Vet.Med.J.,Giza. Vol.44, No.3.(1996): 551-556.

IMMUNODETECTION OF AMPLICAECUM LARVAL HAEMOGLOBIN USING ELISA TECHNIQUE

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SUMMARY

Amplicaecum larvae are a significant parasite infesting Tilapia and some predatory fish species at Nasser Lake since 1988. these laval stages were homogenized and prepared as a crude antigen (structural antigen). The protein concentration was estimated by monitoring the UV absorption spectrum. The positive immune serum anti Amplicaecum larval haemoglobin was prepared and sensitiized in vivo. The highest sensitivity of the haemoglobin was estimated by ELISA technique and amounted to a titer of 1/20 with optical density x 0.554.

INTRODUCTION

The Amplicaecum larvae (Order: Ascaridídea) (Baylis, 1920) are nematode larvae infesting most of the fish species in Nasser Lake. The yellow whitish larvae are easily seen with the naked eye localised in the sinus venosus, opercular and abdominal cavities (Mahmoud et al., 1989). These larvae are not aesthetically pleasing to the public health with great concern that the market ability of infested fish would be lowered.

Today, the serological techniques are extensively used in the diagnosis of nematode infections especially in fish zoonotic diseases (intestinal anisakiasis) (Sakanari, 1990). The definitive diagnosis can be made on the basis of morphologic characteristics of the whole larvae while the immunologic assays support these findings (Sakanari et al., 1988). The haemoglobin (structural antigen) showed a greater antigenic complexity when extracted from larvae (Suzuki et al., 1974). The particular antigenic fractions have been shown to play a major role in stimulating protective immunity, this may be measured by a variety of the conventional immunological tests (Shuji et al., 1986 and Sakanari, et al., 1988). The in vivo preparation of positive immune serum anti Amplicaecum larval haemoglobin is the main objective and new achievement in the present study, while the sensitivity and reactivity of Amplicaecum larval haemoglobin was estimated by ELISA technique.

MATERIAL AND METHODS

Antigen preparation:

The larval haemoglobin (structural antigen) (Suzuki et al., 1974) and (Shuji et al., 1986), was extracted from larvae that were chopped, minced and homogenized in a manual homogenizer in phosphate-buffered saline (PBS) pH 7.5 at 4°C, centrifuged at 3000 rpm twice for 10 min each, the supernatant was collected and the pellet discarded after twice extraction. The supernatant was filtered through Nalgene (0.45 μM, sterile

cellulose acetate disposable syringe filter). The antigen solution was prepared for both immunization and solid phase antigen in enzyme linked immunosorbent assay ELISA.

The preparation of positive immune serum (Hudson and Hay, 1989):

Healthy rabbits weighing 2-3 kg were used in experiment. They were exposed to Amplicaecum larval haemoglobin by intraperitoneal route.All rabbits were bled prior to and on the tenth day post inoculation during 30-45 days. The sera were stored without preservatives at -20°C until tested. The prepared positive sera were sensitized in rabbits by equal volume of Amplicaecum larval haemoglobin mixed with Freund's complete adjuvent (Hoechst Behring Germany, lot No. 377006 A), at dose (1 ml)/week.

ELISA technique (Cain and Susan, 1979; Sakanari et al., 1988) and Hudson and Hay, 1989) was applied in the present study for the immunodetection of both antigens and antibodies. The sensitivity and reactivity of Amplicaecum larval haemoglobin were recorded. The ELISA technique was read on titertek plate reader at wave length 490 nm against blank and negative controls at 15 & 20 min. The estimation of the protein concentration per gram % was recorded with the normal scan mode for pre-bleed sera (normal sera), post bleed sera (positive immune sera), Amplicaecum haemoglobin and Clinostomum haemoglobin through the monitoring of UV absorption spectrum at wave length 280 and 200-500nm (Cecil 3000 instrument) (Hudson and Hay, 1989).

RESULTS

The results are represented in the following tables and plates:

Table (1) ELISA technique showed a higher optical density in the titer 1/20 at 15 min, indicating a positive serodiagnostic reaction in comparison with blank and negative controls.

Table (2): ELISA technique showed a higher optical density in the titer 1/20 at 20 min. indicating a positive serodiagnostic reaction in comparison with blank and negative control.

Plate (1): Positive immune sera were higher when reacted with Amplicaecum larval antigen compared with pre-bleed sera (normal serum) in ELISA.

Plate (2): Positive immune sera were recognized Amplicaecum larval antigen but not Clinostomum larval antigen (Trematoda); ELISA, no sharing epitopis.

Plate (3): Normal scan mode (frequency curve) for pre-bleed sera (normal sera) at wave length 200-550 nm showed 2 peaks.

Plate (4): Normal scan mode (frequency curve) for post bleed sera (positive immune sera) at wave length 200-550 nm showed 6 peaks.

