Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 154 (2008) 167–170
veterinary parasitology
www.elsevier.com/locate/vetpar

Short communication

Anthelmintic activity of *Lippia sidoides* essential oil on sheep gastrointestinal nematodes

A.L.F. Camurça-Vasconcelos [a,*], C.M.L. Bevilaqua [b], S.M. Morais [b], M.V. Maciel [a], C.T.C. Costa [a], I.T.F. Macedo [a], L.M.B. Oliveira [a], R.R. Braga [a], R.A. Silva [a], L.S. Vieira [c], A.M.C. Navarro [c]

[a] *Programa de Pós-Graduação em Ciências Veterinárias/Universidade Estadual do Ceará, Brazil*
[b] *Universidade Estadual do Ceará, Brazil*
[c] *EMBRAPA/CNPC, Brazil*

Received 24 July 2007; received in revised form 13 February 2008; accepted 21 February 2008

Abstract

Medicinal plants have been investigated for their anthelmintic properties and shown to be effective against eggs and larvae of gastrointestinal nematodes. The aim of this study was to evaluate the efficacy of the *Lippia sidoides* essential oil (LsEO) on sheep gastrointestinal nematodes. Initially, 44 naturally infected sheep were divided and treated with 200 $\mu g\ kg^{-1}$ ivermectin and 230 and 283 $mg\ kg^{-1}$ LsEO, respectively, plus the control. Fecal samples were collected from each animal to determine epg at 7, 14 and 21 days after treatment. In another test, 21 sheep were distributed and treated with 200 $\mu g\ kg^{-1}$ ivermectin, 283 $mg\ kg^{-1}$ LsEO and the control, respectively. Seven days after treatment, they were euthanized and necropsied to count and identify the nematodes from the abomasum, small and large intestines. In the first test, the efficacy of 230 and 283 $mg\ kg^{-1}$ LsEO and ivermectin was 38%, 45.9% and 40.2%, respectively, 7 days after treatment, and 30%, 54% and 39.6%, respectively, 14 days after treatment. In the second experiment, the respective efficacy of 283 $mg\ kg^{-1}$ LsEO and ivermectin was 56.9% and 34.4% against *Haemonchus* spp., and 39.3% and 63.6% against *Trichostrongylus* spp.

Keywords: Phytotherapy; Sheep; Gastrointestinal nematodes; *In vivo* tests

1. Introduction

Sheep and goat production is an important source of income to small farmers in Northeast Brazil. However, this activity suffers great economic losses due to gastrointestinal nematode parasites because they could cause mortality and decrease production (Arosemena et al., 1999; Pinheiro et al., 2000). Gastrointestinal nematode control is accomplished using synthetic anthelmintics. However, development of nematode populations resistant to all anthelmintic classes has awakened interest of several researchers to find alternatives for this control (Melo et al., 2003; Sissay et al., 2006). Thus, medicinal plants have been tested around world for their anthelmintic properties.

Lippia sidoides Cham (Verbenaceae) is used in Brazilian folk medicine to treat gastrointestinal disorders (Craveiro et al., 1977; Barraca, 1999) and is very common in Northeast Brazil (Matos, 2002). *L.*

* Corresponding author at: Programa de Pós-Graduação em Ciências Veterinárias/Universidade Estadual do Ceará, Rua Meruoca No. 100, Varjota, Fortaleza, Ceará, CEP 60175-340, Brazil.
Tel.: +55 85 3101 9860; fax: +55 85 3101 9840.
E-mail address: anacamurca@bol.com.br
(A.L.F. Camurça-Vasconcelos).

0304-4017/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2008.02.023

sidoides essential oil (LsEO) have been investigated regarding their anthelmintic properties against *Haemonchus contortus* eggs and larvae (Camurça-Vasconcelos et al., 2007).

Anthelmintic activity in small ruminants can be detected by the fecal egg count reduction test (FECRT), as recommended by Coles et al. (1992), or controlled testing (Wood et al., 1995). FERCT is more rapid and less expensive than controlled testing and thus can be used to choose the effective dose (Taylor et al., 2002), while controlled testing is more reliable and is recommended for dose confirmation assays (Wood et al., 1995). The aim of this study was to evaluate the anthelmintic efficacy of LsEO on gastrointestinal nematodes of sheep, the target species for clinical indications.

