ANTIGENIC RESPONSE OF FREE LIVING NEMATODE *RHABDITIS AXEI* LARVAE AGAINST *TOXOCARA VITULORUM*

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ABSTRACT

The optimum conditions for agar gel precipitation test were determined by using homologous (*Rhabditis axei* antigen vs *Rhabditis axei antibody*), heterlogous (*Rhabditis axei* antigen vs *Toxocara vitulorum* antibody) and vice versa systems. Vernol buffer was replaced by normal saline (pH at 8.5) in the preparation of gel. Precipitation lines were observed against *Toxocara Vitulorum* antigen with antise.a prepared in rabbits against *Rhabditis axei* antigen. Best precipitation lines were seen after 5 days of incubation at 37°C. Similar observations were also recorded by using homologous system. *Rhabditis axei* shares some common antigen(s) with *Toxocara vitulorum*.

INTRODUCTION

Toxocara (T.) vitulorum is a common helminth of neonatal calves (Hayat *et al.*, 1990) transmitted through colostrum of infected dams (Mia *et al.*, 1975). The possibility of control of *T. vitulorum*, therefore, lies in killing the larvae of parasite in dam. The treatment of Toxocara larvae has been reported in the early days of infection with tetramisole hydrochloride (Hossain *et al.*, 1980) and is difficult when they become hypobiotic (Shehada and Herbert, 1984).

An alternate possibility of control is to immunize the dams before the larvae are transferred to the calves. Numerous reports are available on immunization by using antigens from homologous parasites (Huntley and Moreland, 1963; Hogarth-Scott, 1966; Ivey, 1967) but their use has become limited because of resultant hypersensitivity or immunosuppressive effect (Ershov *et al.*, 1974). The use of heterologous antigen is an other area to be explored on wider prospective for this purpose. It has also been reported that soil free-living nematodes, Rhabditis spp, are antigenically related to nematode parasites of man and domesticated animals in respect to amino acids and enzyme contents (Ershov *et al.*, 1974). Such relationship has provided a basis for considering the free-living nematode, Rhabditis as immunizing agents against parasitic nematodes. The present study reports the antigenic response of *Rhabditis axei* against *Toxocara vitulorum* in rabbits.

MATERIALS AND METHODS

Cultivation of *Rhabditis axei* larvae

Soil from canal channels were sprinkled on blood agar and incubated at 37°C with 80 per cent relative humidity for 48 hours for the cultivation of *Rhabditis axei* larvae (Norman *et al.*, 1963). Larvae were concentrated by Baermann's technique and transferred to 20 ml phosphate buffered saline (PBS; pH 7.2) containing 100 IU/ml of streptomycin for axenization (Sommerville, 1966). Axenized larvae were washed thrice in sterile PBS.

Collection and cultivation of infection eggs of *Toxocara vitulorum*

Fresh adult *Toxocara vitulorum* were collected from buffalo calves from the local slaughter house. Adult females were identified and separated. They were washed three times in 0.15 M NaCl and incubated in the same solution at 37°C for 24 to 72 hours. Ten *Toxocara vitulorum* females were cut into half, uteri extruded and transferred to a pestle and mortar in which 5 gms coarse sand was added. The mixture was grounded lightly and 2 per cent formalin was added. The mixture was then centrifuged and supernatant was collected containing the *Toxocara vitulorum* eggs. The supernatant along with 2 per cent formalin was incubated at 28° for 14 to 18 days. At the end of this period, 96 per cent eggs were found to be embryonated. The material was then given 3 washings with PBS and stored at 4°C to stop the growth of *Toxocara vitulorum* larvae (Barriga and Omar, 1992).

Preparation of antigens

a. *Rhabditis axei*

Rhabditis axei larvae were ultrahomogenized for 1 x 4 minutes (with 1 minute interval) in PBS (5 gms in 100 ml). The suspension was subjected to sonification

for 2 x 30 seconds at 8°C. The homogenate was centrifuged at 5000 rpm for 30 minutes. The supernatant was used as antigen.

b. *Toxocara vitulorum*

Embryonated eggs (larvae) of *Toxocara vitulorum* were suspended in PBS (350 larvae/ml PBS) and ultrahomogenized for 1 x 2 minutes at 4°C. The suspension was centrifuged and the supernatant thus collected was used as an antigen.

Protein contents in *Rhabditis axei* and *Toxocara vitulorum* antigens were measured by calorimetric method (Bradford, 1976) adjusted to 1 gm/dl with PBS,. Each antigen was mixed with two parts of Freund's complete adjuvant (Sigma Laboratories, St. Louis, USA).

