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Research paper

Feeding horses with industrially manufactured pellets with fungal spores to promote nematode integrated control

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ABSTRACT

The usefulness of pellets industrially manufactured with spores of parasiticide fungi as a contribution to integrated nematode control was assessed in grazing horses throughout sixteen months. Two groups of 7 Pura Raza Galega autochthonous horses (G-T and G-P) were dewormed pour-on (1 mg Ivermectin/kg bw) at the beginning of the trial, and other group (G-C) remained untreated. The G-P was provided daily with commercial pellets to which was added a mixture of fungal spores during the industrial manufacturing (2×10^6 spores of *Mucor circinelloides* and same dose of *Duddingtonia flagrans*/kg), and G-T and G-C received pellets without spores. The efficacy of the parasiticidal strategy was assessed by estimating the reduction in the faecal egg counts (FECR) and in the number of horses shedding eggs in the faeces (PHR), and also the egg reappearance periods (ERP). Blood analyses were performed to identify the changes in the red and white cell patterns. To ascertain if horses developed an IgG humoral response against the fungi, antigenic products collected from *M. circinelloides* and *D. flagrans* were exposed to the horse sera by using an ELISA.

The faecal elimination of eggs of *Parascaris equorum* and strongyles ceased 2 weeks after treatment in G-T and G-P, thus the values of FECR and PHR were 100%. No *P. equorum*-eggs were detected later, and the strongyle egg reappearance period was 28 weeks in G-P, and 8 weeks in G-T. Strongyle egg-output values remained lower than 300 eggs per gram of faeces in the G-P, whereas numbers between 330 and 772 in G-C and G-T were recorded. Normal values for the erythrocytes, haemoglobin and haematocrit in horses consuming pellets with spores were recorded, and lower than normal in the other groups. Sensitization of horses to the fungal species was disproven. It is concluded that feeding horses with pellets industrially manufactured with fungal spores represents a very useful tool to implement an integrated control of helminths affecting horses. This strategy allows a decrease in their risk of infection, aids in reducing the frequency of anthelmintic treatment.

1. Introduction

As described for other animal species, grazing horses are at high risk of infection by helminth parasites, mainly cestodes and nematodes (ascarids, strongyles and oxyurids) (Lyons et al., 2007; Relf et al., 2013; Rehbein et al., 2013). The possibility also exists that horses on pastures could be exposed to the liver fluke *Fasciola hepatica* and become infected (Arias et al., 2012; Soykan and Oge, 2012; Sanchís et al., 2015). Ingestion of *P. equorum* eggs containing second-stage larvae leads to infection especially in horses younger than 15 months, although infection has been detected in adult horses also (Francisco et al., 2009a,b; Larsen et al., 2011; Burk,

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2013). Infection by strongyles occurs when horses ingest third stage larvae with the herbage, where they can live for at least three months under appropriate conditions (high humidity and warm temperature) (Corning, 2009).

By considering that free-living stages of different parasites (ascarid and trematode eggs, cysticercoid-containing mites, strongyle larvae) can be present in pasture, administration of anthelmintics to the horses provides only a temporary solution. In the last years the intense use of anthelmintic drugs has led to the development of resistance in most of nematode parasitic populations (Reinemeyer, 2009; von Samson-Himmelstjerna, 2012; Canever et al., 2013). According to the selective treatment when horses exceed a predetermined threshold is strongly recommended (Uhlinger, 2007; Francisco et al., 2012; Nielsen et al., 2014).

Some approaches rely on biological control procedures through the distribution of parasiticidal fungi in theenvironment. Favourable results against the larval stages of strongyles by means of the nematode-trapping fungi *Duddingtonia flagrans* or even *Monacrosporium thaumassium* have been reported (Fernández et al., 1999; Araújo et al., 2004). There has been little research conducted on the usefulness of predator (ovicidal) fungi against the eggs of trematodes, cestodes or ascarids, most involving *Pochonia chlamydosporia* (Silva et al., 2010; de Carvalho et al., 2014). Recent investigation showed the ability of *Mucor circinelloides* to destroy the eggs of *Fasciola hepatica* and *Parascaris equorum* in the faeces of infected animals (Arroyo et al., 2016).

Different fungal formulations consisting of oral suspensions and feeding supplements have been assayed (Terrill et al., 2004; Waller et al., 2006; Ojeda-Robertos et al., 2008; Sagüés et al., 2011). Under laboratory conditions, mass mycelia have been embedded in alginate pellets for facilitating the administration of *D. flagrans* or *M. thaumassium* spores to livestock in tropical and temperate regions (Braga et al., 2009; Tavela Ade et al., 2013). Data regarding their inclusion during the industrial manufacturing of commercial pellets are not available.

