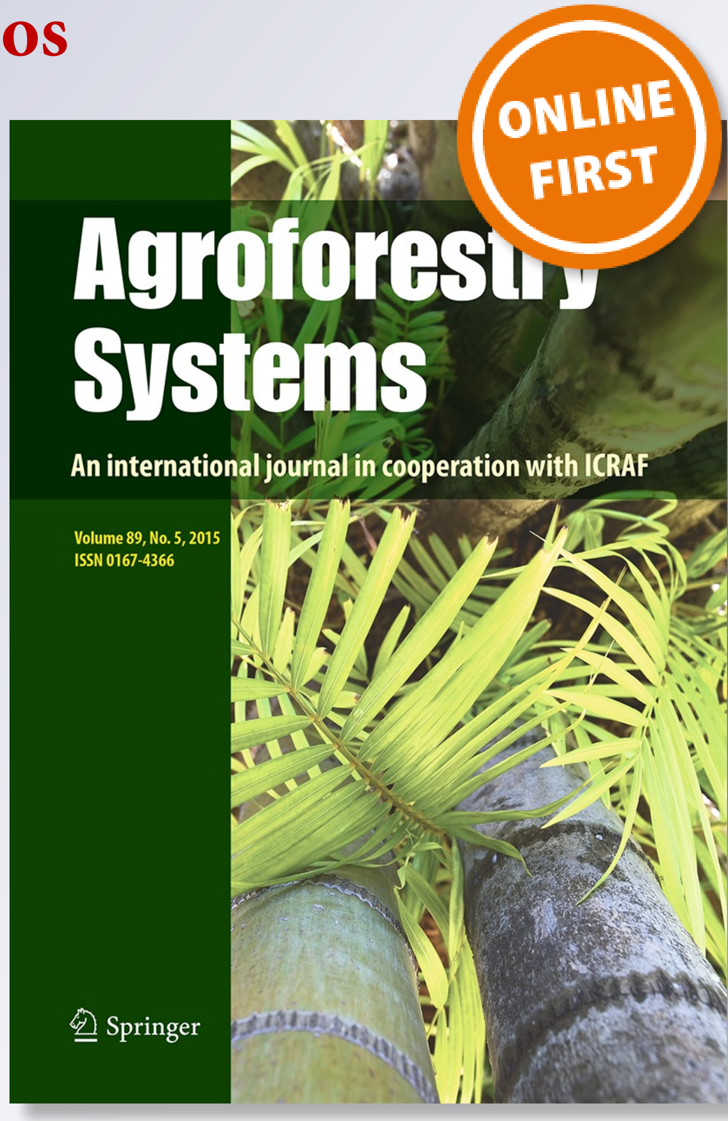
Effect of the integrated livestock–forest system on recovery of trichostrongylid nematode infective larvae from sheep

Eduardo Ferreira Faria, Luciano Bastos Lopes, Daniela dos Reis Krambeck, Douglas dos Santos Pina & Artur Kanadani Campos

Agroforestry Systems
An International Journal incorporating Agroforestry Forum

ISSN 0167-4366

Agroforest Syst
DOI 10.1007/s10457-015-9855-1



Springer

Agroforest Syst
DOI 10.1007/s10457-015-9855-1

Effect of the integrated livestock–forest system on recovery of trichostrongylid nematode infective larvae from sheep

Eduardo Ferreira Faria · Luciano Bastos Lopes · Daniela dos Reis Krambeck · Douglas dos Santos Pina · Artur Kanadani Campos