DISCUSSION

Positive immune sera against the Amplicaecum larval haemoglobin was prepared experimentally

Plate (1): Positive immune sera showed a higher sensitivity when reacted with Amplicaecum larval antigen compared with prebleed sera (normal serum) in ELISA

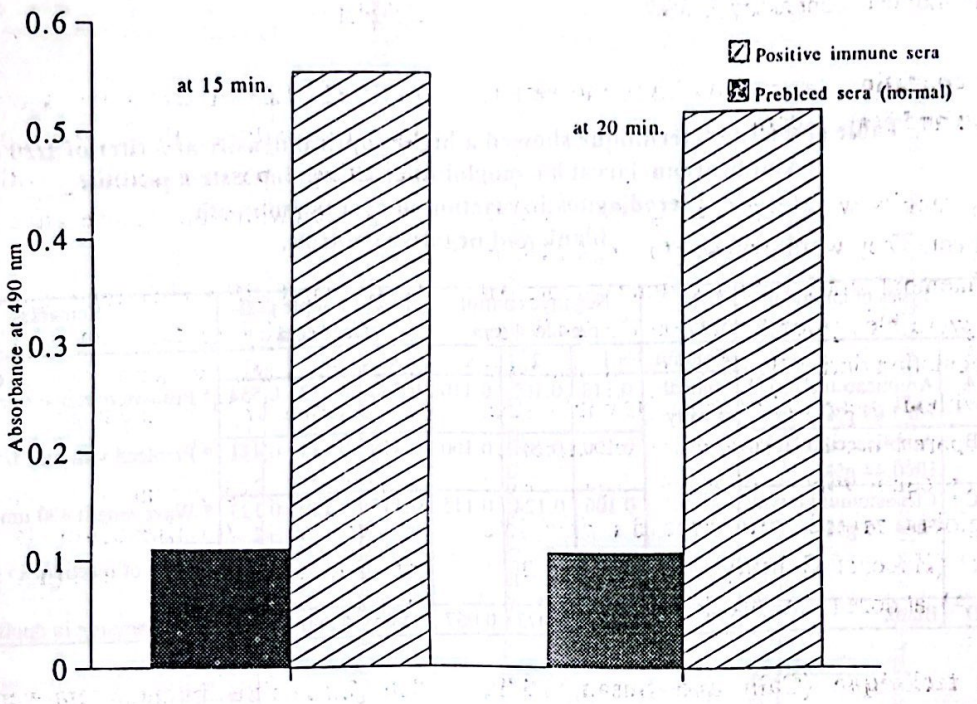

Plate (2): Positive immune sera recognized Amplicaecum larval antigen but not Clinostomum larval antigen (Trematoda) in ELISA, no sharing epitopis

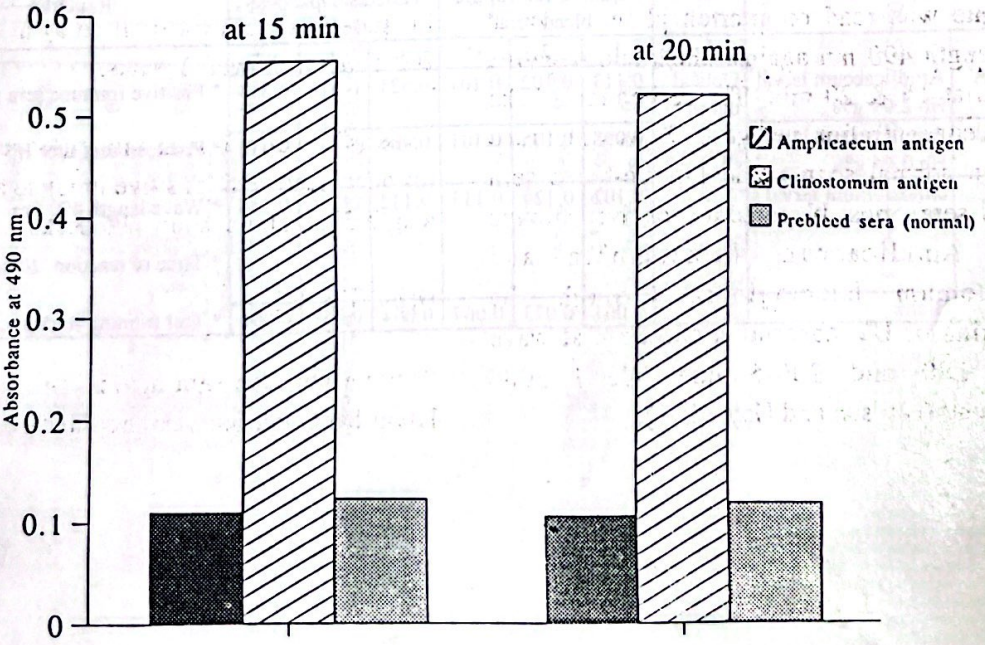

Table (1): ELISA technique showed a higher optical density at a titer of 1/20 of Amplicaecum larval haemoglobin at 15 min indicate a positive serodiagnostic reaction in comparison with blank and negative controls.

