BIOLOGICAL CONTROL OF BOVINE GASTROINTESTINAL NEMATODE PARASITES IN SOUTHEASTERN BRAZIL BY THE NEMATODE-TRAPPING FUNGUS *ARTHROBOTRYS ROBUSTA*.

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SUMMARY: In order to test the viability of using a Brazilian isolate of *Arthrobotrys robusta* in the biological control of bovine gastrointestinal nematode parasites, calves were orally treated with two million conidia of this isolate, twice a week during four months. The calves showed a reduction of 53.81% in the EPG ($p<0.05$) in relation to the non-treated calves (control), and a reduction of 70.45 % ($p< 0.05$) on the number of worms recovered at necropsy of the tracer calves in the last three months of the experiment. The results showed that this isolate of *A. robusta* is a promising agent to be used in the biological control of bovine gastrointestinal nematode parasites.

KEY WORDS: Biological control, nematodes, bovine, *Arthrobotrys robusta.*

INTRODUCTION

Among the factors interfering on the development of cattle raising, helminth infections are a major concern. They cause retarding of animal development, death, and excessive handling expenses, leading to a low herd productivity and, consequently, high economical losses. In Brazil this problem increases as the pastures conditions worsen, mainly in the dry season, or when high stocking rates in certain areas make the infection easier. Generally, most animals present a subclinical infection, due to the acquired immunity, making it more difficult to quantify the effects of such type of infection. In southeastern Brazil, *Cooperia* and *Haemonchus* are the prevalent bovine gastrointestinal nematodes, and account for the variation in the eggs per gram of faeces (EPG) counts. The *Oesophagostomum* genus appears as the third higher prevalence. New approaches to control bovine gastrointestinal nematodes intend to avoid pastures contamination, and consequently the parasite infections. Such strategies together with the antihelminthic treatment could, in the future, help to solve the problems originated from helminthosis. Up to now the alternative research in bovine helminthosis control aimed at vaccine development and breeding of genetically resistant animals. Among these techniques, the biological control using nematophagous fungi appears as one of the most promising possibilities (WALLER & LARSEN, 1993).

Furthermore, they are the nematodes antagonistic organisms widely studied, and almost all of them effectively reduce nematodes population in laboratory. They have also proved their efficacy against nematodes in the pasture (WALLER & LARSEN, 1993). The objective of present study was to assess the viability of an *A. robusta* isolate in the biological control of bovine gastrointestinal nematode parasites, which was selected in previous tests with passages through the gastrointestinal tract (ARAÚJO *et alii* 1996) for field or *in vivo* control of bovine gastrointestinal nematodes.

MATERIALS AND METHODS

Organisms: Infective *Cooperia punctata* larvae (L_3) were obtained from the faeces of calves experimentally infected with 1,000 L_3 per kilogram of live weight. The strain used was kindly supplied by Prof. Maria Cecília Reale Vieira Bressan, Department of Parasitology - São Paulo University (Brazil).

Panagrellus (free living nematodes) were kept in Petri dishes with medium made of moistened and crushed oat flakes. This strain was kindly supplied by Prof. Silamar Ferraz, Department of Phytopathology - Viçosa Federal University. The worms were separated from culture medium using the Baermann apparatus, after 24 hours at room temperature. Then they were kept in hemolysis vials.

An isolate of the nematode-trapping fungus *Arthrobotrys robusta* was cultivated and kept in test tubes containing potato-dextrose-agar 2% (PDA 2%) at 4 °C, in the dark. The cultures were streaked out every four months. This isolated was collected from Brazilian soil and was obtained by the soil sprinkling method of DUDDINGTON (1955) modified by SANTOS *et alii* (1991). To induce the formation of conidia from this isolate, culture disks of about 5 mm were transferred to Petri dishes (diameter 15 cm) containing agar calf faeces. This medium was prepared with 50 g of faeces macerated with distilled water, filtered through a steal filter with 61 µm meshes. The filtered was adjusted at 500 ml, added agar-agar until reaching the concentration of 2 % and autoclaved per 15 min. at 120 °C. One ml of suspension containing 1,000 *Panagrellus* was added to the plates for two days. By the fourth or the fifht day, the dishes became totally covered with the fungi isolates. The conidia and mycelium fragments were removed using a brush, and stocked in small bottles at 4 °C.

Experimental design: The *in vivo* biological control was developed in an experimental farm of the Federal University of Viçosa, in the municipality of Viçosa, Minas Gerais State, Brazil, latitude 20° 45' 20" S, longitude 42° 52' 40" W , 649 m above sea level. The experiment was conducted from September 1st, 1995 to April 1st ,1996.