2. Materials and methods

2.1. General procedures

All proceedings were approved by the Ethics Committee of Universidade Estadual do Ceará. The LsEO was purchased from PRONAT, a company located in the state of Ceará. Male hairless sheep were identified by ear tags and distributed in groups according to the number of epg at EMBRAPA farm in Sobral municipality, in a semi-arid region, at November 2005 and February 2006. Treatment by oral administration with LsEO was during 5 days and ivermectin was by a single oral dose according to the manufacturer's recommendations.

2.2. Fecal egg count reduction test (FECRT)

Forty-four sheep were distributed into four groups ($n = 11$): G1, untreated animals; G2, 200 μg kg^{-1} ivermectin; G3, 230 mg kg^{-1} LsEO; G4, 283 mg kg^{-1} LsEO. Dose calculation of LsEO for each animal was based on alometric calculation (Felippe, 2005) and data used were obtained from a previous study (Camurça-Vasconcelos et al., 2007). Fecal samples from each animal were collected on treatment day 0 and on days 7, 14 and 21 post-treatment to determinate epg by McMaster technique (Ueno and Gonçalves, 1998). Larvacultures were performed with feces using Roberts and O'Sullivan (1950) method.

2.3. Controlled test

Twenty-one sheep were distributed into three groups ($n = 7$): G1, untreated; G2, 200 μg kg^{-1} ivermectin; G3, 283 mg kg^{-1} LsEO. Seven days after oral treatment, the sheep were euthanized and submitted to necropsy to count the worm burden (Wood et al., 1995). The animal gastrointestinal tracts were separated by double ligatures at the abomasum, small and large intestines. Contents of abomasum and small intestine were passed through 0.062 mm aperture sieves and that of large intestine through 0.177 mm. After complete washing in slow running water, material was conserved in Railliet & Henry solution until parasite counting. The worms were recovered and identified from a sample corresponding to 20% of the contents (Ueno and Gonçalves, 1998).

2.4. Statistical analysis

Efficacy was calculated on FECRT according to Coles et al. (1992) and on Controlled test according to Wood et al. (1995). Results were analyzed using ANOVA and Kruskall–Wallis test (a nonparametric method) with 5% significance level. Data were expressed as mean $\pm$ standard error.

3. Results

Efficacy of 230 and 283 mg kg^{-1} LsEO in FECRT 7 and 14 days after treatment ranged from 38% to 30% and from 45% to 54%, respectively. Efficacy of 0.2 μg kg^{-1} ivermectin ranged from 40.2% to 39.6%, 7 and 14 days post-treatment, respectively (Table 1).

Table 2 presents efficacy of LsEO and ivermectin. Ivermectin was more efficient at controlling *Trichostrongylus* spp., while LsEO was more effective against *H. contortus*. However, results were not statistically different ($P > 0.05$).

In FECRT, *Haemonchus* spp. was the most prevalent parasite (97%), followed by *Trichostrongylus* spp. (3%), while in the controlled test, *Haemonchus* and *Trichostrongylus* represented 37.9% and 62.1% of worm burden from untreated sheep, respectively.

4. Discussion

FECRT on sheep infected with *H. contortus* has been used to evaluate the anthelmintic activity of plant extracts like *Artemisia brevifolia* (Iqbal et al., 2004), *Myrsine Africana*, *Albizia anthelmintica*, *Hilderbrantia sepalosa* (Gathuma et al., 2004) and *Khaya senegalensis* (Ademola et al., 2004), presenting a epg reduction of 67.2%, 77%, 89.8%, 90% and 88%, respectively, better than results obtained with LsEO. However, in this study, epg reduction caused by ivermectin was just 39.6% and according to Coles

Table 1
Efficacy (mean percentage ± S.E.) of *Lippia sidoides* essential oil and ivermectin in the fecal egg count reduction test of sheep naturally infected with gastrointestinal nematodes

Days after treatment	Treatment		
	230 mg kg^{-1} LsEO	283 mg kg^{-1} LsEO	Ivermectin[a]
7	38.0 ± 10.7	45.9 ± 10.2	40.2 ± 10.9
14	30.0 ± 11.0	54.0 ± 10.5	39.6 ± 11.9
21	29.8 ± 10.8	22.9 ± 7.9	27.5 ± 11.3

Data were not statistically different ($P > 0.05$).
[a] Dose recommended by manufacturer 0.200 mg kg^{-1}.

