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Why is it important to correctly identify *Haemonchus* species?

Por que é importante a identificação correta das espécies de *Haemonchus*?

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Abstract

Parasitic gastroenteritis caused by *Haemonchus* spp. is a major cause of economic losses in the livestock industry because it impairs weight gain and increases mortality in cattle and small ruminants, especially in tropical and subtropical areas. The proper identification of the various species, as well as knowledge regarding the epidemiology of parasitic gastroenteritis, is essential for the establishment of sustainable strategies of parasite control. This review focuses on the use of easily applied, low-cost parasitological methods of identifying *Haemonchus* species on the basis of their morphology. In most studies carried out in Brazil, the distinctions between *Haemonchus contortus* and *Haemonchus placei* have not been considered. Many reports of *H. contortus*, particularly in cattle, might actually represent *H. placei*. The appropriate identification of species is therefore indispensable. In addition to the measurement of male spicules, new morphological characteristics, such as the synlophe, should be evaluated in order to differentiate between and among species. Measurements of infective larvae in fecal cultures can also indicate the identity of *Haemonchus* species. This approach can be quite useful in studies that do not involve animal sacrifice, such as studies of anthelmintic resistance based on the fecal egg count reduction test.

Keywords: *Haemonchus placei, Haemonchus contortus, Haemonchus similis,* ruminants, morphology.

Resumo

Infecções por *Haemonchus* spp. são uma das principais causas de perda econômica nas criações de ruminantes devido à redução no ganho de peso e mortalidade de bovinos e pequenos ruminantes, especialmente em regiões com clima tropical e subtropical. A identificação precisa das diferentes espécies, bem como o conhecimento sobre a epidemiologia das gastroenterites parasitárias, são fundamentais para a elaboração de estratégias sustentáveis de profilaxia das parasitoses. Essa revisão tem por objetivo central, abordar os principais métodos parasitológicos utilizados na identificação morfológica das espécies, os quais se caracterizam pela facilidade e baixo custo. Na maioria dos estudos realizados no Brasil, a distinção entre as espécies *Haemonchus contortus* e *Haemonchus placei* não tem sido considerada. Vários relatos de *H. contortus*, particularmente em bovinos, podem se tratar na verdade da infecção dos animais por *H. placei*. A identificação correta das espécies é, portanto, fundamental. Além das medidas dos espículos dos exemplares machos, outros detalhes morfológicos, tais como a sínlofe, devem ser avaliados com o objetivo de auxiliar na diferenciação das espécies. Mensurações das larvas infectantes, obtidas em coproculturas, podem também indicar a espécie de *Haemonchus* presente. Esse procedimento pode ser útil especialmente em estudos que não envolvem a necropsia de animais, como é o caso de testes destinados a avaliar a resistência anti-helmíntica em rebanhos.

Palavras-chave: *Haemonchus placei, Haemonchus contortus, Haemonchus similis,* ruminantes, morfologia.

Introduction

Parasitic gastroenteritis caused by *Haemonchus* spp. is a major cause of economic losses in the livestock industry because it impairs weight gain and increases mortality in cattle and small ruminants, especially in tropical and subtropical areas. The prophylaxis of parasitic gastroenteritis relies heavily on anthelmintic treatments. However, their frequent use has led to the appearance of resistant parasite populations, which have been jeopardizing the livestock industry worldwide. There have been many reports of anthelmintic resistance, including resistance to *Haemonchus* species, in small ruminants (ALMEIDA et al., 2010) and cattle (SUTHERLAND; LEATHWICK, 2011). Therefore, the proper identification of the various species, as well as knowledge regarding the epidemiology of parasitic gastroenteritis, is essential for the establishment of sustainable strategies of parasite control.

