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Experimental infection of calves with *Haemonchus placei* and *Haemonchus contortus*: Assessment of parasitological parameters

Flávia Carolina Fávero [a], Carolina Buzzulini [a], Breno Cayeiro Cruz [a], Gustavo Felippelli [a], Willian Giquelin Maciel [a], Bruna Salatta [a], Weslen Fabricio Pires Teixeira [a], Vando Edésio Soares [a], Gilson Pereira de Oliveira [a], Welber Daniel Zanetti Lopes [a,b,*], Alvimar José da Costa [a]

[a] *Faculdade de Ciências Agrárias e Veterinárias, UNESP/CPPAR, Via de acesso prof. Paulo Donatto Castellani, s/n CEP:14884-900, Jaboticabal, São Paulo-Brazil*

[b] *Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia GO 74605-050, Brazil*

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ABSTRACT

The present study evaluated the viability and possible effects of *Haemonchus contortus* infections in experimentally prime infected calves, comparing them to infections by *Haemonchus placei.* Ten male Holstein newborns were used. All calves were individually weighed for subsequent group formation, in which two animals were kept as a control group, inoculated with water (GI); four animals were inoculated with 10,000 third stage (L3) *Haemonchus contortus* larvae (GII); and the remaining four calves were inoculated with 10,000 third stage (L3) *H. placei* larvae (GIII). All experimental animals were necropsied on the 42nd day after inoculation. Based on results obtained by the present study, it can be concluded that bovine calves were susceptible to infections by both *Haemonchus* species (*placei* and *contortus*). *H. contortus* presented an inferior pre-patent period when compared to *H. placei.* No significant difference ($P > 0.05$) was observed between *Haemonchus* burdens recovered from both infected groups (GII and GIII). Moreover, *H. contortus* females maintained an egg production rate similar to *H. placei* females in young animals, which can contribute to pasture contamination by both *Haemonchus* species. This could possibly lead to negative reflexes on helminth control based on a mixed pasture with bovines and ovines, especially when it involves younglings.

1. Introduction

Gastrointestinal endoparasites are amongst the most important health concerns found in bovine herds, affecting the physiological development of young (growth phase) animals (Felippelli et al., 2014). Of all nematode species that affect ruminants, those belonging to the *Haemonchus* genus stand out, mainly due to hematophagy and lesions caused on abomasum mucosa. In order to prevent or minimize production losses caused by these gastrointestinal helminths, the main resources available are anthelmintic treatments (Lopes et al., 2014).

According to Fernandes et al. (2004), an alternative adopted to reduce pasture contamination with infecting larvae of these nematodes is the use of alternate or simultaneous/mixed grazing between different herbivore species (equines, caprines, ovines and bovines). Studies conducted in naturally infected animals created in a grazing system (Amarante et al., 1997) demonstrate the possibility of crossed infections between ovine's *Haemonchus contortus* and bovine's *Haemonchus placei*. However, as time passes, animals usually develop resistance and naturally eliminate helminth species that are not well adapted (Silva et al., 2015). Despite these works conducted by the aforementioned researchers, we are aware of no studies performed with the aim of confirming these hypotheses in young, experimentally prime infected animals. Based on this prerogative, the present study evaluated the viability and possible effects of an infection by *H. contortus* in young calves that were experimentally prime infected in comparison to infection by *H. placei.*

* Corresponding author. Fax: +55 16 3209 2606.
E-mail addresses: wdzlopes@fcav.unesp.br, wdzlopes@hotmail.com (W.D.Z. Lopes).

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2. Material and methods

2.1. Location and animals

This experiment was conducted at the Center for Research in Animal Health (Centro de Pesquisas em Sanidade Animal—CPPAR), FCAV/UNESP, Jaboticabal campus, São Paulo, Brazil.

Ten male Holstein newborns were used. All of them were separated from their mothers at the moment they were born. These animals received colostrum in the first hours of life and were then kept in suspended paddocks, with the objective of avoiding any possibility of helminth infections. The animals received substituted, granulated commercial rations and Tifton until they were 60 days old. After weaning (60 days old), animals were fed for another 30 days with 70% Tifton hay and 30% commercial rations. Water was provided *ad libitum*.

2.2. Experimental group formation and animal inoculation with H. contortus and H. placei and L3 dosages

Animals were randomized into groups when calves were 90 days old. Each animal was individually weighed, and groups were then formed.

