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THE USE OF IDENTIFIED SPECIFIC ANTIGEN FOR SERODIAGNOSIS OF TOXOCARA VITULORUM INFECTION IN BUFFALO - CALVES

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SUMMARY

Fractionation of somatic *Toxoxara vitulorum* antigen was carried out using Sepharose 4 B gel filtration technqiue. The obtained results indicated that 4 fractions were recovered , F_1-F_4. All fractions were tested for their antigenicity by agar gel diffusion and ELISA tests. ELISA values of F_1 & F_2 were relatively similar, while F_4 had no any reactivity with agar gel diffusion and ELISA tests. Fraction 2 was usde as identified specific antigen. This antigen was evaluated for its diagnostic applicability against sera of infected and non infected buffalo-calves by ELISA test. The sensitivity of the test was 100% in diagnosis of confirmed infected calves. Moreover, this test could detect 38.96% of coprologically examined negative calves. It was noticed that the immune response was increased as the intensity of infection had increased.

INTRODUCTION

Toxocara vitulorum (Goeze, 1782; Travassos, 1927) is a highly prevalent parasite of buffalo-calves in tropical and subtropical regions of the world. It is responsible for high morbidity and mortality rates in up to 40 days old buffalo calves (Tawfik, 1970).

The prevalence of *T.vitulorum* can reach 78.8-100% in the absence of field control measures (Selim and Tawfik, 1967; Stark et al., 1983). The control of this parasite is difficult because the larvae migrate in the host tissue where they remain as hypobiotic parasites and they can survive for a long time, (Warren, 1971; Roberts, 1990a). Moreover, larval transmammary passage occurs in calves after their birth (Roberts et al., 1990; Stark et al. 1992; Akyol, 1993) and there is also the possibility of larval placental passage of larvae to faetuses (Mosgovoi and Shikov, 1971; Mia et al. 1975) from infected mothers.

In many instances coprological examinations are not accurate enough . The results of these teshniques are variable without reasons and repeated examinations are required. It depends only upon the presence of eggs . So, in the prepatent period (migratory larval stages), eggs are never found and can not be diagnosed. For these reasons, the use of serodiagnostic tests are very important but, use of crude antigens lacked the desired sensitivity (Amerasinghe et al.,1984; Derbala et al., 1990; Drbala and Zayed, 1992), as well as the posibility of cross reactivity. Detection of specific antibodies requires purified antigenic preparations to avoid such problems (Derbala, 1993). In this paper, separation and identification of *T.vitulorum* antigens were carried out, in a trial to obtain the specific diagnostic antigen. In addition, this antigen was used for the detection of the immune response of buffalo-calves infected with *T.vitulorum* by ELISA test.

MATERIAL AND METHODS

Samples Collection:

One hundred and sixty five buffalo-calf faecal and their corresponding blood samples were collected. These calves slaughtered at the main Cairo abattoir during the winter season of 1996. The faecal samples were examined by the concentration flotation technique (Thienpont et al., 1979). The sera of *T.vitulorum* infected and non infected samples were separated and stored at -20°C till us for serodiagnosis. Negative control sera were obtained from twelve calves proved to be free from any parasite.

Antigen preparation:

Adult *T.vitulorum* worms were recovered from naturally infected calves. The worms were washed several times in phosphate buffer saline, PBS (pH 7.2). The worms were fragmented in Mixer and then homogenized throughly in PBS. The homogenate was centrifuged at 10000g for 30 min. and the supernatant was obtained as a crude soluble antigen (Derbala, 1993). Protein content was determined according to the method described by Bradford, (1976).

Sepharose 4B gel filtration technique:

Isolation of antigenic fractions of *T.vitulorum* crude antigen was performed through Sepharose 4B columns as described by Welch et al. (1983), Ultra-violet absorption of the protein peaks was determined at 280nm. The obtained values were represented in Fig.1. Major protein peaks were pooled and lyophilized separately as partially purified fractions.

Detection of fractions antigenicity:

The antigenicity of these fractions had been tested by agar gel diffusion and ELISA tests against immunized sera prepared in rabbits. These sera were prepared as adopted by Fagbemi et al., (1995). In brief, rabbits were immunized subcutaneously with 40µg of whole-worm antigen in Freund's adjuvant. A boostter dose of 40 µg antigen in Freund's adjuvant incomplete was given on day 14. Second and third booster doses were given on days 21 and 28 and serum samples were collected 4 days after the last immunization.

