Veterinary Parasitology 179 (2011) 277–282

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Short communication

Ability of the fungus *Duddingtonia flagrans* to adapt to the cyathostomin egg-output by spreading chlamydospores

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ARTICLE INFO

Article history:
Received 8 September 2010
Received in revised form 9 February 2011
Accepted 17 February 2011

Keywords:
Nematodes
Duddingtonia flagrans
Biological control
Grazing horses
Nematophagous fungi

ABSTRACT

The analysis of the capability of the nematode trapping-fungus *Duddingtonia flagrans* to adapt to the cyathostomin egg-output in horses was evaluated. Fecal samples from 196 pasturing autochthonous Pura Raza Galega horses were collected from the rectum and then divided according to the egg-output into three groups: ≤300, 310–800 and >800 eggs per gram feces. Four doses of chlamydospores (0.1, 0.2, 0.4 and 0.8 × 10^6/100 g feces) were directly spread onto fecal pats on the ground, remaining one without treatment as control.

Fecal pats confirmed the presence of gastrointestinal nematode larvae belonging to strongylid cyathostomins (*Cyathostomum* and *Gyalocephalus* spp). An overall 94% (95% CI 91, 97) percentage of reduction was obtained, and an increase in the activity of the trapping-fungi simultaneously to the rising in the number of cyathostomin eggs and larvae in the coprocultures was detected. A significantly highest reduction of the cyathostomin L3 in the coprocultures with more than 800 EPG was found, which indicates that *Df* trapping activity is larvae nematode density-dependant.

The present research showed the high biological activity of *D. flagrans* against nematode larvae can adjust to the cyathostomin egg-output, and underlines its efficacy as a practical method for the control of these parasites in grazing horses.

1. Introduction

Some livestock managing involve animals under pasturing conditions, but the possibility for infection by gastrointestinal nematode parasites is notably enhanced under this regime (Francisco et al., 2009). Larvae belonging to several genera (*Strongylus*, *Triodontophorus*, *Gyalocephalus*, *Trichonema*, *Ostertagia*, *Trichostrongylus*, *Haemonchus*, *Chabertia* or *Oesophagostomum*) exit the egg and develop in the environment to attain the infective stage (L3), which are ingested when the animals feed the grass. These parasitic infections reduce the benefits and the inputs afforded (poor gain weight, low reproductive indexes), being chemotherapy the most employed procedure for their control.

Duddingtonia flagrans is a nematophagous fungus which develops traps to capture nematodes present in the soil (Mendoza de Gives et al., 2006). Successful results have been achieved to reduce gastrointestinal nematode infections in horses, calves and sheep under northern temperate, tropical or subtropical climates (Gómez-Rincón et al., 2006; Braga et al., 2009; Campos et al., 2009; Maciel et al., 2010; Silva et al., 2010). This fungus is capable to produce a

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doi:10.1016/j.vetpar.2011.02.014

large quantity of chlamydospores able to resist the passage through the gastrointestinal tract of ruminants and monogastrics, hence different investigations regarding the oral administration of aqueous suspensions have been carried out (Baudena et al., 2000). This practice requires the correct immobilization of the animals, thus the use of multinutritional pellets containing spores of the nematode-trapping fungi *D. flagrans* and *Monacrosporium thaumasium* as vehicles has been checked in sheep (Casillas-Aguilar et al., 2008) and horses (Tavela Ade et al., 2011).

In the last decades an increasing tendency to the abandonment of productive lands has been observed in many European countries. Under the requirement for keeping these properties free of shrubs and bushes and thus for reducing the risk for fire, the presence of 1–2 animals (mainly horses and then donkeys, sheep and goats) is becoming usual for making advantage of <0.5 ha pastures.

