Tropical Vet. 19 (2) 63- 67 (2001)

Effects of Antibiotics, Salt and pH on the Hatchability of *Fasciola Gigantica* Eggs: Short Communication

ADEDOKUN, O. A.; ADEJINMI*, J. O. SADIQ, N. A. and ENIBE,O. F.
Department of Veterinary Microbiology and Parasitology,
University of Ibadan, Ibadan, Nigeria

Keywords: Hatchability, Antibiotic, Salt, pH, *Fasciola gigantica* Egg.

Running Title: Hatchability of *Fasciola gigantica* eggs.

• For Correspondence

Abstract

A study was carried out to examine the effects of various antibiotics, salt concentrations and pH on the hatchability of *F. gigantica* eggs. It was observed that tetracycline concentration of 0.5mg/ml and above inhibited the development and hatching of *F. gigantica* eggs. Salt concentration of 1% gave low hatchability while 2% concentration and above strictly inhibited the hatching of the eggs. Acidic pH of 3-5 inhibited hatching of *F. gigantica* eggs while pH of 7-8 promoted hatching of eggs to above 3.4%.

The possibility of employing the results of this study to fashion out a control measure against fasciolosis in farm animals is discussed.

Introduction

Fasciola is a trematode of domestic ruminants and occasionally man and is the cause of liver fluke disease commonly referred to as hepatic fasciolosis (Blood *et al.*, 1995). It is a parasite of liver and the bile ducts of domestic ruminants. Two species are recognised. *Fasciola hepatica* which is widespread in the temperate areas of the world and *Fasciola gigantica* which is encountered throughout tropical and subtropical areas of Africa and Asia (Soulsby 1982; and Dunn *et al.*, 1991) and is commonly seen in Nigeria (Shillhorn van Veen, 1980).

Economic importance of Fasciola infection is more in sheep resulting in death in acute cases which is rare in cattle (Dunn *et al.*, 1991) Other economic losses in fasciola infection are liver condemnation at meat inspection, chronic loss of weight and predisposition to overwhelming diseases (Hammond and Sewell, 1975). The intermediate hosts are snails of the genus Lymnea (Troncy *et al.*, 1989). For *F. gigantica* the intermediate host is *Lymnea natalensis* in Nigeria (Shillhorn van Veen, 1980) and for *F. hepatica* in temperate areas it is *Lymnea truncatula* (Dunn et al., 1991).

Egg of fasciola has yellow shell with an indistinct operculum and indistinct embryonic cells (Soulsby, 1982; Troncy, 1989). Eggs passed out in faeces of the primary host hatch releasing motile ciliated miracidia in 10-14 days at optimal temperature of 22-28°C with little development occurring below 10°C (Dunn *et al.*, 1991). Sewell (1966) reported that *F. gigantica* eggs do not survive well at low temperature though light appears to be the most important stimulus that activates miracidium and subsequent hatching. Wright (1977) also observed that saline concentrations of up to 0.6% do not have adverse effects on the hatching mechanism of the eggs of *F. hepatica* but higher concentrations of up to 1.0% do have effect.

Laboratory works involving the incubation and hatching of fasciola eggs are often carried

out with incorporation of antibiotics to prevent the growth of bacteria that may adversely affect the hatching of the eggs. Oziegbe (1997) in his preliminary study of effects of antibiotics on hatchability of *F. gigantica* eggs observed that eggs incubated with penicillin-streptomycin combination hatched releasing miracidia while those incubated with tetracycline did not hatch on exposure to light after 21 days. This study was carried out to examine the effects of antibiotics, salt concentrations and pH on the hatchability of *F. gigantica* eggs. The result of this study may be applied in the field for the control of fasciolosis in ruminants.

