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WESTERN BLOTTING AS SERODIAGNOSTIC TECHINQUE FOR DETECTING *ANISAKIS* *sMPLEX* LARVAL HAEMOGLOBIN

By

N.A.M. MAHMOUD*

* Fish Diseases Department, Animal Health Research Inst., Dokki, Giza, Egypt.

SUMMARY

The *Anisakis simplex* larvae are one of the important zoonotic parasites infesting marine water fish. The homogenates of larvae showed a considerable sensitivity and specific reactivity. Moreover, western blotting technique proved that larval haemoglobin of *Anisakis simplex* included specific antigenic properties at 32 KDa relative molecular weight, while sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) 12% characterized 16 heterologue polypeptide bands with molecular weights ranging between 76 and 23 KD a. The total protein in larval haemoglobin (Hb) is calculated through the UV absorbance technique as 1.7 g%.

INTRODUCTION

The *Anisakis simplex* larvae are one of the causative agents of human anisakiasis in countries where raw, smoked, salted or improperly prepared sea fish and squid dishes are commonly eaten (Sakanari, 1990). The larval haemoglobin is the sensitive probe for identification, diagnosis and characterization in Ascarididae species by calculation of their protein concentration and through the migration of charged macromoleculaes under the influence of an electric field used mostly for separation and elution from gels (Laemmli, 1970; Cain and susan, 1979 and Deutscher, 1990). The western blotting technique is rapid and sensitive to transfer the separated proteins from the SDS-PAGE onto the surface of a thin matrix where the proteins are immobilized on the surface of the substrate and are readily accessible by application of an electric field perpendicular to the plane of the gel (Harris and Angal, 1989). The objective of the present study is a better understanding of the function and characterization of larval *Anisakis* haemoglobin for utlization in serodiagnostic techniques.

MATERIAL AND METHODS

A total of 100 larvae of *Anisakis simplex* were isolated and identified from Rockfish (*Sebastes paucispinis*) of Pacific Ocean, San Francisco Bay area. The parasites were washed 5 times with phosphate buffered saline (PBS), homogenized without addition of antibiotic and centrifuged at 1400 rpm for 10-15 minutes under cooling conditions (Suzuki et al., 1974 and Sakanari and McKerrow, 1989). The supernatant was filtered through 0.4 µm sterile single use filter and stored at -70°C in Sarstedt tube 15ml and used as haemoglobin. The total protein is calculated through UV absorbance technique in WL-280 nm (Hudson and Hay, 1989). The haemoglobin is characterized through matching with standard high and low molecular weights (Bio Radm 161.0303 & 0304) ranging between 200-14 KDa (Laemmli, 1970), in Fischer biotech. Vertical and one dimension instrument (Mahmoud and Siam, 1994). The SDS-PAGE 12% is applicable for western blotting technique in Mine transferring blotting wet cell system at 120 volt and 23C

m.Amp. 45 min. using nitrocellulose (NC) paper 0.2 μm (Harris and Angal, 1989). The nitrocellulose matrix is stained with Ponceau S stain (sigma) in 5 min., then washed with distilled water and the (NC) paper was divided into two parts and blocked with 2% gelatin/1hr in tris buffered saline pH 7.5 (TBS) to cover the nonspecific antigen, washed with distilled water, then one part was incubated with prebleed sera and the other part with postbleed of rabbit anti-Anisakis immunoglobulins as primary antisera and kept overnight at room temperature in dilution 1:10,000 in TBS. Blots were washed 3 times, 2 times for 30 min. in Tween (20) tris buffered saline pH 7.5 (TTBS) and then once for 15 min. in TBS, respectively. The (NC) paper of each part was incubated for 2 hr with goat anti-rabbit IgG sera conjugated to horse radish peroxidase 1:100 TBS (Boehringer Mannheim Biochemicals, Indianapolis, Indiana). Blots were washed 3 times in TTBS and then TBS/15 min. then visulaized in 30 min. with 4 chloro-1 naphthol (Sigma Chemical Co, St. Louis, Missouri) 60 mg in 20 ml methanol, 80 ml TBS and 100μl 30% H202 (Sakanari and McKerrow, 1989). Blue dark band indicates positive NC matrix treated with postbleed rabbit anti-Anisakis immunoglobulins, while control is colourless in NC matrix treated with prebleed rabbit sera in 30 min. (Harris and Angal, 1989).

