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CHARACTERIZATION OF *Toxocara canis* and *Toxascaris leonina* A DOG'S INTESTINAL NEMATODES BY SDS-PAGE ELECTROPHORESIS

ISRA'A M. ESSA*

*Dept. of Microbiology & Veterinary Parasitology, College of Veterinary Medicine, University of Basrah, Basrah, Iraq

ABSTRACT

A total of twenty one male and female worms of *Toxocara canis* and thirteen male and female worms of *Toxascaris leonina* were isolated from intestine of stray dogs which necropsy by using strygnine tablet in Basrah city in the southern Iraq. The result of protein concentration was (0.118,m0.131) for male and female of *Toxocara canis* and (0.062, 0.269) for male and female of *Toxascaris leonina*. While, The proteins bands found in both *T. canis* and *T. leonina* was three in both male and female but with different molecular weights, in *T. canis* were varied between (10-100 K. D), while, in *T. leonina* (20-75 K. D).

KEYWORDS: *Toxocara canis*, *Toxascaris leonina*, protein concentration, gel electrophoresis, Protein bands.

INTRODUCTION

The genus *Toxocara* is a common roundworm includes *Toxocara canis* and *Toxocara cati* which infect dogs and cats respectively (Glickman *et al.*, 1986).*Toxocara canis* a cosmopolitan parasite of canines and the major agent of human toxocariasis constitutes a serious epidemiological problem in many countries (Fan *et al.*, 2003). Worldwide surveys of *T. canis* infection occurrence showed that different results. Mundim *et al*. (2001) showed infection rate of *T. canis* was 9.5% when they examined 105 faecal samples from dogs in Brazil. In North Central Colorado, USA, a total of 130 faecal sample from dogs showed that 3.1% was the infection rate with *T. canis* (Hackett and Lappin, 2003). Also, Nobel *et al*. (2004) found that the infection rate with *T. canis* eggs was 8.5% when they examined 224 faecal samples from dogs in Natherland. The major clinical consequences of prolonged migration of *T. canis* larvae in human are visceral larva migrans (*VLM*) and ocular larva migrans (*OLM*) (Despommier, 2003).

T. canis, *T. cati* and *Toxascaris leonina* are nematode round worms of the family Ascaridae, whose adult forms live in the proximal small intestine of their mammalian definitive hosts (Canids and Felids) (Glickman and Schantz, 1981; Glickman et al., 1986).

T. canis infects canids and non-canids in all tropical and temperate regions of the world, while infect human and other mammals as accidental hosts, and it is the primary cause of *VLM* in humans, and toxocariasis is a systemic larval parasitosis that may affect humans of different age or sex (Radman *et al*., 2000).

Al-asadi and Al-emarah (2014) recorded that the nematodes *P. skarjabin* adult worms have protein with molecular weight was 72 KD which detected by *SDS –page* electrophoresis.

Because of there is no studies about proteins content quantitative and qualitative to both worms *T. canis* and *T. leonina*, so, the present study was designed to characterized the proteins of *T. canis* and *T. leonina* adult worms by *SDS-page* electrophoresis for further immunological study and other studies.

Materials and Methods

A total of twenty one male and female worms of *T. canis* male and female worms of *T. leonina* were isolated from intestine of stray dogs which necropsy by using strygnine tablet from different region at Basrah city at the period between June 2012 to June 2013 at the College of Veterinary Medicine in University of Basrah, and the intestine of each dog was open longitudinal, then each nematod found was removed gently and put in petri dish then washed many times with distilled water, each genus of worms were identified according to general and specific characters with taxonomical key (Yamaguti, 1961).

Each worm were mixed with Phosphate buffer saline PBS PH 7.4, then disrupted in a glass-in-glass mortar held homogenizer 4ºC for 1h .The homogenate was kept undisturbed for 24h in refrigerator. Supernatant collected from homogenate was centrifuged in cooling centrifuge at 1200rpm for 15min .Then, the Supernatant was stored as crud solubilized at 20ºC till further use (Mir *et al*., 2008). Protein concentration of crud supernatant were estimated by spectrophotometric assays at 235,280nm according to the method of (Mir *et al*., 2008).

The protein content in mg/ml was calculated by the following equation.

Protein content mg/ml= (A235-A280/2.51).

A= absorbance

Gel electrophoresis technique by sodium dodecyle sulfate is performed for each worm supernatant by using the method (Laemmli 1970). Gel is placed on white paper and calculator the distance traveled by the pigment and the distance traveled by the packet protein, in order

to, extract the relative value of protein immigrant (RF) which was: The distance travelled by protein migration / the distance travelled by pigment.

