Int.J.Curr.Microbiol.App.Sci (2016) *5*(4): xx-xx

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 5 (2016) pp. 969-975**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.504.111

In Vitro Biological Control of Strongyles (*Strongylida*) in Llamas (*Lama glama*) Coming from a Zoo, in Southeastern Brazil

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Keywords

Nematophagous Fungi, Biological Control, Nematodes, Fungi Predators of Nematodes.

Article Info

Accepted:
15 February 2016
Available Online:
10 April 2016

A B S T R A C T

Llamas are ruminant mammals belonging to the camelids family, which also suffer from gastrointestinal parasites. Thus, *in vitro* studies under laboratory conditions were conducted to evaluate the predatory activity of fungal isolates in *in vitro* biological control of gastrointestinal nematode parasites in llamas, coming from a zoo in southeastern Brazil. The species *Duddingtonia flagrans* (AC001 and CG768); *Arthrobotrys conoides* (I40); *Arthrobotrys robusta* (I31); *Monacrosporium sinense* (SF53) and *Monacrosporuim thaumasium* (NF34) were used in the experiment, grown in 2% agar-water culture (AW2%) in 9.0 cm in diameter petri dishes. After seven days of interaction it was observed that all fungal isolates were efficient in the capture and destruction of L3, however, not differing in their predatory activity within each other (p> 0.01), but differing from the control group. At the end of the experiment the following reduction percentages were obtained 87.56% (AC001); 90.45% (CG768); 93.07% (I40); 94.58% (I31); 90.28% (SF53); 92.30% (NF34), in relation to the control group. It was concluded that all fungal isolates were effective in the *in vitro* biological control of L3 nematodes in llamas. However, larger studies, particularly in the field are required toobtain a more effective control of the free life forms of these parasites in Lamasraised in a zooin southeastern Brazil.

Introduction

Fowler and Cubas (2001) mentions that *Lama glama* (LINNAEUS, 1758) belonging to the *Camelidae* family, *Artiodactyla* Order, and *Tylopoda* Sub-order, is a docile animal, easy to manage and can be found throughout North America, Europe and Australia. They are herbivorous mammals that have a wide distribution, adapting to different regions of South America. This designation encompasses both domestic

species like the *Lama glama* as well as wild species.

In this sense, the llamas, which belong to the family of camelids, are ruminant mammals easily adaptable, and can be found in various parts of the world and are known as domesticated animals (Terry, 2009). Their ancestors came from North America millions of years ago and migrated to South America, especially Peru, where they live for a period of about 20-30years, depending on how they are attended to (Murray and Fowler, 2013; Bromage, 2009). Among other features, these animals live in intensely oriented herds, possessing distinct social structure, including hierarchy of command (Andersom *et al.*, 2013).

Regarding the gastrointestinal parasites, interest reported here, ruminants and pseudo-ruminants, especially llamas, are primarily infected by gastrointestinal nematode parasites belonging to the *Strongylida* order, *Strongylidea* family, called strongyles, and among these, highlighting the genres; *Camelostrongylus*, *Trichostrongylus* and *Paralapho strongylus*. Arenas (2007), reports the infection of llamas by *Trichostrongylus colubriformis*, as well as other helminths and external parasites (Cebra, 2014).

According to Fowler (1996), the majority of gastrointestinal parasites which infect these animals produce a gastro-enteropathy capable of protein depletion, which may cause hypoalbuminemia. Enteritis produced by these parasites lead to loss of vital nutrients, such as calcium and phosphorus absorption; affecting the development of the skeleton, and can, in severe cases, lead to the animal's death.

Treatment of helminth infections in domestic ruminantsis performed through the use of anthelmintic, in which in most of the time the results can be disastrous, mainly due to installation of parasitic resistance (Mota *et al.*, 2003). In this sense, the treatment of infections from gastrointestinal nematode parasites in llamas is also performed using anthelminthic drugs with an extrapolated dose of that in domestic ruminants. With this, two "problems" are recognized: empiricism in anthelmintic dosing and performance of drugs only on the parasitic phase in animals, not observing the free-infective stage (Braga and Araújo, 2014).