Eighteen crossbred Holstein x Zebu male calves, six to eight months old, and previously treated with two doses of Albendazole (7.5 mg/kg orally), given with a two weeks interval. The animals grazed on molasses grass pastures (*Melinis minutiflora*) on the first day of September, 1995. Pastures were naturally infested by gastrointestinal helminth stages due the previous grazing by young and adult cattle. After one-month adaptation period, calves were treated again with the same antihelminthic. One week after each calf received by oral route 10,000 L_3 of *C. punctata* orally, according to WOOD *et alii* (1995). Animals were randomly divided in two groups (A and B) with nine calves each and placed in pastures with a stocking rate of 1.5 animal/hectare. In group A, each animal received 2 x 10^6 conidia of *A. robusta* orally twice a week during a four-month period that started together with the region's rainy season (October 7th, 1995) . The criteria for dosage administration was based on a previous work developed by HASHMI & CONNAN (1989). The beginning of the rainy season was determined in a previous work developed by ARAÚJO (1994) and ARAÚJO & BELÉM (1994) at the same experimental farm. In group B (control), the calves didn't receive conidia fungi.

After the administration of L_3 to the calves of group A, faeces samples were collected directly from the rectum of each animal of both groups every fourteen days. In the fecal samples the counting of eggs per gram of faeces (EPG) was determined according to the technique of GORDON & WHITLOCK (1939). Together with EPG counting, larval cultures were done. Twenty grams of faeces were mixed with fragmented vegetal coal and incubated at 26 °C for eight days, in order to collect infective larvae of gastrointestinal nematode parasites. These larvae were identified according to the criteria established by KEITH (1953). Samples of approximately 500 g of pasture were collected in different points of each paddock at every fourteen days. The L_3 were recovered, according to the technique described by LIMA (1989). Five hundred grams of forage were put in a drying stove at 100 °C for three days to assess the dry matter. The data obtained were transformed into number of larvae per kg of dry matter.

At every twenty-eight days, a tracer animal (a crossbred Holstein x Zebu calf, six months old male) was introduced in each group along with the permanent animal population, to measure the pasture contamination by bovine gastrointestinal nematode parasites. Before introduction to the pasture these animals were confined and received two antihelminthics treatments with Albendazole (7.5 mg/kg, orally) at a 14-day intervals. The animals were used seven days after the last treatment. Each animal stood for fourteen days on the paddocks, then were removed and housed in a stall for fourteen days. After it they were sacrificed and the necropsy was performed. Helminths recovering and quantification was done according to COSTA *et alii* (1970). The adult stages were identified according to YAMAGUTI (1961).The identification of the immature stages was done according to DOUVRES (1957).

The meteorological data was collected from a station located in the experimental farm. Monthly averages of minimum, medium and maximum air temperature, monthly rainfall and relative air humidity were recorded.

The results of the EPG total counting, worms recuperated from necropsy and L_3 in the pastures were transformed in logarithmic x+1 to correct for average distortions. The Student test T was used ($p<0.05$).

RESULTS

The monthly average values of EPG counts for the animals of group A (calves which received conidia of *A. robusta* orally) and group B (control calves) are shown in Fig. 1. During the six months of the experiment, EPG counts for group A were greater than those for group B, with statistically significant difference ($p< 0.05$). This difference at the end of six months was 51.9% and 53.81% in the last three months (January; February and March).

The EPG monthly average values obtained from the L_3 percentual participation, after larval culture of the permanent animals of groups A and B, are represented, respectively, in Figs. 2 and 3. For group A, these values show that in October and November, the *Cooperia* prevalence was greater than *Haemonchus* and *Oesophagostomum*. In the following

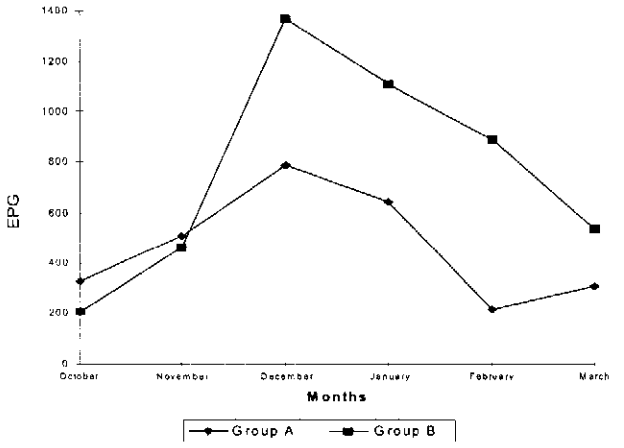

Fig. 1 - Average monthly counting of helminth eggs of the superfamily Strongyloidea per gram of faeces (EPG) of calves treated with *A. robusta* conidia (group A) and in control animals (group B) in the period from October 1995 to March 1996.

