

Comparative susceptibility and pathogeny of Nelore and Holstein-Friesian calves to the experimental infection of *Haemonchus placei* (Place, 1893)

(*Comparação entre patogenia e susceptibilidade de bezerras Nelore e Holandês à infecção experimental com Haemonchus placei (Place, 1893)*)

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ABSTRACT

Two groups of Holstein-Friesian and Nelore calves, five animals each, about nine months old, received, by oral route, 1,000 infective larvae (L₃) per kg of body weight of *Haemonchus placei*. Blood samples were collected by venipuncture, at weekly intervals, from one week before, to eight weeks after infection. Hematological studies comprised the hematocrit, differential leukocyte counts, hemoglobin, fibrinogen and plasma protein determinations. Parasitological examinations covered weekly fecal egg counts (EPG) and worm burden counts at necropsy. Samples of the abomasal mucosa were submitted to gross examination and histopathological studies. Both groups had increasing EPG after the fifth week, with Holstein calves showing higher counts than the Nelore. Holstein calves had anemia and hipoproteinemia from the third week post-infection to the end of the experiment, whereas Nelore calves showed no significant differences in those parameters. Holstein calves had significantly larger worm counts than the Nelore. The gross and histopathological lesions in the abomasum at necropsy were very similar, although macroscopically they look more apparent in the Holstein group. These results showed that Holstein calves are more susceptible to the infection and pathogenic effects of *H. placei* than Nelore calves.

Keywords: *Haemonchus placei*, helminth, calf, Holstein, Nelore

RESUMO

Dois grupos de cinco bezerros, das raças Holandesa e Nelore, com idade aproximada de nove meses, receberam por via oral uma dose única de 1.000 larvas infectantes (L₃) por kg de peso corporal de *Haemonchus placei*. Os animais foram mantidos sob observação clínica após a infecção. Amostras de sangue foram coletadas semanalmente por punção venosa, desde uma semana antes, até a oitava semana pós-infecção. Os estudos hematológicos abrangeram hematócrito, contagem diferencial de leucócitos, determinações de hemoglobina, fibrinogênio e proteínas plasmáticas. Os exames parasitológicos compreenderam exames de fezes semanais com contagem de ovos (OPG) e estimativa de cargas de vermes na necropsia. Amostras de tecido da mucosa do abomaso foram submetidos a estudos histopatológicos. Ambos os grupos mostraram números crescentes de OPG após a quinta semana, sendo que os bezerros de raça Holandesa apresentaram maiores contagens do que os da raça Nelore. Na terceira semana pós-infecção e até o final do experimento, os bezerros da raça Holandesa tiveram anemia e hipoproteinemia, enquanto que os bezerros Nelore não apresentaram alterações significativas desses parâmetros. À necropsia, os bezerros da raça Holandesa apresentaram uma carga de vermes significativamente maior dos que os da raça Nelore. As alterações patológicas visíveis e lesões histopatológicas foram similares, embora macroscopicamente fossem levemente mais evidentes na raça Holandesa. Estes resultados demonstram que bezerros da raça Holandesa são mais susceptíveis à infecção e aos efeitos patogênicos de *H. placei* do que bezerros da raça Nelore.

Palavras-chave: *Haemonchus placei*, helminto, bezerro, Holandês, Nelore

INTRODUCTION

Gastrointestinal nematode infections cause serious losses in cattle throughout the world. *Haemonchus placei* is a common parasite of the abomasum in tropical regions, particularly in Brazil, in very important livestock areas (Honer & Vieira-Bressan, 1992). *H. placei* is a hematophagus helminth causing severe anemia after infection reaches the fourth larval stage (L₄). Infected cattle may therefore, show anemia and low plasma protein concentrations before helminth eggs are passed in the feces. Because of the continuous blood loss, the erythropoiesis in the bone marrow may be affected, increasing the anemia (Harness et al., 1970; Abbott et al., 1984, Jain, 1993).

Although several papers have been published on sheep haemonchosis, only a small number refer to that parasitosis in cattle. It is therefore, of interest to

investigate the pathogenic effects of *H. placei* in two common breeds of cattle in Brazil: Holstein-Friesian and Nelore. The experiment described in this paper investigated the susceptibility of those breeds and the pathogenic effects produced by the experimental infection of *H. placei*.