Larvae (L_3) of *H. placei* and *H. contortus* were kindly donated by Prof. Dr. Alessandro F.T. Amarante (Departamento de Parasitologia do Instituto de Biociências da Universidade Estadual Paulista—UNESP, Botucatu campus, São Paulo). The identification of different *Haemonchus* species used in the present study was performed on both L_3 and adults, based on morphological criteria and PCR criteria previously established by Santos et al. (2014) and Silva et al. (2015).

The number of infecting larvae (inoculum) was defined based on international standards defined by the World Association for the Advancement of Veterinary Parasitology (Wood et al., 1995), which recommends a single infection with approximately 5000–10,000 infecting larvae (L_3). Bovines from groups GII and GIII received approximately 10,000 infecting larvae (L_3) of the respective *Haemonchus* species (Table 1) orally with the aid of a 10 mL syringe. The volume was divided in three consecutive dosages. Each animal belonging to group GI (control) received oral placebo, which consisted of 30 mL of water.

2.3. Fecal collection, parasitological necropsies and helminth species identification

In the five days that preceded inoculation, daily EPG counts (Gordon and Whitlock, 1939 and adapted by Lopes et al., 2014) were conducted to confirm the absence of helminth infections in the experimental bovines. Starting on the 6th post-inoculation day, EPG exams were performed daily, seeking to determine the pre-patent period—to detect the first eggs in fecal samples that originated from each of the studied nematode species.

Euthanasia of all ten experimental calves was performed on the 42nd post-inoculation day (AVMA, 2013). The digestive system of each calf was separated into different anatomical segments (abomasum, small and large intestine). Abomasums were individually subjected to digestion with a pepsin hydrochloric acid solution (Wood et al., 1995). The contents of each segment were fixed in 70.0% alcohol (Borges et al., 2011). Remaining organs were also inspected for the presence of possible helminth species (Wood et al., 1995).

From all parasites recovered, which were subsequently stored in 70% alcohol, 10% of the total amount of specimens of each sex was randomly selected from each animal for analysis.

These parasite samples were measured to obtain their total length. For species classification in females, tails were measured, vulva types were registered, and cuts on the esophagus-intestine junctions were performed in order to count the synlophes (grooves), based on methodology described by Santos et al. (2014) and Silva et al. (2015).

In order to differentiate adult males, cuts of the esophagus-intestine junctions were also performed and spicules and hooks (located in the copulatory bursa) were measured, since these usually present larger dimensions in *H. placei* (Santos et al., 2014; Silva et al., 2015).

2.4. Data analysis

The experimental design used in EPG counts was completely random in a split plot in time scheme, with treatments as main parcels and observation dates as secondary parcels. EPG values underwent a logarithmic transformation (log ($x+1$)) in order to normalize responses homogenize variances.

When evaluating helminth burdens recovered in necropsy, values underwent logarithmic transformations (log ($x+1$)), and a completely randomized design was used.

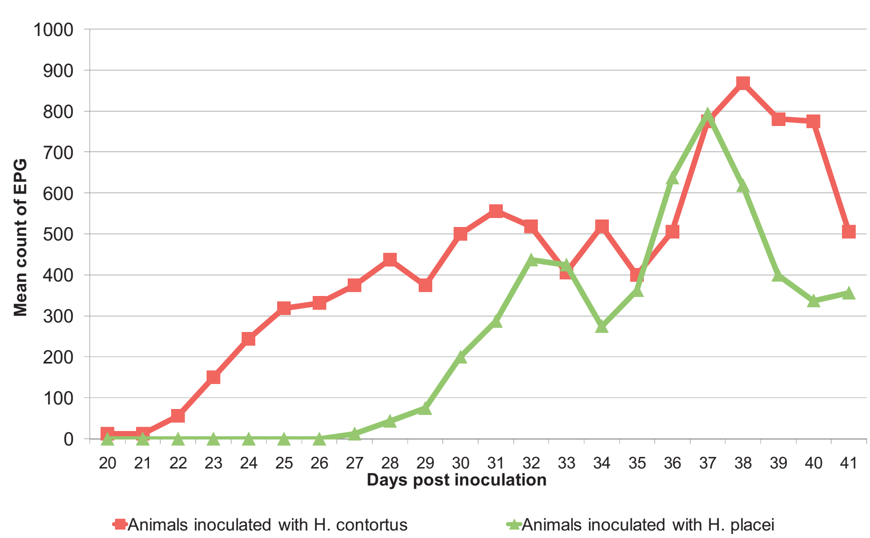

Fig. 1. Egg counts "strongyles" (*Haemonchus*) per gram of feces (EPG) in calves belonging to the two groups infected experimentally.