months, *Oesophagostomum* overcome *Cooperia* and *Haemonchus*, as well as *Haemonchus* overcame *Cooperia*. For group B, *Cooperia* prevalence was always greater than *Haemonchus* and *Oesophagostomum*. *Oesophagostomum* showed a higher prevalence than *Haemonchus*. An exception was found in the month of March for animals of both groups, when *Haemonchus* EPG was higher than *Cooperia* and *Oesophagostomum*. *Trichostrongylus* eggs were found at a low level only in the months of November and December.

The number of worms recovered at necropsy of the tracer animals are shown in Table 1. At the end of six months, the values corresponding to group A tracers showed a difference of 48.9% in relation to tracers of group B ($p < 0.05$) and in the last three months showed a difference of 70.45%. The *Cooperia* genus was the most prevalent followed by the *Haemonchus* and *Oesophagostomum* in the tracers of groups A and B. Immature forms of *Trichuris* were found in animals of both groups, but at low levels.

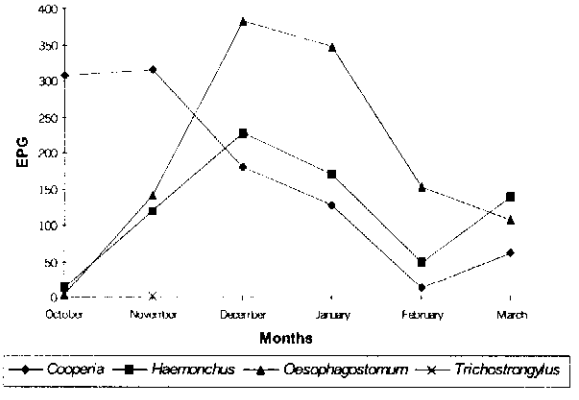

Fig. 2 - Average monthly counting of nematodes eggs per gram of faeces (EPG) recovered from larval cultures, of the calves in the group treated with conidia (group A) in the period from October 1995 to March 1996

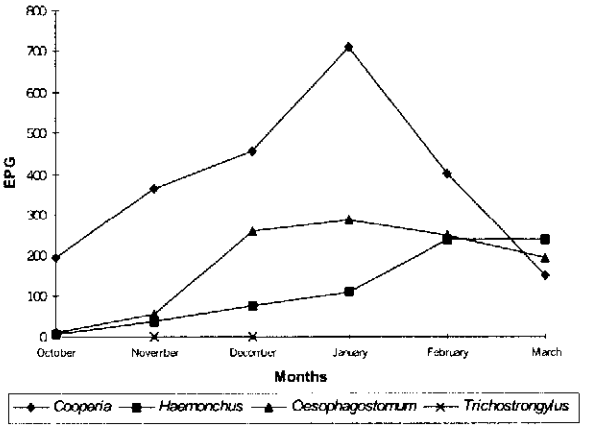

Fig. 3 - Average monthly counting of nematodes eggs per gram of faeces (EPG) recovered from larval cultures of the calves in group B (control) in the period from October 1995 to March 1996.

Table 1 - Number of nematodes recovered from tracer calves wich grazed on pastures of animals treated with conidia of *Arthrobotrys robusta* (group A) and non-treated animals of group B (control) in Viçosa, M.G., Brazil, in the period from October 1995 to March 1996

Nematodes	Oct		Nov		Dec		Jan		Feb		Mar	
	A	B	A	B	A	B	A	B	A	B	A	B
Cooperia	760	940	230	-	10	-	690	2450	590	1230	1160	540
IM Cooperia	20	-	10	-	-	-	-	-	-	-	40	-
Haemonchus	30	280	-	170	-	70	390	90	260	30	-	800
IM *Haemonchus*	-	-	-	-	-	-	10	-	-	-	-	-
Oesophagostomum	80	90	10	60	-	-	10	-	20	10	10	250
IM *Oesophagost.*	-	-	20	-	-	-	-	10	10	-	-	-
IM *Trichuris*	-	20	-	20	-	-	-	-	-	-	-	20

