

## Antigens of the sheep scab mite *Psoroptes ovis*

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**Abstract.** Sheep scab caused by the mite *Psoroptes ovis* (Hering) is a highly contagious disease of sheep. As a first step in developing a mite-derived vaccine for controlling the disease, the soluble antigens in mite extracts which induce an immune response in sheep were identified by electrophoretic and immunoblotting techniques. At least 22 proteins were present in *P. ovis* extracts as revealed by Coomassie Blue staining. Mite-infested sheep serum recognised six antigenic bands in the extracts with approximate relative molecular weights ranging from 12 to 183 kDa. A deeply staining band at 31.2 kDa and another at 41.8 kDa are of particular diagnostic value. Immunoblotting studies showed that there was no cross reactivity between *P. ovis* and two other ectoparasites of sheep in the UK, the sheep louse *Bovicola ovis* (Schrank) and the sheep tick *Ixodes ricinus* L.

The mite *Psoroptes ovis* (Hering) is the cause of sheep scab, a highly contagious disease characterised by scratching and serous exudations with the consequent loss of wool; affected animals cease to feed and become severely debilitated (for a review of sheep scab and its control, see Kirkwood 1986). Although the disease was eradicated from most of the main sheep rearing areas of the world many years ago, it was never eradicated from some countries, while in others, it was reintroduced after a period of successful eradication. Control and eradication of sheep scab has been by treating the mite-infested animals with insecticides, mainly organophosphorus (OP) compounds, and latterly by synthetic pyrethroids and subcutaneous injections of avermectins (Bates et al. 1995). However, populations of *P. ovis* resistant to OP compounds and the synthetic pyrethroid flumethrin have been reported in the UK (Syngle et al. 1995, Clark et al. 1996). Moreover, mites inside the ears can survive plunge dipping (the usual method of administering insecticides) and could serve as potential reservoirs of body infestation (Bates 1996). The ever-present threat of development of insecticide resistance by the mites, the problems associated with environmental pollution by toxic insecticidal compounds and the health hazards to sheep farmers who use OP insecticides (Stephens et al. 1995) have directed attention in the UK to alternative strategies for controlling the mites and the disease. One such strategy is protective immunisation of sheep with mite-derived antigens. This is a unique, innovative, environmentally acceptable method and does not pose any health hazards to animals or man.

Many ectoparasites of livestock induce immunity in their host animals resulting in their protection against

subsequent infestations with the particular parasites (Wikle 1988). Vaccination of animals against ixodid ticks (Opdebeeck et al. 1988, Willadsen and Kemp 1989, Rechav et al. 1992, Riding et al. 1994), the sheep blow fly, *Lucilia cuprina* (East et al. 1993) and the cattle grub, *Hypoderma lineatum* (Baron and Colwell 1991) has been extensively investigated and a genetically engineered vaccine against the cattle tick *Boophilus microplus* has been developed for commercial use (Cobon and Willadsen 1990). No such studies have been reported for *Psoroptes ovis*.

The first step towards developing a mite-derived vaccine to control *P. ovis* is the identification of antigenic mite proteins which induce an immune response in sheep. Sera from infested animals were used for characterising the antigens of North American *P. ovis* from different sources and from the related *Psoroptes cuniculi* from rabbits (Boyce and Brown 1991). Since both species appear to be morphologically identical, reproductively compatible and antigenically similar (Wright et al. 1983, Kirkwood 1986, Evans 1992), *P. cuniculi* mites have been used as an antigen source in serological investigations on bighorn sheep infested with *P. ovis* in the USA (Boyce et al. 1991). Antigens from *P. cuniculi* have also been used for immunisation of rabbits and an immunogen from this species was subsequently isolated and characterised by Uhliř (1992, 1993) as part of in-depth studies on this species. More recently, antigenic proteins from a German strain of *P. ovis* and from three other mite species, *Sarcoptes suis*, *Chorioptes bovis* and *Notoedres cati*, also originating in Germany, were investigated for their cross-reactivity using sera from sheep naturally infested with *P. ovis* (Mathes et al. 1996). We report in this paper the

identification of antigens in a British strain of *P. ovis*, using sera from infested sheep. Apart from their potential as putative vaccines, these antigens are of value in the serodiagnosis of sheep scab.

