Veterinary Parasitology 174 (2010) 85–91

Contents lists available at ScienceDirect

Veterinary Parasitology

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

In vitro infection of sheep lice (*Bovicola ovis* Schrank) by Steinernematid and Heterorhabditid nematodes

P.J. James*, S.E. Hook, P.M. Pepper

Animal Research Institute, Department of Employment, Economic Development and Innovation, 665 Fairfield Road, Yeerongpilly, Queensland 4105, Australia

ARTICLE INFO

Article history:
Received 8 April 2010
Received in revised form 20 July 2010
Accepted 2 August 2010

Keywords:
Entomopathogenic nematodes
Phthiraptera
Trichodectidae
Steinernematidae
Heterorhabditidae

ABSTRACT

Control of sheep lice with conventional pesticides can be compromised by difficulty in contacting lice in the dense water repellent fleeces of sheep, particularly when sheep have not been recently shorn. Entomopathogenic nematodes (ENs) are motile and are able to actively seek out insect hosts. They have particular advantages for the control of pests in cryptic habitats, such as the fleeces of sheep and avoid many of the problems frequently associated with chemical controls. This study investigated whether ENs were able infect and kill *Bovicola ovis* and compared the effectiveness of different species at different temperatures and when applied to wool.

Four species of nematodes, *Steinernema carpocapsae*, *Steinernema riobrave*, *Steinernema feltiae* and *Heterorhabditis bacteriophora* were tested. All were shown to infect and kill lice in Petri dish assays at 30 °C. At 35 °C, the percent infection for *S. carpocapsae* and *S. riobrave* was significantly higher than for the other two species and percent infection by *S. feltiae* was significantly greater than for *H. bacteriophora* ($P < 0.05$). At 37 °C the percent mortality induced by *S. riobrave* was significantly greater than for *S. carpocapsae* ($P < 0.05$). All species were able to locate and infect lice in wool when formulated in water with 8% Tween 80. In wool assays the percent lice infected with nematodes was significantly greater for *S. riobrave* than *H. bacteriophora* at 25 °C, but there were no other differences between species ($P = 0.05$). *S. carpocapsae*, *S. riobrave* and *S. feltiae* caused significantly higher lice mortality than *H. bacteriophora* at both 25 and 35 °C in wool assays, but mortality induced by the three steinernematid species did not differ significantly ($P > 0.05$). It is concluded that of the ENs studied *S. riobrave* is likely to be most effective against *B. ovis* when applied to live sheep because of its greater tolerance to high temperatures and 'cruiser' foraging strategy.

1. Introduction

Sheep lice (*Bovicola ovis*) cause significant economic loss by irritating sheep and reducing wool quantity and quality (James, 2008). Control of sheep lice depends almost exclusively on the application of chemical pediculicides and conventional treatments aim to deliver active concentrations of insecticide to all sites on the sheep where lice survive. As *B. ovis* is a chewing louse and not blood feeding, systemic chemicals are not effective and treatment is usually by immersion dipping, high volume spraying or through the use of high concentration formulations that diffuse through the wool grease in the fleece and on the skin. Difficulty in completely covering the sheep and contacting all lice, particularly when sheep have not been recently shorn, often compromises treatment effectiveness (Lund et al., 2000). In addition, heavy use of chemicals, particularly with long wool treatments, can leave undesirable chemical residues in the wool (Savage, 1998; Morcombe et al., 1999), contribute to the development of resistance in lice populations (James et al., 2008; Levot and Sales, 2008)

* Corresponding author. Tel.: +61 7 3362 9409; fax: +61 7 3362 9429.
E-mail addresses: peter.james@deedi.qld.gov.au, peter.james@dpi.qld.gov.au (P.J. James).

0304-4017/$ – see front matter
doi:10.1016/j.vetpar.2010.08.003

and pose occupational health and safety and environmental risks during application (Littlejohn and Melvin, 1991; Murray et al., 1992).

Entomopathogenic nematodes (ENs) are microscopic worms that invade and kill a wide range of insect species, including human lice *Pediculus humanus* (Weiss et al., 1993; Doucet et al., 1998). Infective juvenile nematodes (IJs) gain entry to an insect's haemocoel through body openings including the mouth, anus, spiracles or in some instances, directly through the insect's cuticle (Bedding and Molyneux, 1982). Once penetration has been achieved they release a toxin and mutualistic bacteria in the genera *Xenorhabdus* or *Photorhabdus*. These bacteria inhibit the insect's immune defences and the growth of other microorganisms and provide food for the developing nematodes. Death of the insect usually ensues within 24–48 h. ENs can be formulated to provide extended shelf life, can be applied through most conventional spray equipment, do not leave chemical residues and are not a health risk for humans or other vertebrates (Akhurst and Smith, 2002; Bedding, 2006). The motility of ENs and their ability to actively seek out insect targets, makes them particularly attractive for use against pests in 'cryptic habitats' which may be hard to reach with conventional pesticides, as is the case with lice in a sheep's fleece. This paper reports the results of a series of laboratory studies conducted to determine if ENs could infect and kill sheep lice and to provide a preliminary assessment of the relative potential of four species of ENs for use in sheep louse control.