Until recently, it was difficult to distinguish between *H. placei* and *H. contortus*, because of their similar morphology. Therefore, many reports of *H. contortus*, particularly in Bovinae, might

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actually represent *H. placei* (HOBERG et al., 2004). This problem is evident in various studies of the epidemiology of *Haemonchus* infection in ruminants in Brazil. The discrepancies among epidemiological studies in cattle can be observed in Table 1. In most such studies, the distinctions between *H. contortus* and *H. placei* were not considered. Some authors followed the criteria, established by Almeida (1935) and by Gibbons (1979), that do not accept *H. placei* as a valid species, whereas others simply referred to specimens found in small ruminants as *H. contortus* and those found in cattle as *H. placei*. It is known that both species can simultaneously infect cattle and small ruminants specially when there are shared pastures (AMARANTE et al., 1997; ACHI et al., 2003; JACQUIET et al., 1998). In studies of anthelmintic resistance, *H. placei* and *H. contortus* were both identified in cattle raised in Brazil (CONDI et al., 2009) and in the USA (GASBARRE et al., 2009). In the latter experiment, the *H. contortus* encountered were well adapted to transmission in cattle, because the pastures under study had not been grazed by small ruminants for more than 40 years. Although considerable advances have been made toward understanding the genomics of nematode parasites, most cases of haemonchosis occur in the tropics, where modern technology is typically unavailable due to its high costs. Therefore, the use of traditional parasitological methodology continues to be an effective tool in the diagnosis and epidemiological study of parasitic gastroenteritis. This review focuses on the use of easily applied, low-cost parasitological methods of identifying *Haemonchus* species on the basis of their morphology.

Species of *Haemonchus*

One of the first detailed reviews of *Haemonchus* spp. was published by Almeida (1935). On the basis of the male copulatory structures (bursa and spicules), the author classified 10 *Haemonchus* species as valid (Table 2). The author did not mention *H. placei*, and the specimens obtained from cattle were designated *H. contortus*. Later, Gibbons (1979) also established a list of valid *Haemonchus* spp., comprising nine species. The classification was based on the number and arrangement of the longitudinal cuticular ridges and male characteristics such as dorsal ray, distal ends of spicules, and gubernaculum. The author considered *H. placei* and *H. contortus* it to be synonymous and therefore excluded *H. placei* from the list of valid species (GIBBONS, 1979). However, in a subsequent study, co-authored by Gibbons (HOBERG et al., 2004), *H. placei* was recognized as a valid species. In that study, the authors performed a phylogenetic analysis of 25 morphological characteristics and established a list of twelve valid *Haemonchus* species (Table 2). Of those twelve species, three have been reported in ruminants raised in Brazil: *H. contortus*, *H. similis*, and *H. placei*.

Morphological and Cytogenetic Differences Among Species

Among the parasite species that infect domestic ruminants, male specimens of *H. similis* are easily distinguished, because they present spicules that are shorter than those observed for *H. placei* and *H. contortus*, whereas the distances of the barbs to distal end of the spicules of *H. similis* are longer (Table 3).

Although various studies have confirmed the distinctions between *H. placei* and *H. contortus*, there is still some debate regarding their individual identities. In a recently published veterinary parasitology textbook, Taylor et al. (2010, p. 59) stated:

> Until recently, the sheep species were called *H. contortus* and the cattle species *H. placei*. However, there is now increasing evidence that these are the single species *H. contortus* with only strain adaptation for cattle and sheep.

Table 1. Prevalence of *Haemonchus placei*, *H. contortus*, and *H. similis* in cattle raised in different regions of Brazil. Means of infection intensity[a] are in parentheses.

