THE EFFECTS OF SOME DETERGENTS ON THE DEVELOPMENTAL STAGES OF HORSE STRONGYLES (NEMATODA)

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During the past several years a search for possible anthelmintics has been carried out in our laboratory, using the developmental stages of horse strongyles. The technic has been described by Levine (1950). By its use, several compounds were discovered which, when mixed with horse feces, killed the parasites developing in the feces, or at least prevented the development of larvae. In order to learn which stages these compounds affected, a study was begun on eggs and larvae that had been separated from feces and washed clean. Because some of the compounds were not water-soluble, detergents were added to disperse them. It was then observed that the detergents themselves were toxic. The present paper reports the effects of two detergents, Triton NE and Tergitol 7, on the developmental stages of horse strongyles in water.

Materials and Methods

The strongyle eggs and larvae used in this study were obtained from feces from horses on the University of Illinois farm. The strongyles of horses belong to the nematode family Strongylidae. Three species of the genus *Strongylus* are known as large strongyles. McIntosh (1951) lists 34 species of 7 genera of small strongyles. Most of these are quite similar both morphologically and biologically, and the adults can be differentiated only with difficulty. The eggs and young larvae of most species cannot be differentiated.

Four stages of the strongyle life cycle occur in manure. The egg contains only a few cells when passed, but embryonates rapidly. A first-stage larva hatches after about a day and feeds on bacteria and other material in the feces. It molts in another day to the second-stage larva, which also feeds on bacteria. After two to four days it molts to the third-stage, infective larva. This larva does not feed; it retains the cast skin of the second-stage larva as a sheath which protects it against desiccation and chemical agents.

The eggs used in this study were obtained from fresh feces by flotation with saturated sodium chloride solution. They were placed in a Bureau of Plant Industry (BPI) watch glass 1 inch in diameter, and washed five times with tap water. They were then picked out with a micropipette, placed in another BPI watch glass, and 0.6 ml. of the test solution was added. The watch glass was covered with a round coverslip and sealed with white petrolatum. Since the capacity of these watch glasses

TABLE 1.—EFFECT OF TRITON NE ON WASHED HORSE STRONGYLE EGGS.

	Percent Triton	No. tests	No. eggs per test	Results		
					1 day	3 days
Percent eggs embryonated.....	0.5	8	14.5	Mean	76	78[b]
				s	12.8	12.8
	Control	8	13.5	Mean	88	90[b]
				s	9.4	5.8
Percent eggs hatched.....	0.5	8	14.5	Mean	23[a]	72
				s	13.4	18.9
	Control	8	13.5	Mean	65[a]	87
				s	26.7	9.5

[a] Difference significant at 1% level.
[b] Difference significant at 5% level.

is about 1 ml., oxygen for development was obtained from the 0.4 ml. of air remaining under the coverslip.

The larvae were obtained from feces which was incubated at room temperature. First-stage larvae were obtained after incubation for 1 day, second stage larvae after incubation for 2 days, and third stage larvae after incubation for 6 days. The technic of incubation was that described by Levine (1950). A cheesecloth bag containing 4 gm. feces was suspended in a 2 oz. wide-mouth glass bottle, which was then placed in a dark cupboard for the proper number of days. The larvae were collected by a modification of the Baermann technic.

After incubation, the bag containing feces was placed in a small funnel to which a piece of rubber tubing closed by a pinchclamp had been attached. Warm water was added to cover half the bag, and the preparation was allowed to stand for two hours. The water was then drained into a centrifuge tube, centrifuged for 5 minutes at 1900 r.p.m., and 0.5 ml. was removed from the bottom of the centrifuge tube with a pipette and placed in a Syracuse watch glass. This water contained most of the larvae. They were washed by adding 5 ml. of tap water and mixing. The larvae were then picked out with a micropipette, transferred to 0.6 ml. of water in a BPI watch glass and mixed again. In setting up the tests, the larvae were again picked out with a micropipette, transferred to another BPI watch glass, 0.6 ml. of the test solution was added, and the preparations were sealed with a coverslip as described above.