Braga *et al.*, (2007), Larsen (1999), and Araújo (1999, 2000b), affirmed that the biological control of nematodes performed by nematophagous fungi is a promising alternative and have shown satisfactory results. Araujo and Braga (2015) also reported that the use of natural antagonists among these nematophagous fungi, in the environmental control of infective nematode larvae of gastrointestinal parasitescan be successfully used. On the other hand, literature is sparse regarding an alternative control ofnematodeL3 in llamas, being this a tool to be used in the control of these parasites in zoo animals. Thus, this study aimed to evaluate the *in vitro* predatory activity of nematophagous fungi on strongylids of Llamas (*Lama glama*) coming froma zooin southeastern Brazil.

Materials and Methods

Fungi

The nematophagous fungi *Duddingtonia flagrans* (AC001 and CG768), *Monacrosporium thaumasium* (NF34), *Monacrosporium sinense* (SF53), *Arthrobotrs robusta* (I31), and *Arthrobotrys conoides* (I40) were used. These isolates are from the Parasitology Laboratory of the Federal University of Viçosa, Minas Gerais, Brazil.

Animals

The llamas are from the Zoo Park das Montanhas, in the municipality of Marechal Floriano/ES. Parasitological exams were performed in 100% of the animals, from which was found that all animals were infested with parasitic nematodes. Eight animals were studied, being 03males and 05 females. The animals receive industrialized feed, specific for ruminants, as well as hay and; weighed between140 and180Kg. They live in habitats, created by the zoo, for them. Use of the animals as a source for research was authorized by the zoo and approved by the Ethics Committee of the University Vila Velha.

Obtainment of Fungal Conidia

Culture disks of 4mm in diameter were extracted from the fungal isolates maintained in test tubes containing 2% corn-meal-agar (2% CMA) and transferred to 9.0cm in diameter Petri dishes containing 20 ml of 2% potato dextrose-agar, kept at25°C,in the dark for10 days. After growth of the isolates, novel 4mm diameter culture disks were transferred to Petri dishes of 9.0 cm diameter containing 20 ml of2% water-agar (2% WA) in which was added 1 mL of distilled water containing 1,000 *Panagrellus* sp. larvae, daily for a period of 21 days for fungal conidia formation induction. When complete fungal growth was observed, 5ml of distilled water was added to each Petri dish, and conidia and mycelial fragments were removed according to the technique described by Araujo *et al.,* (1993).

Obtainment of Gastrointestinal Nematode Parasites L3 of Ilamas

Fresh feces were collected directly from the rectum of the llamas from the zoo located in the municipality of Marechal Floriano, Espírito Santo, Brazil. Following, from these fecal samples were taken about2gfor performance of parasitological stool tests (egg count per gram of feces) to measure the degree of infection. There after, fecal cultures were prepared with approximately 20g of feces and they were incubated in a BOD incubator for a10 day interval (Gordon and Whitlock, 1939). After this period, using the Baermann technique, 3,500 larvae were extracted and identified according to Keith (1989). Analyses were performed in the laboratory of the University Vila Velha, in the period from July 25- 31 2014.

In vitro Assay

Seven groups were formed in 9.0cm in diameter Petri dishes containing 20ml of2% water agar, six treated groups and a control group, being performed 6replicates for each group. The Petri dishes were previously marked in fields of 4 mm in diameter. In the treated groups each Petri dish contained 500 strongyles L3and 500 conidiaof the fungal isolatesAC001, CG768, NF34, SF53, I31 and I40in 2% WA, and the control group (without fungi) contained only500L3on in the dishes with 2% WA (Mota *et al.,* 2002). During seven days, every 24 hours, 10 random fields of 4mm in diameter from each dish of the treated and control groups were observed under a light microscope at10x objective, counting the number of non-preyed L3in each one. At the endof seven days, non-preyed L3 were recovered from the content of the Petri dishes using the Baermann apparatus with water at42°C (Braga *et al.,* 2010).