MATERIALS AND METHODS

Ten worm-free calves, five Holstein and five Nelore, nine months old, were kept on slatted-floor pens from birth to the beginning of the experiment. Calves received colostrum after birth and dried milk up to the weaning (50 to 60 days). Furthermore, they were fed with a commercial concentrate and Coast Cross grass (*Cynodon dactylon*) hay. Water was given *ad libitum*.

Animals were randomly assigned to two groups composed by five Holstein and five Nelore calves. Each animal received by oral route 1,000 infective larvae (L₃) of *H. placei* per kilogram of body weight on day zero. Blood and fecal samples were collected, at one week intervals, from day zero until the eighth week (W8) post-infection. Animals were individually weighed on the same occasions. The calves were sacrificed and the abomasum removed for parasitological and histopathological studies on day 56. Animals were daily monitored regarding their feeding behavior, aspect of conjunctiva and fecal consistence. Blood samples (5ml) were collected by venipuncture in tubes containing ethylenediaminetetraacetic acid (EDTA). Counts of red blood cells (RBC) and white blood cells (WBC), were carried out in an electronic cell counter (CELL CC510). Differential leukocyte counts were carried out in smears stained by the Leishman method. Hemoglobin concentration (Hb) was determined by the cyanomethemoglobin method and the packed cell volume was determined by the microhematocrit method. Plasma protein and fibrinogen concentrations were estimated by refractometry according to Jain (1986). Infective larvae belonged to a strain kept since 1987, in the Department of Parasitology, Institute of Biomedical Sciences of the University of São Paulo, Brazil. Third stage larvae (L₃) recovered from fecal cultures obtained from calves infected with *H. placei* were used as inoculum two weeks after collection. Ten 0.01ml samples of water containing L₃ were counted to estimate the required volume of the established inoculum of 1,000 L₃ per kg of body weight of each calf. Helminth eggs in feces were counted according to a modified method of Gordon & Whitlock (1939).

Samples of the total abomasum volume (10%) were fixed with 10% formalin for further worm counts. Then, the organ was incubated at 42°C in saline solution 0.9% for six hours. Larvae and nematodes detaching from the mucosa were recovered after filtering the incubated liquid through a fine sieve.

The internal surface of the abomasum was macroscopically inspected at necropsy. Tissue specimens were fixed in 10% buffered formalin before paraffin inclusion. Slices of 5-6µm stained by hematoxylin-eosin were examined under light microscopy.

The results were compared by ANOVA in a multiple range test (Vieira & Hoffmann, 1989), studying the interaction between breed and time. The *F* test and the probability level were calculated and when the level was significant ($P < 0.05$), the contrasts between pairs of means were evaluated by the Tukey's test.

RESULTS

The mucous membranes of the eye and mouth were pale in the last weeks and Holstein calves moved less actively, became weak and apathetic. No diarrhea or submandibular edema were observed.

The hematological abnormalities observed after infection were more apparent in Holstein calves (Fig. 1). In the third week (W3) they showed the following mean values: erythrocytes $4.47 \times 10^6/\mu\text{l}$; Hb 7.98g/dl; packed cell volume (PCV) 24%; mean corpuscular volume (MCV) 52.68 fL (femtoliter = μm^3) and mean corpuscular Hb concentration (MCHC) 32.58%, denoting a normochromic normocytic anemia. The Nelore calves showed a slight decrease of those parameters throughout the experimental period but had no anemia. The mean values of this group were: erythrocytes $6.68 \times 10^6/\mu\text{l}$; Hb 12.82g/dl; PCV 38%; MCV 52.8fL and MCHC 33.7%. The differences between Holstein and Nelore calves were statistically significant ($P < 0.05$) along the experimental period.

The mean values of WBC and differential leukocyte counts of both groups showed no significant differences ($P > 0.05$). There was an increase of WBC in the first week after infection with mean value of $12.72 \times 10^3/\mu\text{l}$.

