A study on in vitro culture of *Trichuris ovis* in different physiological solutions at constant temperature, 37°C

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Abstract The primary aim of in vitro culture of whipworms (*Trichuris ovis*) is to provide artificial conditions under which the life cycle of the parasites completed outside the host under controlled conditions. The physiological solutions used for the present study were sodium chloride (0.64%), Ringer's solution, Tyrode's solution, and Lock–Lewis solution. Parasites were collected from freshly slaughtered intestine of the host. The recovered parasites were washed with running tap water after that with normal saline. After washing parasites were put in four petridishes containing different physiological solutions. Observations were recorded after interval of every 8 h. The hundred percent survival of *Trichuris ovis* was observed at 32, 40, and 48 h in NaCl (0.64%), Ringer's, Tyrode's, and Lock–Lewis solution, respectively in case of both male and female parasites. In sodium chloride solution (0.64%) cent percent mortality was observed after 64 h of incubation in males and in case of females it was observed 72 h. In Ringer's solution cent percent mortality was observed after 72 in males and in females it was observed 80 h. In Tyrode's solution cent percent mortality was observed after 88 h in males and 96 h in females. In Lock–Lewis solution cent percent mortality was observed after 96 h in case of both the male and female parasites. Present study could be used to understand the effects of various drugs on the above parasites and also other intra-intestinal parasites.

Keywords *Trichuris ovis* · Ringers solution · Tyrode's solution · Lock–Lewis solution

Introduction

The in vitro cultivation of nematode parasites of vertebrates is an important tool which might ultimately be used in the study of many aspects of nematode biology, and in the elucidation of some of the complex factors which are involved in the parasite–host relationship (Paul and Jones 1956).

Parasitic infections account for hundreds of million of dollars in annual losses and medication costs in live stock and poultry industry throughout the world (Gamble et al. 1986).

Helminthes infection affects the socio-economic structure of a society, through its harmful effects on the physical and intellectual development of children as well as on the productivity of adult members of the society. (Stephenson 1987).

Parasite *Trichuris* adults were whitish in colour which were easily recognized by their characteristic, slender, long thread like anterior portion, and a much thicker posterior end. Heavy infections with whipworms are not common but may be seen in very young lambs or during drought conditions when sheep are fed on the ground. The complex life cycles of most of the parasites require to maintain an ordered route of development, and tend to adapt rapidly to a variety of environmental factors, which involves a complex interaction between intrinsic and extrinsic factors. The intrinsic factors may include the nutritional state, the degree of morphological development and an internal clock. The extrinsic factors include temperature and carbon dioxide level (Tsuji and Fujisaki 1994).

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Several studies conducted on in vitro culture/survival of the parasitic helminthes were of Hall 1971; Narien et al. 1995; Narien 1980; Kuchler 1928; Weinland 1901; Weinstein and Jones 1959; Tayler and Baker 1968; Dauschies and Watzel 1999; Hata 1978 and Hata et al. 1980. In vitro culture of nematodes parasites is important because of their medical, veterinary, and economic importance, extensive work has been carried out on the *Ostertagia* could never live for more than 24 h in sodium chloride solution and with addition of (0.23) percent calcium chloride solution the above parasite could live for 3 days (Davey 1938).

Studies of in vitro cultivation/survival of parasitic helminthes depend upon their evaluation on criteria for the recognition of the degree of development taking place under a particular set of environmental conditions.

Therefore a study on in vitro cultivation/survival of *Trichuris ovis* in different physiological solutions at constant temperature was proposed. The primary aim of culture of *Trichuris ovis* is to provide artificial conditions under which the life cycle of the parasites completed outside the host under controlled conditions.

Materials and methods

Experiments were conducted using the following physiological solutions

Sodium chloride solution (0.64%), Lock–Lewis solution, Ringers solution, and Tyrode's solution.

Adult parasites were collected from the intestine of freshly autopsied vertebrate host by making incisions in the intestine longitudinally with the help of blunt end of scissors.

Live male and female parasites after rinsing using running water and normal saline culture in experimental petridishes containing sterile four physiological solutions (sodium chloride solution (0.64%), Lock–Lewis solution, Ringer solution, and Tyrode's) at 37°C were incubated. Sterile media was changed daily and readings were recorded at the interval of every 8 h. The entire experiment was repeated three times.

Results

Survival experiments for *Trichuris ovis* were conducted thrice using different physiological solutions at 37°C constant temperature. The following observations were reported.

Sodium chloride solution

The maximum survival of male *Trichuris ovis* was observed at 32 h in sodium chloride solution (0.64%). After incubation of 40, 48, and 56 h, rate of mortality was 30, 60, and 80%. Mortality rate of the female species was 30, 60, and 70%. After incubation of 64 h, 90% female species perished. The cent percent mortality was recorded after 64 h in males and in case of female nematodes it was 72 h (Table 1; Graph 1a, b).

Ringer solution

In Ringer solution, maximum survival was observed at 32 h both in case of males and females parasites. After 40, 48, 56, 64 h mortality rate was, 40, 60, 80 and 90%, in males while in case of females after 40, 48, 56, 64, and 72 h, it was 40, 60, 70, 90 and 90%, respectively.

