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VIABILITY OF *TAENIA SAGINATA* EGGS IN VARIOUS MEDIA USING METHYLENE BLUE TECHNIQUE

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ABSTRACT

Taenia saginata is an economically significant parasite in human beings responsible for high morbidity in beef consuming regions of the world. This study using methylene blue staining technique was aimed to observe the influence of various media on the longevity of the viability of its eggs. Ten T. saginata gravid segments were collected from non-treated patients and were put in separate dishes containing 0.9% NaCl solution, 5% dextrose, sludge containing soil, leaves and water (naturally obtained) and sewage from septic tank. A rough estimate of 110000 to 150000 eggs was put in each five separate dishes. Viability was checked with 1% solution of methylene blue staining at weekly intervals. In the week first, all the eggs from all the media were found live, but in second week, the egg viability diminished. The maximum number of days for viability of T. saginata eggs has been achieved of 0.9 % NaCl medium followed by 5% dextrose, then sludge and sewage with 651, 203, 77 and 63 days, respectively. We conclude that eggs of T. saginata could retain viability according to its surrounding media and can remain viable for quite longer under the prevalent environmental conditions in Kashmir valley. However, further studies to prove these assumptions are recommended.

Keywords: Kashmir, Media, Methylene blue, Taenia saginata, Viability,

I.INTRODUCTION

Taenia saginata is an economically significant parasite in human beings responsible for high morbidity than mortality especially in beef consuming regions of the world. It completes its life history in two hosts- the ultimate one are the humans (adult parasite is present) and the intermediary are cattle (larvae-cysticercusbovis is present) including cow (*Bos taurus*); buffalo (*Bos buffelus*); Zebu (*Bos indicus*), Yak (*Bos grunniens*). The eggs of *T. saginata* are spherical, brown and are provided with a double layered shell. The external shell is thin, transparent and is frequently not retained with the egg. The inner shell containing a thick embryophore is brown,

radially striated and measures 31 to 43 cm in diameter [1]. It contains an oncosphere with 3 pairs of hooklets. About 80,000 eggs may be present in single proglottid, the eggs of *T. saginata* are morphologically indistinguishable from other *Taenia* species. The eggs retain their viability even up to 8 weeks on the exposed pastures and are infected to cattle only (WHO 3rd report on zoonosis control) have reported viability of *T. saginata* after 159 days. In sewage (16 days), septic tank (40 days), laboratory anaerobic condition (200 days), activated sludge (42 days) [2].

II.MATERIAL AND METHODS

Media used: Ten *T. saginata* segments were collected from non-treated patients and were identified by counting uterine branches [3-4]. These segments were then put in separate dishes containing different media. The media include, 0.9% NaCl solution, 5% dextrose, sludge containing soil, leaves and water (naturally obtained) and sewage from septic tank.

For each dish containing separate media two segments were first opened with sharp needles, razors and then put into these separate dishes, a rough estimate of 1, 10,000 to 1, 50,000 eggs were put in each five separate dishes. Viability was checked with methylene blue staining at weekly intervals.

Methylene blue staining technique (Knaus and Lange, 1987): One gram of methylene blue powder was dissolved in 100 ml of distilled water to make 1% solution of methylene blue.

Weekly the four dishes containing *T. saginata* eggs in their respective media were first stirred with a sterilized glass rod and at random one drop of this suspension was placed on the slide following one drop of methylene blue solution, then after few seconds a cover glass was laid on it. Then eggs were observed under microscope without any concentration or floatation technique as the suspension of various media was concentrated with known quantity of *T. saginata* eggs. The eggs taking stain were considered as non-viable and non-staining eggs as viable [5]. The percentage of viable eggs was calculated by counting total number of non-staining eggs by total number of eggs seen [6].

The similar technique was also employed to observe the viability of *T. saginata* eggs which were fed to two local fowl; kept in separate cages for two weeks after inoculation of *T. saginata* two gravid segments each. Following the inoculation of segments, coprological examination was done to evaluate the role of birds in the dispersal of eggs and dissemination of infection.

III.RESULTS AND DISCUSSION

In the first week of examination of eggs placed in four different media, all the eggs from all the media were found live in methylene blue. After one more week the number of egg viability was found diminishing (Table 1). The maximum number of days for viability of *T. saginata* eggs has been achieved in laboratory conditions of 0.9 % NaCl medium followed by 5%dextrose, then sludge and sewage with 651, 203, 77, and 63 days respectively.

Our results can be correlated with [2], who reported that, eggs may remain viable for several weeks or months in sewage, rivers, and on pasture. The results are also in agreement with [7] with certain variations i.e., the first two media of NaCl 0.5 % and dextrose 5 % have not been reported till date for influencing viability of *T. saginata* eggs as reported in current study. This study revealed that in spite of minor sewage and sludge treatments there remains every apprehension of infection of locally raised cattle due to *T. saginata* as if only one taeniasis patient exposes his faeces to the field, pasture or water body, thousands of bovines can get infected. It is significant to note that eggs have been shown to survive almost all stages of sewage treatment [7]. Eggs may remain viable for 71days in liquid manure, 16 days in city sewage, 33days in river water and 159 days in open pastures [8]. It has been found that the eggs may remain alive on pastures for at least eight weeks and on dry sunny pastures for 14 $^{1}/_{2}$ weeks in Australia [9].

It appears that modern sewage purification methods do not efficiently remove *T. saginata* from the final effluent and that prolonged setting is necessary before effluent is used to irrigate cattle pastures if *Cysticercus bovis* infection is to be reduced to very low levels. [10] reported that the eggs of all taenids are sensitive to desiccation and temperature. In temperate zone, such as those where the summer and winter air temperature range from 10^0C to 20^0C and -20^0C to -10^0C respectively, survival as measured by infectivity, is in the order of 100-200 days and >200 days respectively.

Kashmir being a temperate zone and favours the longevity of the viability of eggs of *T. saginata.* This is attributed to be a good reason for the infection in cattle, sewage treatment is as almost negligible in this region and cattle straying a common practice. The viability of *T. saginata* eggs attained in artificial media of 5% Dextrose and 0.9% NaCl solution under laboratory conditions suggest that in saline solution viability was attained maximum and more than three times than that of Dextrose solution thus evaluating that decomposition had occurred early in all other media other than saline medium where it got differed due to saline concentration which invariably influenced the longevity and the viability of eggs. The viability of eggs demonstrated in experimental fowl by examining their faeces through methylene blue technique revealed that eggs obtained where 100 % viable and suggest that birds act as good source for the dissemination of eggs. Birds and cattle being coprophagous animals acted as a good source of disseminator and infection respectively as also observed by [11] who found gulls playing role in the dispersal of *T. saginata* eggs.

IV.FIGURES AND TABLES

Table 1. Representing Viability of Eggs in Various Media

Medium	Conditions	Number of weeks of viability of eggs	Percentage of eggs viable in the last week	Survival of eggs in days
NaCl Solution (0.9%)	Laboratory conditions (15-25^{o}C)	93	01	651
Dextrose (5%)	- do-	29	0.5	203
Natural Sludge containing soil, leaves and water	Natural 18-32^{o}C	11	5	77
Sewage of septic tank	Natural	09	05	63

V.CONCLUSION

We conclude from this experimental study that eggs of *T.saginata* could retain viability according to its surrounding media and under the prevalent environmental conditions in Kashmir valley these can remain viable for quite longer. However further studies to prove these assumptions are recommended.

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