The eggs and larvae were observed daily. Eggs were considered dead if, after failing to embryonate, they began to disintegrate. Several criteria were used to determine whether the larvae were alive. Dead larvae lay motionless and straight or only slightly curved, they were darker than live ones, and their internal structures became progressively less distinct and finally disintegrated.

The effects of Triton NE and

TABLE 2.—EFFECT OF TRITON NE ON WASHED HORSE STRONGYLE LARVAE.

	Percent Triton	No. tests	No. larvae per test	Percent larvae alive			
					1 day	2 days	3 days
1st stage larvae.....	0.5	6[b]	13.5	Mean	77	41[a]	32[a]
				s	31.1	29.6	21.3
	Control	6[b]	12.2	Mean	99	93[a]	95[a]
				s	2.6	5.7	3.4
2nd stage larvae.....	0.5	4	13.5	Mean	83	87	57
				s	13.7	10.8	27.9
	Control	4	13.8	Mean	96	96	85
				s	3.5	3.5	11.7
3rd stage larvae.....	0.5	6	16.0	Mean	100	98	95
				s	0	3.3	3.0
	Control	6	15.7	Mean	99	98	94
				s	3.0	3.5	6.2

a Difference significant at 1% level.
b Only 4 tests were carried to 3 days.

Tergitol 7 were determined against eggs and larvae in the first, second, and third stages. Triton NE is manufactured by Rohm and Haas Co. It is a 33% aqueous solution of the monoisoctyl phenyl ether of polyethylene glycol, which has an average molecular weight of 633. Tergitol 7 is manufactured by Carbide and Carbon Chemicals Co. It contains 25% of the sodium sulfate derivative of 3,9-diethyltridecanol-6, 15% of the mono butyl ether of diethylene glycol as a coupling agent, and 5% of a mixture of sodium sulfate and sodium chloride, all dissolved in water. The first compound is the active detergent.

RESULTS

The effects of 0.5% Triton NE on horse strongyle eggs are given in table 1. This concentration of Triton NE is equivalent to 263×10^{-5} M. active ingredient. There was no significant difference in embryonation between the treated and control eggs after one day, but after three days 78% of the treated eggs had embryonated, as against 90% of the controls. Since the unembryonated eggs later died, this difference represents a 13% kill. It was significant at the 5% level.

Egg hatching was slowed down but not prevented. After one day only 23% of the treated eggs had hatched, as against 65% of the controls. However, after three days there was no significant difference in hatching percentages.

The effects of 0.5% Triton NE on the larvae are given in table 2. The compound killed first-stage larvae slowly. It had no significant effect after one day, but after two days 41% of the larvae were alive as against 93% of the controls, and after three days 32% of the treated larvae and 95% of the controls were

TABLE 3.—EFFECT OF TERGITOL 7 ON WASHED HORSE STRONGYLE EGGS.

	Percent Tergitol	No. tests	No. eggs per test	Results		
					1 day	3 days
Percent eggs embryonated.....	0.1	3	22.3	Mean	0[a]	1[a]
				s	0	2.4
	Control	2	19.5	Mean	85[a]	87[a]
				s	3.0	6.0
	0.01	3	20.0	Mean	83[a]	83[a]
				s	1.0	1.0
	Control	2	19.5	Mean	97[a]	100[a]
				s	1.0	0
Percent eggs hatched.....	0.1	3	22.3	Mean	0	0[a]
				s	0	0
	Control	2	19.5	Mean	3	85[a]
				s	2.6	8.5
	0.01	3	20.0	Mean	0	42[a]
				s	0	1.7
	Control	2	19.5	Mean	0	97[a]
				s	0	3.0

a Difference significant at 1% level.

alive. Thus the compound produced a 56% kill after two days and a 66% kill after three days. It had no significant effect on second- and third-stage larvae.

The effects of Tergitol 7 on the eggs are given in table 3. A concentration of 0.1% Tergitol 7 is equivalent to 70×10^{-5} M. of the sodium sulfate derivative of 3,9-diethyltridecanol-6, while a concentration of 0.01% is equivalent to 7×10^{-5} M. Egg embryonation was almost completely prevented by 0.1% Tergitol 7. No eggs had embryonated after one day, and only 1% had embryonated after three days. The unembryonated eggs died. A concentration of 0.01% was made less effective, allowing 83% to embryonate.

