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COMPARATIVE ANALYSIS OF ASCARIDIAL PARASITIC CRUDE ANTIGEN BY SDS-PAGE AND UV ABSORBANCE TECHNIQUES

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SUMMARY

Ascaridial nematodes are among the well-known parasites infesting fish, birds and mammals. Crude antigen was extracted from *Amplicaecum* larvae (AL_3), *Ascaridia galli* (adult worm-AG) and *Neoascaris vitulorum* (adult worm-AV). The characterization of these parasitic nematodes revealed relative homologous polypeptide chains (MW 25.5 KDa) common to the three parasitic species, whereas the UV absorbance scan mode wave length (WL 200/550 nm) as related to the absorbance (AB) value zero-2 recorded an indicative frequency curve. The protein concentration in *Amplicaecum, Ascaridia* and *Neoascaris* figured up to 5.47 g%, 4.93 g% and 2.20 g%, respectively.

INTRODUCTION

Ascaridial crude antigen has been used in serodiagnostic techniques where the functional protein evidenced self markers of antigenic properties (Morris, 1991). The specific parasitic protein molecule of crude antigen can be identified through sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and the characterization of protein appeared in specific heterogeneous polypeptide chains whcih indicated the relative molecular weights (Sakanari and McKerrow, 1990, Xie et al., 1992 and Mahmoud et al., 1993). The protein concentration and characterization may serve as an initial step toward the development of vaccine and may contribute to the general understanding of host-helminth interaction (Suzuki, 1973). The family Ascaridiae comprises several species of parasites that are capable of infesting man, animals and aquatic life (Soulsby, 1982). Moreover, it can produce marked tissue damage and immunopathologic reactions (Morris, 1991). Migration of larval Ascarididae have induced acute pathological lesion in paratenic host either animal, aquatic life or human other than final host (Ilan Paperna, 1980 and Essa and Hafex, 1982).

A plot of wave length against the degree of absorption for a given substance results in a curve composed of a number of peaks. The shape of this absorption curve or absorption specturm is characteristic for configuration and composition of a molecular species, the absorption spectrum, therefore, can be used for identification of a substance (Campbell et al., 1963). The present study is a trial to prove whether Ascarididae parasitic nematode crude antigen shares features common to three species of this family. Moreover, it is aimed to determine the protein concentraiton in the crude antigen of the three parasitic species to be applied as an aid in immunodiagnostic techniques striving to the development of a poly- and mono-valent antisera as well as the possible production of a specific vaccine.

MATERIAL AND METHODS

A total number of 30 larval and adult worms related to *Amplicaecum* type larvae (AL_3), *Ascaridia galli* (AG) and *Neoascaris vitulorum* (AV) were used in this study. The Ascarididae parasitic species were procured from freshwater fish *Tilapia* species, chicken and cattle and washed with PBS. The parasites were homogenized in porcelain mortar then centrifuged at 14000 RPM for 5 minutes. The supernatant was filtered through 0.45 Mm Nalgene cellulose acetate No. (190245). The precipitate was discarded and the supernatant was used as crude antigen (functional protein) (Sakanari and McKerrow, 1990). The protein con-

centration was evaluated by UV absorbance technique at wave length (WL 280 nm) and factor of dilution 10. The absorbance (Ab) scan mode was recorded with wave length 200-550 nm in relation to absorbance value zero to 2 for the haemoglobin (Hb) of the 3 parasitic species. (Campbell et al., 1963) using (CECIL 3000 instrument). The crude antigen was running through SDS-PAGE (Laemmli, 1970). The method of detection was done in 1 dimension vertical slab gel, 10% SDS-PAGE 1 mm in thickness, 10 cm2 in diameter and the protein was denaturated with B-mercaptoethanol at 100°C/5 minutes. The electric power was adjusted firstly at 90 volts then 120 volts, 10 mA in 60 minutes with standard protein molecular weight marker (MW) as quality control (Rainbow mix, Code RPM 756, Amersham). The gel was stained with Coomassie brilliant blue stain (R 250 Sigma) then decolourized and fixed. The molecular weight of heterogeneous polypeptide chains and number of protein subunits were recorded according to Hudson and Hay (1989).

RESULTS

The results are recorded in the following tables and figures:

1- Electrophoretic characterization of the crude antigen of *Amplicaecum* larvae (AL_3), *Ascaridia galli* (AG and *Neoascaris vitulorum* AB) adult worms at the molecular level, Table (1), Fig. (1).