2. Materials and methods

2.1. Nematodes and lice

Four species of nematodes were tested against *B. ovis*. These were *Steinernema carpocapsae* (ALL strain), *Steinernema feltiae* (NJ), *Steinernema riobrave* (RGV) and *Heterorhabditis bacteriophora* (Otio). Initial stocks of nematodes were sourced from EcoGrow Environment Pty Ltd (Westgate, NSW, Australia), a commercial supplier of ENs, and nematodes used in the assays were maintained in our laboratory by rearing through *Galleria mellonella* according to standard methods (Poinar, 1979). Nematodes were stored at 20 °C in spring water (Mt Franklin®, Sydney, Australia) aerated with aquarium bubblers after emergence from *G. mellonella* larvae and all assays conducted with nematodes within 2 weeks of collection.

All lice were collected directly by vacuum suction from source sheep that had been held at the Animal Research Institute, Yeerongpilly, Queensland without chemical treatment for 6 years (Animal Ethics Approval ARI 055/2004) and all assays commenced within 24 h of lice collection.

2.2. Treated surface (TS) assays

2.2.1. TS assay 1

Assays were conducted with the four nematode species and with controls (no nematodes) in 50 mm glass Petri dishes containing a single layer of Whatman No1 filter paper. Nematode suspensions were mixed to contain 1000 nematodes per ml in commercial spring water (Mt Franklin®, Coca-Cola Amatil, Sydney Australia), agitated to disperse the nematodes and 0.5 ml of the suspension applied to the centre of the filter paper from a pipette with widened aperture. Ten mixed sex adult lice were then added to each Petri dish and held in darkness at either 30 or 35 °C for 24 h at which time mortality of the lice was assessed. There were three replicates for all treatment and control groups.

For inspection, lice from each group were rinsed twice in deionised water to remove any adhering nematodes and individually inspected under a microscope to ensure no nematodes were still present on the integument. They were then mounted on a microscope slide in a drop of normal saline under a coverslip and examined for movement of nematodes within. If no nematodes were obvious, pressure was applied to the coverslip to rupture the louse exoskeleton and the released body contents and haemolymph carefully inspected.

2.2.2. TS assay 2

A second Petri dish assay was conducted at 32 and 37 °C with *S. carpocapsae* and *S. riobrave*, which were the most effective species in infecting lice in the first experiment. A slightly different system was used because of the high mortality experienced in control lice in Experiment 1 and the experiment was conducted at higher temperatures to more adequately span the range of temperatures likely at the skin surface of sheep (Lee et al., 1941). Aliquots of 0.3 ml of nematode suspension mixed at 1000 nematodes per ml in mineral water were pipetted onto 50 mm × 50 mm pieces of unbleached calico held tight in 50 mm Petri dishes by 47 mm internal diameter metal rings. The nematodes were distributed approximately equally among five sites, one at the centre of the test arena and four at evenly spaced positions approximately 1 cm inside the ring circumference. Ten mixed sex adult lice were added to each dish and mortality assessed 24 h later. In this assay the Petri dishes were used without lids and held in darkness at 65% RH in a humidity controlled incubator. There were three replicates for the controls and each species at both temperatures. Dead lice were squashed under a coverslip to confirm nematode infection.

2.3. Infection of lice in wool

In preliminary studies it was determined that without a wetting agent, droplets of water that formed on or between wool fibres trapped the nematodes, preventing them from moving along the fibres to infect lice. Sometimes lice also got caught in the droplets, which caused significant non-nematode induced mortalities. A series of preliminary tests determined that 8% Tween 80 (Tw80) allowed good wetting of wool fibres without droplet formation and had no effect on nematode viability.

2.3.1. Wool assay 1

To assess the relative effectiveness of *S. carpocapsae*, *S. feltiae*, *S. riobrave* and *H. bacteriophora* in infecting and killing lice in wool, groups of naturally aligned wool fibres (staples) plucked from shorn fleece were cut to