Table 1 Percentage of mortality of male and female *Trichuris ovis* in different physiological solutions at 37°C

Time interval in hours	NaCl solution (0.64%)		Ringer solution		Tyrode' solution		Lock–Lewis solution	
	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)
8	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0
40	30	30	40	40	0	0	0	0
48	60	60	60	60	30	30	0	0
56	80	70	80	70	50	40	40	30
64	100	90	90	90	70	60	60	50
72	–	100	100	90	80	80	90	80
80	–	–	–	100	90	80	90	80
88	–	–	–	–	100	90	90	90
96	–	–	–	–	–	100	100	100

Graph 1 **a** Percentage of mortality of male *Trichuris ovis* in different physiological solutions at 37°C. **b** Percentage of mortality of female *Trichuris ovis* in different physiological solutions at 37°C

Cent percent mortality was recorded after 72 h in male parasites while in case of females it was 80 h. (Table 1; Graph 1a, b).

Tyrode solution

The maximum survival observed in Tyrode's solution was at 40 h for both male and female parasites. After 48 h mortality rate recorded was 30% in case of males and female parasites. After interval of, 56, 64, 72, and 80 h the mortality rate recorded in males was 50, 70, 80, and 90%, respectively. While in case of females it was 40, 60, 80, 80, and 90% after the period of 56, 64, 72, 80, and 88 h.

Cent percent mortality was observed after 88 h in male parasites while in case of females, it was 96 h (Table 1; Graph 1a, b).

Lock-Lewis solution

The maximum survival observed in Lock-Lewis solution was 48 h in both the cases. After 56, 64, 72, 80, and 88 h the mortality was recorded 40, 60, 90, 90, and 90%, respectively in males whereas in case of females mortality was recorded 30, 50, 80, 80, and 90%.

The cent percent mortality was observed after 96 h both in males and females (Table 1; Graph 1a, b).

Discussion

Studies on in vitro cultivation of parasitic helminthes depend on their evaluation on criteria for the recognition of the degree of development taking place under a particular set of environmental conditions. The survival of nematode

parasites is still a problem because of their minute size and also because of their varied habitat and variety of hosts.

The present study is focused on the survival *Trichuris ovis* of vertebrate host in different physiological solutions i.e., sodium chloride solution (0.64%), Ringer solution, Tyrode's solution, and Lock-Lewis solution at 37°C constant temperature was studied. The maximum survival was reported in Lock-Lewis solution and minimum in sodium chloride solution. The maximum survival for male and female parasites of *Trichuris ovis* was observed 96.

Oestertagia could never live for more than 24 h in sodium chloride solution and with addition of 0.23 percent calcium chloride the above parasite could live for 3 days (Davey 1938). The histotropic fourth stage (L_4) is valuable tool for in vitro applications, e.g., for screening of drugs for physiological and immunological studies (Daughschies 1995, 9196). They described the in vitro cultivation procedures for *Oesophagostomum dentatum* using pig blood serum and triptase. In liver and yeast they could develop fourth stage larvae at 40°C.

In vitro studies have been used to understand the development of parasitic stage out side the host body. The development of in in vitro conditions of the free living larvae of *Strongylus venzuenlensis* was studied by temperature shift (Tsuji and Fujisaki 1994) they observed that infective larvae developed to the parasitic state in Dulbecco's modified eagle medium at 25 and 35°C and observed changes in cuticle and bud like head.

Singh et al. (2004) cultured the *Ascaridia galli* a fowl nematode under constant condition of temperature in different physiological conditions and found that among different physiological solutions used for the study Lock-Lewis solution was found most suitable for culturing nematode parasites.

In vitro cultures were also used by Sampson et al. (1998) to develop bovine lung worm, *Dactylocallus viviparus*. Sampson et al. cultured larvae's in a new born calf serum at 39.5°C and they observed that the larvae could develop up to third stage. Weinland (1901) used 1% sodium chloride for his experiment and found that addition of sodium bi carbonate (Na_2CO_3) made no difference but the addition of carbon di oxide increased the period of survival of *Ascaris lumbricoides*.

Hata (1978) and Hata et al. (1980) cultured *Metastrongylus apri* third stage larvae for 10 weeks in NCTC 109 supplemented with 20% calf serum, 200 units/ml of penicillin and 100 Rg/ml of streptomycin, with a gas phase of 5% CO_2 in air at 37.5°C.

Warburton and Zelmer (2010) evaluate potential for survival in a vertebrate host, Dauer larvae seven species of rhabditid nematodes in vitro for 72 h post exposure.

It is concluded that the Lock–Lewis solution is the most suitable media to culture nematodes parasites of different vertebrates. Furthermore, the study reveals that drug used on the parasites and also other intra-intestinal parasites is Lock–Lewis solution. The longer the period of survival the easier it is to perform experiments to find the comparative effects of various anthelmintics, regarding various histopathological changes, drug designing, genetic studies and also for molecular studies.

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