Types of larval Hb		Titer 1/20	Negative control pre-bleed sera			Tested sample post-bleed sera			Remarks
		Column	1	2	$\bar{x}$	3	4	$\bar{x}$	
A	Amplicaecum larval Hb 2.09 g%	Optical density	0.118	0.102	0.110	0.556	0.552	0.554	* Positive immune sera titer 1/5
B	Amplicaecum larval Hb 0.44 g%		0.100	0.107	0.100	0.418	0.444	0.431	* Prebleed sera titer 1/5
C	Clinostomum larval Hb 4.75 g%		0.106	0.124	0.115	0.117	0.129	0.123	* Wave length 490 nm * Time of reaction 15 min
D	Blank		0.061	0.073	0.067	0.065	0.060	0.062	* Test running in duplicate

Table (2): ELISA technique showed a higher optical density at a titer of 1/20 of Amplicaecum larval haemoglobin at 20 min indicate a positive serodiagnostic reaction in comparison with blank and negative controls.

Types of larval Hb		Titer 1/20	Negative control pre-bleed sera			Tested sample post-bleed sera			Remarks
		Column	1	2	$\bar{x}$	3	4	$\bar{x}$	
A	Amplicaecum larval Hb 2.09 g%	Optical density	0.113	0.102	0.107	0.521	0.516	0.518	* Positive immune sera titer 1/5
B	Amplicaecum larval Hb 0.44 g%		0.098	0.105	0.101	0.405	0.432	0.418	* Prebleed sera titer 1/5
C	clinostomum larval Hb 4.75 g%		0.102	0.124	0.113	0.112	0.125	0.118	* Wave length 490 nm * Time of reaction 20 min
D	Blank		0.061	0.073	0.067	0.064	0.059	0.061	* Test running in duplicate

Plate (3): Normal scan (frequency curve) for prebleed sera (normal sera) at wave length 200-550 nm showed 2 peaks.

Plate (4): Normal scan mode (frequency curve) for postbleed sera (positive immune sera) at wave length 200-550 nm showed 6 peaks.

in rabbits according to the methods of Hudson and Hay (1989). In the present study, the two fold dilution of Amplicaecum larval haemoglobin reacted specifically at a titer 1/20 with the positive immune sera 1/5 in PBS using ELISA technique the recommended a single wave length 490 nm and serodiagnostic reaction within 15 and min (Tables 1 and 2) (Cain and Susan, 1979; Shuji et al. (1986). The highest optical density (O.D) of the tested larval haemoglobin was recorded by a vertical photometry system (Dynatech mircoplate reader) as 0.316 at 15 min and 0.351 at 20 min within two fold serial dilution. A titer of 1.20 of Amplicaecum larval haemoglobin recorded the highest optical density x 0.554 in comparison with the blank and negative control at 15 and 20 min., respectively in the test proper (Plates 1 and 2). The protein concentration (Suzuki et al., 1974) of Amplicaecum larval haemoglobin amounted to 0.44 g% and 2.09g% showing a positive reactivity and sensitivity with the prepared positive sera (Tables 1 and 2). The Clinostomum larval haemoglobin (Trematoda) amounted to 4.75g% indicating a negative reaction with the pre pared positive immune sera in comparison with the blank and negative control that proved no sharing epitopis between it and Amplicaecum larvae (Tables 1 and 2 and Plate 2). The significance of the prepared positive immune sera in laboratory (Plate 4) against the Amplicaecum larval haemoglobin emphasized a good candidate in tracing the accidental infestation in human, animals and birds when a planning scheme recommended to explore the biological cycle and zoonotic importance of the parasite in future.

REFERENCES

Baylis, H.A. (1920): On the classification of the Ascaridae. 1. The systematic value of certain characters of the alimentary canal. In systema Helminthum: The nematodes vertebrates, Part 3, Interscience Publishers, New York.

Cain, G.D., Susa, W. (1979): Immunochemical detection of Anisakine nematode larvae. Manual of Methods and Procedures. Department of Biology, University of Iowa, Iowa, USA: 52242: 1-70.

Hudson, L. and Hay, e.C. (1989): Practical Immunology. 3rd ed. Blackwell scientific Publications, London.

Mahmoud, N.A.M.; Ibrahiem, M.M. and Abdel-Gawad, A.F. (1989): Acquisition of Ascarididea larvae by tilapia nilotica and T. galilae in High dam Lake. Agric. Res. Rev., 67 (2): 261-270.

Sakanari, J.A. (1990): Anisaki from the Platter to the Microfuge. Parasitology Today, 6 (10): 323-327.

Sakanari, J.A.; Margarita, L.; Thomas, L. Deardroff, Richard B. raybourne, James H.M. and Gordon, F. (1988): Intestinal Anisakiasis. A case diagnosed by morphologic and immunologic methods. American J. of Clinical Pathology, 90 (1): 107-113.

Shjui Takahashi, Noriyuki sato and Hajime Ishikuro (1986): Establishment of monoclonal antibodies that discriminate the antigen distribution specifically found in Anisakis larvae (type I). The Journal of Parasit. 72 (6): 960-962.

Suzuki, T.; Sato, Y.; Yamashita, T.; Sekikawa, H. and Otsura, M. (1974): Anisakiasis: Preparation of stable antigen for indirect fluorescent antibody test. Exp. Parasit. 35: 418-429.