Veterinary Parasitology 203 (2014) 173–183

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Primary and secondary experimental infestation of rabbits (*Oryctolagus cuniculus*) with *Sarcoptes scabiei* from a wild rabbit: Factors determining resistance to reinfestation

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ARTICLE INFO

Article history:
Received 4 September 2013
Received in revised form 9 February 2014
Accepted 3 March 2014

Keywords:
Sarcoptes scabiei
Experimental infestation
Route of infestation
Immune response
Protection
Rabbit

ABSTRACT

Studies of sarcoptic mange and immunity are hampered by lack of mite sources and natural infestation models. We have investigated the clinical and pathological signs, specific IgG response and acquired immunity in naïve New Zealand White rabbits (*Oryctolagus cuniculus*) experimentally infested with *Sarcoptes scabiei* originally isolated from a clinically affected free-living European wild rabbit. Twenty rabbits were infested using two methods, direct contact for a 24 h period with a seeder rabbit simulating the natural process of infestation and application of a dressing holding approximately 1800 live mites on each hind limb (foot area) for a 24 h period. Eight weeks post infestation, rabbits were treated with ivermectin and infestation cleared. Eight weeks later seventeen previously infested and four uninfested naïve controls were then re-exposed to the same *S. scabiei* variety using the same methods and followed for another 8 weeks. The progress of the disease was markedly more virulent in the animals infested by contact, indicating that the effective dose of mites managing to thrive and infest each rabbit by this method was higher. Nevertheless, infestation by contact resulted in partial protection to reexposure, rabbits developed high non-protective antibody titres upon reinfestation and presented severe clinical signs. However, rabbits reinfested by dressing developed lower IgG titres, and presented high levels of resistance to reinfestation, which might be due to induction of a strong local cellular response in the inoculation point that killed the mites and resulted in a lower mite effective dose, with subsequent reduced lesion development. Statistical analysis showed that sex, method of infestation and previous exposure are key factors determining the ability of rabbits to develop immunity to this disease. The rabbit-mange model developed will allow the further study of immunity and resistance to this neglected pathogen using a natural host system.

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http://dx.doi.org/10.1016/j.vetpar.2014.03.001
0304-4017/

1. Introduction

Sarcoptic mange is a highly contagious skin condition caused by infestation with the ectoparasite *Sarcoptes scabiei* which infests humans and a wide range of domestic and wild animals (Zahler et al., 1999).

The immune response to *S. scabiei* infestation has been studied in human (Walton et al., 2010; Mounsey et al., 2013) and animal species (Bornstein and Zakrisson, 1993; Arlian et al., 1994; Bornstein et al., 1995; Arlian et al., 1996; Little et al., 1998; Arlian and Morgan, 2000; Sarasa et al., 2010; Rodríguez-Cadenas et al., 2010). Research by Walton et al. (2010) suggests that clinical severity is associated with differences in the type and magnitude of antibody and cellular responses to scabies proteins. Naïve New Zealand rabbits inoculated with *S. scabiei var. canis* in the ear canal showed inter-individual variability in the immunological responses to *S. scabiei*, with 65% of the rabbits that experience heavy experimental infestations developing immunologically mediated resistance to reinfestations (Arlian et al., 1994). As both immune and non-immune hosts developed scabies-specific elevated antibody levels humoral immunity was not protective, suggesting that resistance to reinfestation was associated with a cell-mediated response (Arlian et al., 1994). Likewise, Tarigan (2002) showed that goats sensitized with *S. scabiei var. ovis* acquired protective immunity, lesions in sensitized goats developed rapidly and then waned slowly, whereas naïve goat lesions progressed slowly but steadily. Similar results were obtained by Arlian et al. (1996) using dogs ($n = 8$) sensitized with *S. scabiei var. canis* which showed clinical signs that peaked at 24 days and then waned. In contrast, Little et al. (1998) showed that the clinical response, severity of the disease and relative numbers of mites per cm^2 of skin of previously infected ($n = 3$) and naïve ($n = 3$) red foxes did not differ, previously infested foxes developed strong immediate hypersensitivity reaction to *S. scabiei* but did not exhibit resistance to re-infestation.