Received: 19 January 2015 / Accepted: 4 October 2015

Abstract Trichostrongylids nematodes are capilliform worms that parasitize the gastrointestinal tract of ruminants that frequently cause serious injuries such as severe gastroenteritis and acute anemia. Sheep breeds are highly susceptible to these parasites, including adult animals as well. In sheep herds, this parasitic disease is traditionally controlled through the use of antihelminthics; however, some possible auxiliary methods of control have been suggested, and among them is the adoption of the integration livestock–forest system. The aim of the present study was to evaluate comparatively the recovery of trichostrongylid nematode larvae (L3) from sheep in a integration livestock–forest system and in grass monoculture, also analyzing the climatic influence in the four seasons of the year. The study was carried out from December 2013 to September 2014 in the experimental field of Embrapa Agrosilvopastoral located in the municipality of Sinop/MT, Brazil. In each season of the year, each treatment received thirty samples of feces weighing 20 g and containing approximately 60,000 eggs of trichostrongylid nematodes. At the end of 14 days, the remaining feces from the soil surface, as well as soil below the deposition area, and the adjacent forage near the feces were collected and taken to the laboratory where the number of infective larvae per kilogram of dry matter (L3/kg DM) in each collected material was determined. The recovery of L3 larvae was possible in all seasons and in all samples collected. There was a significant interaction between treatments and seasons of the year ($p < 0.05$). The silvopastoral system showed greater counts of L3/kg DM in the forage during the spring, summer, and winter collections. The feces collected in the winter were the material with highest counts of L3/kg DM in the study, with 30,199 in silvopastoral area, and 22,020 in grass monoculture, which differed significantly ($p < 0.05$). The soil also showed the same response, with 6112.74 L3/kg DM in silvopastoral system and 4847.56 L3/kg DM in grass monoculture, which were also significantly different ($p < 0.05$).

Keywords Integrated system · Sheep farming · Parasitic infection

E. F. Faria · D. dos Reis Krambeck
ICAA-UFMT, Av. Alexandre Ferronato, no. 1200, Setor Industrial, Sinop, MT, Brazil

L. B. Lopes (✉)
Embrapa Agrosilvopastoral, Sinop-MT, Rodovia MT, no. 222, Km 2,5, Zona Rural, Sinop, MT, Brazil
e-mail: luciano.lopes@embrapa.br

D. dos Santos Pina
Department of Animal Science, ICAA- UFMT, Av. Alexandre Ferronato, no. 1200, Setor Industrial, Sinop, MT, Brazil

A. K. Campos
Department of Veterinary, UFV, Av. Peter Henry Rolfs, s/n - Campus Universitário, Viçosa, MG, Brazil

Published online: 13 October 2015

Introduction

Trichostrongylids nematodes are capilliform worms that usually parasitize the gastrointestinal tract of ruminants causing serious injuries with considerable morbidity and mortality rates, in addition to great economic losses to livestock farming. Sheep breeds are highly susceptible to these parasites, including adult animals as well. Sheep may suffer serious infections involving multiple genera of trichostrongylid nematodes, and animals infected by these parasites may develop different symptoms and clinical cases. Some of these noteworthy conditions are the acute anemia caused by worms of the genus *Haemonchus* and the severe gastroenteritis caused by worms of the genus *Trichostrongylus* (Monteiro et al. 2011).

Taylor et al. (2010) stated that constant monitoring of the number of eggs count per gram of feces (EPG) of sheep to evaluate the parasitic load and rigorous deworming practices could provide the control of infestations by trichostrongylid nematodes. These authors also claimed that different management strategies can be used efficiently to prevent and/or control infections by these parasites.

The integration livestock–forest system has been pointed as a possible alternative method to control these parasites due to this system's ability to attract a greater soil fauna that would include predators of helminthes species and competitors for substrate, thereby reducing the parasitic load available in the environment (Soca et al. 2002; Auad and Carvalho 2011).

This type of productive system may provide benefits to animals, mainly with regard to the thermal comfort, through lower incidence of solar radiation, milder temperatures, and greater moisture retention (Garcia and Andrade 2001; Balbino et al. 2011). However, these more favorable microclimatic conditions may also foster the development and maintenance of nematode larvae in the environment.

Given this scenario, the aim of this study was to evaluate comparatively the recovery of trichostrongylid nematodes infective larvae originating from sheep hosts between the systems of integration livestock–forest and conventional pasture with grass monoculture.

