Morphometric study of eggs of Fasciola hepatica treated with Closantel

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Abstract

The trematode *Fasciola hepatica* is spread worldwide causing important losses in livestock and is also an expanding zoonotic disease. Chemotherapy is fundamental for its control, yet triclabenzadol resistance is appearing, thus closantel can be a valid alternative. It is effective against mature and immature stages yet it does not exhibit ovicidal activity *in vitro* and the ovicidal effects *in vivo* at present are undetermined.

The Egg Hatch Assay is a valid test to evaluate ovicidal activity and was implemented to evaluate the effect of closantel; morphometrical study was included as part of the characterization of the treated eggs. Sheep were inoculated with *Cullompton* strain of *F. hepatica*; eggs were recovered from the gall bladder of control and closantel treated (subcutaneously and orally) at 12, 24, and 36 hours post treatment. Measures were taken on 50 eggs from each were found in length; size; width and shape. No statistically significant differences were found in length and size between control and experimental groups, for another hand significant differences were detected in width and shape. Eggs exposed to Closantel tend to be more elliptical. It is worth highlighting that al the length, width and size of the control eggs are considerably greater than previous descriptions for normal *F. hepatica* eggs in sheep and other domestic and wild species. This could be a characteristic of the *Cullompton* strain, yet studies should be done to assess if these large eggs are isolated strains or there is greater variability in egg morphology than previously supposed.

Objectives

To evaluate the effect of the anthelmintic closantel in the morphology of the egg of Fasciola hepatica exposed to such drug

Methods

Eight parasite-free Corriedale weaned lambs were orally inoculated with 200 metacercariae (*Cullompton* strain) of *F. hepatica.* Infection was confirmed 16 weeks later by the presence of eggs in faeces. The lambs were separated in 3 groups consisting of:

- **Group 1** (control) (n = 2) untreated animals,
- **Group 2** (n=3) treated subcutaneously (SC) with Closantel (10 mg/kg bw)
- **Group 3** (n=3) treated orally with Closantel (10 mg/kg bw) .

At 12, 24 and 36 h post-treatment, the lambs was stunned and exsanguinated immediately. Animal procedures and management protocols were approved by the Ethics Committee according to Animal Welfare Policy (act 087/02) of the Faculty of Veterinary Medicine, UNCPBA, Argentina. Eggs were collected by puncture of the gallbladder and were washed (3x) with tap water and conserved in dark at 4ºC until use.

Eggs (n> 50 per group) were measured using an ocular micrometer attached to 10X optical microscope with 10X objective. In all analyzed eggs the following measurements were carried

I) Width, the central area perpendicular to the axial axis,
II) Length, a centerline from one pole to the other,
III) Size, was determined by multiplying the length by the width of each egg and
IV) Shape was obtained by dividing the length by the width.

From the collected data, arithmetic mean and standard deviation were obtained. To compare results between groups, Bonferroni Multiple Comparisons Test was used and differences were considered significant if p< 0,05.

Results

The mean values were smaller for width and size where and greater for length and shape between control and treated groups, (Table 1). yet there were significant differences only in size and shape (Fig. 1). Eggs exposed to closantel tend to be more elliptical, which is reflected in the greater value of shape (Fig. 2).

Eggs (n > 50)		Media± SD (μ) LENGTH	WIDTH	SIZE	SHAPE
Control		149,78±6,98	94,66±10,67	14204,95±1917,58	1,60±0,18
12 h	Sc	152,68±6,80	88,74± 7,10	13561,04±1379,62	1,73±0,14
	Oral	151,88±5,46	91,36±8,61	13875,66±1420,21	1,68±0,16
24 h	Sc	154,55±4,60	88,38±3,58	13655,61±622,86	1,75±0,09
	Oral	148,32±5,35	85,06±8,10	13623,45±1340,39	1,76±0,24
36 h	Sc	152,99±7,34	89,36±5,63	13677,98±1174,60	1,72±0,13
	Oral	153,20±6,68	87,75±5,89	13449,85±1144,34	1,75±0,13

Table 1: Morphometric pattern analysis of *F. hepatica* eggs. Effect of Closantel (10mg/Kg bw) subcutaneous (SC) and Oral at 12, 24 and 36 h post treatment.