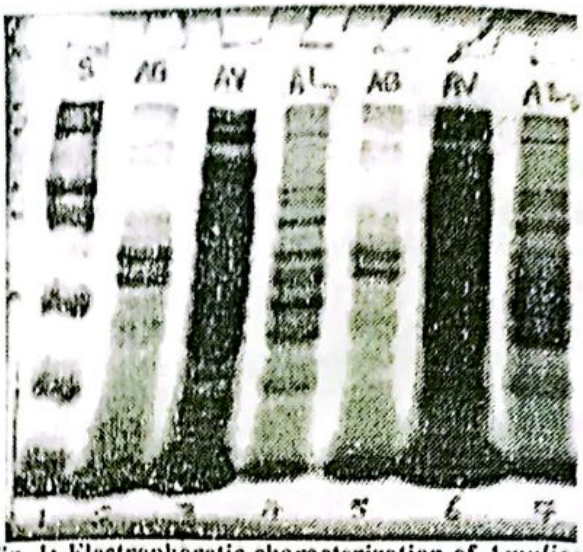

Fig. 1: Electrophoretic characterization of *Amplicaecum* larvae (AL3) , *Ascaridia galli* (AG) and *Neoascaris vitulorum* (AV) crude antigen at the molecular level by SDS-PAGE.

2. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SS-PAGE) technique, Table (2).

3. Absorbance scan mode (frequency curve) of *Amplicaecum* larvae (AL_3) haemoglobin through UV absorgance (AB) techique showing specific curve with three peaks of (Ab) at wave length 214.5, 258-5 and 405nm (Instrument CECIL, CE 3040 Cambridge-England), Plate (1).

4. Absorbance scan mode (frequency curve) of *Ascaridia galli* (AG) adult worm haemoglobin

Table (1): Electrophoretic characterization of *Amplicaecum* larvae, *Ascaridia galli* and *Neoascaris vitulorum* adult worms crude antigen at the molecular level.

Haemoglobin species	Lane No.	Heterogeneous polypeptide bands	Relative molecular weight (MW/KDa)															
			69	50	49	36.5	34.5	32.5	33	27.5	26	*25.5	23	20.5	20	17.5	15	10
1. *Amplicaecum* larvae L_3	4.7	9	-	1	-	1	-	1	1	1	-	1	-	-	1	1	1	-
2. *Ascaridia galli* (adult worm)	2.5	2	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-
3. *Neoascaris vitulorum* (adult worm)	3.6	11	1	1	1	-	1	-	-	-	1	1	1	1	-	1	1	1

* Homologous sharing polypeptide bands between the 3 different Ascaridilidial parasitic haemoglobin at molecular level.

Table (2): Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique.

Haemoglobin species	Lane No.	Protein concentration microgram/microliter	Distilled water microliter	Sample buffer microliter	Total sample microliter
1. *Amplicaecum* larvae L_3	4.7	5.47	17	5	25
2. *Ascaridia galli* (adduli worm)	2.8	4.93	16	5	25
3. *Neoascaris vitulorum* (adult worm)	3.6	2.20	11	5	25

Technical specification
Instrument: Vertical slab gel 10% SDS-PAGE.
Time 60 minutes
Electire power. 90/120 volts. 20 mA.

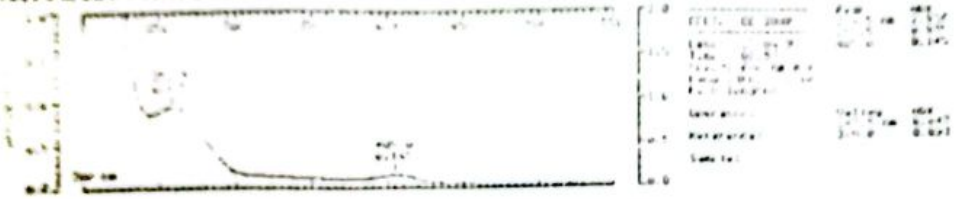

Plate (1): Absorbance scan mode (frequency curve) of *Amplicaecum* larvae (AL_3) haemoglobin through UV absorbance technique showing 3 peaks

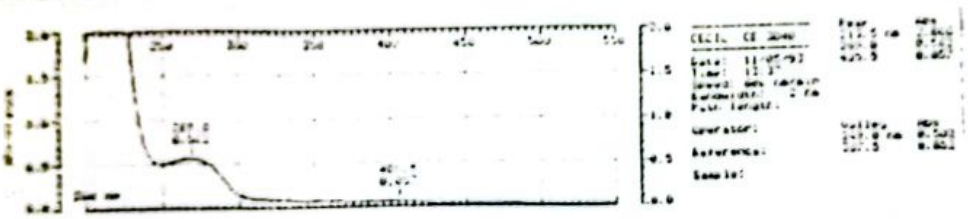

Plate (2): Absorbance scan mode (frequency curve) of *Ascaridia galli* (AG) haemoglobin through UV absorbance technique showing 3 peaks.