Statistical Analysis

The average of strongyles L3 was calculated. Data were interpreted statistically by analysis of variance at significance levels of 1 and 5% probability (Ayres *et al.,* 2003). The L3 predation

efficiency compared to the control was evaluated by the Tukey test at 1% probability. The mean, standard deviation, and the Tukey test were calculated using the Biestat3.0 software. Subsequently, the reduction percentage of the average L3 was calculated according to the following formula:

Reduction%= (average L3 recovered from control – average L3 recovered from the treatment) x 100

Average L3 recovered from control

Results and Discussion

After seven days of interaction between larvae and the fungal isolates, a significant difference ($p < 0.01$) in the mean number of larvae recovered per dish was observed in all treatments. After analyzing each isolate and their interaction with L3, in this study, it was possible to observe that all fungal isolates were efficient in reducing larvae (Table1) and at the end of the experiment the following average reduction percentages were recorded: AC001(87.56%); I31 (94.38%); I40(93.07%); CG768(90.45%); SF53(90.28%); NF34(92.30%), Figure2.

The fungus *D. flagrans* (AC001) demonstrated efficacy on L3. The average larvae count in the control group were higher than those of the AC001 group (treatment group), with difference ($p<0.01$) being observed. The average reduction percentage of L3 was 87.56%. These data corroborate with other studies that analyzed the predatory activity of this isolate on strongyles of domestic ruminants (Silva *et al.*, 2011).

Braga et al (2009b) presented an assay where they formed four groups: *D. flagrans*, *M .thaumasium*, *A. robusta*, and the control group, to evaluate the predatory ability of these fungi. In the end, the isolate *D. flagrans* demonstrated better performance compared to other isolates used and the control group, observing a significant reduction of 97.5% in the average number of L3 recovered. Significant differences ($p<0.01$), in the number of L3 recovered from fungal isolates compared to the control group was seen. In the present study, a reduction of87.56% compared to the control group was observed. On the other hand, a difference between the mean and standard deviation of 1.06±0.95 was observed from day 1in the treated group and 8.12±4.11 in the control group. Comparing on the 7th day, an average and deviation of 0.1±0.3 was observed in the treated group and 7.18± 4.1in the control group. Analyzing such data it is observed that there is an average reduction in the treated group and an even lower reduction in the control group. In this sense, Braga *et al.*, (2010) stated that from the seventh day of analysis, there is a moisture decrease in the dishes and so the larvae use their motility to migrate to the periphery seeking higher humidity, which makes visualization, and counting of these larvae difficult, justifying this larvae reduction in the control group.

Regarding the fungus *A. conoides* (I40) used in this a assay, the reduction percentage was93.07%, being this result considered satisfactory as forits predation. It can be said that predation was very efficient in the use of this fungus. It's possible to observe that the averages from the control group are much larger than the treated group (I40), demonstrating a large predation of the fungus *A. conoides* on L3 larvae. Andaló *et al.*, (2008) demonstrated the effectiveness of *A.conoides* predation on L3 of gastrointestinal parasites of ruminants.
Studies show that other subspecies of *Arthrobotrys* have been used very

effectively in the biological control of nematode infective larvae. Araújo *et al.*, (2012) and Larsen (2000) stated that in the group of predators the genres *Arthrobotrys, Duddingtonia* and *Monacrosporium* stand out as for control of nematode larvae in the environment. It was observed, in the present study, the action of *A. Robusta* (I31) as its predation on infective larvae (L3). The percentage reduction results were satisfactory and demonstrate the predation effectiveness of *A. robusta*onL3.

