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Horizontal transmission of fungal infection by *Metarhizium anisopliae* in parasitic *Psoroptes* mites (Acari: Psoroptidae)

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Abstract

Ectoparasitic mites of the genus *Psoroptes* cause mange in a wide range of domestic and wild vertebrate hosts. The mite infestation may be localised in the ear of the host, or be more generalised over the body. In the latter case, infestation may be debilitating and often fatal, particularly in domesticated cattle, sheep and goats. At present, control depends on the use of organophosphate or pyrethroid plunge dips or injectable endectocides. A series of in vivo bioassays are described here to further evaluate the use of an entomopathogenic fungus, *Metarhizium anisopliae* (Metschnikoff) as a biological control agent for *Psoroptes* mites. Mites were obtained from the ears of rabbits (*Psoroptes ovis* (Hering) syn. *Psoroptes cuniculi*). High levels of mortality (>94%) were observed in all life-cycle stages when exposed to a 1×10^8 conidia ml^{-1} suspension; there were no significant differences in infection between the different life-cycle stages. The treatment of eggs, however, had no effect on hatch rate. The horizontal transmission of the pathogen was considered by placing live uninfected mites in experimental chambers with an infected cadaver, but preventing direct contact using a mesh cage which would allow the passage of conidia but exclude direct physical contact. None of the uninfected mites showed signs of mycosis-induced mortality, indicating that direct contact with the cadaver was required for infection. Uninfected live mites were then brought into direct contact with an infected cadaver by a single touch of the mite to the cadaver (<1 s), or indirectly by allowing the live uninfected mite to walk over a piece of filter paper from which an infected cadaver had been removed. Both treatments resulted in fungal infection in approximately 40% of the live mites. The implications of these results for the transmission of *M. anisopliae* between infected *Psoroptes* mites, when used as a biological control agent, on an infected host is discussed.

Keywords: *Psoroptes*; Biological control; Fungus; Mites; *Metarhizium*; Sheep scab; Horizontal transmission

1. Introduction

Mites of the genus *Psoroptes* (Acari: Psoroptidae) are obligatory ectoparasites that infest a range of mammalian hosts. The genus is distinguished by the presence of relatively long, jointed pre-tarsi (Babcock and Black, 1933; Sanders et al., 2000; Sweatman, 1958). The mites are non-burrowing and feed superficially on a lipid emulsion of skin cells, bacteria and lymph on the host skin, produced as a result of a hypersensitivity reaction to the presence of antigenic mite faecal material (Blake et al., 1978; Sinclair and Kirkwood, 1983). Infestation may be chronic or even sub-clinical and localised, usually in the ear of the host. Or it may be acute and more generalised over the entire body, described as psoroptic mange (Bates, 1999). The taxonomy of the mites in this genus is confused, with mites located in different parts of the body or on different hosts traditionally given different species names; however, little good evidence exists to support this taxonomy (Bates, 1999; Evans, 2004; Zahler et al., 1998, 2000).

The most clinically and economically important infestations of *Psoroptes ovis* (Hering), occur on sheep. The first indication that a sheep may have an infestation is the rough appearance of the wool and abnormal animal

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activity, including persistent biting and scratching (Babcock and Black, 1933). Small pustules form which expand and rupture, exuding lymph and serous fluids that rapidly congeal to form yellow crusts. The lesions cause severe irritation to the sheep making them restless and nibble and rub at the infested areas (Berriatua et al., 2001; Corke and Broom, 1999). This self-trauma results in wool loss, skin damage and weight loss and if left untreated can lead to epileptiform fitting when handled and ultimately death due to dehydration, pneumonia or bacterial septicaemia (Downing, 1936; Roberts and Meleney, 1971; Tarry, 1974).

Psoroptic mange is commonly treated using organophosphate or pyrethroid plunge dips or injectable macrocyclic-lactone endectocides. However, concerns over potential health and environmental effects of neurotoxic insecticides and logistic, cost and practical difficulties associated with endectocide use, have stimulated interest in the identification of alternative control methods.