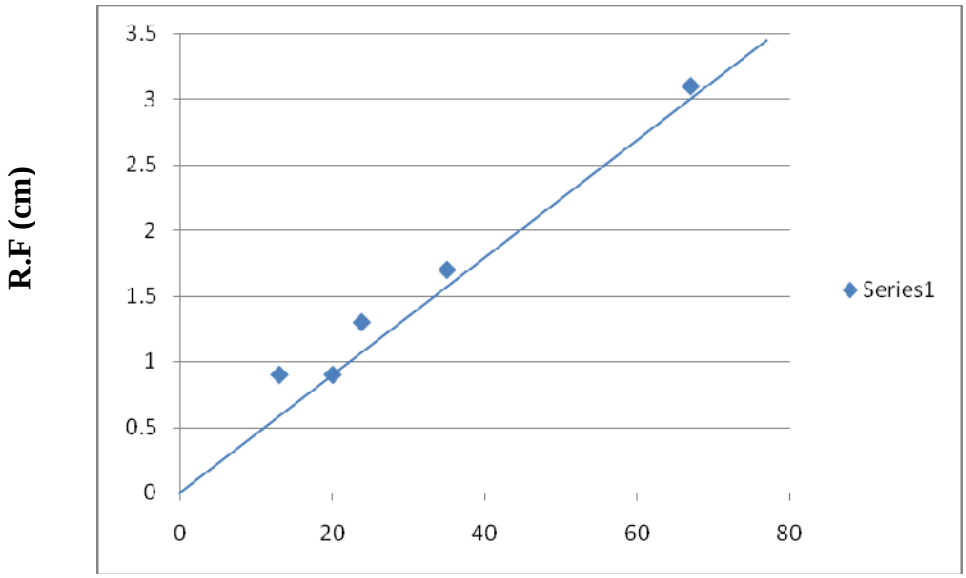

Figure 1. The linear equations of stander proteins

Results and Discussion

The result of protein concentration was differences between male and female and between the two different genus, T. canis found that the protein concentration in male (0.118) while, in female (0.131), but in T. leonina was (0.062, 0.269) for male and female respectively. No significant differences were found.

The curve of standard proteins under this study was: Bovine serum albumin (67 K.D), Procine pepsin (35K.D), Trypsein (23.8 K.D), Alkaline Phosphatase (13 K. D), Peptone (20 K. D) and the relative distance as in (table, 1).

Table 1. Standard proteins with molecular weights

PROTEIN	SOURCE	MW (Da)	R.F (cm)
Bovine serum albumin	bovine serum	67.000	3.1
Procine pepsin	porcine stomach	35.000	1.7
Trypsein	soybean	23.8000	1.3
Alkaline Phosphatase	rabbit muscle	13.000	0.9
Peptone		20,000	0.9

The proteins bands found in both *T. canis* and *T. leonina* was three bands in both male and female but with different molecular weights, in *T. canis* was varied between (10-100 K. D), while, in *T. leonina* (20-75 K. D) (table 2) and (Fig. 2).

Table 2. Protein bands and molecular weights with relative distance for both *T. canis*, *T. leonina*

Parasite	R.F (Cm)	M.W. (Da.)
T. canis	0.5	10
	4.5	90
	5	100
T. leonina	1	25
	2.5	50
	3.5	75

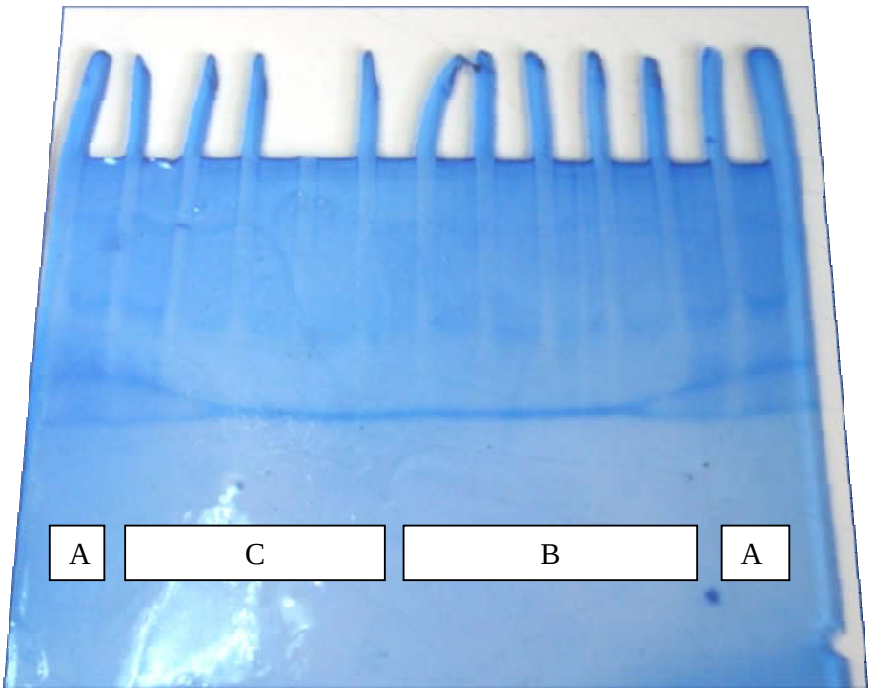

Figure 2. The protein bands from *T. canis* (B) and *T. leonina* (C) protein by gel electrophoresis technique (A):bands of stander protein

Cats and dogs are the natural hosts of the parasitic disease, called toxocariasis, it is produced by nematodes *Toxocara* and *Toxascaris* genera and *T. canis* a dog ascarids being the most frequent (Schantz and Glickman, 1981).

Infection with parasites induces a variety of immunological alterations in the hosts (Wang *et. al.*, 1995), so, it must be know about these parasites as a protein concentration and the molecular weight or by other words know about the quantitative and qualitative of proteins inside parasites.

The protein concentration found under this study varied between male and female, in female looks like high in male with regard to authors unpublished information and data, the reproductive system and vitelline glands and ova cause a high proteins amount and molecular weights.

The current study including electrophoresis of proteins which were isolated from adult worms of T. canis and T. leonine showed three bands only found d in both which molecular weight varied between (10-100, 20- 75 K.D) respectively.

Few studies were done about isolation protein from different parasites and each parasite has bands with number and molecular weight which different from others for examples, Al-Azizz (2010) recorded two bands from adult of *Taenia hydatigena* with molecular weight (22, 70 KD). Kara and Doganay (2005) found in *Cysticercus tenuicollis* two clear bands with molecular weight 38 and 42.5 KD. Al-Emarah (2007) showed results of molecular weight vary between 80.408 to 102.047 from seven different zoonotic parasites. Al-Bander (2012) showed four bands from the nematodes *Paeabronem skarjabani* which isolated from sheep**.**

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