Table.1 Means and Standard Deviations of Ilama Infective Nematode Larvae (L3) Daily Count in Petri Dishes of the Treated Groups with the Fungi *Duddingtonia flagrans* (AC001 andCG768) *Monacrosporium thaumasium* (NF34), *Monacrosporium sinense* (SF53) *Arthrobotrys robusta* (I31) and *Arthrobotrys conoides* (I40) as well as Control Group after Seven days of Interaction

Fungi isolates	Interaction days (1 to 7 days)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
AC001	1.6 [a] ±0.9	1.2 [a] ±0.7	1.3 [a] ±1.4	1.07 [a] ±0.9	0.73 [a] ±0.4	0.16 [a] ±0.4	0.1 [a] ±0.3
I40	0,87±0,6[a]	0.73 [a] ±0.6	0.67 [a] ±0.7	0.13 [a] ±0.3	0.63 [a] ±0.6	0.67 [a] ±0.2	0.03 [a] ±0.1
I31	0.8±0.6[a]	0.70 [a] ±0.6	0.88 a ±0.6	0.23 [a] ±0.4	0.33±0.5[a]	0.10 [a] ±0.3	0.06 [a] ±0.2
CG768	0.90 [a] ±1,0	1 [a] ±0.7	0.83 [a] ±0.6	0.92±0.7[a]	1 [a] ±2.7	0.12 [a] ±0,3	0.1 [a] ±0
SF53	0.52 [a] ±0,7	0.70 [a] ±0.7	1.05 [a] ±0.8	0.88 [a] ±0.7	0.72 [a] ±0.6	0.15 [a] ±0.3	0.66 [a] ±0,2
NF34	1.03 [a] ±0.9	0.98 [a] ±0.8	0.72 [a] ±0.8	0.35 [a] ±0.6	0.65 [a] ±0.5	0.12 [a] ±0.3	0.0 [a] 7±0.2
Control	8.12 [b] ±4.1	7.01 [b] ±3.6	7.7 [b] ±4.5	7.05 [b] ±3.7	6.73 [b] ±3.8	7.43 [b] ±4.3	7.18 [b] ±4.1

Means followed by the same lower case letters do not differ statistically (p>0.01). Tukey Test

Figure.1 A Trap Produced by Nematophagous Fungi; B-Third Stage Larvae of Gastrointestinal Nematode Parasite of Llamas (White Arrow) and C-L3 Gastrointestinal Nematode Parasite of Llamas Captured by the Fungus (Black Arrow)

