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Biological characterization and pathogenicity of three *Haemonchus contortus* isolates in primary infections in lambs

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ABSTRACT

The biological characterization and differential pathogenicity of three isolates of *Haemonchus contortus*, one autochthonous (Aran 99) and two allochthonous (Moredun Research Institute, MRI, and Merck Sharp and Dohme, MSD) were studied by primary experimental infection of Manchego lambs. Thus, six female lambs (5.5 months old) were infected with 12,000 L_3 larvae of each helminth isolate. Parasitological (pre-patent period, parasite egg shedding dynamics), biopathological (packed cell volume (PCV), haemoglobin concentration, plasma proteins, serum pepsinogen) and zootechnical parameters (live weight gain, thoracic perimeter) were measured throughout the study. After sacrifice (85 days post-infection (pi)), lamb carcasses were inspected for parasite burden and development (establishment rate, male/female ratio, degree of parasite development), and the average carcass weight of the experimental groups was compared.

The autochthonous combination (Manchego lambs—Aran 99) had a longer pre-patent period (28 days) and a significantly different pattern of egg elimination (maximum elimination on day 80 pi). The establishment rate and parasite burden (average values of 8.18% and 988 adult helminths, respectively) were both low, with no significant differences between isolates. There were no significant differences in parasitic nematode development in terms of size and weight (1264.66 μm and 149.45 μg for male worms and 2093.33 μm and 411.46 μg for females, respectively), although Aran 99 females weighed less ($p < 0.05$).

All isolates induced a slight but significant reduction of PCV values from day 23 pi onwards. Inter-isolate differences were found, with the effects in the case of MSD being more pronounced. Variations of serum protein levels were minimal in all lamb groups. The live weight gain of MSD- and Aran 99-infected animals was significantly lower ($p < 0.05$) than for MRI-infected lambs and uninfected control animals. Carcass yield from the lambs infected with the autochthonous isolate (Aran 99) was lower. The MSD isolate therefore showed a higher comparative pathogenicity.

1. Introduction

Haemonchus contortus is a gastric nematode that infects ruminants, particularly sheep and goats. This helminth species has a direct life cycle, with free-living pre-parasitic stages, and its successful transmission is dependent on the prevailing environmental conditions (Veglia, 1915). This parasite can be found all over the world, although infections are more prevalent and intense in areas with warm and humid climates. Indeed, haemonchosis is one of the most significant limiting factors for the health and productivity of small ruminants in these regions (Radostits et al., 2000).

H. contortus infections can follow different clinical courses, ranging from chronic cases in older animals with low parasite burdens to acute and often fatal outbreaks in young animals or those not previously exposed to the

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parasite. The pathophysiology of these infections includes different digestive tract disorders, such as loss of appetite, intestinal motility and flow alterations, increased gastric pH, and impaired energy and protein metabolism (Holmes, 1987; Fox, 1997; Hoste, 2001). The main pathogenic mechanism, however, is related to haematophagous feeding of the pre-adult and adult stages on the abomasal mucosa, which leads to anaemia, hypoproteinaemia, oedema and death in heavily infected animals (Rowe et al., 1988; Rahman and Collins, 1990).

The closed-structure of many sheep and goat herds, the different environmental conditions in those areas of the world where the nematode is present, and the extreme variability of the management practices employed (feeding, anthelmintic use, treatment strategies, herd and breed age structures) create obstacles for genetic exchange, thus favouring the appearance of discrete populations of *H. contortus* (Troell et al., 2003, 2006a). The free movement of goods, people, services and capital, including the free movement of animals subject to temporary community restrictions, is one of the main cornerstones of EU policy. The sheep population in the EU exceeds 90 million, therefore the movement of (presumably parasitized) animals between different geographic locations creates new parasite–host combinations by bringing previously separated parasite isolates into contact with each other. It is clear that the co-existence of populations of gastrointestinal nematodes, including *H. contortus*, with distinct phenotypic characteristics (e.g. biological requirements, anthelmintic resistance, virulence and immune response elicited) will have a significant impact on herd management, including control measures. To date, there have been relatively few experimental studies using combinations of *H. contortus* sheep-isolates (Newton et al., 1995; Aumont et al., 2003; Gatongi et al., 2003; Troell et al., 2006b). More recently, Hunt et al. (2008) found both genotypic and phenotypic differences amongst *H. contortus* isolates in Australia.