Table 2
Efficacy (mean percentage ± S.E.) of *L. sidoides* essential oil and ivermectin on worm burden of sheep naturally infected with gastrointestinal nematodes

Nematodes	283 mg kg^{-1} LsEO	200 mg kg^{-1} IVM	Control
Haemonchus spp.	56.9 ± 10.7	34.4 ± 10.3	16.930 (37.9%)
Trichostrongylus spp.	39.3 ± 11.7	63.6 ± 10.2	27.715 (62.1%)
Total	43.7 ± 11.8	50.3 ± 8.7	44.645 (100%)

Data were not statistically different ($P > 0.05$). Control = untreated sheep.

et al. (1992), anthelmintic resistance is present if egg count reduction is less than 95%. Thus, nematode population of sheep used in the FECRT was resistant to ivermectin. This is a common occurrence in Brazil (Vieira and Cavalcante, 1999; Melo et al., 2003). Nine native plants of Ceará State, Brazil, and *Azadirachta indica* were used in sheep harboring natural gastrointestinal nematode infections with no reduction of worm burden (Vieira et al., 1999; Costa et al., 2006).

Use of LsEO could be justified even with efficacy lower than 95% in situations where synthetic anthelmintics might be inadvisable, such as at organic farms, treatment of animals during milk production period, for sheep flocks presenting anthelmintic resistant nematode populations, or when cost is a factor.

In a previous study, efficacy of LsEO against mouse nematodes was 70% (Camurça-Vasconcelos et al., 2007). In addition, low cost of essential oil has stimulated anthelmintic testing on sheep nematodes. However, validation model of plants with anthelmintic activity using mouse intestinal nematodes has some disadvantages, such as the fact that nematode habitats are quite different in mice versus sheep and goats. The relationship between nematode habitat and drug site absorption determine the greater or lesser drug efficacy (Hennessy, 1997). Different efficacy obtained on mice and sheep nematodes can be explained by mechanism of distribution and biotransformation of essential oil in a poligastric or monogastrical animal species. However, efficacy test on mouse nematodes can help researchers extrapolate the doses to be used on sheep and goats.

Efficacy of *L. sidoides* essential oil on *H. contortus* was 56.9%, on sheep whose nematode population was resistant to ivermectin. Therefore, the alternative use of plants may be a useful tool associated with other methods to control gastrointestinal nematodes of small ruminants.

Acknowledgements

This work received financial support from CNPq, FUNCAP and EMBRAPA/PRODETAB. We would like to thank the EMBRAPA/CNPC, and Felipe Cavalcante Machado and Helena Araújo da Ponte.

References

Ademola, I.O., Fagbemi, B.O., Idowu, S.O., 2004. Evaluation of the anthelmintic activity of *Khaya senegalensis* extract against gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. Vet. Parasitol. 122, 151–164.

Arosemena, N.A.E., Bevilaqua, C.M.L., Girão, M.D., Melo, A.C.F.L., 1999. Seasonal variations of gastrointestinal nematodes in sheep and goats from semi-arid area in Brazil. Rev. Méd. Vét. 150, 873–876.

Barraca, S.A., 1999. Manejo e produção de plantas medicinais e aromáticas. Net, ESALQ/USO. Piracicaba. Available at http://www.ciagri.usp.br/planmedi/alecrim-pimenta.html (accessed on March 18, 2006).

Camurça-Vasconcelos, A.L.F., Bevilaqua, C.M.L., Morais, S.M., Maciel, M.V., Costa, C.T.C., Macedo, I.T.F., Oliveira, L.M.B., Braga, R.R., Silva, R.A., Vieira, L.S., 2007. Anthelmintic activity of *Croton zehntneri* and *Lippia sidoides* essential oils. Vet. Parasitol. 148, 288–294.

Coles, G.C., Bauer, C., Borgsteede, F.H.M., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P.J., 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.): methods for detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44, 35–44.