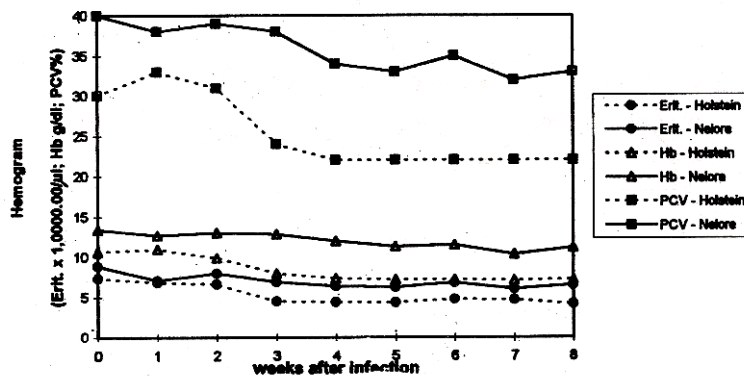


Figure 1. Mean values of the number of erythrocytes ($\times 10^6/\mu\text{l}$), hemoglobin concentration (g/dl) and Packed Cell Volume (PCV - %) of Nelore and Holstein calves infected with 1,000 *L3 Haemonchus placei*/kg of body weight.

The mean absolute values of neutrophils, lymphocytes and monocytes remained similar in both groups. However, eosinophilia (Fig. 2) occurred in the W1 and W2 post-infection, with the Nelore calves showing smaller ($P < 0.05$) values than the Holstein.

The mean values of plasma protein concentrations of Nelore calves were significantly ($P < 0.05$) greater than those of the Holstein along the experimental period. The small protein concentration of 4.6g/dl (Fig. 3) observed among Holstein calves on W5 characterizes hypoproteinemia.

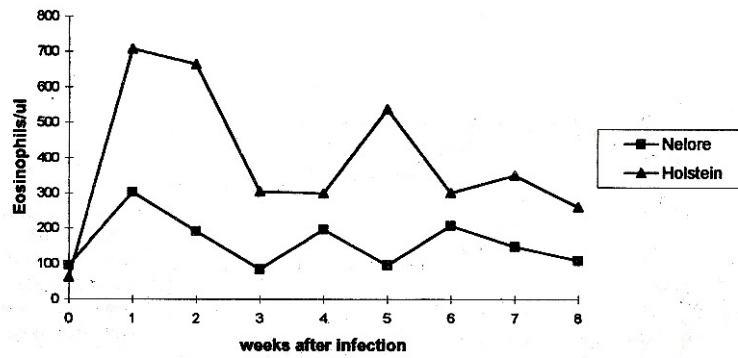


Figure 2. Mean values of the absolute number of eosinophils in Nelore and Holstein calves infected with 1,000 *L3 Haemonchus placei*/kg of body weight.

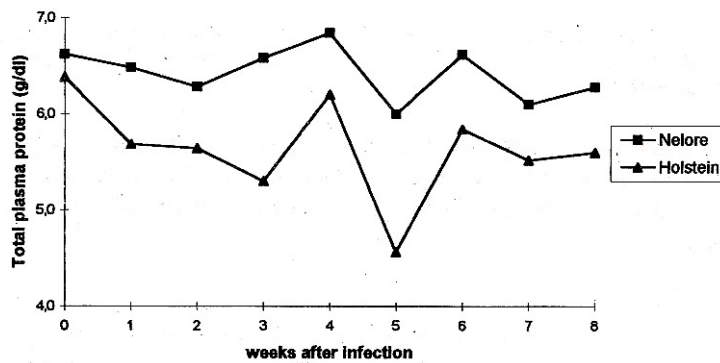


Figure 3. Mean values of total plasma protein concentration (g/dl) of Nelore and Holstein calves infected with 1,000 *L3 Haemonchus placei*/kg of body weight.

Before infection, both groups showed normal concentration of plasma fibrinogen (346-574mg/dl in the Nelore and 326-594mg/dl in the Holstein group). After infection, these values showed significant ($P<0.05$) differences between the two breeds. Only on W4 (28 days after infection) there was an increase of fibrinogen concentration in Holstein calves.

