Res. Vet. Sci. 43, 34–35.
- Wozniak, E.J., Butler, J.F., Zam, S.G., 1996. Immune recognition of *Ornithodoros* tick (Acari: Argasidae) salivary antigens by anti-*Psoroptes cuniculi* antibodies. J. Parasitol. 82, 375–378.