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In vitro Effects of Sodium Chloride and Calcium Carbonate on the Development and Survival of *Haemonchus contortus*

M. AHSAN MUNIR, ZAFAR IQBAL AND MUHAMMAD NISAR KHAN
Department of Veterinary Parasitology, University of Agriculture, Faisalabad–38040, Pakistan

ABSTRACT

Studies were conducted to investigate the effects of Sodium Chloride (NaCl) and Calcium Carbonate ($CaCO_3$) on the development and survival of *Haemonchus (H) contortus*. The egg hatching started from day 2 post treatment (PT) at all concentrations of NaCl. However, there was a gradual decrease in hatching with increasing concentrations of NaCl and the total number of eggs hatched was lower in NaCl treated groups compared with control. Rare eggs hatched to L_1 in 10.0% $CaCO_3$ concentrations. This indicated destructive effects of $CaCO_3$ on eggs and larvae of *H. contortus*. The eggs of *H. contortus* hatched to L_1, L_2 and L_3 in 2.5% $CaCO_3$ solution. However, per cent hatching and subsequent moulting to L_2 and L_3 was lower as compared to control. It was concluded that NaCl and $CaCO_3$ can be used on farm premises and pastures in safer doses to prevent development of eggs of *H. contortus* and their transmission to the animals.

Key Words: *Haemonchus conortus*; Sheep; Anthelmintic; Farms; Pastures; NaCl; $CaCo_3$

INTRODUCTION

Haemonchus species are abomasal nematode parasites infecting almost all ruminants. The parasite is of high economic significance due to its blood sucking habit and worldwide distribution (Whitlock, 1966; Preston & Allonby, 1979). The estimated production losses due to haemonchosis has been estimated at Rs. 31.43 million per annum in sheep and goats slaughtered at Faisalabad abattoir (Iqbal *et al.,* 1993). Although substantial efforts have been made to treat the *Haemonchus* infected animals using anthelmintics, yet development of resistance in parasites against anthelmintics and chemical residual/toxicity problems limit their scope (Kaemmerer & Butenkotter, 1973; Waller, 1987; Van Wky & Malan, 1988). Therefore, alternate strategies for the control of this deadly disease are in focus research areas. This paper reports the results of a study aimed at investigating the physiological/osmoregulatory effects of sodium chloride (NaCl) and calcium carbonate ($CaCO_3$) on the developmental stages of *Haemonchus (H) contortus.* The main objective of the current study was to provide the farmers with chemicals/agents which possess inhibitory/lethal properties against the developmental stages of worms on soil/pastures to prevent their transmission.

MATERIALS AND METHODS

Collection of adult *H. contortus.* Abomasa of sheep, slaughtered at Faisalabad abattoir were collected, incised longitudinally and examined for the presence of adult *H. contortus* (Maqsood *et al.,* 1996). The worms present in ingesta or attached to the abomasal epithelium were picked manually using artery forceps and placed in a bottle containing PBS (pH 7.2). Female worms were separated from males by grossly witnessing the blood filled intestine spirally coiled around white ovary giving an appearance of barber's pole worm. The female worms were washed thrice in PBS (pH 7.2).

Extraction of *H. contortus* eggs. The worms were transferred to 0.9% normal saline solution, incubated at 37°C for 24 hours and ova laid by them were collected by sedimenting them using slow centrifugation. Inoculi containing 25,000 eggs in 10 mL of distilled deionised water were prepared for further use.

Preparation of medium for culture. The culture medium was prepared by mixing sheep faeces, soil and supernatant derived from ovine gastric contents in 25 g: 25 g: 8 mL ratio as follows: The faeces of sheep were broken up finely, using a large pestle and mortar, mixed with equal quantity of soil (low porosity sandy loam) and sterilised at 15 lb pressure 121°C using an autoclave. The ovine gastric contents were obtained from slaughtered sheep. The contents were filtered through gauze, centrifuged and the clear supernatant sterilized by filtration (0.2 µm, Millipore). The supernatant fluid from sheep was stored at –20°C before use.