Fecal egg counts showed a continuous EPG increase in both groups along the time (Fig. 4). After the W5 post-infection, the mean EPG values of Holstein calves were significantly ($P<0.05$) greater than those of Nelore calves. At W8 the mean values of Holstein reached levels close to 4,500 EPG, while those of Nelore never raised above 500 EPG.

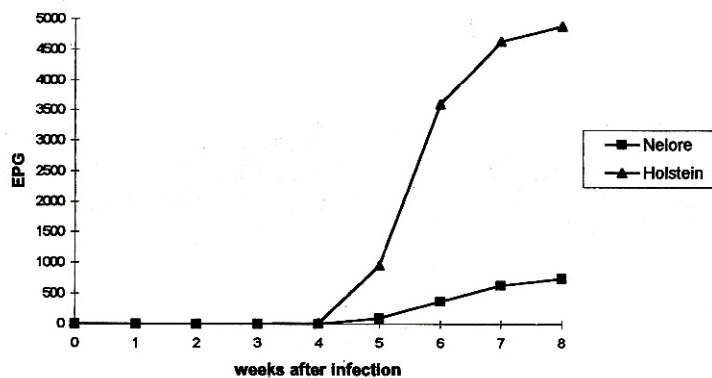


Figure 4. Mean values of egg counts per gram of feces (EPG) of Nelore and Holstein calves infected with 1,000 *L3 Haemonchus placei*/kg of body weight.

The mean estimated numbers of *H. placei* recovered at necropsy were $6,702 \pm 1,772$ and $23,086 \pm 742$ for Nelore and Holstein calves, respectively. These worm burdens were therefore, significantly ($P<0.05$) different and much smaller in the Nelore calves.

There were no differences among the animals of the two breeds regarding weight gain. However there was a trend to interaction between breeds and the analyzed weeks ($F=5.89$ and $P<0.10$). In both breeds, body weights increased along the time with a highly significant ($P<0.01$) difference. Body weights of Holstein calves became quickly stabilized and on day 56 (W8), both groups showed similar body weights: 175 and 178kg to Nelore and Holstein, respectively.

Edema and petechiae were apparent during the gross examination of the abomasal mucosa. These lesions were more evident in Holstein calves. Histological examinations of the abomasal mucosa of both groups showed integrity of the secretory epithelium with some eroded focal areas. Enlargement of glands and some cysts were observed in most animals. Inflammatory changes were related to the presence of a diffuse mononuclear infiltrate composed by plasmacytes and mastocytes. There was a slight presence of eosinophils in the inflammatory infiltrate. The observed diffuse inflammatory infiltrate containing mainly mononuclear and few polymorphonuclear leukocytes, and vascular alterations were limited to the interstitial edema of the submucosa, associated or not to vascular congestion (Fig. 5).

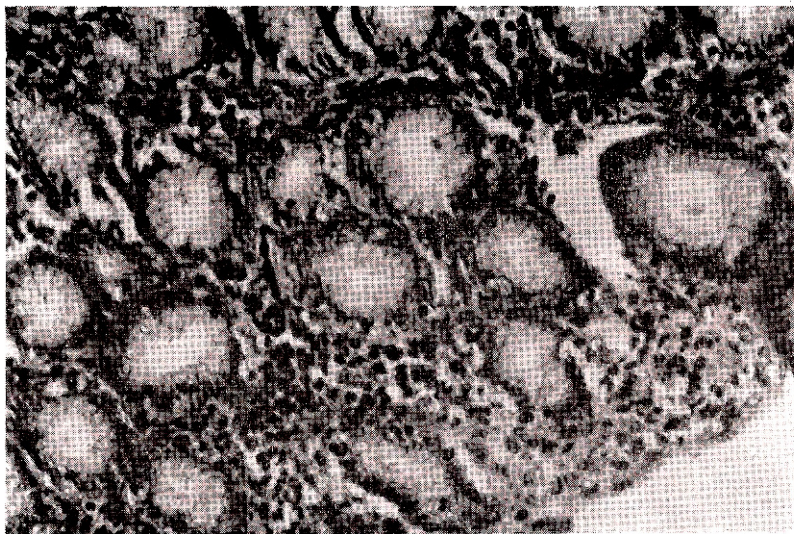


Figure 5. Microscopic section of the abomasal mucosa of a Holstein calf with diffuse inflammatory infiltrate containing mainly mononuclear and few polymorphonuclear leukocytes. EH. 400X.