Costa, C.T.C., Bevilaqua, C.M.L., Maciel, M.V., Camurça-Vasconcelos, A.L.F., Morais, S.M., Monteiro, M.V.B., Farias, V.M., Silva, M.V., Souza, M.M.C., 2006. Anthelmintic activity of *Azadirachta indica* A. Juss against sheep gastrointestinal nematodes. Vet. Parasitol. 137, 306–310.

Craveiro, A.A., Fernandes, A.G., Andrade, C.H.S., Matos, F.J.A., Alencar, J.W., 1977. Óleos essenciais de canelas silvestres regionais. Cienc. Cult. 29, 445.

Felippe, P.A.N., 2005. Sistema alométrico ou cálculo de dosagem por taxa metabólica do animal. Disponível em http://www.ib.unicamp.br/institucional/ceea/calculo.doc (acesso out/2005).

Gathuma, J.M., Mbaria, J.M., Wanyama, J., Kaburia, H.F.A., Mpoke, L., Mwangi, J.N., 2004. Efficacy of *Myrsine africana*, *Albizia anthelmintica* and *Hilderbrantia sepalosa* herbal remedies against mixed natural sheep helmintosis in Samburu district, Kenya. J. Ethnopharmacol. 91, 7–12.

Hennessy, D.R., 1997. Modifying the formulation or delivery mechanism to increase the activity of anthelmintic compounds. Vet. Parasitol. 72, 367–390.

Iqbal, Z., Lateef, M., Ashraf, M., Jabbar, A., 2004. Anthelmintic activity of *Artemisia brevifolia* in sheep. J. Ethnopharmacol. 93, 265–268.

Matos, F.J.A., 2002. Farmácias Vivas: sistema de utilização de plantas medicinais projetado para pequenas comunidades, 4a ed., Fortaleza, Ceará, Brasil. Editora UFC, 267 p.

Melo, A.C.F.L., Reis, I.F., Bevilaqua, C.M.L., Vieira, L.S., Echevarria, F.A.M., Melo, L.M., 2003. Nematódeos resistentes a anti-helmínticos em rebanhos de ovinos e caprinos do estado do Ceará, Brasil. Cienc. Rur. 33, 339–344.

Pinheiro, R.R., Gouveia, A.M.G., Alves, F.S.F., Haddad, J.P.A., 2000. Aspectos epidemiológicos da caprinocultura cearense. Arq. Bras. Med. Vet. Zootec. 52, 534–543.

Roberts, F.H.S., O'Sullivan, P.J., 1950. Methods for egg counts and larval cultures for strongyles infecting the gastrointestinal tract of cattle. Aust. J. Agric. Res. 1, 99–102.

Sissay, M.M., Asefa, A., Uggla, A., Waller, P.J., 2006. Anthelmintic resistance of nematode parasites of small ruminants in eastern Ethiopia: exploration of *refugia* to restore anthelmintic efficacy. Vet. Parasitol. 135, 337–346.

Taylor, M.A., Hunt, K.R., Goodyear, K.L., 2002. Anthelmintic resistance detection methods. Vet. Parasitol. 103, 183–194.

Ueno, H., Gonçalves, P.C., 1998. Manual para diagnóstico das helmintoses de ruminantes, 4ª ed. JIICA, Tokyo, Japan, 143 p.

Vieira, L.S., Cavalcante, A.C.R., 1999. Resistência anti-helmíntica em rebanhos caprinos no Estado do Ceará. Pesq. Vet. Bras. 19, 99–103.

Vieira, L.S., Cavalcante, A.C.R., Pereira, M.F., Dantas, L.B., Ximenes, L.J.F., 1999. Evaluation of anthelmintic efficacy of plants available in Ceará State, North-east Brazil, for the control of goat gastrointestinal nematodes. Rev. Méd. Vét. 150, 447–452.

Wood, I.B., Amaral, N.K., Bairden, K., Duncan, J.L., Kassai, T., Malone Jr., J.B., Pankavich, J.A., Reinecke, R.K., Slocombe, O., Taylor, S.M., Vercruysse, J., 1995. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). Vet. Parasitol. 58, 181–213.