Experimental design. The culture medium was added to 32 culture dishes, each inoculated with 25,000 eggs of *H. contortus* in 10 mL water and assigned to four concentrations (each having three replicates) of NaCl and $CaCO_3$ as follows:

A1= Distilled water (control); A2= 2.5% NaCl solution spray; A3= 2.5% NaCl solution spray; A4= 2.5% NaCl solution spray; B1= Distilled water (control); B2= 5% NaCl solution spray; B3= 5% NaCl solution spray; B4= 5% NaCl solution spray; C1= Distilled water (control); C2= 7.5% NaCl solution spray; C3= 7.5% NaCl solution spray; C4= 7.5% NaCl solution spray; D1= Distilled water (control); D2= 10% NaCl solution spray; D3= 10% NaCl solution spray; D4= 10% NaCl solution spray; E1= Distilled water (control); E2= 2.5% $CaCO_3$ solution spray; E3= 2.5% $CaCO_3$ solution spray; E4= 2.5% $CaCO_3$ solution spray; F1= Distilled water (control); F2= 5% $CaCO_3$ solution

spray; F3= 5% $CaCO_3$ solution spray; F4= 5% $CaCO_3$ solution spray; G1= Distilled water (control); G2= 7.5% $CaCO_3$ solution spray; G3= 7.5% $CaCO_3$ solution spray; G4= 7.5% $CaCO_3$ solution spray; H1= Distilled water (control); H2= 10% $CaCO_3$ solution spray; H3= 10% $CaCO_3$ solution spray; H4= 10% $CaCO_3$ solution spray

The cultures were stirred gently daily, covered with lids and incubated at 27°C (relative humidity 75%) for eight days. Three aliquots each of one gram medium from different places of each culture dish were taken randomly at alternate days up to eight days of culture. The aliquots were dissolved in d
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on eggs and larvae of *H. contortus*. The eggs of *H. contortus* hatched to L_1, L_2 and L_3 in 2.5% $CaCO_3$ solution. However, per cent hatching and subsequent moulting to L_2 and L_3 was lower (4.8%) as compared to control (47.2%). In 5.0% $CaCO_3$ concentration, however, only 4.4% L_2 were found and only rare L_3 were recorded. Likwise, in 7.5 and 10% $CaCO_3$ rare eggs hatched and developed to L_3 by day 8 PT in contrast to the hatching and development in control groups, which ranged from 45.0 to 48.6% in control groups (Table III & IV).

Studies on *in vitro* cultivation of *H. contortus* have

Table III. Effect of 2.5 and 5% $CaCO_3$ solution on hatching, moulting and survival of eggs and larvae of *Haemonchus contortus*

Days PT	2.5% $CaCO_3$ (Control)				5% $CaCO_3$ (Control)			
	Eggs/ml	L_1	L_2	L_3	Eggs/ml	L_1	L_2	L_3
0	500 (500)	– (–)	– (–)	– (–)	500 (500)	– (–)	– (–)	– (–)
2	298 (59)	7.8 (72.4)	– (6.4)	– (–)	88 (78)	R (72.8)	(7.8)	– (–)
4	52 (R)	4.6 (38.4)	5.2 (43.2)	R (20.4)	22 (22)	4.6 (45.2)	R (42.8)	R (22.4)
6	R (–)	R (12.4)	5.6 (7.8)	4.8 (38.8)	R (–)	R (12.6)	4.4 (9.8)	R (37.0)
8	– (–)	R (R)	4.2 (R)	R (47.2)	– (–)	R (R)	R (R)	R (46.8)

PT= Post-treatment; R= < 20

Table IV. Effect of 7.5 and 10% $CaCO_3$ solution on hatching, moulting and survival of eggs and larvae of *Haemonchus contortus*

Days PT	7.5% $CaCO_3$ (Control)				10% $CaCO_3$ (Control)			
	Eggs/ml	L_1	L_2	L_3	Eggs/ml	L_1	L_2	L_3
0	500 (500)	– (–)	– (–)	– (–)	500 (500)	– (–)	– (–)	– (–)
2	93 (72)	R (65.8)	– (8.4)	– (–)	21 (84)	– (72.6)	– (5.6)	– (–)
4	33 (R)	4.8 (50.2)	R (42.6)	R (24.4)	R (R)	– (44.0)	– (42.8)	– (23.6)
6	R (–)	R (11.8)	R (13.2)	R (37.4)	R (–)	R (19.4)	– (18.0)	– (35.8)
8	– (–)	R (R)	R (R)	– (48.6)	– (–)	R (R)	R (R)	R (45.0)