RESULTS AND DISCUSSION

The present study revealed the presence of 16 heterologue polypeptide chains through the characterization of larval *Anisakis simplex* haemoglobin in SDS-RAGE 12% (Table 1) and Fig. (1). The electroimmunodiagnostic techniques of protein through SDS-PAGE are sensitive for diagnosis and identification, a finding supporting the methods described by Laemmli (1970). Moreover, two relative molecular weights at 25.5 and 36.5 KDa were demonstrated in the larval *Anisakis simplex* haemoglobin (Table 1 and Fig. 1), which were comparable with those of Ascarideal haemoglobin of adult stage *Neoascaris (Toxocara) vitulorum,* adult stage *Ascaridia galli* and larval stage of *Amplicaecum* infesting cattle, poultry and aquatic life, respectively. These results agree with the previous findings of Mahmoud et al. (1993) and Mahmoud and Siam (1994) who recorded a strong relationship between the antigenic properties of Ascarideal species. A relative molecular weight at 32 KDa through the application of western blotting serodiagnostic technique indicated the presence of a sensitive and specific antigenic polypeptide band from 16 heterologue polypeptide chains in SDS-PAGE, thus matching with the methods carried out in enzymatic excretory and secretory (ES) products described by Harris and Angal (1989) and Sakanari and McKerrow (1989). The application of specific rabbit anti-*Anisakis simplex* immunoglobulins as suggested by Curtis and Merrill (1967) revealed a sensitivity for detection of *Anisakis* haemoglobin (specific antigen) that was transferred onto nitrocellulose matrix and is indicated with dark blue band, The dark blue band at molecular level 32 KDa was pronounced in nitrocellulose matrix indicating a positive reactivity of *anisakis* haemoglobin anti-anisakis immunoglobulins in the presence of goat anti-rabbit sera conjugated with horse radish peroxidase and 4 chloro-1-naphthol as indicators (Fig. 2).

From the present study, it is evident that the western blotting is proved to be a rapid and sensitive technique in the detection of the parasitic haemoglobin and its enzymatic production in the tissues of seawater and freshwater fish infested with Ascaridiaeal parasites as well as in biopsy of tissues in Anisakiasis (Sakanari and McKerrow, 1990).

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Table (1): The number of heterologue polypeptide chains of larval *Anisakis simplex* haemoglobin in relation to the relative molecular weights in SDS-PAGE.

Haemoglobin species	Heterologue polypeptide chains	Relative molecular weight (MW/KDa)							
		76	65	56	53	50	47	41	39.5
Anisakis simplex larvae	16	1	1	1	1	1	1	1	1
		37	36.5	34	32	31	27.5	25.5	13
		1	1	1	1	1	1	1	1

Table (2): The calculation of larval *Anisakis simplex* haemoglobin (Hb) through running in SDS-PAGE immunoelectrophoretic techniques.

Haemoglobin species	Protein concentration (microgram/microliter)	Distilled water (microliter)	Sample buffer (microliter)	Total sample (microliter)
Anisakis simplex larvae	17	19	5	25

Technical specification:

* Instrument: Vertical slab gel, 12% SDS-PAGE, 8x9 cm^2 x 1.5 mm.
* Time: 60 minutes.
* Electric power: 85/105 volt, 20mAmp
* Type: Fischer biotech one dimension