## MATERIALS AND METHODS

### Preparation of mite extracts for soluble antigens

*Psoroptes ovis* mites (Cornish strain) fed on calves and stored at -70°C were used for preparing soluble antigens. Calf reared mites were used because mites raised on sheep have been shown to give false positive reactions in ELISAs (Wassall et al. 1987) and non-specific staining of antigenic bands in immunoblots using sheep serum (Mathes et al. 1996). The mites were taken out of storage, weighed, thawed, and "cleaned up" by washing in cold phosphate-buffered saline (PBS; Dulbecco's A, Oxoid), pH 7.2 containing 1% Tween 80. After two more washes in PBS, the mites were dried by placing on filter paper and transferred to a glass tissue grinder (or porcelain mortar for larger masses of mites) standing on ice. The mites were crushed to a fine powder in a small amount of liquid N<sub>2</sub>; crushing in liquid N<sub>2</sub> was done two more times and extraction buffer (PBS with 0.05% Tween 20 and 0.1mM of the protease inhibitor PMSF) added to give an approximate 10% (w/v) suspension. This was then sonicated on ice four times, 15 seconds at a time at 18 µ with 1 min intervals. The homogenate was centrifuged at 50,000 g for 1 hr at 4°C. The supernatant was removed, filtered sequentially through 0.45 µm and 0.22 µm filters and stored at -70°C. The protein content estimated by the Bradford assay (Bradford 1976) was 2.5 to 3.0 mg/ml.

To determine the specificity of the *P. ovis*-sheep sera reactions, soluble antigen extracts were also prepared from two other sheep ectoparasites in the UK, the sheep tick, *Ixodes ricinus*, and sheep louse, *Bovicola ovis*, for testing with *P. ovis* infested sera. *B. ovis* (nymphs and adults) were from sheep chronically infested with a research farm strain of sheep lice; larvae of *I. ricinus* were from fed females ticks collected from sheep on a farm. The method of preparing the extracts from *B. ovis* and from *I. ricinus* was essentially the same as for the mites. The protein content of the tick extract was estimated as 6.4 mg/ml and of the sheep louse extract as 0.63 mg/ml.

### Antisera

The infestation procedures are those routinely employed at the Central Veterinary Laboratory at Weybridge, UK. Naïve sheep (Dorset × Suffolk/Welsh mountain × Charolais) of mixed sex, aged six months or over with no history of previous exposure to ectoparasites were infested with 25 ovigerous females of *P. ovis*. An area of wool was plucked from the withers leaving a bare abraded area of skin; the mites were placed directly on the skin and the area circled by stockmarker ring. A second infestation with a similar number of mites was done three weeks after the primary infestation. The animals were not prevented from grooming. Live adult females found on the periphery of the lesion were counted and the area of the lesion measured every week. Blood samples for serology were taken before infestation (pre-infestation sample) and every week till the seventh week when the experimental infestations were usually terminated, but some animals were kept for longer periods, up to 15 weeks post

infestation. Anti-*P. ovis* serum for comparative studies on immunoblotting and ELISAs was from a naturally infested sheep with patent sheep scab; the animal had no history of previous exposure to ectoparasitic infestations. Anti-*B. ovis* serum was obtained from a sheep with a chronic heavy infestation with *B. ovis* following repeated infestations during a period of four months. During and prior to the infestation, the animal was free of other ectoparasitic infestations.

### Electrophoretic separation of soluble proteins

Extracts from *P. ovis* mites, sheep ticks and sheep lice, containing soluble proteins were separated by SDS-PAGE on 12% uniform gels according to the method of Laemmli (1970) using the Mini-Protean system (Bio-Rad). Each lane was loaded with 3 µg of protein; one lane had prestained low range SDS-PAGE molecular weight standards (Bio-Rad). All the samples were boiled for 5 min in SDS sample buffer containing 5% 2-mercaptoethanol and placed on ice for 5 min before loading on to the gels. The gels were run at 120 V until the tracker dye bromophenol blue had reached the bottom of the gels.

For visualisation of proteins in *P. ovis*, 10 µg protein per lane of the extracts were electrophoresed on 10% uniform gels and the gels stained with Coomassie Brilliant Blue R250 stain (Bio-Rad).