Materials and methods

Experimental field

The project was established in the experimental field of Embrapa Agrosilvopastoral located in the municipality of Sinop/MT, Brazil 11°51′43″S, 55°35′27″W; 384 m altitude. The Köppen classification characterizes the climate in the region as Aw tropical with rains concentrated in the summer, and dry winter. The study area consists of paddocks covered with *Brachiaria brizantha* cv Piatã, in which the following treatments were evaluated: treatment A: grassland monocultured in full sun; and treatment B: integration livestock–forest system (LFI) with triple rows of *Eucalyptus urograndis* (H13 clone) spaced 15 m apart. The areas evaluated in the study had not been utilized previously by any other animal category or specie. Historically, the land was destined exclusively to rice crop and cotton. Based on the previous use of the land, there is a very slight chance of contamination by trichostrongylid nematodes at the time of this study. Likewise, no animal had access to the evaluated paddocks during the period of the experiment and in the 2 years before.

Obtaining feces with trichostrongylid nematode eggs

Samples of feces with trichostrongylid nematode eggs were obtained from 10 naturally infected crossbred sheep belonging to the Veterinary Parasitology Department of Federal University of Mato Grosso, *Campus* Sinop. Stool samples from these animals were prepared regularly according to the technique described by Ueno and Gonçalves (1998) to check the maintenance of trichostrongylid nematode populations. Three days before the field inoculations, feces collection was started twice daily to reach an appropriate volume. It was calculated the mathematical average of those samples and based on this result, it was calculated the amount of feces necessary to obtain approximately 60,000 eggs of trichostrongylid nematodes. In this way, each excreta portion contained the amount of eggs necessary to inoculation, but in a variable volume according each repetition. Fecal material was then identified, stored under refrigeration, and protected against dehydration for subsequent inoculation in both treatments. To standardize the inoculum, feces from uninfected isolated animals, analyzed previously, were incorporated until reach 20 g.

Experimental modules

Aiming to standardize the pasture height, before each deposition of feces, the deposition area in both treatments was mowed for the pasture to have a standard height 30 cm. In these areas, 30 modules per treatment were marked by numbered flags; those were the sites of deposition of the feces through the pasture.

Deposition of feces

Fecal depositions occurred during the studied period with approximately 90 day interval, so each treatment would receive deposition in each of the four seasons of the year. Feces were deposited on the soil through the grass in each one of the previously tagged modules on December 1st 2013, February 26th, May 29th, and August 29th of 2014, categorizing spring, summer, fall, and winter, respectively.

Material collection and laboratory tests

Fourteen days after depositing feces, on December 14th, March 10th, June 11th, and September 11th, respectively, to the deposition schedule, samples of remaining feces in the soil surface as well as the soil 2 cm below those feces and pasture adjacent with 10 cm radius delimited by a metal hoop were collected in three different times (06h00, 12h00, and 18h00). The hoop diameter was determined based on the report that approximately 90 % of the larvae do not migrate sideways further than 10 cm from the feces, as described by Skinner and Todd (1980) cited by Rocha et al. (2008). The larvae were extracted from these samples by the technique of Baerman modified from Ueno (Ueno and Gonçalves 1998). After 24 h in Baerman's apparatus, the samples (forage, soil, and feces) were placed in paper bags with known weight and later dried in an oven at 100 °C for 24 h to determine the dry matter, in accordance with Silva and Queiroz (2002). The recovered larvae were counted under optical microscopy and then the number of infective larvae per kilogram of dry matter (L3/kg DM) was obtained. The larvae recovered from the samples were identified according to Ueno and Gonçalves (1998), which made it possible to monitor the contamination by other nematodes.

Statistical analysis

The study was designed in a completely randomized, in a 2×4 factorial arrangement with two treatments and four sampling seasons. The interaction between treatments and seasons of the year was analyzed in the three types of material evaluated: forage, soil, and feces. Data underwent logarithmic transformation (Log X) and were then analyzed through the PROC MIXED procedure of SAS System®. For better understanding, the arithmetic means are presented in the tables.