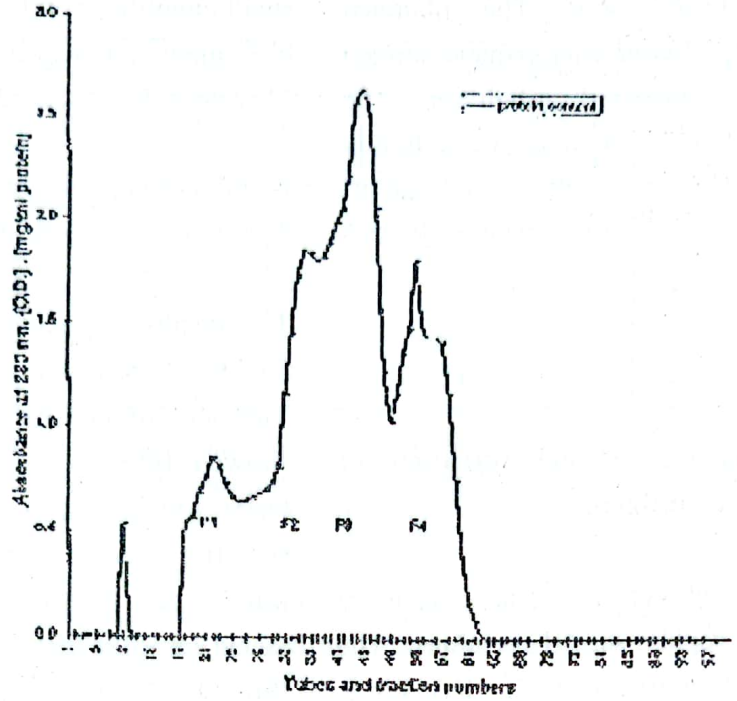

Fig.1: Protein elution profile of *Toxocara vitulorum* somatic antigen fractionated by Sepharose 4B

Serodiagnosis of *T. vitumorum* by ELISA test using identified fraction:

ELISA technique was applied to evaluate the antibodies against *T.vitulorum* in relation to the degree of infection . ELISA test was performed as described by Voller et al. (1976) with some modifications. The optimal amount of antigenic fraction, sera and conjugate (Horseradish peroxidase (HRP) conjugated anti-bovine IgG, product No.A-7414 (Sigma Co.) were determined by standard checkerboard titrations. ELISA plates were coated with antigen (50µl/well) in coating buffer (0.05 M carbonate buffer pH 9.4) and incubated overnight at 4°C. After washing three times with phosphate buffer saline 0.015 M (pH 7.2) containing 0.05 Tween 20 (PBS-T + 0.02% NaN3). 200µl of blocking buffer (1% BSA in coating buffer) were added to each well and incubated for 1 hr at room temperature. The plates washed 3 times with PBS-T. 50 µl of serially diluted serum were pipitted in each well and incubated for 2 hrs at 37°C, followed by washing 5 times with PBS-T. 50 µl/well 1/250 diluted conjugate were added. Then washed 3 times with PBS-T. 50 µl of substrate [1 mg Orthophenylene-diamine, Sigma, Co./1 ml substrate buffer pH 9.6] were added to each well, incubated for 15-30 min. at room temperature. The reaction was stopped by addition of 50µl stopping solution (1 N. Na OH) to each well. The optical density (O.D.) was read at 490 nm. using Dynatech Immunoassay system. Background readings were automatically substracted from the values of experimental wells by blanking the

plate reader against well that contained all reagents except tested sera. The obtained antigenic fractions of *T.vitulorum* somatic antigen were tested againt immunized rabbit sera. The serum samples of heavily, moderately and lightly infected buffalo calves were also tested against identified antigenic fraction using ELISA technique.

RESULTS

Results of Sepharose 4B gel filtration of *T.vitulorum* somatic antigen:

Crude *T.vitulorum* antigen was fractionated by filtration through sepharose 4B columns. The antigen produced four fractions (F_1 - F_4), Fig.1. Antigenic reactivity of these fractions showed that F_1 , F_2, & F_3 were positive against sera of immunized rabbits using agar gel diffusion test, while the F_4 had no reactivity. Testing of the same fractions using ELISA test proved that F_1 & F_2 had relatively similar ELISA value (O.D. value 0.293±0.02 & 0.292±0.02) respectively, (Table 1). However, fraction, F_1 was obtained in small quantities compared with F_2. Comparatively less intense reaction was observed with F_3. Therefore, F_2 was used as diagnostic antigen.

Results of coprological and ELISA diagnosis of *T. vitulorum* infecting buffalo-calves:

The results of faecal examination revealed that 53.33% of calves were infected with *T.vitulorum*. The sera of confirmed positive faecal samples showed 100% positive immune response, when tested with ELISA test against F_2 fraction as specific selected antigen. Moreover, the test proved that 38.96% (Table 2) of the negative faecal samples were positive . So, the results of serological test revealed 71.52% positive calves.

The results displayed in table (3) showed that ELISA value was higher in heavily infected infected calves (0.336±0.038) than the other two groups (moderately, 0.311±0.009 and lightly infected 0.288±0.017, respectively).

Table (1): Antigen activity of fractions recovered from *T. vitulorum* somatic antigen.