The use of entomopathogenic fungi for the control of insect pests was considered around the turn of the 20th century, but with little commercial success. The most commonly investigated entomopathogenic fungi are species of *Metarhizium* and *Beauveria* (Deuteromycotina: Hyphomycetes), as they have a wide geographic spread and host range. These entomopathogenic fungi have been widely considered for biological control of agricultural pests (Gillespie and Moorhouse, 1989; Van der Geest et al., 2000), but only a small number of studies have considered their potential against parasites of animals, particularly ticks (Kaaya et al., 1996; Kirkland et al., 2004). In mites, *Metarhizium anisopliae* (Metschnikoff) Sorokin, has previously been shown to give mortalities of up to 71% in adult female *Psoroptes* mites in vitro (Smith et al., 2000) and fungal infections shown to be transmitted from infected cadavers to live uninfected mites (Brooks and Wall, 2001). This previous work suggested that the fungal pathogen, sporulating on infected cadavers after the death of mites on the ovine host, may serve as an ongoing source of the pathogen and hence sustain the control effect after the initial application of conidia to a sheep. Substantive strain differences in pathogenicity of *M. anisopliae* against *Psoroptes* mites at a range of temperatures has been demonstrated (Brooks et al., 2004). The aim of the present study therefore, was to consider the mechanism through which horizontal transmission of *M. anisopliae* may occur and to extend the previous studies to examine the effects of this pathogen against other life-cycle stages of *Psoroptes*.

2. Materials and methods

2.1. Mites

Mites were collected from scabs removed from the ears of two infected New Zealand white rabbits. These mites are described here as *P. ovis* (syn. *Psoroptes cuniculi*) since genetic studies do not support a distinction between *Psoroptes* isolated from sheep and rabbits (Evans, 2004; Zahler et al., 1998, 2000). After removal from the host, the scabs were placed into clean plastic jars and the mites allowed to wander up the sides of the jars from where they were removed a few minutes later using a fine paintbrush. Different life-cycle stages were identified (Sanders et al., 2000) and separated into sterile 1.5 ml eppendorf tubes until required; all mites were used in experimental trials within 6 h of collection.

2.2. Fungal strain: maintenance and growth

Metarhizium anisopliae, strain ARSEF 4556, which had been isolated originally from a *Boophilus* spp. tick, was obtained from the USDA-ARS collection. The isolate was cultured at 25 °C on Sabouraud dextrose agar plus yeast (SDAY) (Oxoid, Basingstoke, UK). Conidia were collected from 10- to 14-day-old plates by adding 10 ml sterile 0.05% Tween 80 (BDH Chemicals, Poole, UK) and the surface of the culture agitated, using a sterilised loop, to bring the spores into suspension. The conidial suspension was pipetted from the plate and the spore concentration calculated using an Improved Neubauer Haemocytometer (Weber Scientific International, Middlesex, UK). The conidial suspension was diluted using 0.03% Tween 80 to a concentration of 1×10^8 conidia ml^{-1}. The lower concentration of Tween 80 was used in the final dilutions to reduce any toxic effects of the detergent on the mites.

2.3. Pathogenicity to different life-cycle stages

Mites were maintained and monitored in experimental chambers constructed from $35 \times 75 \times 6$ mm glass blocks with a 20 mm diameter hole through the centre. Two pieces of 42.5 mm diameter Whatman No. 1 filter papers (Whatman International, Maidstone, UK) were placed centrally over the 20 mm diameter hole in the glass block and pressure used to create a shallow indentation. 500 µl of ovine serum was then pipetted onto the filter papers and either 500 µl of a 1×10^8 conidia ml^{-1} suspension or 500 µl of 0.03% Tween 80 was also added. Twenty mites were selected at random from the eppendorf tubes and placed in the centre of the indentation in the filter paper and a glass microscope slide was placed over the top and clamped at either end using foldback clips. The chambers were maintained at 30 °C and 95% r.h. and 400 µl serum was added each day to the filter paper to prevent the filter papers drying out. Experimental chambers were set-up containing adult females, adult males, or male nymphs. In addition adult male *Psoroptes* mites attach and guard female nymphs; it is assumed that this allows copulation at the point of adult moult. Twenty attached pairs of adult males with female protonymphs and twenty attached pairs of adult males with