PT= Post-treatment; R= < 20

Days PT	2.5% NaCl (Control)				5% NaCl (Control)			
	Eggs/ml	L_1	L_2	L_3	Eggs/ml	L_1	L_2	L_3
0	500(500)	–(–)	–(–)	–(–)	500(500)	–(–)	–(–)	–(–)
2	340(59)	11.2(70.0)	–(5.6)	–(–)	126(65)	9.2(68.8)	–(6.4)	–(–)
4	128(R)	16.0(39.6)	6.8(45.2)	R (19.2)	29(R)	11.6(41.2)	9.8(40.8)	R(20.4)
6	R(–)	8.4(10.4)	10.8(6.4)	7.6 (36.8)	R(–)	R(9.8)	8.4(8.4)	6.4(38.4)
8	–	R	6.4 (R)	8.4 (43.8)	–(–)	R(R)	4.4(R)	R(43.0)

PT= Post-treatment; R= < 20

Table II. Effect of 7.5 and 10% NaCl solution on hatching, moulting and survival of eggs and larvae of *Haemonchus contortus*

Days PT	7.5% NaCl (Control)				10% NaCl (Control)			
	Eggs/ml	L_1	L_2	L_3	Eggs/ml	L_1	L_2	L_3
0	500(500)	–(–)	–(–)	–(–)	500 (500)	–(–)	–(–)	–(–)
2	72(74)	13.2(64.4)	–(8.4)	–(–)	32 (74)	5.2 (69.6)	–(5.2)	–(–)
4	32(R)	9.8(47.8)	10.4 (44.6)	R (21.8)	R (R)	R (50.4)	4.4 (40.8)	R (25.8)
6	R(–)	R(11.2)	4.4 (11.2)	4.2 (36.4)	R(–)	R (12.4)	R (18.4)	4.2 (34.4)
8	–(–)	R(R)	R (R)	R (46.6)	–(–)	R (R)	R (R)	R (43.0)

PT= Post-treatment; R= < 20

concentrations. This indicated destructive effects of $CaCO_3$ *Toxocara vitulorum*. He reported maximum (96%)

embryonation of eggs in silt clay loam and organic soils followed by sandy loam soil (59.25%), clay loam soil (50%), formalin (46.42%) and farm yard manure (22.38%), respectively. He concluded that the composition of soil has a definite impact on the embryonation of the eggs of *Toxocara vitulorum.*

The influence of soil moisture and porosity on the transformation of *H. contortus* eggs was also investigated by Singhal *et al.* (1983). For this purpose, they infected six different types of soils from India with ova of *H. contortus* from sheep to give a concentration of 500 epg. Soils of low porosity (sand and sandy loam I) favoured maximum development of eggs to larvae at 30% moisture, compared to 34 and 38% moisture in highly porous sandy loam and sandy clay loam, and 36 and 40% moisture in medium porous sandy loam II and sandy clay.

A gradual decrease in hatching of *H. contortus* eggs and moulting of larvae with increasing concentrations of NaCl and $CaCO_3$ suggested adverse effects of increasing salinity on hatching as well as moulting of larvae. The delayed or blocked hatching of *H. contortus* eggs may be attributed to the affect of salts on pH of the soil media not favorable for hatching or development of larvae.

There are some enzymes also which are required for the hatching process of parasite eggs. It is speculated that delayed or blocked hatching of the eggs of *H. contortus* could also be due to disturbance in the secretion of these enzymes or loss of their function due to salt stress. Many cytoplasmic enzymes are altered during salt stress (Greenway & Osmond, 1972; Bruggeman & Janiesch, 1989; Khan *et al*., 1989; Maugal *et al*., 1989; Garcia-Carieno & Ochoa, 1991). It is understood that eggs undergo some active metabolic processes for hatching and further development. Nitrogen metabolism has also been reported to be affected by salt stress.

CONCLUSIONS

NaCl and $CaCO_3$ solutions delayed hatching and development of eggs of *H. contortus* at lower concentrations and proved lethal at higher (>5-10 %) concentrations. These chemicals may be used on farm premises or on pastures in 5-10% concentrations for the control of Haemonchosis. However, further studies are suggested for standardization of doses and mode of application of these agents.

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