Results and discussion

Based on the obtained results, it was found that the interaction between treatments and seasons of the year was significant ($p < 0.05$) regarding the number of larvae recovered for all evaluated materials. It was also found that it was possible to recover infective larvae in all samples in the four periods of collection.

Expressive counts of L3/kg DM were obtained in the forage in the spring and summer collections, which correspond to the rainy season in the north region of Mato Grosso State. This result is coherent with those published by other authors (Lima et al. 1997; Niezem et al. 1998; Rocha et al. 2007).

The highest count of L3/kg DM in pasture was obtained in the collection during the fall in the grass from the conventional system: 5870.20 L3/kg DM ($p < 0.05$). However, the integration livestock–forest system showed superiority in the counts ($p < 0.05$) as compared with the other seasons, as can be seen in Table 1. Based on the conditions observed in this study, the result of integration of livestock–forest demonstrated that this system can increase the chances of an animal ingest infective larvae of trichostrongylid nematodes. However, it is important to highlight that this study has specific environment conditions, with a dry and rainy season very well defined. Besides, integrated livestock–forest systems can be designed in different ways, wherein each strategy can lead to distinct microclimate conditions.

Table 1 Mean number of trichostrongylid nematode infective larvae per kilogram of dry matter recovered from forage in collections performed in the four seasons of the year, 14 days after deposition of feces samples from sheep with trichostrongylid nematode eggs through the pasture and in a integration livestock–forest system (LFI) and pasture in full sun

System	Season of the year				Standard error of the mean
	Spring	Summer	Fall	Winter	
ILF	4889.68Ab	5667.87Aa	4607.28Bb	749.09Ac	292.59
Full sun	3887.76Bb	2924.45Bc	5870.20Aa	385.85Bd	

Means followed by the same letter do not differ ($p < 0.05$). Lowercase letters compare values in the rows and uppercase letters compare values in the columns

The recovery of infective larvae in the winter sampling, which corresponds to the peak of the dry season in the Northern Mato Grosso State, was significantly lower than in the other seasons ($p < 0.05$). This result corroborates those found by Ndamukong and Ngone (1996), who also observed greater recovery of larvae during the rainy season. The aforementioned authors consider rain as the climatic factor of greatest importance for the development and survival of larvae in the field.

The absence of rain may bring parasite transmission to a halt during the driest months (Rocha et al. 2007), and sharp peaks in the abundance of larvae on herbage can be seen after periods of rainfall (Sissay et al. 2007). Authors as Krecek et al. (1990) and Niven et al. (2002) have suggested that a film of moisture is needed for the migration from dung onto herbage, and thus, larval migration would only be possible either just after rains or when dew is found on grass leaves. According to Boom and Sheat (2008), the different climatic conditions between seasons of the year may lead to a selection pressure, thereby interfering with the variability among the species of trichostrongylid nematodes recovered in the environment.

Khadijah et al. (2013) attributed great importance to the soil moisture-retention ability for the maintenance of the life of infective larvae. The present study also detected this phenomenon, with the presence of larvae in the superficial soil layers in all months of collection. The largest numbers of L3/kg DM in the soil ($p < 0.05$) were recovered in the month corresponding to the peak drought in the studied region. These results can be observed in Table 2.

The fecal material displayed the greatest recovery of L3/kg DM in the winter collection for both treatments ($p < 0.05$), averaging 30,199 in the integrated system, and 22,020 in the conventional system, which differed significantly from each other ($p < 0.05$). Results are described in Table 3.

The greater concentration ($p < 0.05$) of L3/kg DM in the remaining fecal material in winter demonstrates that this material can also serve as reservoir for the permanence of these larvae in the field until the precipitation rates are favorable to their maintenance in the pasture. This result was consistent as that obtained by Silva (2007), who also observed the presence of larvae in sheep feces in June (winter) in a study similar to the current one. The contaminated stools may provide the ideal environment for these helminth larvae, ensuring the appropriate moisture and temperature for the maintenance of life until this condition is reestablished in the environment (Starke et al. 1992; Lima et al. 1997; Stromberg 1997; Almeida et al. 2005). Amarante et al. (1996) found an expressive number of infective larvae in the pastures even during a severe drought period with monthly precipitation below 23 mm between June and August. The authors attributed part of this effect to the survival of eggs and larvae under favorable microclimatic conditions in the fecal environment.