Location, state	n	*H. placei*	*H. contortus*	*H. similis*	Reference
		Prevalence (MI)	Prevalence (MI)	Prevalence (MI)	
19 counties in SP, MG, GO, and MT	38	-	7.9% (192)	63.2% (621)	Machado et al. (1979)
13 counties in MT and MS	65	-	23.9%	76.1%	Grisi and Nuernberg (1971)
Campo Maior, PI	24[b]	-	96% (1378)	92% (764)	Girão et al. (1985)
	24[c]	-	84% (1304)	96% (1139)	
Itaju do Colônia region, BA	48	-	72.9% (493)	81.3% (483)	Santana et al. (1989)
Nhecolandia, MS	45	-	71.1% (96)	88.9% (1854)	Catto and Ueno (1981)
Formiga, MG	76	100% (3896)	-	29.0% (160)	Santos et al. (2010)
Seropédica, RJ	98	96-100% (559-1720)	-	-	Pimentel Neto and Fonseca (2002)
Jaboticabal, SP	42	97.6% (2010)	-	21.4% (175)	Borges et al. (2001)
Irapuã, SP	40	-	12.5% (8)	75.0% (179)	Rodrigues et al. (1985)
Ilha Solteira, SP	6	-	83.3% (151)	83.3% (463)	Zocoller et al. (1983)
Northwestern, SP	48	33.3%	-	33.3%	Bresciani et al. (2001)
São Carlos region, SP	74	-	100% (988)	43.2% (116)	Oliveira and Matsumoto (1985)
Santa Maria, RS	12	91.7% (631)	-	66.7% (374)	Santiago et al. (1975)
Itaqui, RS	18	83.3% (382)	-	16.7% (57)	Santiago et al. (1975)

n = number of animals; SP = São Paulo; MG = Minas Gerais; GO = Goiás; MT = Mato Grosso; MS = Mato Grosso do Sul; BA = Bahia; PI = Piauí; RJ = Rio de Janeiro; [a]MI (mean intensity) = Total number of parasites of each species/number of hosts infected with that parasite; [b]9–12 months of age; [c]20–24 months of age.

This is inconsistent with other data in the literature. There is considerable morphological, biological, and genetic evidence of the existence of both species. In this context, there are key differential aspects.

1. *Synlophe*

The system of longitudinal surface cuticular ridges, known as synlophe, shows significant differences among species: In both sexes of *H. contortus*, the synlophe extend significantly beyond midbody, whereas they end near midbody in both sexes of *H. placei* (LICHTENFELS et al., 1986). In addition, the synlophe system of *H. contortus* has 30 ridges in the region of the posterior half of the esophagus, whereas *H. placei* and *H. similis* have 34 (LICHTENFELS et al., 1994).

2. *Spicules and spicule barbs*

Despite occasional anomalies, male characteristics are more reliable and suitable as a means of species identification than are female characteristics. Because numbers of the vulvar flaps and their shapes are variable and dependent on several factors, they cannot be relied upon for specific and generic differentiation (GIBBONS, 1979).

Table 2. Valid *Haemonchus* species, according to Almeida (1935), Gibbons (1979) and Hoberg et al. (2004).

Almeida (1935)	Gibbons (1979)	Hoberg et al. (2004)
H. contortus	*H. contortus*	*H. contortus*
H. longistipes	*H. longistipes*	*H. longistipes*
H. similis	*H. similis*	*H. similis*
*H. lunatus**		
H. bedfordi	*H. bedfordi*	*H. bedfordi*
H. vegliai	*H. vegliai*	*H. vegliai*
H. mitchelli	*H. mitchelli*	*H. mitchelli*
*H. bispinosus**		
*H. cervinus**		
H. lawrencei	*H. lawrencei*	*H. lawrencei*
	H. dinniki	*H. dinniki*
	H. krugeri	*H. krugeri*
		H. horaki
		H. placei
		H. okapiae

*Synonymous with *H. contortus*, according to Gibbons (1979).

Male parasites identified as *H. contortus* present spicules and spicule barbs that are smaller than are those of *H. placei*. These differences were consistent in specimens collected in several regions of the world, such as Australia (ROBERTS et al., 1954), North America (HERLICH et al., 1958; LICHTENFELS et al., 1994), South America (SANTIAGO, 1968; AMARANTE et al., 1997) and Africa (JACQUIET et al., 1997; ACHI et al., 2003). Measurements taken by Lichtenfels et al. (1994) from specimens selected from different geographic regions and from several host species are presented in Table 3 and demonstrate consistent differences among *Haemonchus* species.

