Rev. prod. anim., 27 (2): 2015

TECHNICAL NOTE

Cyathostome Larvae Exsheathment Inhibition Assay

Lester A. Aguilera-Valle and Anay Delgado-Martínez

Faculty of Agricultural Sciences, University of Camagüey, Cuba

lester.aguilera@reduc.edu.cu

INTRODUCTION

Exsheathment of L_3 is a vital process in the life cycle because it is a transition from a free life form into parasitic states (Hertzberg *et al.,* 2002). Studies on exsheathment kinetics have emphasized on the fact that any disrupting factor, or toxic compounds, might hinder the parasite settlement on the host (Dakkak *et al.*, 1981).

In vitro methods provide ways for quick sieving of various extracts from plants with anthelminthic potential; and they also help analyze the possible mechanisms involved in active compound-parasite interactions (Katiki *et al.*, 2011).

The larval exsheathment inhibition assay (LEIA) has been commonly used to evaluate the anthelmintic effect of extracts from different tanniferous plant species (Katiki *et al.*, 2011; Macedo *et al.*, 2012; Alonso-Díaz *et al.*, 2008), especially against *Haemonchus contortus* and other ovine nematodes. This assay has never been tested in Cyathostomes, but due to growing occurrence of anthelminthic resistance in these parasite species (Kaplan, 2004), searching for new alternatives, (plants with anthelminthic properties) is an imperative. Accordingly, the aim of this paper is to support LEIA in Cyathostomes.

DEVELOPMENT

Raw aqueous extracts from *D. cinerea* (sickle bush) leaves and bark were used to perform the assay. The plant contains large amounts of tannins (Roig, 1974), and has been used as vermifuge in humans (Dalzield, 1948).

The active larvae were collected by coproculture, using a 25 µm sieve, washed twice with PBS 1 x, and centrifuged (4 000 rpm for 2 min), until a 140 larvae/ml concentration was achieved. Later, 600 µl of the solution were mixed with 400 µl of raw aqueous extracts from the leaves and bark in three 15 ml Falcon tubes, for the treatment groups; 400 µl PBS were used for the control, making a final volume of 1 ml. The incubation time was 3 h, at 27° C. Then the larvae were washed three times with PBS, centrifuged (4 000 rpm), and made sheath by contact with sodium hypochlorite (1 %) in a 1/150 dilution with PBS (Bahuaud *et al.*, 2006). The exsheathment behavior was monitored every 10 min (0; 10; 20; 30; 40; 50 and 60 min) using a microscope (40 x); for every reading, 100 µl were used; exsheathment was stopped with Lugo solution. The control the treatment groups had four replicas.

A paired T Student was made to determine the difference in larval percent, between the control and the treatment groups. Graph Pad Prism 5.0.0. was used for statistical analysis.

In the control group, 100 % exsheathment was observed at minute 60; whereas exsheathment was inhibited by both extracts, 75.95 % and 87.4 %, for the leaf and bark extracts, respectively (see Table).

These results coincide with reports made by Macedo *et al.* (2012; Katiki *et al.* (2011); Alonso-Díaz, Torres-Acosta, Sandoval-Castro and Hoste (2011); Alonso-Díaz *et al.* (2008), for *Haemonchus contortus* and other ovine nematodes, on which tannin-rich plants inhibited L_3 larvae exsheathment.

CONCLUSIONS

This is the first report made on the use of LEIA on the Cyathostomes species, supporting its use in the form of in vitro tests to evaluate the anthelminthic effects of tannin-rich plant extracts on Cyathostome species.

REFERENCES

ALONSO-DÍAZ, M. A.; TORRES-ACOSTA, J. F. J.; SANDOVAL-CASTRO, C. A. y HOSTE, H. (2011). Comparing the Sensitivity of Two *in Vitro* Assays to Evaluate the Anthelmintic Activity of Tropical Tannin Rich Plant Extracts Against *Haemonchus Contortus*, *Veterinary Parasitology, 181*, 360-364.

ISSN 2224-7920

ALONSO-DÍAZ, M. A.; TORRES-ACOSTA, J. F. J.; SANDOVAL-CASTRO, C. A.; AGUILAR-CABALLERO, A. J. y HOSTE, H. (2008). *In Vitro* Larval Migration and Kinetics of Exsheathment of *Haemonchus contortus* Larvae Exposed to Four Tropical Tanniferous Plant Extracts, *Vet. Parasitol*., *153*, 313-319.

BAHUAUD, D.; MARTÍNEZ-ORTIZ, C.; CHAVEAU, S.; PREVOT, F.; TORRES-ACOSTA, F.; FOURASTE, I. *et al.* (2006). Effects of Four Tanniferous Plant Extracts on the *In Vitro* Exsheathment of Third-Stage Larvae of Parasitic Nematodes, *Parasitology*, *132* (4), 545-554.

DAKKAK, A.; FIORAMONTI, J. y BUENO, L. (1981). *Haemonchus contortus* Third Stage Larvae in Sheep: Kinetics of Arrival into the Abomasum and Transformation during Rumino-Omasal Transit. *Res. Vet. Sci.*, *31*, 384-385.

HERTZBERG, H.; HUWYLER, U.; KOHLER, L.; REHBEIN, S. y WANNER, M. (2002). Kinetics of Exsheathment of Infective Ovine and Bovine Strongylid Larvae *In Vivo* and *In Vitro*, *Parasitology*, *125*, 65-70.

KAPLAN, R. M. (2004). Drug Resistance in Nematodes of Veterinary Importance: a Status Report, *Trends Parasitol.*, *20*, 477-481.

KATIKI, L. M.; CHAGAS, A. C. S.; BIZZO, H. R., FERREIRA, J. F. S. y AMARANTE, A.F.T. (2011). Anthelmintic Activity of *Cymbopogon martinii*, *Cymbopogon schoenanthus* and *Mentha piperita* Essential Oils Evaluated in Four Different In Vitro Tests, *Veterinary Parasitology*, *183*, 103-108.

MACEDO, I.; BEVILAQUA, C.; DE OLIVEIRA, L.; CAMURCA-VASCONCELOS, A.; MORAIS, S. M.; MACHADO, L. y *et al.* (2012). *In vitro* Activity of *Lantana camara*, *Alpinia zerumbet*, *Mentha villosa* and *Tagetes minuta* Decoctions on *Haemonchus contortus* Eggs and Larvae, *Veterinary Parasitology*, *190*, 504-509.

DALZIELD, J. M. (1948). *The Useful Plants of West Tropical Africa*. The Crown Agents for the Colonies. Westminster, Londres.

ROIG MESA, J. T. (1974). *Plantas medicinales, aromáticas y venenosas de Cuba*. La Habana, Cuba: Editorial Ciencia y Técnica.

.

Received: 23-6-2015
Accepted: 25-7-2015

Table. Effect of raw aqueous extracts of *D. cinerea* on L_3 Cyathostome exsheathment process

Extract	Concentration (mg/ml)	Exsheathment percent at minute 60 (means ± D.T.).
D. cinerea (leaves)	4	24.05 ± 1.42 a
D. cinerea (bark)	6.8	12.6 ± 0.48 a
Control (PBS)	-	100 ± 0

a: $P < 0.05$