Materials and Methods

Fasciola egg collection, treatment and standardization

Gall bladders were collected from cattle with natural fasciolosis, slaughtered at the Bodija Municipal abattoir in Ibadan. The contents of the gall bladder were emptied into a beaker with 100ml of distilled water and allowed to sediment for about one hour. After sedimentation the supernatant was decanted and more distilled water added. The eggs were washed thrice by centrifugation at low speed. For proper identification (Soulsby, 1982) a few drops of the sedimented eggs were placed on a glass slide, covered with slip and examined under the light microscope using X10 objective lens. The eggs were resuspended in 3ml of distilled water and the concentration of eggs were determined using the modified Neaubeur's counting chamber.

Antibiotic Salt and pH Preparation

Serial dilutions of the following antibiotics: ampiclox, tetracycline, procaine penicillin, streptomycin and 1:1 ratio of penicillin-streptomycin were prepared for each of the antibiotics to obtain concentrations of 0.05, 0.1, 0.5 and 1.0mg/ml. Sodium chloride solutions were prepared to obtain the following: 1, 3, 5, 7 and 10% concentrations in distilled water. HCL at pH 3 and 5 and NaOH at pH 7, 7.5, 8 and 10 were prepared from stock of concentrated HCL and NaOH as described by Harper (1982) at room temperature.

Incubation and hatching of Eggs

Drops of suspension containing 250 eggs were placed in test tubes containing 1ml of each dilution of the antibiotics, salt or pH solutions. The same number of eggs placed in 1ml. of distilled water served as control. The mouth of the tubes were stuffed with cotton wool to allow for aeration. The tubes were arranged in a test tube rack and kept in a dark cupboard at room temperature. The tubes were examined regularly from day 7 for hatching. The emergence of miracidia was observed under the disecting microscope and their number counted.

Results

Microscopic examination of the egg showed the characteristic pollar operculum of the yellowish coloured oval egg as described by Soulsby (1982).

The results obtained before and after incubating the eggs of *F. gigantica* in a dark cupboard at room temperature using antibiotics, salt or pH solutions are presented in Table 1 and 2. At 0.05 and 0.1mg/ml antibiotic concentrations there was high percentage hatchability with ampiclox and penicillin-streptomycin. The percentage hatchability in each of the antibiotic solutions decreased with increase in the concentration of antibiotics from 0.5 - 1.0mg/ml particularly with ampiclox. No hatching was observed with tetracycline even at 0.05mg/ml concentrations. In the salt solutions it was only in the 1.0% concentration that 0.4% hatching was observed. Above this concentration no hatching of eggs was noted. In pH solution *F. gigantica* eggs hatched only in pH range of 7-8.

Table 1: Percentage hatachability of *F. gigantica* eggs incubated with different antibiotics

	Samples	pH Evaluation	No. of eggs before hatched/ml	No. of miracidia hatched/ml	% hatched/ml
a.	**Antibiotics**				
i.	(0.05mg/ml)				
	Pen. strep.	6.8	250	39	15.6
	Ampiclox	6.7	250	43	17.2
	Streptomycin	6.5	250	9	3.6
	Pro. Penicilin	6.6	250	40	16
	Tetracycline	3.8	250	7	2.8
ii.	0.1mg/ml				
	Pen. strep.	6.6	250	30	12.0
	Ampiclox	6.6.	250	28	11.2
	Streptomycin	6.4	250	18	7.2
	Pro-Penicilin	6.5	250	40	16
	Tetracycline	3.5	250	5	2.0
iii.	(0.5mg/ml)				
	Pen. strp.	6.5	250	32	12.8
	Ampiclox	6.0	250	0	0
	Streptomycin	6.0	250	15	6
	Pro-Penicilin	5.8	250	52	20.8
	Tetracycline	3.1	250	0	0
iv.	(1mg/ml)				
	Pen. strep.	6.2	250	10	4
	Ampiclox	5.8	250	0	0
	Streptomycin	5.9	250	0	0
	Pro-Penicilin	5.8	250	25	10.0
	Tetracycline	3.0	250	0	0

* Pen = penicillin strep = streptomycin

Table 2: Percentage Hatchability of *F. gigantica* eggs incubated with salts of different concentrations and at different pH of suspending medium.