Pelleted feeds are frequently provided for horse nutrition because of the presence of all the ingredients in every pellet ensures a complete and balanced ration. In previous works, the ability of the spores of *M. circinelloides* (ovicidal) or *D. flagrans* (larvicidal) to survive the industrial manufacturing of pelleted feed without losing their activity has been described (Arias et al., 2015; Arroyo et al., 2016). Considering the presence of parasitic eggs and larvae in thepasture, it was decided to investigate their combined effect against both.

In the current investigation, the preventive effect on nematode infection by feeding horses with pellets manufactured with a mixture of spores of *M. circinelloides* and *D. flagrans* was evaluated.

2. Material and methods

2.1. Area of study

This study was carried out in Castro Riberas de Lea (Lugo, northwest Spain, 43°15′83″N – 7°05′0″W).

2.2. Fungal specimens

Two fungal species with proven parasiticidal activity were utilized in the current study, *Mucor circinelloides* (ovicidal) and *Duddingtonia flagrans* (larvicidal) (Cortiñas et al., 2015; Arias et al., 2015). With the aim to obtain their spores, both fungi were simultaneously cultured in a submerged medium (COPFr; patent Nr PCT/ES2014/070110) for 1.5–2 months at room temperature until reaching a concentration higher than 1.10^8 spores/L medium (Arias et al., 2013).

2.3. Experimental design

Twenty-one autochthonous Pura Raza Galega mares (2–8 yr) were randomly divided into three groups of 7 each. These are indigenous horses feeding natural pastures in forests and wooded areas (a regime called silvopasturing). Due to difficulties in their handling, deworming consists of the pour-on administration of macrocyclic lactones (Francisco et al., 2009a,b).

The horses of group G-P were dewormed in August 2014 (1 mg Ivermectin pour on/kg bw, Noromectin 0.5%, Norbrook Laboratories, UK) and provided daily pelletswith fungal spores; G-T was also dewormed in August 2014 (1 mg Ivermectin/kg bw pour on) and received daily pelleted feed without fungal spores; G-C remained without treatment as control and was given daily concentrate without fungal spores.

Horses were maintained in three different 3 Ha fenced meadows provided with drinkers, feeders and wooden shelters. Water was available ad libitum, and a quantity of 2.5 kg of concentrate given daily to each horse. The equines were supplemented with wheat straw and barley when the grass was scarce (December to February and July–August).

2.4. Pelleted horse feed

Horses belonging to G-C and G-T were given a commercially available pelleted feed (*ProHorse Club*®, Nanta, Padrón, Spain), which contains cereal grains and by-products, oil seeds and derivatives, sugar cane processing by-products, minerals, forages and amino acids. The analytical composition comprises crude protein (14%), crude fat (2.9%), crude fibre (12.5%), Calcium (1.5%), Phosphorus (0.65%), Sodium (0.53%), Magnesium (0.54%), vitamin A (10000 UI/kg), vitamin D3 (1500 UI/kg) and vitamin E (42 UI/kg).

Every 3 months, one batch of concentrate was inoculated with spores of *M. circinelloides* and *D. flagrans* and provided to the G-P horses. After milling the feed ingredients, a dosage of 2×10^6 spores of each fungus was added per kilogram of meal in the feed mixer, and the complete blend conditioned by injecting steam (75 °C for 90 s) before entering the pelletizer. The final product was cooled, dried and finally packed into 25 kg bags.

2.5. Coprological probes

Faeces were collected monthly directly from the rectum, and 5 g analysed by using the saline flotation test (ρ= 1.20 g/L), with a sensitivity of 30 eggs per gram (EPG) (Francisco et al., 2009a,b). Then, a quantity of 15–20 g faeces from every horse in each group were mixed and pooled, and finally incubated for 20 days at 22–25 °C. Four replicates were performed for each group. Third-stage larvae (L3) were collected by means of the Baermann technique, observed under a light microscope and identified according to morphological keys (MAFF, 1986).

2.6. Evaluation of the efficacy

The efficacy of the parasiticide procedures was measured by estimating the reduction of the faecal egg counts (FECR) as well as of the number of horses shedding eggs in the faeces (PHR) (Francisco et al., 2012):

$$\text{FECR}(\%) = [1-(\text{FEC}_{\text{post-treatment}}/\text{FEC}_{\text{pre-treatment}})]\times 100$$

$$\text{PHR}(\%) = [1-(\text{number of positive horses}_{\text{post-treatment}} / \text{positive horses}_{\text{pre-treatment}})]\times 100$$