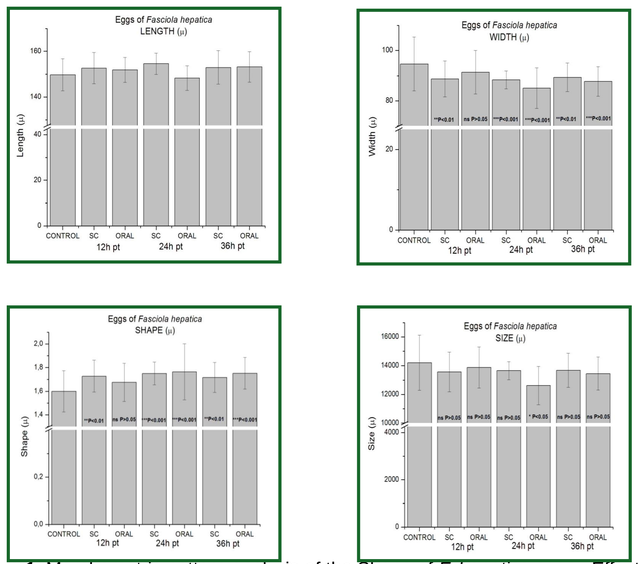

Figure 1: Morphometric pattern analysis of the Shape of *F. hepatica* eggs. Effect of Closantel (10mg/Kg bw) subcutaneous (SC) and Oral at 12, 24 and 36 h post treatment (pt)

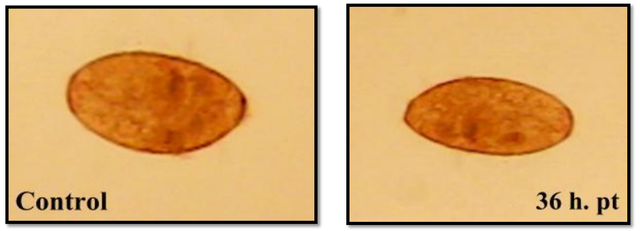

Figure 2: Eggs of *Fasciola hepatica.* Control and 36 h post treatment (Closantel (10mg/Kg bw). Eggs exposed to the effect of the drug tend to be more elliptical

Conclusions

The observed modifications in the morphology of the eggs post treatment can be accounted for due to the effect of closantel on the fluke´s reproductive system. Moreover is worth highlighting the observed dimensions of the eggs in the control group. The length, width and size are greater than previous descriptions for normal *Fasciola hepatica* eggs in sheep and other domestic and wild species including existing reports for Argentina, Bolivia, Georgia and Egypt, France, Spain and Portugal. These reports describe eggs of approximately 130μ in length and 72μ in width, quite smaller than what we have observed (Table I).

There are reports of unusually large strains of *F. hepatica* eggs in sheep and cattle, similar to our findings. Further morphological studies should be done to assess if these large eggs are isolated strains or there is greater variability in egg morphology than previously supposed.

References

1. Hanna, R.E.B., Scarcella, S., Solana, H., McConnell, S., Fairweather, I., 2012. Early onset of changes to the reproductive system of *Fasciola hepatica* following in vivo treatment with triclabendazole. Veterinary Parasitology 184, 341-347.

2. Valero MA, Perez-Crespo I, Periago MV, Khoubbane M, Mas-Coma S. 2009.- Fluke egg characteristics for the diagnosis of human and animal fascioliasis by *Fasciola hepatica* and *F. gigantica*. Acta Trop 2009; 111: 150-159urnal Article, *Name of Journal*

3. Maes, L., Lauwers, H., Deckers, W., Vanparijs, O., 1988. Flukicidal action of closantel against immature and mature *Fasciola hepatica* in experimentally infected rats and sheep. Research in veterinary science 44, 229-232.

4. Fantozzi C, Sidoti L, Neira G, Mercado C, Cuervo P & Mera y Sierra R, 2012. Phenotypic analysis of eggs of *Fasciola hepatica* recovered from Creole goats, in western Argentina. In: *XI European Multicolloquium of Parasitology*, July 2012 Cluj-Napoca, Rumania.

5. Jansen J. New cases of Fasciola hepatica with very large eggs. International Journal for Parasitology 1972 2:281-282