### Immunoblotting of separated antigenic proteins

The proteins separated on the gels were transferred to nitrocellulose (NC) membranes of pore size 0.45 µm (Schleicher and Schuell) according to the method of Towbin et al. (1979). Blotting was done for 35 min at 15 V using the Semi-Dry Transblot system (Bio-Rad). After washing in 0.05% Tween 20 in PBS (PBST), unbound sites on the blots were blocked with 3% pig gelatin in PBST. The blots were then incubated for 45 min at room temperature on a rocking platform with naïve sheep serum, *P. ovis* infested serum and sera from sheep infested with sheep lice to determine specificity of the *P. ovis* antigens. All sera were used at a dilution of 1 : 250. Antigen-antibody reactions were visualised using as the second antibody, donkey anti-sheep IgG (whole molecule, Sigma) conjugated with alkaline phosphatase and used at a dilution of 1 : 5,000. The substrate used was 5-Bromo-4-Chloro-3-Indolyl Phosphate/Nitro Blue Tetrazolium (BCIP/NBT, Sigma). The blots were scanned with a densitometer using "Quantiscan" software (Microbial Systems Ltd.) to determine the position of the major antigenic peaks and estimate their relative molecular weights.

### ELISA

Ninety-six well microtitre plates were coated with soluble proteins from extracts of cattle-raised *P. ovis*, from *B. ovis* and from *I. ricinus* in carbonate/bicarbonate coating buffer pH 9.6 at the rate of 0.25 µg/100 µl per well. The plates were incubated for 40 min at 37°C, and stored overnight at 4°C. After washing the following morning, the plates were blocked with 3% pig gelatin in PBS containing 1% Tween-20 (PBST). Antisera were used at a dilution of 1 : 250 in PBST containing 1% pig gelatin. The second antibody was rabbit anti-sheep IgG conjugated with horseradish peroxidase used at a dilution of 1 : 10,000. The chromogen was O-phenylenediamine dihydrochloride (OPD, Sigma) and the reaction was stopped after 10 min in the dark at 37°C with 2.5 M sulphuric

acid. The plates were read at 492 nm using a Labsystems Multiscan Multisoft plate reader. Each ELISA included pooled sera from known negative sheep as a control. Ratios of optical density reading of test serum/negative serum of 2 or above were considered positive (Voller et al. 1980).

## RESULTS

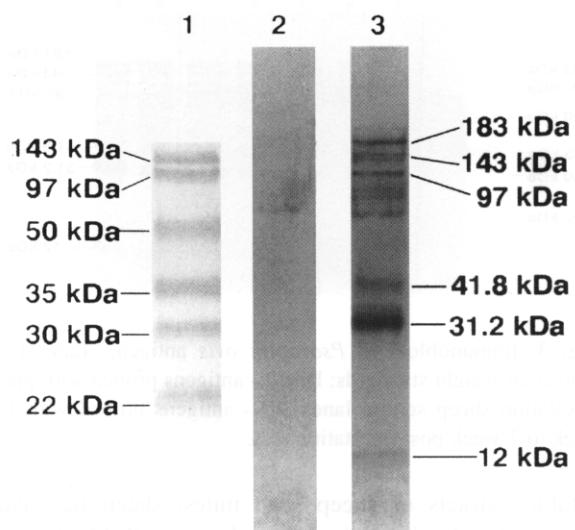
### SDS-PAGE

Coomassie Blue stained gels of electrophoresed *Psoroptes ovis* extracts revealed at least 22 protein bands with estimated relative molecular weights ranging from approximately 12 kDa to more than 120 kDa.