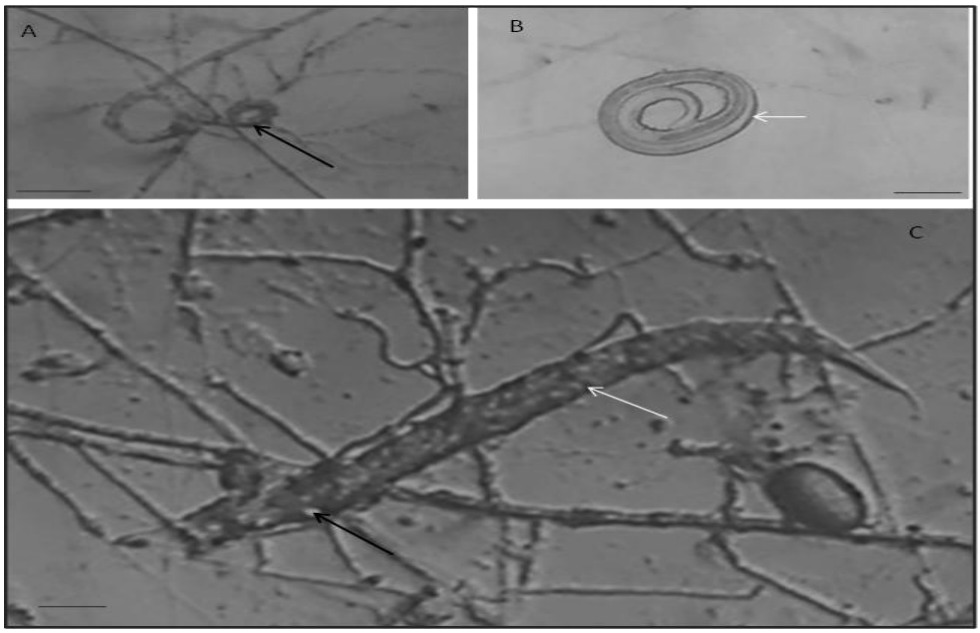
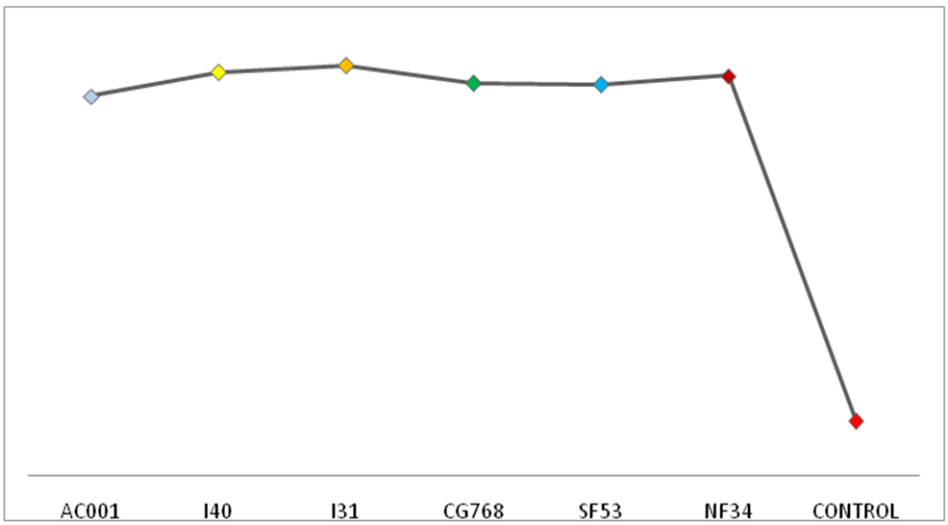


Figure.2 Average Percentage of Recovery (L3) in Petri Dishes in the Groups Treated with the Nematophagous Fungi and in the Control After 7days



Predatory ability of the nematophagous fungus *M. sinense* (SF53) on strongyles infective larvae (L3) was also analyzed in laboratory conditions in this assay. In the end,it was observed that there was a 90.28% average reductionofL3. Braga *et al* (2011) also observed the predatory ability of this fungus in laboratory testing, however obtaining 62.50% reduction on L3. The daily averages remained consistently lower in relation to the control group ($p < 0.01$) for this assay.

Other experiments may corroborate with this study, for example, the experiment performed by Campos (2006), which studied the use of the fungus *M. sinense* in the control of nematodes in cattle. The reduction percentage of infective larvae *in vitro* varied between 90.6 and 100%. Comparing to the experiment conducted it's realized that this variation also continued, but between 83.36 and 97.98%, being able to say that the reduction of larvae was satisfactory in the use of *M. sinense* for the biological control of nematodes found in llama feces.

The fungus *M. thaumasium* was used to evaluate its effectiveness in predation of strongyles found in llama feces in this assay. After seven days of observation and analysis, a 92.30%percentage reduction of infective larvae was obtained. Similar results were found in other studies. Silva *et al.*, (2013) conducted an experiment evaluating the effect of various nematophagous fungi in the control of infective nematode larvae (L3) after gastrointestinal transit in cattle. At the end of this study, *M. thaumasium* showed higher predation activity than the others, having a 98.3% reduction percentage. This result is in agreement with the present study, showing high efficacy of this fungus in the biological control of strongyles when infecting llamas. The lowest reduction percentage achieved in the seven-day period was 86.20%. Use of this isolate as a biological control for strongyles in llamas can be quite significant in treating these parasites in llamas. The results obtained in the present study showed differences ($p < 0.01$) in the daily L3 count compared to the control group.

