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Research paper

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, resulting in an elicitation of specific serum IgE levels in week 2 PC significantly higher than those demonstrated after primary infection. WB analysis revealed one additional IgG-reactive band (180 kDa) 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.

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 antibody ELISA to

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

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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). 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, 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 characterize 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 with *S. scabiei* var. *ovis* mites (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 $\mu g\,kg^{-1}$ 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 2000 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 $900 \times g$ for 15 min the sera were removed and stored at −20 °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 30 °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 −20 °C until being processed as it is described next. 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 $5500 \times g$ for 5 min the supernatant (mite extract) had been removed, its protein concentration measured by the