Egg hatching was also affected. No eggs treated with 0.1% Tergitol 7 hatched. Only 42% of the eggs treated with 0.01% Tergitol 7 hatched; of the 83% of eggs which had embryonated after three days, only 51% hatched. The larvae in the remainder died before they could get out of the eggshell.

The effects of Tergitol 7 on the larvae are given in table 4. The first-stage larvae, the most sensitive, were all killed in one day by 0.01% of the detergent. All second stage larvae were killed in one day by 0.1% Tergitol 7. There was no significant difference (at the 5% level) between the second stage larvae treated with 0.01% Tergitol 7 and the controls after one day, but after two days only 9% and after three days only 1% of the treated larvae remained alive. Taking into account the mortality in the controls, these

TABLE 4.—EFFECT OF TERGITOL 7 ON WASHED HORSE STRONGYLE LARVAE.

	Percent Tergitol	No. tests	No. larvae per test		Percent larvae alive		
					1 day	2 days	3 days
1st stage larvae.....	0.1	3	20.0	Mean	0[a]		
				s	0		
	Control	2	15.0	Mean	93[a]		
				s	7.0		
	0.01	3	22.0	Mean	0[a]		
				s	0		
	Control	2	21.5	Mean	98[a]		
				s	2.5		
2nd stage larvae.....	0.1	3	20.0	Mean	0[a]		
				s	0		
	Control	2	20.0	Mean	100[a]		
				s	0		
	0.01	3	26.7	Mean	81	9[a]	1[a]
				s	2.2	4.2	1.9
	Control	2	26.5	Mean	96	96[a]	94[a]
				s	4.5	4.5	3.0
3rd stage larvae.....	0.1	3	23.3	Mean	6[a]	0[a]	
				s	3.6	0	
	Control	2	26.5	Mean	100[a]	100[a]	
				s	0	0	
	0.01	3	23.3	Mean	100	100	100
				s	0	0	0
	Control	2	19.0	Mean	97	97	97
				s	2.5	2.5	2.5

a Difference significant at the 1% level.

figures represent 89% and 99% kills, respectively. The third-stage larvae were much more resistant than those in the first two stages. They were not affected by 0.01% Tergitol 7, but 0.1% of the compound killed 94% in one day and 100% in two days.

DISCUSSION

Tergitol 7 was much more toxic for horse strongyle developmental stages than Triton NE. The two detergents are of different types, Triton being non-ionic, and the active detergent ingredient of Tergitol being anionic. However, further tests will be necessary to determine whether the toxic effect of the Tergitol is due to the sodium sulfate derivative of 3,9-diethyltridecanol-6, to the mono butyl ether of diethylene glycol, which is non-ionic, or to both.

Jaskoski (1950, 1951) has studied the effects of anionic detergents on the eggs of the common pig roundworm, *Ascaris lumbricoides*. He found that 5.0% Duponol 80, Aresklene 400, Areskap 100 and Aresket 375 inhibited cleavage.

The present study was carried out

on washed eggs and larvae. Further work would be desirable to determine whether Tergitol 7 retains its activity in feces and in soil.

Summary

In a study of the effects of detergents on washed eggs and larvae of the small strongyles of horses, 0.5% Triton NE was found to kill 13% of the eggs and to slow down the hatching of the remainder. It killed 56% of first stage larvae after two days exposure, and 66% after three days, but had no significant effect on second- and third-stage larvae.

Egg embryonation was almost completely prevented by 0.1% Tergitol 7, and significantly decreased by 0.01% of the detergent. No eggs hatched in 0.1%, and 51% of the embryonated eggs hatched in 0.01% of the detergent. All first-stage larvae were killed in one day by 0.01% Tergitol 7. All second-stage larvae were killed in one day by 0.1% of this detergent; 0.01% did not affect them significantly in one day, but killed 89% and 99% after two and three days, respectively. Ninety-four percent of third stage larvae were killed by 0.1% Tergitol 7 in one day, and 100% in two days; 0.01% did not harm them.

References

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