IM - Immature Stage A- Group A B- Group B

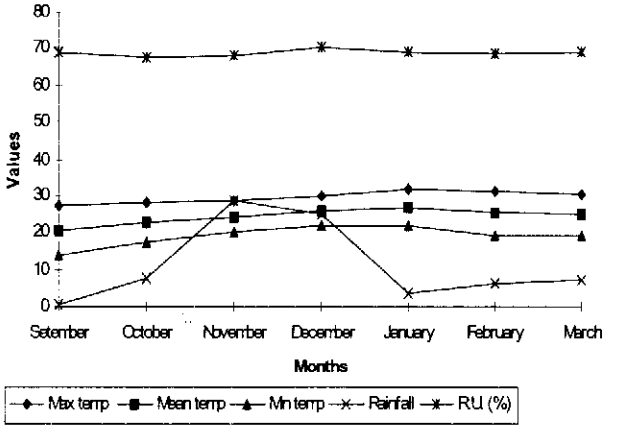

Fig. 4 - Average minimum (min temp), medium (mean temp) and maximum temperatures (max temp)(°C); and monthly rainfall (mm) and relative humidity (R.U.) (%) in Viçosa, Minas Gerais State, Brazil during the period from September 1995 to March 1996.

Table 2 - Counting of infective larvae per kg of dry pasture collected in pastures with calves treated with conidia of *Arthrobotrys robusta* (group A) and non-treated of group B (control) in Viçosa, M.G., Brazil, in the period from October 1995 to March 1996.

Months	*Cooperia*		*Haemonchus*		*Oesopha gostomum*		*Tricho strongylus*	
	A	B	A	B	A	B	A	B
October	-	-	-	-	-	-	-	-
November	528.89	709.29	78.65	114.87	-	-	-	6.76
December	50.00	39.13	9.44	-	9.44	-	-	-
January	11.50	-	-	-	-	-	-	-
February	27.90	38.46	5.62	32.97	13.96	-	-	-
March	67.66	87.94	36.14	93.00	87.28	-	-	-

A- Group A B- Group B

The number of L_3 per Kg of pasture dry matter is depicted in Table 2. There was no statistically significant difference ($p>0.05$) of these values among the pastures of animal from the groups A and B. The number of L_3 recovered from the pastures of the group B animals was higher, the *Cooperia* genus being the most prevalent in both pastures.

The meteorological data relative to temperature, rainfall and air relative humidity from September 1995 until March 1996 are shown in Fig. 4.

DISCUSSION

The results presented shown that the isolated E of *A. robusta* tested is a promising agent to be used in the biological control of bovine nematodes parasites. The oral administration of conidia to the animals presented a pastures infestation control of 70.45% (Table 1) in the last three months of the rainy season (January; February and March). This was reflected in the animal's EPG with a 53.81% reduction (Fig. 1). Furthermore, it is very important to select nematophagous fungi in each place, because many times they can be effective, or even be found in a given ecological niche.

The use of nematophagous fungi in the biological control of animal helminth parasites can reduce pasture contamination, acting directly in the environment. HASHMI & CONNAN (1989) and WOLSTRUP *et alii* (1994) used this method, mainly in the beginning of the infective period, when pasture conditions were adequate to animal grazing, and at the same time were favorable to the development of helminths eggs and larvae, consequently increasing pasture contamination.

Besides, in the region where the present study was conducted, the seasons of larger rainfall are those of the best pasture conditions, and highest mean temperatures.The development of fungi in the environment would be then favored by high temperatures. Generally, the optimum temperature for development of *Arthrobotrys* range between 20 and 29 °C (TOLMSOFF, 1959; COOKE, 1963; OLTHOF & ESTEY, 1965; MONOSON, 1968, 1971; PANDEY, 1973; AL-HAZMI *et alii*, 1982; MITSUI, 1983, 1985; GRONVOLD, 1989; GRONVOLD *et alii*, 1985 and NAVES & CAMPOS, 1991). However, VIRAT & PELOILLE (1977) investigating the capture of *H. contortus* L_3 by *A. oligospora* in ovine faeces, found that capture efficiency was greater between 15 and 22 °C. According to FEDER (1963), generally the optimum temperature for trap formation and for the nematodes capture is lower than the temperature for optimum micelial growth.

According to WALLER & LARSEN (1993), the application of fungi in the nematode biocontrol helps the chemical control and should be administered not only when there is a prediction of greater pasture infestation by helminths eggs and larvae, but also when there would be better conditions for the fungi growth at the environment, preventing in this way the clinical parasitism and productivity losses, yet supplying a sufficient number of larvae to allow these animals to develop a naturally acquired immunity.