It has been suggested that the mechanism involved in the development of acquired resistance against *S. scabiei* in goats is related to IgE responses as animals with a strong IgE response upon rechallenge were resistant to reinfestation (Tarigan and Huntley, 2005; Rodríguez-Cadenas et al., 2010). This is in conflict with the apparent lack of any protective effect of the extremely high IgE levels in human crusted scabies indicating that the functional role of IgE in scabies immunity is not clearly resolved (Mounsey et al., 2013). Recent studies in Iberian ibexes have shown that previous exposure to the mite and sex appear to affect the IgG response to infestation (Sarasa et al., 2010).

Clinical outbreaks of sarcoptic mange have been recently reported for the first time in the European wild rabbit in Spain (Millán, 2010; Navarro-Gonzalez et al., 2010), which may be considered as a threat to the conservation of the wild rabbit and their predators. Sarcoptic mange can also be a problem on rabbit farms decreasing rabbit productivity (Rosell et al., 2000). *S. scabiei* infestation in rabbits causes heavy scratching and considerable weight loss (Soulsby, 1982; Aiello et al., 1998). Severe infestation leads to anaemia and leucopenia, the rabbits becoming lethargic with death taking place within a few weeks (Rosell et al., 2000).

Experimental infestation of 10 seronegative wild-caught European rabbits with *S. scabiei* from a naturally infested wild rabbit resulted in only three rabbits producing specific antibodies, two of which developed lesions (Millán et al., 2013). This has led us to develop a rabbit mange model in domestic rabbits using experimental infestation with the specific variety of *S. scabiei* obtained from its closely related natural free-living host in order to better understand the host reaction during a natural (albeit experimental) infestation.

The purpose of this study was to deepen our knowledge of the pathogenesis of rabbit sarcoptic mange and the factors determining disease outcome in naïve New Zealand White rabbits experimentally infested with *S. scabiei* obtained from a wild rabbit. The effect of previous exposure, method of infestation (by contact or by means of a dressing) and sex on the immunological responses to *S. scabiei* was analyzed.

2. Methods

2.1. Experimental design

Thirty, 3 month old scabies-free New Zealand White rabbits of 2.5–3 kg were housed individually in 0.47 m^2 wire cages, which allows free movement of three rabbits, in rooms with air conditioning and ventilation and were provided with food and water *ad libitum*. Animals were kept under observation during an acclimatization period of two weeks. Two different methods of infestation were used: (i) 10 rabbits (5 females and 5 males, C401–C405 and C411–C415 respectively, Table 1) were infested by direct contact for a 24 h period with a seeder rabbit, experimentally infested with *S. scabiei* from a wild rabbit, in order to simulate the natural route of infestation. Preliminary experiments showed successful transmission of the mite to 100% of rabbits if groups of two rabbits at a time were placed in the seeder rabbit cage for a 24 h period, and (ii) 10 rabbits (5 females and 5 males, C406–C410 and C416–C420, Table 1) were infested by means of a dressing, each holding approximately 1800 live mites placed on each previously shaved hind limb (foot area) and worn for 24 h (no dressings were removed mechanically by the animals). We chose the foot area as mange lesions in rabbits have most frequently been observed in the limbs.

Additionally, 4 rabbits (2 males and 2 females, C421–C424, Table 1) were maintained as a non-infested control group. The chronology of the experimental phases was as follows: primary infestation was allowed to progress for 8 weeks, at week 8 post infestation (PI) all rabbits (except the control group) were treated with ivermectin (Ivomec, Merial) by two subcutaneous injections, given one week apart at 200 μg/kg of body weight, and animals were allowed to fully recover for 8 weeks. At week 16 PI all pre-infested rabbits and the four naïve controls (one female and one male for each method) were challenged with the same *S. scabiei* variety using the same methodologies as for primary infestations. After challenge, rabbits were monitored for another 8 week period (to a