DISCUSSION

The experimental infection by *H. placei* produced biochemical and hematological changes in both breeds. These changes, however, were much more apparent in Holstein calves. These animals had reduction on the number of RBCs, hemoglobin and PCV after W3 post-infection. These parameters remained low up to W8, configurating a normochromic normocytic anemia. This condition is usually a consequence of a slow and continuous loss of blood (Gennari et al., 1995) caused by the hematophagus habits of *H. placei*, which starts in this helminth, at the beginning of the fourth larval stage (L₄). These observations agree with those of Harness et al. (1970) and Abbott et al. (1984), who have worked with *H. contortus* and *H. placei* in sheep and cattle, respectively. They also agree with Silvermann et al. (1970) and Shoo & Wiseman (1986), who suggest that reduction of the hemoglobin provided a cue for the identification of the initial stage of haemonchosis. The changes in PCV confirmed the observations of Santos Filho (1994) and Vieira Bressan et al. (1992) in cases of severe or light haemonchosis, respectively. The anemia was confirmed by clinical evaluation, with Holstein calves showing apathy and pale mucous membranes.

Nelore calves did not show signs of anemia, despite a slight curtailment of blood elements, which might be justified by the parasitic burden of *H. placei* in the abomasum of these calves. The absence of clinical signs do not agree with the observations of Harness et al. (1970), although denotes a smaller breed susceptibility.

The hipoproteinemia observed from W3 until W8 post-infection, was also more intense in Holstein calves. Albumin loss through the stomach lesions might have caused this abnormal condition. It could also be associated to the impaired capacity of those calves to digest and absorb nutrients. Bremmer (1981) and Abbott et al. (1984) observed this problem in sheep haemonchosis; Baker et al. (1993) reported the same observation in the ostertagiosis of calves.

Plasma levels of fibrinogen had some fluctuation on W4, with Holstein calves showing increase of this globulin. The inflammatory process produced on the abomasal mucosa by fixation of most adult *H. placei* was probably responsible for it.

No noticeable changes occurred on the number of leucocytes nor in the differential counts of neutrophils, lymphocytes, basophils and monocytes in calves of either group. Eosinophilia was detected in Holstein calves on a

compatible level with the helminth burden of the abomasum. Eosinophilia occurs as a result of larval migration throughout the tissues, in a direct relationship between the contact surface of the parasite or its metabolic products and the host. Harness et al. (1970) also observed a feeble eosinophilia in calves infected with similar levels of *H. placei*.

The weekly evaluation of EPG in fecal examinations draw attention to the significant increase occurring after W5 post-infection in both breeds. Similar findings were reported by Santos Filho (1994) in Holstein calves also infected by *H. placei*. As the means of egg counts or the number of helminths is related to the pathogenic effects, Holstein calves that showed the highest egg counts had serious anemia. It is of interest to notice that the anemia was detectable through the hematological changes on W3, whereas the diagnosis of helminthosis by fecal examinations could only be confirmed on W5. This, according to Urquhart et al. (1990), is attributed to the development of a dorsal lancet in the buccal cavity of the immature stages of *H. placei*. This appendage fits *H. placei* with early hematophagus capability.

Although the same inoculum level was used in both breeds, a large helminth burden became established only in Holstein calves. Nelore calves had a small worm burden and did not show clinical signs. These results are similar to those reported by Santos Filho (1994) who used the same infective dose producing severe infection. Gennari et al. (1991) found a much smaller worm burden, associated to a slight reduction of the packed cell volume. The difference should be attributed to the inoculum that contained only 500 L₃ per kg of body weight.

For the first time, the microscopic lesions produced in the abomasum of cattle by *H. placei* are reported. The detailed study of these lesions showed no significant differences between Holstein and Nelore calves, although the macroscopic aspect of the lesions was more visible in Holstein calves. The histopathologic studies showed integrity of the secretory epithelium with some eroded focal areas. Enlargement of glands and some cysts were observed in most animals. Inflammatory changes were related to the presence of a diffuse mononuclear infiltrate composed by plasmacytes and mastocytes. There was edema, congestive changes and mononuclear infiltration in the submucosa. These alterations were also observed by Hunter & Mackenzie (1982) in sheep infected by *H. contortus*.