This was the first report of the *in vitro* use of nematophagous fungi in biological control

of strongyles in llamas, however, certain premises must be suggested and among them, the need for *in vivo* studies and in environmental conditions for the purpose of comparing possible decrease of infection reoccurrence by these nematodes and as well as a tool to be used in the future in llamas raised in zoos.

Acknowledgment

The authors would like tothank CNPq, Capes, Fapes and Fapemig for financial support and grant concession.

References

Andaló, V., Moreira, G.F., Maximiniano, C., Moino, A., Campos, V. P.,2008. Suscetibilidade de *Heterorhabditis amazonensis (Rhabditida: Heterorhabditidae*) a Fungos Predadores de Nematóides. Universidade Federal de Lavras. Nematologia Brasileira, v. 32(3).

Anderson, D. E.,Jones, M. L.,Miesner, M. D.,2013.Veterinary Techniques for Llamas and Alpacas. Oxford: Willey-Blackwell.

Araújo, J. V., Stephano, M. A. Sampaio, W. M., 1999.Passage of nematode-trapping fungi through the gastrointestinal tract of calves. Veterinarski Arhiv Journal, v. 2, p.6978.

Araújo, J. V.,S tephano, M. A., Sampaio, W. M., 2000b.Effects of temperature, mineral salt and passage through the gastrointestinal tract of calves on sodium alginate formulation of *Arthrobotry srobusta*, a nematode-trapping fungus. Revista Brasileira de Parasitolologia Veteterinária, v. 9, p. 55-59.

Araujo, J. M., 2012. Avaliação de fungos nematófagos sobre larvas infectantes de *Strongyloides westeri*e ovos de *Toxocaracani*.(Tese)Universidade Federal de Viçosa. Minas Gerais.

Arenas, R.E.Z., 2007. Estudio de la fauna parasitaria deltracto gastrointestinal de laliebreeuropea (*Lepus Europaeus*, Pallas 1778), proveniente de Estancias de lasprovincias de Última Esperanza y Magallanes, XIIªRegión, Chile. Universidad Austral del Chile (tese).

Braga, F. R.; Araujo, J. V.; Campos, A. K.; Carvalho, R. O.; Tavela, A. O.; Maciel. A. S., 2007.Observação *in vitro* da ação dos isolados fúngicos *Duddingtonia flagrans, Monacrosporium thaumasium* e *Verticillium chlamydosporium* sobre ovos de *Ascaris lumbricoides* (Lineu, 1758). Revista da Sociedade Brasileira de Medicina Tropical, v.40 (3):356-358, mai-jun.

Braga, F. R.; Araujo, J. V.; Carvalho, R. O.; Araujo, J. M.; Silva, A. R.; Campos, A. K., 2009. Controle *in vitro* de larvas infectantes de ciastomíneos (*Nematoda: Cyathostominae*) de equinos utilizando os fungos predadores *Duddingtonia flagrans, Monacrosporium thaumasium e Arthrobotrys robusta*. Ciência Animal Brasileira, v. 10, n. 3, p. 887-892.

Braga F. R.; Silva, A. R.; Carvalho, R. O.; Guimarães, P. H. G.; Fujiwara, R. T.; Araujo, J. V.; Frassy, L. N.,2010.*In vitro* predatory activity of the fungi *Duddingtonia flagrans, Monacrosporium thaumasium, Monacrosporium sinense and Arthrobotrys robusta on Ancylostoma ceylanicum* third-stage larvae.Veterinary Microbiology, v.146. 183–186.