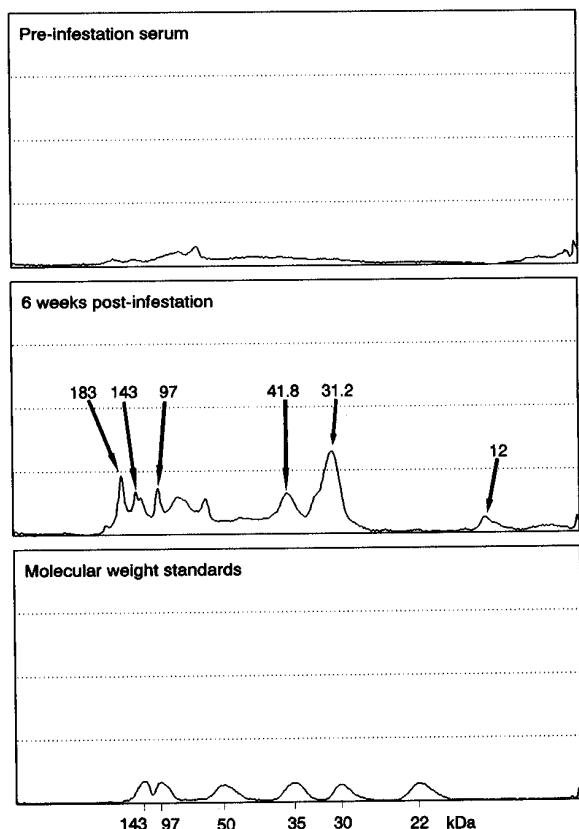
### Immunoblots

The "Quantiscan" software provided a reliable estimate of the relative molecular weights of the antigenic bands. Sera from three sheep infested with the Cornish strain of *P. ovis* recognised eight antigenic bands in soluble whole body extracts of Cornish *P. ovis* (Fig. 1). Two of these with relative molecular weights between 50 kDa and 97 kDa are non-specific since they were recognised by pre-infestation serum and a pool of naive sera used as a negative standard. The remaining six antigens are considered specific and were recognised only by infested sheep serum although there was some sample to sample variation in the time of appearance of antibodies to the different bands. The six antigens had approximate relative molecular weights of 183, 143, 97, 41.8, 31.2 and 12 kDa (Figs. 1 and 2). The 183, 143 and 97 kDa bands were sharp and narrow while the 41.8 kDa and 31.2 kDa bands were slightly wider and stained more deeply than the others. Sera from experimental sheep infested individually with three other strains of *P. ovis*, the Gwynned, Dartmoor and Norfolk strains, as well as sera from six naturally infested sheep with clinical sheep scab, also recognised the antigens in Cornish *P. ovis* extracts. The 143 kDa antigenic band appeared to be a doublet of two sharp and narrow bands. The 41.8 and 31.2 kDa antigens are of particular diagnostic value since they are clearly visible and well separated from the other bands.

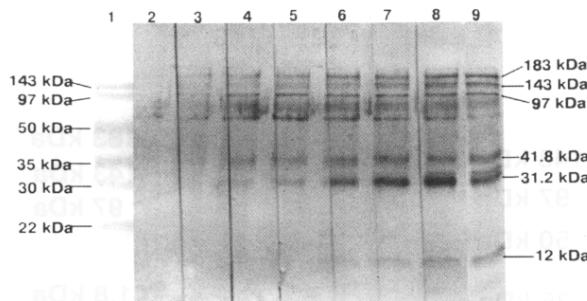
The kinetics of antibody production to these antigens is shown in Fig. 3. Sheep sera taken as early as two weeks after infestation detected these bands and by 5 weeks infested sera stained clearly all six antigenic bands. There was some evidence that antibodies to the 12 kDa antigen developed later, at approximately 4 weeks post infestation, suggesting that development of antibodies to this particular antigen required a prolonged exposure to it. Sera from some infested sheep taken as late as 15 weeks post infestation, still recognised all six antigens, although staining of the 41.8 kDa and 31.2 kDa bands tended to be fainter; because of this these two bands probably have only a limited diagnostic value in low-grade chronic infestations.



**Fig. 1.** Immunoblots of *Psoroptes ovis* antigens. Lane 1 - molecular weight standards; lane 2 - antigens probed with pre-infestation sheep serum; lane 3 - antigens probed with 6-week post infestation sheep serum.



**Fig. 2.** "Quantiscan" profiles of *Psoroptes ovis* antigens probed with pre-infestation sheep serum, 6-week post infestation sheep serum, and of molecular weight standards.



**Fig. 3.** Immunoblots of *Psoroptes ovis* antigens. Lane 1 - molecular weight standards; lane 2 - antigens probed with pre-infestation sheep serum; lanes 3-9 - antigens probed with 1-week to 7-week post infestation sera.

### ELISA

Results using sera from sheep infested with sheep scab mites (*P. ovis*) and sheep lice (*Bovicola ovis*), and soluble extracts of sheep scab mites, sheep lice and sheep ticks (*Ixodes ricinus*) are shown in Table 1 with corresponding results from immunoblots. *P. ovis*-infested serum gave positive reactions with *P. ovis* as well as *B. ovis* and *I. ricinus* extracts, although none of the antigenic bands stained by *P. ovis*-infested serum in *P. ovis* extracts were seen in *B. ovis* or *I. ricinus* extracts. *B. ovis*-infested serum gave a positive reaction only with *B. ovis* soluble extracts. It did not recognise *P. ovis* specific antigens in *P. ovis* extracts.