REFERENCES

- ABBOTT, E.M., PARKINS, J.J., HOLMES, P.H. Studies on pathophysiology of chronic ovine haemonchosis in Merino and Scottish Balckface lambs. *Parasitology*, v.89, p.585-596, 1984.
- BAKER, D.G., BRUSS, J.L., GERSHWIN, I.J. Abomasal interstitial fluid to blood concentration gradient of pepsinogen in calves with type-1 and type-2 ostertagioses. *Am. J. Vet. Res.*, v.54, p.1294-1298, 1993.
- BREMNER, K.C. The pathophysiology of parasitic gastro-enteritis of cattle. In: SYMONS, L.E.A.; DONALD, A.D., DINEEN, J.K. *Biology and control of endoparasites*. Sydney: Academic Press, 1981. P.277-289.
- GENNARI, S.M., VIEIRA BRESSAN, M.C.R., ROGERO, J.R. et al. Pathophysiology of *Haemonchus placei* infection in calves. *Vet. Parasit.*, v.38, p.163-172, 1991.
- GENNARI, S.M., VIEIRA BRESSAN, M.C.R., ROGERO, J.R. et al. Pathophysiology of *Haemonchus placei* in calves: Effects of dietary protein and multiple experimental infection on worm establishment and pathogenesis. *Vet. Parasitol.*, v.59, p.1199-1126, 1995.
- GORDON, H.M., WHITLOCK, H.V. A new technique for counting nematode eggs in sheep faeces. *J. Council Sci. Ind. Res. Aust.*, v.12, p.50-52, 1939.
- HARNESS, E., FITZSIMMONS, W.M., SELLWOOD, S.A. Experimental *Haemonchus placei* infection in calves. *J. Comp. Pathol.*, v.80, p.173-179, 1970.
- HONER, M.R., VIEIRA-BRESSAN, M.C.R. Nematódeos de bovinos no Brasil. Situação atual da pesquisa, 1991. *Rev. Bras. Parasitol. Vet.*, v.1, p.67-79, 1992.
- HUNTER, A.R., MACKENZIE, G. The pathogenesis of a single challenge dose of *Haemonchus contortus* in lambs under six months of age. *J. Helminthol.*, v.56, p.135-144, 1982.
- JAIN, N.C. *Essentials of veterinary hematology*. Philadelphia: Lea & Febiger, 1993. 417p.
- JAIN, N.C. *Schalm's veterinary hematology*. 4th ed. Philadelphia: Lea & Febiger. 1986. 1221p.
- SANTOS FILHO, P.J. *Avaliação do metabolismo da água corpórea e do balanço hídrico e do nitrogênio em bezerras infectados com Haemonchus placei (Place, 1893) Ransom, 1911*. São Paulo: Instituto de Ciências Biomédicas, 1994. 72p. Dissertação (mestrado).
- SHOO, M.K., WISEMAN, A. Changes in serum pepsinogen and concentration in calves infected with *haemonchus contortus*. *Res. Vet. Sci.*, v.41, p.125. 1986.

- SILVERMANN, P.H., MANSFIELD, M.E., SCOTT, H.L. *Haemonchus contortus* infection in sheep: effects of various levels of primary infections on nontreated lambs. *Am. J. Vet. Res.*, v.31, p.841-857, 1970.
- URQUHART, G.M., ARMOUR, J., DUNCAN, J.L. et al. *Parasitologia veterinária*. Rio de Janeiro: Guanabara Koogan, 1990. 306p.
- VIEIRA BRESSAN, M.C.R., GENNARI, S.M., ABDALLA, A.L. Body composition of calves infected with *Haemonchus placei* estimated by tritiated water technique. *Rev. Bras. Parasitol. Vet.* V.1, p.17-21, 1992.
- VIEIRA, S., HOFFMANN, R., *Estatística experimental*. São Paulo: Atlas, 1989. 179p.