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Author(s): Bert E. Stromberg, John C. Schlotthauer and Gary A. Conboy

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## The Efficacy of Closantel Against *Fascioloides magna* in Sheep

Bert E. Stromberg, John C. Schlotthauer, and Gary A. Conboy, Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota 55108

Closantel(N-[5-chloro-4[(4-chlorophenyl)-cyanomethyl]-2-methylphenyl]-2-hydroxy-3,5-diiodobenzamide), a new, salicylanilide anthelmintic discovered at Janssen Pharmaceutica, Beerse, Belgium, by Janssen and Sipido (1977, U.S. Patent No. 4,005,218) has been found to have lethal activity against several species of nematodes, trematodes and arthropods (Van den Bossche et al., 1979, *Arch. Int. de Physiol. et de Biochim.* **87**: 851-852; Guerrero et al., 1983, *Vet. Parasit.* **12**: 71-77). Efficacy against adult and larval stages of *Fasciola hepatica* and *F. gigantica* has been previously demonstrated and the information summarized (Guerrero, 1983, *Prev. Vet. Med.* In Press); closantel seems to be especially effective against parasites which are either in close contact with circulating blood or that are hematophagus (Guerrero et al., 1982, *J. Parasit.* **68**: 616-619). The present study was undertaken to determine if Closantel was effective in killing larvae of *Fascioloides magna* in sheep, a major economic problem in Minnesota and several other states in the USA.

Twelve sheep (ewes) less than 12 mo old, mean weight of 38.7 kg, were purchased from a farm in an area known not to have liver flukes. It was believed that animals this age and size accurately reflect the age and size at which sheep on pasture would naturally be exposed to and become infected with *F. magna* for the first time. Sheep were maintained in indoor pens and held for 4 wk prior to infection. The sheep were fed a balanced ration of hay and concentrate throughout the experiment and received water ad lib. During this time fecal examinations were performed on all sheep on arrival and they were treated with thiabendazole, 50 mg/kg (Omnizole, Merck). Two weeks later fecal examinations were performed and some were still found to be positive for helminths. All animals were treated with levamisole at a dose of 8 mg/kg (Levasole, Pitman-Moore, Inc.). All animals were found to be negative for intestinal helminths at the start of the experiment.

Animals were weighed and separated into 2

equal groups of approximately the same size and weight. The mean weights of the 2 groups were 39.1 and 39.0 kg. Group 1 was randomly selected to be the infected-unmedicated group and Group 2 the infected-medicated group. On day 0 all sheep were inoculated orally with 100 viable metacercariae of *F. magna* suspended in saline. The metacercariae originated from *F. magna* ova collected from livers of Minnesota white tailed deer and were harvested following conversion in the snail *Lymnaea columella* (Baldwin Enterprises, Monmouth, Oregon). The viability of the metacercariae were checked visually for activity and by infecting guinea pigs. Eight weeks after inoculation the animals in Group 2 were treated orally with 20 mg/kg of closantel. All sheep were necropsied 16 wk after infection (8 wk post-treatment).

At the time of inoculation with *F. magna* all sheep in both groups were healthy and remained healthy after infection and treatment. All sheep gained weight throughout the study, however 1 animal in the untreated control group died acutely during the fourteenth week post-infection with no prior signs of illness. Necropsy revealed lesions in this animal that were indicative of infection with *F. magna* and that she died of acute pulmonary hemorrhage induced by fluke migration. Thirteen immature *F. magna* were recovered from the abdominal and thoracic cavities, liver and lung parenchyma.

No adverse reactions were observed in any of the sheep after treatment with closantel. Two of the ewes in the treated group lambed after treatment and produced normal healthy lambs.

Sixteen weeks after infection the remaining 11 sheep were stunned with a captive bolt and exsanguinated. Necropsy involved examining the lungs and peritoneal organs for flukes and evidence of fluke-induced lesions. The liver (or other affected organs) was cut in 1 cm slices and examined for flukes. The tissue was then incubated in warm (37 C) saline (0.15 M NaCl) for at least 3 hr and both tissue and saline were then examined for the presence of flukes. The number

TABLE I. Comparison of the liver pathology score, total number of flukes recovered and the total weight of the flukes recovered for each animal in the infected and control groups.

Infected/Untreated				Infected/Treated			
Animal number	Liver score	Flukes recovered	Fluke weight (mg)	Animal number	Liver score	Flukes recovered	Fluke weight (mg)
471	4	4	459	467	0	0	—
472	3	5	1,156	469	0	0	—
473	3	3	331	470	0	0	—
475	4	15	3,679	474	0	0	—
1065	4	13	3,897	1067	0	0	—
1066	4	13	2,219	1068	0	0	—
Means	3.7	8.8	1,956.8		0	0	—
± SD	0.5	5.4	1,570.2		—	—	—

of flukes recovered from each animal is listed in Table I. In an attempt to evaluate and compare the pathology of the sheep, a subjective evaluation and score (0 to 4+) was made for each animal. A score of 0 indicated little or no evidence of fluke infection, while a score of +1 was evidence of fluke infection but with little or no pathology. The scores of 2+, 3+ and 4+ described the degree of pathology, with 2+ where some lesions were found in various organs, generally located in a single organ (primarily the liver). A score of 3+ was a more significant degree of pathology in the liver, sometimes with evidence of flukes in other organs. The score of 4+ indicates extensive pathology, the liver showing multiple and extensive lesions and often with the involvement of other organs (i.e., lungs, kidneys, spleen). The individual pathology scores are presented in Table I as well as the total weight of the flukes.

Significant and extensive lesions caused by in-

fection with *F. magna* were seen in the infected-untreated control group. However, in spite of this pathology, the animals continued to gain weight and appear quite healthy. This may not have continued as other investigators have reported that sheep will survive about 6 mo before flukes will cause acute illness and death (Foryet and Todd, 1976, *J. Parasit.* **62**: 26–32). All sheep in the experiment including the treated animals showed some evidence of infection, indicating a successful challenge infection.

The efficacy of closantel when used orally at a dose rate of 20 mg/kg in controlling infection with *F. magna* in sheep was 100%. No flukes were found in the liver or any other organ of the treated animals. The degree of pathology in the closantel treated sheep was minimal. There is a need for a highly efficacious flukicide like closantel, since a single fluke has the potential to kill a sheep. This work was supported in part by a grant from Pitman Moore, Inc.

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## Phospholipids of Cercariae and Adult *Schistosoma mansoni*

Bruce W. Young and Ronald B. Podesta, Membrane Biology Laboratory, Department of Zoology, University of Western Ontario, London, Ontario N6A 5B7, Canada

Previous reports on the phospholipid content of *Schistosoma mansoni* have been qualitative with the exception of the quantitation of 4 phospholipid classes by Young and Podesta

(1982, *Mol. Biochem. Parasit.* **5**: 165–172) and the incorporation of isotopically labelled phospholipid precursors by Meyer et al. (1970, *Biochim. biophys. Acta* **210**: 257–266). Cercariae of