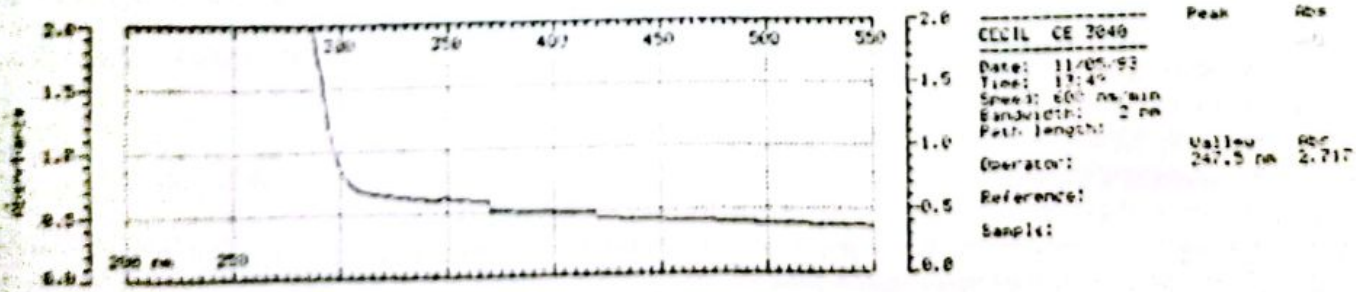

Plate (3): Absorbance scan mode (fequency curve) of *Neoascaris vitulorum* (AI.3) haemoglobin through UVabsorbance technique showing 3 peaks.

through UV absorbance (Ab) technique showing specific curve with three peaks of (Ab) at wave length 213.5, 269 and 405.5, (Instrument CECIL, CE 3040, Cambridge-England), Plate (2).

5. Absorbance scan mode of *Neoascaris vitulorum* (AV) adult worm haemoglobin through UV absorbance (Ab) technique showing specific curve with no peak of (Ab), (Instrument CECIL, CE 3040, Cambridge-England), Plate (3).

DISCUSSION

The present study characterized the haemoglobin (Hb) of 3 Ascaridial parasitic species recovered from fish, poultry and cattle in Egypt through immunodiagnostic electrophoretic techniques. The results of SDS-PAGE vertical slab at the molecular level Fig. (1), coincided with those of the methods described by Sakanari and McKerrow (1990), Xie et al. (1992) and Mahmoud et al. (1993). The comparative analysis in Ascaridial parasitic crude antigen in the present study proved a unique structure in each parasitic nematode species. These figures amounted to 9, 2 and 11 heterogeneous polypeptide chains for *Amplicaecum* type larvae (AL_3), *Ascaridia galli* (adult stage AG) and *Neoascaris vitulorum* (adult stage AV), repectively (Fig. 1 and Table 1). However, the crude antigen shares many features in common, where it contains a homologous polypeptide chain at the molecular level 25.5 KDa, a finding that supports the report of Morris (1991). The immunodiagnostic electrophoretic technique through SDS-PAGE proved a relationship between *Amplicaecum* larvae (AL_3) and *Neoascaris vitulorum* at the molecular level 15, 17.5 and 50 KDa (Table 1 and Fig. 1) Moreover, another relationship between *Ascaridia galli* and *Neoascaris vitulorum* at the molecular level 26 KDa was noticed (Table 1 and Fig. 1). The absorbance (Ab) scan mode was recorded in frequency curve for the Ascaridial parasitic crude antigen in each of the three parasitic species. This curve is proved to be in the form of indicative and characteristic plot of wave length against the degree of absorpton. This result substantiates what has been reported by Campbell et al. (1963). The shape of this absorption curve or absorption spectrum is characteristic for configuration and composition of a molecular crude antigen species as shown in Plates (1, 2,3). The protein concentration was found to be 5.47 g%, 4.93g%, and 2.20 g% in AL_3, AG and AV, respectively. These results coincided with average percentage recorded by Mahmoud et al.(1993) using the applicable methods of Campbell et al. (1963) and Hudson and Hay (1989) in estimating the protein concentration through UV absorbance techniques. The present study has proved the main relationship and specification between crude antigen of the different ascarids which can be applied in immunodiagnostic techniques for the development of poly- and mono-valent antisera as well as the possible production of a specific vaccine for prophylaxis and control of ascaridiidiasis in human and animal hosts.

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