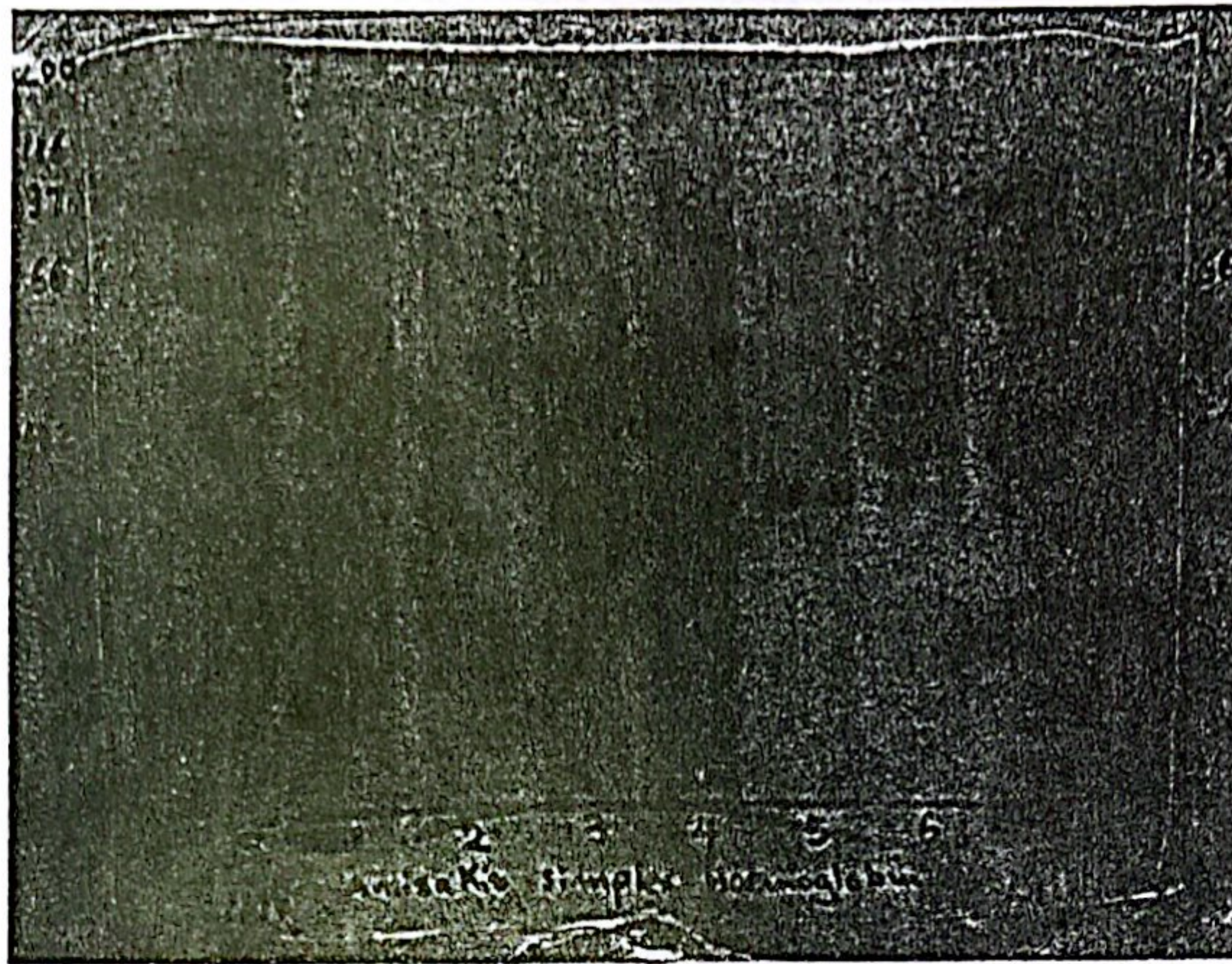

Fig. (1): The Characterization of Larval *Anisakis simplex* Hb in SDS-PAGE in relation to standard molecular weights.