In order to identify individual male worms of *Haemonchus* species, Achi et al. (2003) described a linear discriminate function combining three measures of male spicules:

$$DF = 0.0016\ TL + 0.128\ THr + 0.152\ THl - 9.97 \quad (1)$$

where DF is the discriminate function, TL is the total length of the spicule, THr is the distance from the tip to the barb of the right spicule, and THl is the distance from the tip to the barb of the left spicule. Species identification was established as follows:

- DF < 0.63: *Haemonchus contortus*
- 0.63 < DF < 3: *Haemonchus placei*
- DF > 4: *Haemonchus similis*

In conclusion, the measurements of the spicules should be the first method employed to identify *Haemonchus* species, which easily allows the identification of *H. similis*. Overlap between measurements of *H. placei* and *H. contortus* can occur, casting doubt upon the correct identification. In this case, the enumeration of the longitudinal surface cuticular ridges (synlophe) can help distinguish between *H. contortus* and *H. placei*. The former has 30 ridges in the region of the posterior half of the esophagus, whereas the latter has 34. Synlophe studies rely on the analysis of histological preparations. However, in our laboratory, we demonstrated that, with the use of a sharp needle under a stereomicroscope, it is possible to cut a transverse section of the body at the esophageal-intestinal junction, i.e., a slice of *Haemonchus*, which can be examined under microscopy for enumeration of the cuticular ridges (unpublished data). Lichtenfels et al. (1986) also studied cross-sections of *Haemonchus* specimens obtained by free-hand cuts with a cataract knife.

3. *Infective larvae*

Infective larvae of *H. placei* are longer and more robust than are those of *H. contortus* (ROBERTS et al., 1954; SANTIAGO 1968), although *H. similis* infective larvae are shorter than either

Table 3. Morphometrics of male specimens of *Haemonchus contortus*, *H. placei*, and *H. similis.* Measurements are ranges followed by means in parentheses.

Morphological variable	*H. contortus*	*H. placei*	*H. similis*
Body length, in mm, range (mean)	11.1-17.0 (13.1)	10.9-18.9 (14.4)	7.3-10.1 (8.8)
Gubernaculum length, in µm, range (mean)	195-255 (221)	210-270 (245)	142-198 (179)
Spicule length, in µm, range (mean)	383-475 (425)	438-511 (481)	304-389 (341)
Right spicule barb length*, in µm, range (mean)	37-48 (42)	45-60 (55)	62-81 (71)
Left spicule barb length*, in µm, range (mean)	19-24 (22)	22-32 (27)	41-65 (54)

*Distances from spicule barbs to distal tips of spicules. Table adapted from Lichtenfels et al. (1994).

(SANTIAGO, 1968). In a detailed study of the biology and morphology of *Haemonchus* species, Santiago (1968) measured the infective larvae of *Haemonchus* and the mean values are presented in Table 4. Therefore, it is possible to have an indication about the identity of *Haemonchus* species based on the measurements of infective larvae present in fecal cultures. This approach can be very useful in studies that do not involve animal sacrifice, as in those of anthelmintic resistance based on the fecal egg count reduction test.

Table 4. Morphometrics of infective larvae of *Haemonchus contortus*, *H. placei*, and *H. similis*.

Species	Total larvae length, in µm	Sheath tail length*, in µm
	mean (SE)	mean (SE)
H. contortus	681.7 (2.3)	73.6 (0.53)
H. placei	784.6 (2.5)	99.2 (0.70)
H. similis	611.1 (5.4)	57.3 (0.8)

*Distance between the tip of the larval tail and the end of the sheath tail. Table adapted from Santiago (1968).

4. *Chromosome morphology*

The first detailed study of the cytology of ovine and bovine strains of *Haemonchus* was carried out in Australia by Bremmer (1955). The author observed that all *H. contortus* chromosomes (the autosomes and the X chromosome) presented similar morphology, whereas the X chromosome of *H. placei* differed from the autosomes in terms of morphology, the X chromosome being larger. Such differences in chromosome morphology were also observed in other studies (LE JAMBRE; ROYAL, 1980; AMARANTE et al., 1997).