The availability of an *H. contortus* isolate obtained in central Spain (Aran 99) (Domínguez-Toraño et al., 2000) led us to test the apparent adaptation of host–parasite sympatric combinations (Aumont et al., 2003) using Manchego lambs and this isolate. Two combinations with older, widely used *H. contortus* isolates subpassaged in experimental animals (MRI and MSD) were included for comparative purposes. Immunological, parasitological, and physiological parameters were determined in order to study the host–parasite adaptation. The results obtained point to the existence of certain inter-isolate differences related to parasite infectivity and development (duration of pre-patent period, faecal egg output and helminth development). Isolate-dependent biopathological and zootechnical effects (packed cell volume, haemoglobin, live weight gain, carcass yield) were also found.

2. Materials and methods

2.1. Parasite isolates

Three *H. contortus* nematode isolates were used, namely MSD, provided by Merck Sharp and Dohme-AGVET, Spain, and maintained in our installations by using subpassages in helminth-free lambs since 1987; MRI (Moredun Research Institute, Edinburgh, Scotland); and Aran 99, obtained from naturally infected sheep from Aranjuez (Madrid, Spain; Domínguez-Toraño et al., 2000). Infective larvae (L_3) were obtained from faecal cultures (26 °C, 10 days) of monospecifically infected donor lambs for all three isolates. These larvae were then separated from the faecal material using the Baermann technique, cleaned and stored at 4 °C in tap water until use.

2.2. Lambs and experimental design

Twenty-four 2-month-old female Manchego lambs were obtained from a local closed-structure-type flock (Villarrobledo, Castilla-La Mancha). The animals were kept in stables used for experimentation (Veterinary School, Madrid) under helminth-free conditions. Feed consisted of hay, mineral supplement and water *ad libitum*. Commercial pelleted food was also provided, with the amount increasing with age (400, 500 and 600 g/animal/day). When the lambs were 5.5 months old, they were stratified on a weight basis into four comparable groups of six animals each. The lambs in each infected group received a single dose of 12,000 L_3 of an *H. contortus* isolate (MSD, MRI, Aran 99) by means of oro-oesophageal administration. A further group was kept as an uninfected control. All lambs were slaughtered on day 85 post-infection (pi) at a local abattoir (MAFASA, Getafe, Madrid).

2.3. Parasitological parameters

Faecal egg elimination (eggs/g) was checked by taking samples directly from the rectum twice a week. Sampling commenced before infection and continued on a daily basis from day 12 pi until the animals reached patency, whereupon samples were taken two or three times a week. The results from the last 3 days sampled were used for the indirect determination of the relative fertility of the females. Coprological analysis was carried out by using a modified McMaster method (MAFF, 1971), where each egg counted represents an elimination of 50 eggs/g. After slaughter, the abomasa were refrigerated and transferred to our department, where they were opened and washed with a 0.9% saline solution and the adult helminths collected (Gómez-Muñoz et al., 1998). After washing, the abomasa were placed in warm water for 4 h to recover the L_4 from the gastric mucosa; the washing solution was sedimented (2 h) and stored in 5% formaldehyde (Amarante et al., 1999) for subsequent analysis. An aliquot containing 10% of the adult parasites retrieved was fixed with 5% formaldehyde. The adult parasites obtained from this aliquot were classified by sex and counted to determine the recovery rate (% of the dose administered) and the female/male ratio (Coadwell and Ward, 1982). The relative fertility was estimated by dividing the average of the eggs/g values obtained during the last three days of analysis by the estimated number of females recorded, and expressed as eggs/g/female/day (Gómez-Muñoz et al., 1998). The length of the adult nematodes was determined for 40 females and 40 males from each lamb. These helminths were dried and weighed until