There is a lack of information about the efficacy of the addition of the chlamydospores directly onto the pasture. The possible influence of the numbers of eggs passed by feces on the efficacy of *D. flagrans* spores to form trapping devices in feces remains also untested. In the current work, the utility of the direct administration of spores on the pasture was evaluated, and their ability to adapt to different cyathostomin egg-output numbers also. An annual coprological survey was conducted to get a useful knowledge about the risk periods for infection in horses.

2. Materials and methods

2.1. Area of study

The current research was developed in SW Europe (Galicia, Spain) (42°20′–43°45′N, 6°49′–8°00′W), an agricultural area where cattle livestock represents the main farm activity.

2.2. Annual variations of nematode egg-output and on climatic parameters

To determine the kinetics of nematode egg-output and the highest risk periods for infection, a herd of 25 indigenous Pura Raza Galega (PRG) horses in a farm in Lugo (NW Spain) was monthly sampled throughout 2008.

Data corresponding to the maximal temperature, minimal temperature, rainfall, and relative humidity were monthly obtained from 2 automated meteorological stations to establish the climatic pattern.

All the management of the animals has been carried out in accordance with the EC Directive 86/609/EEC for animal experiments.

2.3. Design of the study of the efficacy of D. flagrans

According to the results from the previous experience, fecal samples were collected between June and October 2009 from 196 indigenous Pura Raza Galega (PRG) grazing horses in several farms located at different sites interspersed within this area, and the distance among the farms is 20–100 km approx.

By taking into account previous investigations concerning the cut-off egg-output values to establish the anthelmintic treatments the horses were grouped in the basis of the counts of strongyle eggs per grams (EPG): G-1 (≤300 EPG), G-2 (310–800), G-3 (>800 EPG) (Nielsen et al., 2006; Uhlinger, 2007).

2.3.1. Coprological techniques

Fecal samples were individually collected from the rectum of the horses and analyzed by the coprological flotation method. Five grams of each fecal sample were processed (by duplicate) by using the copromicroscopical flotation technique (MAFF, 1986), with a sensitivity of 10 eggs per gram of feces. The counts of nematode eggs were expressed as counts of EPG.

The microscope analysis of fecal samples was performed and additional blind samples were run to diminish possible technique errors.

To gain more information about the different genera/specie of the strongyles affecting the horses, fecal samples were cultured for 10–15 days at 22–24 °C to allow the development of eggs to infective larvae. Once larvae showed the typical morphological features of L3, they were collected by means of the Baermann procedure (Osterman Lind et al., 1999; Kuzmina et al., 2006) and taxonomically identified according to Lichtenfels et al. (1998). The counts were expressed as numbers of larvae/gram (LPG).

2.3.2. D. flagrans chlamydospore production

The Mexican strain of *D. flagrans* FTHO-8 (CENID-PAVET, INIFAP-MEXICO) was used and chlamydospores were produced, harvested and managed according to Llerandi-Juárez and Mendoza de Gives (1998).

2.3.3. Efficacy of D. flagrans

The efficacy of *D. flagrans* against nematode larvae was measured by comparing the number of L3 recovered from fecal pats with (LPGDR) and without *D. flagrans* chlamydospores (LPG). For every horse, three pats were prepared by leaving 50 g feces/each in plastic trays (50 cm × 50 cm) placed in the pastures fed by the horses.

The spores of *D. flagrans* were diluted in an aqueous solution and added to two pats, and the other remained as control. Four doses of 0.1 (D1), 0.2 (D2), 0.4 (D3) and 0.8×10^6 (D4) spores/100 g feces were employed. Three replicates were considered in all cases.

The efficacy in the larval reduction by the fungal action was determined as follows:

$$\text{Larval reduction (LR \%)} = \left(\frac{\text{control mean L3} - \text{fungus mean L3}}{\text{control mean L3}}\right) \times 100$$

Twelve days after the addition of the chlamydospores, the pats were collected and analyzed by using the flotation and the Baermann techniques.

2.4. Statistical analysis

Considering that EPG and LPG counts are not normally distributed, these data were presented as the quartiles 1,