International Journal of Veterinary Sciences and Animal Husbandry 2019; 4(3): 22-24

ISSN: 2456-2912
VET 2019; 4(3): 22-24

www.veterinarypaper.com
Received: 15-03-2019
Accepted: 17-04-2019

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The effect of tetracycline on the development and hatchability of *Fasciola gigantica* eggs following variable periods of incubation

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Abstract
Liver fluke, *Fasciola gigantica* is a digenetic trematode belonging to the family fasciolidae. Found in the liver of ruminants and pigs, it causes the disease Fascioliasis. Adult flukes expel eggs in hosts' faeces. In this study, parasite eggs were extracted from gall bladders of Fascioliasis infested cattle, washed with distilled water, and viewed under microscope to confirm presence of eggs. Five milliliters of the egg sediment was dispensed into 14 labeled test tubes. Into three of the tubes was dispensed 1.5mls of pen-streptomycin, while 1.5mls of 2500ug/ml dilution of tetracycline was dispensed into the rest. All the tubes were incubated in dark cupboard at room temperatures (27-31˚C). Samples from different test tubes were observed at days 5,10,15,20 and 21. Samples incubated with tetracycline showed no evidence of zygote development while those incubated with pen-streptomycin did, with some eggs hatching and releasing miracidia.

Keywords: Eggs, *Fasciola gigantic*, incubation, miracidia, tetracycline

1. Introduction
The liver fluke, *Fasciola gigantica* is a digenetic trematode that belongs to the family fasciolidae. It is found in the liver of cattle, sheep, goats, and pigs, where it causes the disease Fascioliasis. Infestation with the parasite has profound effect on growth rate and productivity of ruminants, making it economically significant [1]. The local breeds of cattle have been found to have lower prevalence rates and fluke burdens than exotic breeds and crosses of local and exotic breeds [2].
F. gigantica is predominant in the tropical regions of the world [3]. The snail intermediate host for the parasite in Nigeria is *Lymnaea natalensis*, an aquatic snail and the only lymnaeid snail encountered here [4]. Apart from this species, there are other lymnaeid snails (intermediate hosts), such as *Lymnaea viridis*, *L. columella*, *L. cousin*, *L. ollula*, and *L. auricularia rubiginosa* [5]. The adult flukes expel eggs through the feces of the definitive host. Miracidia are hatched from the eggs after 16-20 days [6], from where they penetrate into the lymnaeid snails and then develop into the sporocysts, rediae, and cercariae. Cercariae separate from the snails and are encysted to become metacercariae on vegetation (infective stage). The metacercariae can then be found on vegetations [7]. These vegetations are important sources of fodder for the definitive hosts. The life cycle gets completed when the definitive host ingests vegetation containing the metacercariae.
In studies involving the parasite in Nigeria, the eggs are mainly incubated with penicillin-streptomycin combination as antibiotic, they would hatch releasing miracidia at the end of the incubation period. The aim of the present study is to incubate *F.gigantica* eggs with tetracycline added as antibiotic for variable number of days and determine the effect of the antibiotic on zygote development and eggs hatchability to release miracidia.

2. Materials and Methods
2.1 Sample Collection and Preparation
The gall bladders used for this study (five in number) were obtained from the condemned liver of cattle infested with Fascioliasis after post mortem inspection at the Bodija abattoir, Ibadan,

Oyo State Nigeria. The bladders were excised from the livers, wrapped in aluminum foil and transported in a cooler packed with ice to the laboratory. Eggs from the parasite were recovered from the bile after the bladders were incised with scalpel blade. They are washed several times with distilled water in a crucible to reduce the viscosity of bile and for easy sedimentation of eggs. Drops of sediment placed on a glass slide were examined under stereomicroscope, where elliptiform large eggs operculate at one pole and yellowish in color were observed. Into 14 test tubes labeled 1-6 and $7^{A,B}$-$10^{A,B}$ were dispensed 5mls of the sediment each.

2.2 Preparation of Tetracycline Dilutions

Two capsules of tetracycline (250mg) were used in the study. The 500mg of tetracycline was added to 10ml of distilled water and shaken to dissolve. One milliliter (1ml) of this solution was added to 9 ml of distilled water to give 5000ug/ml and then 5ml of this solution was added to 5ml of distilled water to give a concentration of 2500ug/ml regarded as the minimum inhibitory concentration.

2.3 Egg Incubation and Examination

One and half milliliter (1.5 ml) of penicillin- streptomycin antibiotic was added to test tubes labeled 1-3, while equivalent volume (1.5 ml) of the minimum inhibitory concentration of tetracycline was added to the ones labeled 4-6 and $7^{A,B}$-$10^{A,B}$. All the test tubes were partially covered with cotton wool to allow for aeration. The 14 test tubes were placed on test tube rackets and incubated in dark cupboards at room temperature (27-31˚C; av. 29˚C). The first three test tubes (labeled 1-3) containing pen-streptomycin and the test tubes labeled 4-6 incubated with tetracycline were placed close to each other in the dark cupboard for 21 days. The remaining test tubes labeled $7^{A,B}$-$10^{A,B}$ were also placed in the same dark cupboard.