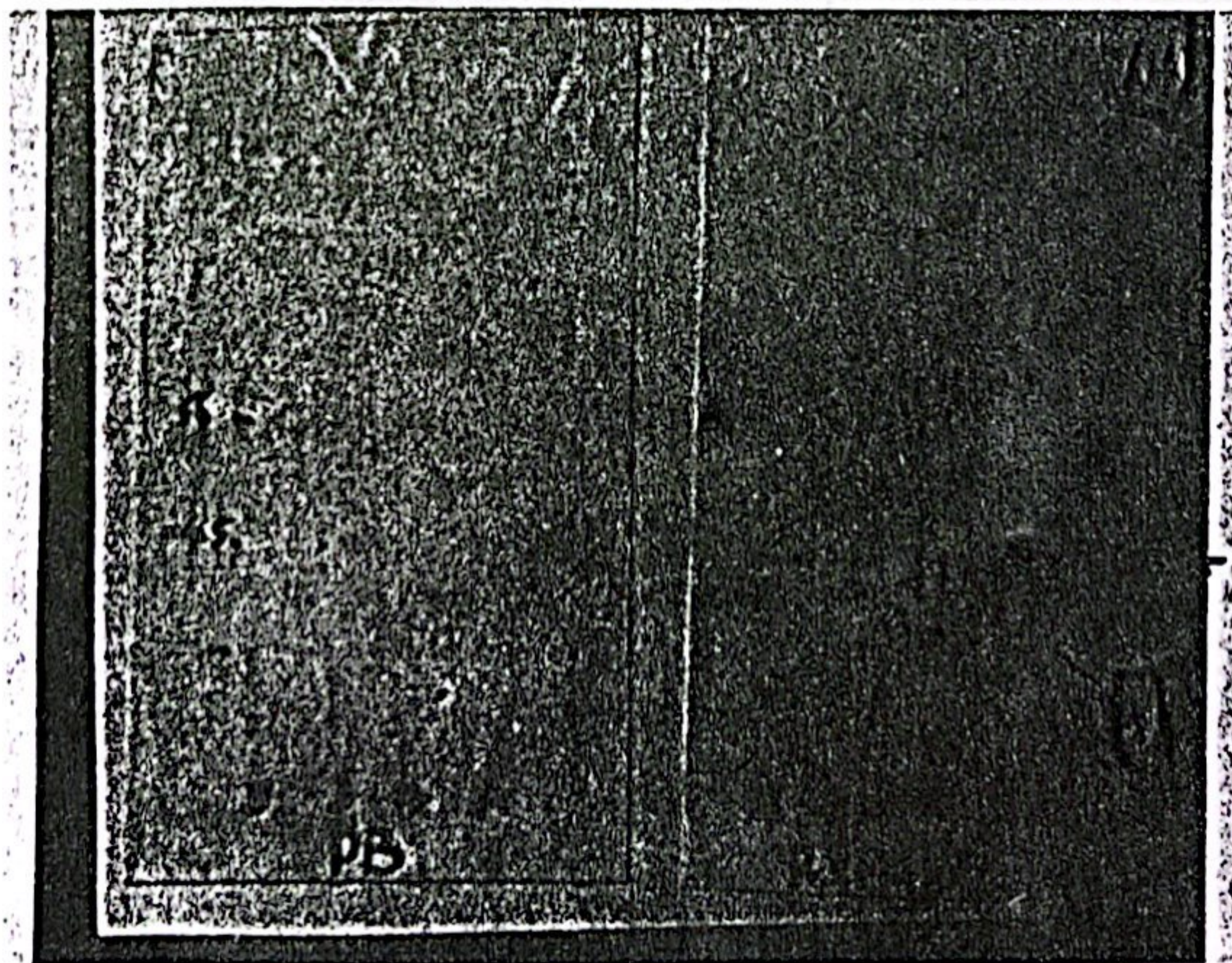

Fig. (2): The western blotting technique indicating specific antigenic chain at 32 KDa relative molecular weights in nitrocellulose matrix.

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