Samples	No. of eggs before % hatched/ml	No. of miracidia hatching/ml	hatched/ml
Salt Solutions			
0%	250	18	7.2
1%	250	1	0.4
3%	250	0	0
5%	250	0	0
7%	250	0	0
10%	250	0	0
pH Solutions			
3.0	250	0	0
5.0	250	0	0
7.0	250	1.8	7.0
7.5	250	14	5.4
8.0	250	9	3.5
10.0	250	0	0
Distilled Water (Control)	250	43	17.0

Discussion

The relatively high percentage hatchability of *F. gigantica* eggs observed with 0.05 and 0.1mg/ml concentration of ampiclox and penicillin-streptomycin might probably be due to low concentration of the antibiotics. Tetracycline is acidic (Brander and Pugh, 1979) and this probably may be a factor that prevented hatching of *F. gigantica* eggs with tetracycline as observed in this study.

Streptomycin (an amininoglycoside) was found to inhibit hatching of *F. gigantica* eggs at 1mg/ml while ampiclox inhibits at 0.5mg/ml concentration. This observation might be due to the pH of these antibiotics at this concentration.

Tetracycline is metabolised in the liver, its bile concentration may be as high as 30 times that of the blood (Ozeigbe, 1997). *F. gigantica* are found in the bile ducts of ruminants where they shed their eggs into the gall bladder. When animals are treated with tetracycline and its concentration becomes very high in the bile it may inhibit the development of *Fasciola* eggs. Even though the alkaline nature of bile secretions may render inactive the acidity of high bile concentration of tetracycline in the gall bladder, a transient decrease in bile pH would adversely affect the development of Fasciola eggs. It is therefore suggested that in-depth pharmacological study be carried out on tetracyclines so as to find other derivatives of the drug that can act within the alkaline range of gall bladder environment. The destruction

of the eggs before they are excreted with faeces could serve as a control measure for fasciolosis in ruminants.

References

Bertran, G. K. Basic and clinical Pharmacology. (Appleton and Lang Publishing pp. 641-642 1992).

Blood, D. C., Henderson, J. A. and Rudostits, O. M. Veterinary Medicine. A Textbook of Diseases of Cattle, Sheep, Pigs and Horses. (5th Ed. ELBS and Bailliere Tindal London 1995).

Brander, G. C. and Pugh. D. M. Veterinary Applied Pharmacology and Therapeutics. The English language Book Society and Bailliere Tindal London pg 361-374.

Dunn, A. M. Urquhart, G. M. , Armour, J.; Duncan, J. L. and jenning, F. W. Veterinary Parasitology(©) (Longman group U.K. Ltd. 4th Ed. pg. 98-109 1991).

Hammond, J. A. and Sewell, M. M. H. Experimental infections of cattle with *F. Gigantica* and number of Parasites recovered after varying period of infection Trop. Anim. Hlth. prod. 7(2): 105-113 (1975).

Harper, H. A. Review of physiological chemistry (15th ed. Langt medical Publications 553pp 1982).

Ozeigbe, S. D. The effect of Tetracycline on the development of the miracidia of *Fasciola gigantica* Undergraduate proj. Fac. of Vet. Med. University of Ibadan (1972).

Sewell, M.M. H. The Pathogenesis of facioliasis. Bri Vet. Rec. 78(3) 98-105 (1966).

Shillhorn van Veen, T. W. Dynamics of *Lymnea natalensis* population in the Zaria area (Nigeria) and the relation of *F. Gigantica* infection. Acta tropica 6: 183-194 (1980).

Soulsby, E. J. L. Helminths, Arthropods and Protozoa of Domestic Animals 7th ed. (London bailliere Tindall 1982).

Troncy, P. M. Manual of Tropical Vet. Parasitology; Hepatic and renal helminths., pg. 65-72.

Wright, C. A. Flukes and snails. Science of Biology Series No.4 (c) (George Allen and Unwin Ltd. Pg. 64-68 1971).