Egg samples were taken from test tubes ($7^{A,B}$-$10^{A,B}$) and observed at variable periods of 5, 10, 15 and 20 days respectively for zygote development under a stereomicroscope. Eggs from test tubes labeled 1-3 and 4-6 were examined at day 21 of incubation.

3. Results

The egg samples taken from test tubes 7A and 7B at day 5 of incubation, showed no zygote development when observed under the microscope after exposure to light. The same was observed for test tubes 8 (A and B) and 9 (A and B) at days 10 and 15 of incubation respectively. Eggs observed from test tubes 10 (A and B) also showed no development at day 20 (table.1).

Table 1: The Effect of Tetracycline on The Development of *F. gigantica* Eggs Following Varying Lengths of Incubation.

Sample No.	Length of Incubation (Days)	Vol. of Antibiotic used (ml)	Zygote Development
7A	5	1.5	Absent
7B	5	1.5	Absent
8A	10	1.5	Absent
8B	10	1.5	Absent
9A	15	1.5	Absent
9B	15	1.5	Absent
10A	20	1.5	Absent
10B	20	1.5	Absent

Egg samples taken from test tubes labeled 1-3 incubated with pen-streptomycin were examined under microscope at 21 day of incubation after exposure to light. Miracidia were observed, some still attached to the eggs. However, samples of egg taken from test tubes labeled 4-6 incubated with tetracycline showed no zygote development when examined under microscope (Table.2).

Table 2: Effects of Penicillin-Streptomycin combination and Tetracycline on The Development of *F. gigantica* Eggs at Day 21 of Incubation

Sample No.	Antibiotics Used(1.5ml)	Egg Development at 21 Days of Incubation
1	Penicillin-Streptomycin	Miracidia Present
2	Penicillin-Streptomycin	Miracidia Present
3	Penicillin-Streptomycin	Miracidia Present
4	Tetracycline	Miracidium Absent
5	Tetracycline	Miracidium Absent
6	Tetracycline	Miracidium Absent

4. Discussion

The present study showed that eggs of *F. gigantica* incubated in test tubes in to which tetracycline was added as antibiotic in a dark cupboard at room temperatures, did not hatch to release miracidia on exposure to light after 21 days. When examined under a stereomicroscope no zygote development was observed. The eggs incubated in test tubes examined at days 5, 10, 15 and 20 also did not show any evidence of zygote development.

The phenomenon of failure of eggs to develop and hatch when incubated in the presence of tetracycline relates to its mode of action. This is consistent with the studies of [8.9] which showed that tetracyclines inhibit protein syntheses in the mitochondria due to their interactions with the ribosomal subunits in these organelles. Since protein synthesis is of key importance in the development of *F. gigantica* zygotes, these zygotes are susceptible to tetracycline inhibitory activities. Reports from [10, 11] showed that tetracycline is broad-spectrum, bacteriostatic and effective against a wide range of microbes including bacteria, protozoa and helminthes.

It has been reported that mitochondria are organelles that are derived from free-living proteobacteria acquired by eukaryotic cells via endosymbiosis [12]. Accordingly, the eukaryotic mitochondria possess ribosomes that are similar to those found in bacteria. Consequent to these ribosomal similarities, it is believed that the anti-protozoal activity of tetracycline is achieved through its interactions with the mitochondrial ribosomes of these parasites in manners comparable to the interactions with bacterial ribosomes [13]. Meanwhile, it has been established that two processes appears to be required for these antibiotics to gain access to the ribosomes; passive diffusion through hydrophilic pores and energy dependent active system [14].

The anti-eukaryotic activity of tetracyclines has been linked to their weak inhibition of protein synthesis at the 80S ribosomal sub units [15]. Despite the fact that different studies reported same interaction sites for tetracyclines, [16] showed that the apparent sites for drug interaction in the ribosome may not necessarily reflect the actual binding site. Following their binding, tetracyclines prevent accesses of amino-acryl tRNA (transfer ribonucleic acid) to the acceptor site on the mRNA (messenger ribonucleic acid). This prevents the addition of amino acid to the growing peptide chain providing an explanation for the bacteriostatic effects of these antibiotics as reported by [17]. Furthermore, tetracyclines are known to have

acidic PH in aqueous solution, and may therefore have the ability to prevent the hatching of the eggs *in vitro* [18].
Conversely, the eggs incubated with penicillin streptomycin combination for 21 days hatched. Miracidia were observed on examination under microscope, the mechanisms of action of penicillin and streptomycin being different from those of tetracyclines.

5. Conclusion and recommendation
This study showed that tetracyclines prevent the hatching of *F. gigantica* eggs incubated with them for variable number of days. Eggs examined under microscope after 21 days of incubation did not show zygote development and did not hatch releasing miracidia. The inability of eggs to hatch is attributed to the ability of tetracyclines to gain access in to the eggs and inhibit embryo development by interfering with protein synthesis mechanism. It is recommended that further studies be carried out to ascertain ways of harnessing therapeutic benefits from this phenomenon.

6. Acknowledgments
The authors would like to thank the laboratory staff of the Department of Veterinary Parasitology, University of Ibadan who assisted and provided some of the facilities used for the study.

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