The maintenance of the viability of eggs and larvae of trichostrongylid nematode in sheep feces, the disperse elimination of scybala (covering a large area during defecation), and the time of permanence of the feces in the field, which could be observed for a period of at least 50 days according to Yeates et al. (2007), are characteristics that demonstrate the potential of this material for environmental contamination.

The summer sampling was the only one to show a response pattern regarding the counts of infective larvae. In this collection, the counts of L3/kg DM in forage were 5.66787 in the integration livestock-forest system and 2924.45 in the system with grass monoculture. The expected response was thus observed

Table 2 Mean number of trichostrongylid nematode infective larvae per kilogram of dry matter recovered from soil in collections performed in the four seasons of the year, 14 days after deposition of feces samples from sheep with trichostrongylid nematode eggs through the pasture and in a integration livestock–forest system (LFI) and pasture in full sun

System	Season of the year				Standard error of the mean
	Spring	Summer	Fall	Winter	
ILF	2259.64Ac	2731.61Bb	1131.47Bd	6112.74Aa	130.28
Full sun	2421.28Ac	3336.56Ab	2301.59Ac	4847.56Ba	

Means followed by the same letter do not differ ($p < 0.05$). Lowercase letters compare values in the rows and uppercase letters compare values in the columns

Table 3 Mean number of trichostrongylid nematode infective larvae per kilogram of dry matter recovered from feces in collections performed in the four seasons of the year, 14 days after deposition of feces samples from sheep with trichostrongylid nematode eggs through the pasture and in a integration livestock–forest system (LFI) and pasture in full sun

System	Season of the year				Standard error of the mean
	Spring	Summer	Fall	Winter	
ILF	3839.85Ac	1638.84Bd	5316.97Bb	30199.00Aa	922.06
Full sun	4025.79Ac	2578.18Ad	6140.71Ab	22020.00Ba	

Means followed by the same letter do not differ ($p < 0.05$). Lowercase letters compare values in the rows and uppercase letters compare values in the columns

when these data were confronted with those of the reservoir media, with mean values of 2731.61 and 3336.56 for soil, and 1638.84 and 2578.18 for feces in the integrated and conventional systems, respectively, which demonstrates an inverse proportionality. The pairing of these data indicated a shift in the concentration of infective larvae from the reservoir media to the forage when the environment provides better moisture levels for their maintenance.

According to Dijk and Morgan (2011), the migration behavior of trichostrongylids appears to be the result of two trade-offs. Travel in a horizontal plane is determined by a trade-off between energy reserves and the probability of ingestion by a host. In a vertical plane, optimum travel has to balance exposure to damaging environmental influences and the probability of ingestion. As continuous larval movement is spatially random, the proportion of a larval population present on herbage is fairly constant in time and declines with the rate of larval death in the soil reservoir.

Based on the results, new management strategies can be developed for a better use of systems integrating livestock and forest. Among these, noteworthy practices are the constant monitoring of the number of eggs per gram of feces and strategic deworming of the more susceptible categories as suggested by Taylor et al. (2011). The management with different animal species aiming to decontaminate the field based on the principle of parasitic specificity can also be an option. According to Silva et al. (2014), the species *H. placei* and *H. similis* are highly specific for cattle, while *H. contortus* is highly specific for sheep. The reduced contamination of pastures by trichostrongylid nematode larvae through the adoption of managements with different species has been described in the literature, wherein studies conducted in different forages and with different arrangements may be in a regime of succession between cattle and sheep or even with the two species grazing together in the same area with good results of decontamination (Fernandes et al. 2004; Torres 2008; Torres et al. 2009). However, the risk of bacterial or viral diseases transmission among different species cannot be neglected and must be evaluated for each property.