In a study involving cross-breeding between *H. placei* and *H. contortus*, Bremmer (1955) obtained fertile hybrid females, which were also seen, particularly in sheep, in a natural, mixed infestation, when cattle, sheep, or goats grazed the same pasture. These hybrids, however, appeared only in small numbers and were never seen in animals with pure infestations, suggesting that there is some fertility barrier. In Brazil, the identification of *Haemonchus* species based on the chromosome morphology of specimens matched the identification of the same specimens based on length of spicule barbs (AMARANTE et al., 1997).

In hybridization experiments, Le Jambre (1979) observed that F1 males obtained by mating *H. contortus* males with *H. placei* females were sterile. Female hybrids from these generations had a low level of fertility when backcrossed to males of either parent species. Cytological studies of the hybrid males indicated that sterility was due to various types of meiotic disturbance and that spermatogenesis was arrested during metaphase I. In addition, many hybrid males produced by backcrossing hybrids with parental species had grossly deformed spicules.

Haemonchus Species in Ruminants in Brazil

In studies involving small ruminants in Brazil, *H. contortus* has been reported to be the major species infecting the abomasums of sheep and goats (Amarante, 2009; Vieira et al., 2009). *H. placei* was observed in sheep only when tracer lambs were placed on pasture grazed simultaneously by sheep and cattle (AMARANTE et al., 1997; ROCHA et al., 2008).

Haemonchus similis is frequently found in cattle in Brazil (Table 1) and has not been detected in sheep, even when they share pastures with *H. similis*-infected cattle (SANTIAGO, 1968; AMARANTE et al., 1997; ROCHA et al., 2008). Likewise, in Martinique (French West Indies), lambs sharing pastures with *H. similis*-infected cattle did not become infected (GIUDICI et al., 1999). In the Ivory Coast, a few specimens of *H. similis* were found in naturally infected sheep that shared pastures with cattle in the Savannah (ACHI et al., 2003). These findings confirm the high specificity of *H. similis*, which almost exclusively parasitizes cattle. One exception was reported in wild ruminants in Brazil. In six species of deer (Cervidae) in the states of Mato Grosso do Sul and São Paulo, the prevalence of *H. similis* infection was found to be 45.2% (NASCIMENTO et al., 2000). In that same study, the reported prevalence of *H. contortus* infection was 76.2%. It is of note that those authors did not attempt to differentiate between *H. placei* and *H. contortus*. Therefore, it is possible that the parasites identified as *H. contortus* in deer were in fact *H. placei*, because of the identification problems previously mentioned.

H. similis was reported as the dominant species in the abomasums of cattle raised in locations with high annual mean temperatures (>23 °C). In Brazil, various studies have been conducted in such locations, including the states of Mato Grosso and Mato Grosso do Sul (GRISI; NUERNBERG, 1971; CATTO; UENO, 1981), as well as the cities of Tupi Paulista, in the state of São Paulo (ROCHA et al., 2008), and Governador Valadares, in the state of Minas Gerais (LIMA, 1998). In certain areas of the states of São Paulo (BORGES et al., 2001), Minas Gerais (SANTOS et al., 2010), Paraná (BRICARELLO et al., 2007), and Rio Grande do Sul (SANTIAGO et al., 1975), where the annual mean temperatures are lower, *H. similis* is also found, but *H. placei* is the predominant species (Table 1).

Studies carried out in Brazil routinely demonstrate the presence of *H. similis* in cattle. One exception was an experiment conducted in the state of Rio de Janeiro and involving dairy cattle, in which the authors found only *H. placei*, with high worm burdens, especially from April to September (PIMENTEL NETO, 1976).

Final Remarks

For most Brazilian states, there are no consistent epidemiological data related to haemonchosis in ruminants. Given the enormous economic importance of the disease to the Brazilian livestock industry, this is an unacceptable situation. The problem is aggravated by the absence of standardized methodology to differentiate between *H. contortus* and *H. placei.* Therefore, the appropriate identification of species is indispensable. In addition to the measurement of male spicules, new morphological characteristics, such as the synlophe, should be included for differentiation between species. In addition, molecular markers can be developed to identify the species based on DNA extracted from eggs or from third stage larvae obtained from cultures. If possible, the parasite collections evaluated in previous studies should also be reanalyzed with these new approaches in order to clarify this issue.

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