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RESEARCH ARTICLE

CHARACTERIZATION OF A SPECIFIC PURIFIED PROTEIN FRACTION FOR DIAGNOSIS OF *Cephalopina* NASAL MYIASIS IN CAMELS IN SAUDI ARABIA

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ABSTRACT

Cephalopina titillator is a common parasite that infest camels all over the world and causes nasal myiasis. The infestation is diagnosed only on post-mortem examination of the animal after slaughtering. The goal of the present study was to isolate and characterize a special protein fraction extracted from the larvae of the parasite, which can be used for sensitive and specific diagnosis of early infestation in living camels in Saudi Arabia. Four soluble antigens were extracted from the proximal and distal ends, the surface tegument, and the excretory-secretory (ES) products of living first-, second- and third-stage larvae. Fractionation of the antigens using SDS-PAGE revealed seven protein fractions from the anterior-end antigens with molecular weights (MWs) of 76-85 kD, 58 kD, 47-49 kD, 37-45 kD, 35 kD, 30-32 kD and 23-25 kD. We used a Western blot technique to identify the protein fractions extracted from different larval stages in the sera of infested camels and non-infested camels. The protein fraction with a MW of 58 kD had the highest sensitivity, specificity and validity among all of the protein fractions. This protein is present in large enough quantities in the anterior end of larvae, particularly in the third-stage of development, that the antigenic fractions may be used for diagnosis of parasitic infestation in living camels.

Key words: *Cephalopina titillator*, Camel, Protein fractions, EITB, Saudi Arabia, Nasal myiasis.

INTRODUCTION

Cephalopina titillator causes nasal myiasis in camels throughout the world, but this parasitic infestation is most frequently associated with camel farms in tropical countries where camels are raised (Oryan *et al*., 2008). Myiasis causes weakness, emaciation, growth disturbance and decreased productivity in infested animals (Otranto, 2001). The rate of infestation of *Cephalopina titillator* varies from one country to another, with a prevalence of 46.7% in Iraq (Gegory *et al*., 2004) and 41% in Riyadh, Saudi Arabia (Alahmad, 2002). The prevalence is 67.6% in the same locality and 52% in the eastern area of the Kingdom (Al-Hafof) (Amin, 2005). These data were obtained as from slaughterhouses that investigated the camel heads for research purposes: no serological means are used to diagnose infestation in pre-slaughter camels (Otranto, 2001). Due to the nature of the camel breeding system and practices in various localities, scientific papers concerning the pre-slaughter diagnosis of nasal myiasis in camels are scarce, particularly in the area of serology. Data are available concerning similar parasites such as those reported by (Otranto, 2001) who mentioned that several recently improved immunodiagnostic techniques could be used for diagnosis of myiasis in living animals instead of the traditional post-mortem inspection. (Escartin-Pena and Bautist-Garfias, 1993) used double immune diffusion (DD), counter immune electrophoresis (CIE), thin layer immunoassay (TIA), haemagglutination (HA), and diffusion-in-gel ELISA (DIG-ELISA) to diagnose equine stomach myiasis; DIG-ELISA and TIA were the most sensitive (93.9%). (Goddard *et al*., 1999) used ELISA with crude *Oestrus ovis* antigens to diagnose sheep nasal myiasis and proved that the sensitivity and specificity reached to 97.4% and 97.6%, respectively, compared to post-mortem examination of slaughtered heads. Moreover, (Alcaide, 2005) showed that excretory-secretory (ES) antigens offer a more specific of infestation using ELISA than other antigens of the same parasite. In addition, antigens from first-stage larvae were more sensitive than antigens from later larval stages. Moreover, there is a positive correlation between the number of larvae and the level of IgG in the host's body. However, little information is available about the response of the immune system during myiasis. (Roelfstra *et al*., 2009) identified 14 protein bands using fractionated larval antigens from *Gastrophilus equi* larvae. Only three bands in the range of 17.9-37.8 kD were specific and sensitive in diagnosing an infestation in equines using an electro-immuno transfer blot (EITB) technique. therefore, we aimed to characterize specific and sensitive protein fractions from different stages of camel nasal flies (*Cephalopina titillator*)

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using SDS-PAGE and EITB techniques; we also aimed to evaluate the use of these fractions in the diagnosis of myiasis in living camels.

MATERIAL AND METHODS

We collected *Cephalopina* larvae from the upper part of the nasopharyngeal cavity of freshly slaughtered infested camel heads from Buraidah in the Qassim region of the Kingdom of Saudi Arabia. We identified the larvae according to protocols described by (Zumpt, 1965; Soulsby, 1986).we also collected blood and fecal samples from camels with infested heads and from randomly selected pre-slaughter camels.