Braga, F. R.; araujo, J. M.; Silva, A. R.; Araujo, J. V.; Carvalho, R. O.; Melo, A. L.; Tavela, A. O.; Silva, M. E.; Fernandes, F. M.,2011. Destruição de larvas infectantes de *Strongyloides venezuelensis* pelos fungos *Duddingtonia flagrans, Arthrobotrys robusta e Monacrosporium sinense*. Revista da Sociedade Brasileira de Medicina Tropical, v.44 (3):389-391, mai-jun.

Braga, F. R.; Araujo, J.

V.,2014.Nematophagous fungi for biological control of gastrointestinal nematodes in domestic animals. Applied Microbiology and Biotechnology, 98 (1): 71-82, jan.

Bromage, G.,2009.Llamas and Alpacas. A guide to management. Wiltshire: Crowood Press.

Campos, A. K.,2006.Fungos nematófagos no controle de nematóides gastrintestinais de ruminantes. (Tese). Universidade Federal de Minas Gerais. Belo Horizonte.

Cebra, C; Anderson, D. E.; Tibary, A.; Van saun, R. J.; Jonhson, L. W.,2014.Llama and Alpaca care: Medicine, Surgery, Reproduction, Nutrition, and Herd Health, 1st edition, Canada: Elsevier.

Fowler, M. E.,1996. Husbandry and diseases of camelids. Scientific and Technical Review of the Office International des Epizooties. 15 (1), 155-169.

Fowler, M. E.; Cubas, Z. S., 2001.Biology, Medicine, and Surgery of South American Wild Animals. Iowa: Iowa University Press.

Gordon, H. McL; Whitlock, A.V.,1939. A new technique for counting nematode eggs in sheep feces. Journal Council Scientific Industry Research Australia, v. 12: 50-52.

Larsen, M.,1999. Biological Control of Helminths. International Jounal of Parasitology, v.29, p.139-146.

Larsen, M., 2000. Prospects for controlling animal parasitic nematodes by predacious microfungi. Parasitology, v.120, p. 121-131.

Mota, M. A.; Campos, A. K.; Araújo, J. V., 2002.Evaluation of the predatory capacity of the fungi *Arthrobotrys robusta* and *Monacrosporium thaumasium* submitted to different preservation methods against gastrointestinal parasitic nematodes of bovines. Revista Brasileira de Parasitologia Veterinária, v.11, n.1, p.13-17.

Murray, E.; Fowler, M. E., 2013. Alpaca and Llama and Behavior and this implications for illness detection. Camelid Symposium. University of California.

Silva. M. E.; Araujo, J. V.; Braga, F. R.; Soares, F. E. F.; Rodrigues, D. S.,2013. Controle de larvas infectantes de nematóides gastrintestinais de novilhas por diferentes isolados dos fungos nematófagos. Revista Brasileira de Parasitolologia Veterinária, v. 22, n. 1, p. 78-83, jan-mar.

Terry, J. A.,2009. The use of *Duddingtonia flagrans* for gastrointestinal parasitic nematode control in feces of exotic *Artiodactylids* at Disney's Animal Kingdom. Louisiana State University. (Master Science). Louisiana, MA.

How to cite this article:

Maria Gorett Ramos Rodrigues, Filippe Elias Freitas Soares, Emy Hiura, Anderson Rocha Aguiar, Carolina Magri Ferraz, Dominik Lenz, Tatiana de Souza Barbosa, Maria Cristina Valdetaro Rangel, Leandro Abreu da Fonseca, Tracy Ferreira Lacerda, Gracilene Maria Almeida Muniz Braga, Fabio porto Sena, Jackson Victor Araújo and Fabio Ribeiro Braga. 2016. In Vitro Biological Control of Strongyles (Strongylida) in Llamas (Lama glama) Coming from a Zoo, in Southeastern Brazil. *Int.J.Curr.Microbiol.App.Sci*. 5(4): 976-983.
doi: http://dx.doi.org/10.20546/ijcmas.2016.504.111