**Table 1.** Antigen/antibody reactions between *Psoroptes ovis*, *Bovicola ovis* and *Ixodes ricinus* assayed by ELISA and immunoblotting

Antigen/antisera <sup>†</sup>	ELISA ratio*	<i>P. ovis</i> specific antigenic bands**
<i>P. ovis/P. ovis</i>	3.30	Yes
<i>P. ovis/Bov. ovis</i>	1.63	No
<i>Bov. ovis/Bov. ovis</i>	4.54	No
<i>Bov. ovis/P. ovis</i>	2.63	No
<i>I. ricinus/Bov. ovis</i>	-0.73	No
<i>I. ricinus/P. ovis</i>	2.10	No

<sup>†</sup> *P. ovis* - *Psoroptes ovis*; *Bov. ovis* - *Bovicola ovis*; *I. ricinus* - *Ixodes ricinus*

\* Ratio of infested/naive sera OD values. Values greater than 2.0 are considered positive

\*\* Presence of one or more *P. ovis* specific antigenic bands in immunoblots

### DISCUSSION

We found that at least 22 proteins were present in *Psoroptes ovis* extracts as revealed by Coomassie Blue staining. This is lower than the 35 to 36 protein bands reported by Boyce and Brown (1991) and Mathes et al. (1996) and may be attributed to the differences in the methods employed. There are also some differences as

well as similarities, in the number of antigens and their relative molecular weights recognised in *P. ovis* extracts from North America, Europe and the UK by sera from *P. ovis* infested sheep. However, without further in-depth studies, it is not possible to ascribe any significance to this. In investigations on the antigen-antibody relationship between North American *P. ovis* from bighorn sheep, mule deer and cattle and *P. cuniculi* using sera from the different animals as probes, Boyce and Brown (1991) found that the sheep antiserum recognised over 30 antigens in *P. ovis* extracts, antigen recognition being especially strong in the 8-12, 26-34 and > 97 kDa regions. Boyce et al. (1991) obtained similar results by probing *P. cuniculi* extracts with *P. ovis* infested bighorn sheep serum; the serum reacted with at least seven (in some cases up to 20) different antigens ranging from 12-164 kDa. The authors concluded that antigens in the 12-34 kDa range were of particular diagnostic value in detecting *P. ovis* infestations in sheep. At least 24 antigens ranging from 10 kDa to 170 kDa were detected in extracts from a German strain of *P. ovis* probed with *P. ovis* infested sera by Mathes et al. (1996), major antigens being present in the 15, 44, 130 and 170 kDa regions; the *P. ovis* infested sheep serum also detected antigens at 39, 41, 55, 148, 154 and 170 kDa in extracts of *P. ovis* and three other mites, *Sarcoptes suis*, *Chorioptes bovis* and *Notoedres cati*. Non-specific staining of a 67 kDa antigen in all four mite extracts was seen with specific pathogen free (naive) sheep serum. The non-specific bands seen by us were also in this molecular weight area. In our experiments, *P. ovis* infested sheep serum recognised six antigens in *P. ovis*. Of these, the 183 kDa antigen has not been seen by previous workers although a dominant antigen of 170 kDa was present in the German *P. ovis*. The nearest to our 143 kDa antigen is the 148 kDa antigen of German *P. ovis*. The 97 kDa antigen of North American *P. ovis* was seen by us also in *P. ovis*, but is not mentioned as a major antigen of the German *P. ovis*. The 41.8 kDa antigen in our *P. ovis* is most probably the same as the 41 kDa antigen of all 4 German mite species, including *P. ovis*; no antigen of a similar molecular weight has been reported for North American *P. ovis*. The 31.2 kDa antigen which stained most intensively in our studies is not mentioned as a dominant antigen in the German *P. ovis*. We found that this and the 41.8 kDa antigen, because of their staining intensity and distance from other antigenic bands, were of diagnostic value. The 31.2 kDa antigen in particular, was a useful marker for detecting sheep scab in sheep before they developed clinical illness.

The antigenic cross-reactivity between *P. ovis*, *P. cuniculi*, other ectoparasitic mite species, the house dust mite *Dermatophagoides pteronyssinus* and the tick *Dermacentor variabilis* have been well documented

(Den Hollander and Allen 1986, Stewart and Fisher 1986, Mathes et al. 1996). However, there was no cross-reactivity between *P. ovis* and the tick *Dermacentor hunteri* (Boyce et al. 1991). It has been pointed out that these cross reactions have to be taken into consideration when devising diagnostic methods or developing vaccines (Mathes et al. 1996). We found that in immunoblotting studies, there was no cross reactivity between *P. ovis*, and two other ectoparasites of sheep in the UK, the sheep louse *Bovicola ovis* and the sheep tick *Ixodes ricinus*. However, in ELISAs, *P. ovis* infested sheep serum reacted positively with both *B. ovis* and *I. ricinus* native antigens, suggesting that where concurrent infestations of mite, louse and tick occur, immuno-blotting (using denatured antigens) and not ELISA (using native antigens) is of specific diagnostic value.

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