The employment of nematophagous fungi for nematode biocontrol in the rainy period, as shown in the present study, would prepare pastures for the dry period, for what concerns about helminthic infestation. According to FURLONG *et alii* (1985), the critical period for calves in this region is the dry period, due the number of larvae available in the pastures and the smaller food availability due to poor pasture conditions.

The *Cooperia* predominance in relation to the other helminth genera was found in the number of nematodes recovered at necropsy of the tracer animals (Table 1), in the countings of infective larvae per Kg of pasture dry matter (Table 2) and in the faeces of group B permanent animals (control), as well as in the faeces of group A permanent animals (calves who received *A. robusta* conidia orally) during October and November. This result probably reflects the greater resistance of the *Cooperia* free life stages to the climatic variations and dry conditions, and the greater migratory capacity of these larvae (REINECKE, 1960), besides the smaller requirements of pluvial precipitation in relation to *Haemonchus* and *Oesophagostomum* larvae (ROBERTS *et alii* 1952) and the initial infection of the permanent calves with *C. punctata*.

The *Oesophagostomum* predominance in the larval cultures of group A permanent calves beginning in December 1995 (Fig. 2), in relation to the other helminth genera, and the similarity with *Oesophagostomum* EPG curve in group B animals (Fig. 3) (no statistically significant difference ($p>0.05$)), suggests that this genus was less predated by the fungi in relation to the other helminth genera. However, further specific *in vitro* and *in vivo* tests with *Oesophagostomum* are needed in order to confirm such results. Besides, the studies so far developed using fungi on biocontrol of animal nematode parasites, were carried out in temperate climate countries, in which this genus is rare, due to the climatic conditions, according to WILLIAMS & MAYHEW (1967). NANSEN *et alii* (1988) when testing the

capacity of inducing the *A. oligospora* trap formation by L_3 of nine species of animal nematode parasites, observed that this ability for L_3 of bovine *Cooperia oncophora* and *Ostertagia ostertagi*, ovine *C. curticei* and *H. contortus*, and equine *Cyathostoma* was greater and faster than for L_3 of swine *O. dentatum* and *O. quadrispinulatum*, mouse *Nematospiroides dubius* and bovine lungs *Dictyocaulus viviparus*. The authors comment that these results suggest that nematode species with greater motility are better trap formation inducers. However, all nematode species were effectively predated by this *A. oligospora* isolate in a previously grown colony that already had traps.

The results were analyzed based on the full experimental period and the last three months. If the results were analyzed separately in order to overestimate them, and only the ones which were more relevant were mentioned, we would have: 77.49% difference between the treated group and control group in February, 99.21% difference in the pasture infestation, verified in necropsies of tracer animals and of 69.33% in the number of L_3 per pasture dry matter, both for January. Besides, pasture stocking rate was constant, the animals only received mineral supplementation under pasture conditions, the pasture infestation was evaluated through pasture tests as well as through tracer animals. The fungi doses administered orally, were known and without variation (two million conidia twice a week, for each animal, during four months). The various existing techniques to evaluate the number of larvae in the pasture by forage collection vary in efficiency, and many times can not simulate the natural population existing in the pastures (LANCASTER, 1970). In the present study, there were no statistically significant differences ($p>0.05$) in the amount of L_3 recuperated per kg of pasture dry matter, between treated animals and control group, as found in the tracer animals, what could be explained by the commentaries above.

WOLSTRUP *et alii* (1994) mention that it seems to happen an improvement in the use of *D. flagrans* in the nematode biocontrol, when compared with *Arthrobotrys* isolates, specially for *A. oligospora*. However their conclusions were based on the use of its own isolate, and variations in the passage through the gastrointestinal tract and even in the predatory capacity of different isolated can occur.

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SUMÁRIO

Com o objetivo de testar a viabilidade do uso de um isolado de *Arthrobotrys robusta* oriundo de solo brasileiro no biocontrole de nematódeos parasitos gastrintestinais de bovinos, bezerros tratados com dois milhões de conídios deste isolado, administrado por via oral, duas vezes por semana durante quatro meses, apresentaram em relação a bezerros não tratados (Controle), 53,81 % de redução no OPG ($p< 0,05$) e 70,45 % ($p< 0,05$) sobre o número de vermes recuperados das necrópsias de animais traçadores nos últimos três meses do experimento. Os resultados apresentados demonstram que este isolado de *A. robusta* é um promissor agente a ser utilizado no biocontrole de nematódeos parasitos gastrintestinais de bovinos na região estudada.

PALAVRAS-CHAVE: Controle biológico, nematódeos, bovino, *Arthrobotrys robusta*

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