Conclusion

The integration livestock–forest system can increase the chances of recontamination of sheep by trichostrongylid nematode infective larvae, as higher counts of L3/kg DM were found in the forage as compared with the grass monoculture system in the samples collected in the spring, summer, and fall under the conditions of the experimental arrangement established in Northern Mato Grosso State. Once again, integrated livestock–forest systems can be designed in different ways, in each strategy the microclimate conditions can change significantly, interfering with biology of parasites. Anyway, management strategies aiming at controlling nematodioses in sheep managed under livestock–forest systems should be evaluated so that they can be used more efficiently.

It is suggested to assess the parasitic dynamics of nematodes in integration livestock–forest system so that management strategies can be evaluated for ruminant species based on results achieved. Evaluating other arrangements of integration livestock–forest systems with different spacing between rows and the use of other arboreal species will be important for future recommendations. The integrated systems are dynamic, and may follow different arrangements, thus providing diversified microclimatic and environmental conditions that would be potentially sufficient to obtain different results from those of the present study.

References

Almeida LR, Castro AA, Silva FJM, Fonseca AH (2005) Desenvolvimento, sobrevivência e distribuição de larvas infectantes de nematoides gastrintestinais de ruminantes, na estação seca da Baixada Fluminense. Revista Brasileira de Parasitologia Veterinária 14:89–94

Amarante AFT, Padovani CR, Barbosa MA (1996) Contaminação da pastagem por larvas infectantes de nematódeos gastrintestinais parasitas de bovinos e ovinos em Botucatu-SP. Revista Brasileira de Parasitologia Veterinária 5:65–73

Auad AM, Carvalho CA (2011) Análise faunística de coleópteros em sistema silvipastoril. Ciência Florestal 21:31–39

Balbino LC, Cordeiro LAM, Silva VP, Moraes A, Martinez GB, Alvarenga RC, Kischel AN, Fontaneli RS, Santos HP, Franchini JC, Galerani PR (2011) Evolução técnológicae arranjos produtivos de sistemas de integração Lavoura-Pecuária-Floresta no Brasil. Pesquisa Agropecuária Brasileira 46:1–12

Boom CG, Sheath GW (2008) Migration of gastrointestinal nematode larvae from cattle faecal pats onto grazable herbage. Vet Parasitol 157:260–266

Dijk JV, Morgan ER (2011) The influence of water on the migration of infective trichostrongyloid larvae onto Grass. J Parasitol 138:780–788

Fernandes LH, Seno MCZ, Amarante AFT, Souza H, Belluzzo CEC (2004) Efeito do pastejo rotacionado e alternado com bovinos adultos no controle da verminose em ovelhas. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 56:733–740

Garcia R, Andrade CMS (2001) Sistemas silvipastoris na Região Sudeste. SISTEMAS AGROFLORESTAIS PECUÁRIOS: Opções de sustentabilidade para áreas tropicais e subtropicais. Embrapa Gado de Leite, Anais, pp 173–187

Khadijah S, Kahn LP, Walkden-Brown SW, Bailey JN, Bowers SF (2013) Soil moisture influences the development of *Haemonchus contortus* and *Trichostrongylus colubriformis* to third stage larvae. Vet Parasitol 196:161–171

Krecek RC, Murrell KD, Douglass LW (1990) Effects of microclimatic variables on the availability and movement of third-stage larvae of *Ostertagia ostertagi* on herbage. Onderstepoort J Vet Res 57:133–135

Lima WS, Fakuri E, Guimarães MP, Malacco MA (1997) Dinâmica das helmintoses de bovinos de leite na região metalúrgica de Minas Gerais. Revista Brasileira de Parasitologia Veterinária 6:97–103

Monteiro SG, Oliveira AR, Silva CLG, Fortes FS, Santos HT, Araújo JV, Luque JL, ÓDwyer LH, Sangioni LA, Molento MB, Rodriges MLA, Carneiro ME, Menezes RCAA, Bittencourt VREP (2011) Parasitologia na Medicina Veterinária. Roca, São Paulo