Antigens	Rabbit antisera	
	Agar gel diffusion test	ELISA activities (O.D. mean values at 490 nm±S.D.
Fraction No. 1	+	0.293±0.02
Fraction No. 2	+	0.292±0.02
Fraction No. 3	+	0.268±0.03
Fraction No. 4	-	0.178±0.08
Control	-	0.120±0.08

Table (2): Resutls of coprological and serological diagnosis (ELISA) of *T. vitulorum* infecting buffalo-calves.

No. of examined samples	Coprological examinations				ELISA activities			
	Positive		Negative		Positive		Negative	
	No.	%	No.	%	No.	%	No.	%
88	88	100	0	0	88	100*	0	0
77	0	0	77	100	30	38.96**	47	61.04
Total 165	88	53.33	77	46.66	118	71.52***	47	28.48

* Sero-positive percentage of confirmed infected calves
** Percentage of sero-positive samples from the negative examined calves.
*** Sero-positive percentage of the total examined calves.

Table (3): Optical density (O.D) variation among positive serum samples according to the degree of infection detected by ELISA test.

No. of tested samples	Degree of infection	Mean O.D. values at 490 nm.±S.D.
45	+++	0.336±0.038
12	++	0.311±0.009
31	+	0.288±0.017
Control	--	0.037±0.0006

+++ = Heavy infection 400-500 EPG.
++ = Moderate infection 200-300 EPG
\+ = Light infection up to 200 EPG.

DISCUSSION

The multiplicity of parasitic antigens might be considered as the major difficulities in immunological studies of parasites. So, little contributions had been reported on the use of the diagnostic partially specific antigen. *Toxocara vitulorum* somatic antigen has been fractionated into small units and partially purified after filtrationn through Sepharose 4B columns chromatography technique.

The present study described the use of the partially purified antigen (fraction) which revealed a high level of antigenic proportion in the immunodiagnosis of *T.vitulorum*. The

preliminary analysis of parasite extract resulted in four fractions (F_1 - F_4) Fractions (F1 & F2) were found to be the most antigenic fractions when, tested with agar gel diffusion and ELISA tests. Similar results were recorded with slight variation in the total fraction numbers and their antigenicity. Safar et al. (1992) obtained six fractions from each *Ascaris vitulorum* and *Ascaris lumbricoides*, after chromatographic analysis using . Sephacryl S-300 was performed on protein extract from both species. They added that no cross reactivity was recorded between *T.canis* and these species using a partially purified antigens. Moreover , Kandil (1994) separated 3 antigenic fractions from *Ostertagia circumcincta* larval extract by an ion exchange chromatography DEAE-cellulose technique. In addition Wedrychowicz (1984) revealed that 6 and 5 fractions were obtained from 4th & 5th. *O.circumcincta* larval somatic extracts using Sephadex G. 200 columns. In each larval stage of this parasite 2,3 & 4 and 1,2 & 4 fractions revealed high levels of precipitating and haemagglutinating antibodies. Moreover, in 1980, the same author showed that *Strongyloides papillosus* larvae produced only 3 fractions, where only two of them (1 & 2) had immunological activities.

The preparation of *Toxoxara vitulorum* antigen of high purity is often laborious and difficult. The results of this investigation described for the first time depends on the use of specific antigen fraction in the serodiagnosis of *T.vitulorum*. Affinity chromatography to parasite using Sepharose 4 B offered a method for the purification of *T.canis* specific antigen, (Welch et al., 1983). Such results supported the present work. The sensitivity of ELISA test reached 100% using specific antigen, fraction when comparing the results of (Derbala & Zayed, 1992) where crude antigen was used in serodiagnosis of the same parasite. Evaluation of partially purified *T.vitulorum* antigen confirmed that this antigen was more sensitive than the crude extract. Moreover , the test could detect 38.96% from negative infected calves. This results suggested that the parasitic stages may be in the prepatent period. Similar finding reported that IHA showed a much greater sensitivity with purified picture than with crude product of *O.circumicincta* (Wedrychowicz, 1984). probably, this high sensitivity might be attributed to the use of purified antigen.

The levels of detected antibodies depending on the intensity of infection with *T.vitulorum* were compared. The ELISA titres showed a significant response as the intensity of infection had increased. Such finding agreed with the results obtained by Fernando et al., (1989) when they described the antibody response of the calves with *T.vitulorum* gel diffusion and ELISA tests. However, Rajapakse et al. (1994), found that the titres of antibodies in the serum of calves measured by ELISA showed inverse correlation with the maximum egg count. Moreover, Barriga and Omar (1992) showed that antibodies rose slowly during the primary infection with *T.vitulorum* and remained stable during the second infection. This pattern suggests that antigens stimulate immunoeliminated circulating

antibodies during the first infection.

Further purification might be necessary for obtaining high purified antigens with high satisfactory results.

It could be concluded that serological tests probably provide the practical means of specific pre-mortem diagnosis of *T.vitulorum* infection. Moreover, the use of ELISA test might be of great value in the control and epidemiological studies of this important and economic parasite.

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