Experimental design

Twenty-seven rabbits of about the same age, sex and body weight were procured from the local market and maintained in the Department of Veterinary Parasitology, University of Agriculture, Faisalabad. All the rabbits were rendered free from parasites by using velbazen solution (1:10). The parasite-free status of the animals was confirmed through regular faecal examination for two weeks before using them for immunological work. Rabbits were divided into three equal groups viz. A, B and C. Group A was further subdivided into three subgroups i.e. A1, A2 and A3. Each subgroup was injected 0.4, 0.8 and 1.0 ml of the *Toxocara vitulorum* antigens, respectively. Similarly, group B was subdivided into subgroups i.e B1, B2 and B3 and injected *Rhabditis axei* antigen at the same dose rate as that of *Toxocara vitulorum*, respectively. Group c served as control, and was injected with adjuvant and PBS. All the rabbits were given the same boosting dose of respective antigen after 14 days of the 1st injection. Controls were similarly injected with adjuvant and PBS 14 days of the 1st injection.

Blood sampling

Blood samples (2 ml) without anticoagulant were collected from each animal prior to inoculation and on day 14, 21, 35 and 72 post inoculation. Serum was separated and stored at -20°C for further use.

Agar gel precipitation test (AGPT)

Noble agar (Difco laboratories, Detroit, Michigan. USA) 1.5 per cent prepared in normal saline, pH 8.5, was used in the test (Huntley and Moreland, 1963). Hyperimmune serum prepared against crude antigens of *Toxocara vitulorum* and *Rhabditis axei* (Collee *et al.*, 1992) was used as a positive control for the respective antigens. The plates were incubated at 37°C in a humid chamber and were examined for precipitation lines.

RESULTS AND DISCUSSION

AGPT has been frequently used by employing the veronal buffer for the preparation of gel (Olson *et al.*,

Table 1: Antigenic response of immunized rabbits against *Toxocara vitulorum* vs *Toxocara vitulorum*

Groups	Subgroup	Dose (mL)	Serum Samples Post-inoculation (days) 14	21	35	72
A	A1	0.4	-	-	-	-
	A2	0.8	-	+*	+++	+++
	A3	1.0	-	-	-	-
B	B1	0.4	-	-	-	-
	B2	0.8	-	+*	-	-
	B3	1.0	-	-	-	-
C	C1	0.4	-	-	-	-
	C2	0.8	-	-	-	-
	C3	1.0	-	-	-	-

+++ = Clear and descriptive lines. +* = Precipitation lines but not obvious.
- = No precipitation lines.

Table-2 Antigenic response of immunized rabbits against *Rhabditis axei* vs *Toxocara vitulorum* or vise versa.

Groups	Subgroup	Dose (mL)	Serum Samples Post-inoculation (days)			
			14	21	35	72
	A1	0.4	-	+*	+++	+++
A	A2	0.8	-	-	-	-
	A3	1.0	-	-	-	-
	B1	0.4	-	+*	+++	+++
B	B2	0.8	-	-	-	-
	B3	1.0	-	-	-	-
	C1	0.4	-	-	-	-
C	C2	0.8	-	-	-	-
	C3	1.0	-	-	-	-

+++ = Clear and descriptive lines. +* = Precipitation lines but not obvious.
- = No precipitation lines.

1960; Huntley and Moreland, 1963). The constituents of the vernol buffer are very expensive and are not available locally. In the present project, vernol buffer was successfully replaced by normal saline by adjusting the pH at 8.5 with 0.1 N NaOH/0.1 HCl for AGPT.

Precipitation lines were observed against Toxocara vitulorum from the sera of the rabbits injected with 0.4 ml of *Rhabditis axei* antigen and vice versa, after 35 days from the first injection (21 days from the booster dose). On the other hand, rabbits injected with 0.8 and 1.0 ml of the Rhabditis antigen showed no precipitation lines in their sera (Table 1). This may be due to the excessive antibody levels or antigen concentration at the zone of equivalence that no precipitation lines were observed. Precipitation lines were also not seen in either of the case on days 14 and 21 post-inoculation (Tizzard, 1992). Similar observations have also been recorded by using homologous system. However, the precipitation lines were only seen in rabbits immunized with 0.8 ml of either antigen (Table 2). The precipitation lines persisted up to 72 days post-inoculation from the first injection in both the homologous and heterologous systems.

Best precipitation lines were observed after 5 days of incubation at 37°C. Although, these lines were formed after 3 days at 37°C but were not conspicuous and demarkable. These observations slightly deviated from those of Fernando (1968) who observed the precipitation lines after 4 days at 37°C and 14 days at 26-28°C by using homologous system. These variations may be due to the differences in material used for the preparation of agar. In the current study, agar was made in normal saline by adjusting the pH at 8.5 instead of vernol buffer.

It may be concluded that *Rahditis axie* share some common antigenic properties with *Toxocara vitulorum*. Hence, this free living parasite may be employed to immunize the animals against *Toxocara vitulorum* infection.

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