Ndamukong KJ, Ngone MM (1996) Development and survival of *Haemonchus contortus* and *Trichostrongylus* sp. on pasture in Cameroon. Trop Anim Health Prod 28:193–198

Niezen JH, Charleston WAG, Hodgson J, Miller CM, Aghorn TS, Robertson HA (1998) Effect of plants species on the larvae of gastrointestinal nematodes which parasitise sheep. Int J Parasitol 28:791–803

Niven P, Anderson N, Vizard AL (2002) Trichostrongylid infections in sheep after rainfall during summer in southern Australia. Aust Vet J 80:567–570

Rocha RA, Bricarello PA, Rocha GP, Amarante AFT (2007) Recuperação de larvas de *Trichostrongylus colubriformis* em diferentes estratos de *Brachiaria decumbens* e *Panicum maximum*. Revista Brasileira de Parasitologia Veterinária 16:77–82

Rocha RA, Rocha GP, Bricarello PA, Amarante AFT (2008) Recuperação de larvas infectantes de *Trichostrongylus colubriformis* em três espécies de gramíneas contaminadas no verão. Revista Brasileira de Parasitologia Veterinária 17:227–234

Silva BF (2007) Migração vertical das larvas infectantes de *Haemonchus contortus* em capim Braquiária (*Brachiaria decumbens*). Dissertação de Mestrado, Universidade Estadual Paulista

Silva MRL, Amarante MRV, Bresciani KDS, Amarante AFT (2014) Host-spcificity and morphometrics of female *Haemonchus contortus*, *H. placei* and *H. similis* (Nematoda: Trichostrongylidae) in cattle and sheep from shared pastures in São Paulo State, Brazil. J Helmintol 4:1–5

Silva DJ, Queiroz AC (2002) Análise de alimentos: métodos químicos e biológicos. Editora UFV, Viçosa

Sissay MM, Uggla A, Waller PJ (2007) Epidemiology and seasonal dynamics of gastrointestinal nematode infections of sheep in a semi-arid region of eastern Ethiopia. Vet Parasitol 143:311–321

Skinner WD, Todd KS Jr (1980) Lateral migration of Haemonchus contortus larvae on pasture. Am J Vet Res 41:395–398

Soca M, Simón L, Sánchez S, Gómez E (2002) Dinámica parasitológica em bostas de bovinos bajo condiciones silvopastoriles. Agroforesteria em lãs Américas 9:33–34

Starke WA, Zocoller MC, Machado RZ, Montenegro EL (1992) Helmintíases em búfalo II- Sobrevivência de larvas de nematódeos parasitos de búfalos jovens nas fezes depositadas em pastagens no município de Selvíria, MS., nos períodos secos e chuvosos. Revista Brasileira de Parasitologia Veterinária 1:7–15

Stromberg BE (1997) Enviromental factors influencing transmission. Vet Parasitol 72:247–264

Taylor MA, Coop RL, Wall RL (2010) Parasitologia Veterinária. Guanabara Koogan, Rio de Janeiro

Torres SEFA (2008) Recuperação de larvas infectantes (L3), em sistemas de pastejo de ovinos isolado, combinado e alternado com bovinos, no período das águas. Dissertação de Mestrado, Universidade de Brasília

Torres SEFA, Mcmanus C, Amarante AFT, Verdolin V, Louvandini H (2009) Nematódeos de ruminantes em pastagem com diferentes sistemas de pastejo com ovinos e bovinos. Pesquisa Agropecuária Brasileira 44:1191–1197

Ueno H, Gonçalves PC (1998) Manual para diagnostico de helmintoses em ruminantes. Japan International Cooperation Agency, Tokyo

Yeates GW, Skipp RA, Gray RAJ, Chen LY, Waghorn TS (2007) Impact on soil fauna of sheep faeces containing a range of parasite control agents. Appl Soil Ecol 35:180–389