Preparation of crude larval antigens

larvae collected from each animal were washed several times using phosphate buffered saline (PBS; pH 7.4). We extracted four soluble antigens from living first-, second- and third-stage larvae namely: anterior end (pre-enteric region), posterior end (the respiratory opening and tegumental ring), surface tegument (after removing the internal layers) and ES products.

Selected crude larvae antigens preparation

Three crude antigens were prepared from each group of larvae: a proximal part, a posterior part, and external body tegumental antigens. We used the technique for crude antigen preparation described by (Cheng and Ko, 1991), with slight modifications. Briefly, the tissues were homogenized in PBS and the contents were sonicated using a Cole-Parmer ultrasonic homogenizer under 150 watt interrupted pulse output at 50% power cycle in an ice bath. The suspension was centrifuged at 10,000 rpm at 4^oC for 1 h. The supernatant was collected and dialyzed overnight in a refrigerator against PBS (pH 7.2) using a dialysis membrane (6-8 KD molecular weight (MW) cut-off). We measured the protein contents according to the process described by (Lowry *et al*., 1951), and stored them at -70^oC until use.

ES product antigens

Cephalopina larvae ES antigens were prepared from living active larvae according to the protocol described by (El-Bahy, 2002), with modifications. The clean active larvae were incubated (2 larvae/1ml) for 3 h at 37^oC in PBS (pH 7.4). The supernatant was separated by centrifugation at 5000 rpm at 4°C for 1 h. The sample was dehydrated using polyethyleneglycol in a molecular porous membrane tubing with a MW cut-off of 6-8 KD (Spectrum Medical Inc., Los Angeles, CA). The protein content was determined and stored at -20°C.

Hyper-immune sera

Rabbit hyper-immune mono-specific-sera were prepared against the previously-prepared antigens according to the process described by (Langely and Hillyer, 1989). We administered an initial subcutaneous injection of equal volume of Freund`s complete adjuvant and three consecutive intramuscular injections of equal volumes of Freund`s incomplete adjuvant over 60 days. We used the double gel diffusion test to evaluate the level of anti-bodies produced before collection from the vaccinated rabbits.

Fractionation of *Cephalopina* larval antigens using SDS-PAGE

The prepared antigens were resolved using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 1.5 mm thick gels, according to the process described by (Laemmli, 1970). We used 12% polyacrylamide gel slabs in Tris-glycine buffer (pH 8.3) under reducing conditions. The stacking gel was 5% acrylamide prepared in 12.5 mM Tris-HCL buffer (pH 6.7) (Sigma Chemical Co.). We used pre-stained low MW standard (Sigma SDS-100B). The comb was adjusted to provide one small well for the standard and one large well for the sample.

Electrophoretic transfer of protein fractions onto nitrocellulose sheet

The electrophoretic transfer of fractionated proteins onto a nitrocellulose (NC) sheet for EITB was performed according to (Towbin *et al*., 1979) using transfer buffer (25 mM tris-base, 192 mM glycine, 20% (v/v) methanol; pH 8.3). The transfer was completed overnight at 10V, 100 mA at 4°C. A longitudinal strip from the side of the NC membrane that contained the whole fractionated antigens was removed and used for immunoreaction. The relative MWs of the visualized bands were estimated from the reference MW standard curve.

Determination of specific protein fractions using EITB

According to the process described by (Towbin *et al*., 1979), We used an EITB technique to determine the specific diagnostic fractions of *Cephalopina* larvae. Longitudinal NC strips (15 x 0.5 cm) were removed and reacted against *Cephalopina*- hyper-immune rabbit sera specific for each antigen, We tested *Cephalopina* infested camels and negative control samples in a 1:100 dilution with 0.5ml of sera per strip. We used horseradish peroxidase conjugated anti-Protein A (Sigma Immunochemicals) in a 1:1000 dilution in 3% bovine serum albumin/PBS as the conjugate; We used 4-chloro-1-naphthol as the substrate. Fractions that reacted with positive reference sera and did not react with negative control sera were considered specific protein fractions. The strips of NC were returned to their original positions on the NC sheet to determine the size of the protein fractions. We evaluated the specificity of each fraction according to (Rokni *et al*., 2006). We determined the ability of the tested fraction to detect its target antibody from different antibodies of other parasites using the following equation:

Specificity % = (T – P) / T X100 / 100.

where T = the number of tested sample and P = the number of +Ve samples.

Sensitivity is the ability of the antigenic fractions to detect its target antibody in samples infested by the parasite at standard serum dilutions (% of positive sera among the total number of the positive samples).

Evaluation of fecal samples

We examined fecal samples using at least one method, including the direct smear method according to (WHO, 1983) and the concentration floatation technique using saturated sodium chloride solution described by (Wattal *et al*., 1986), to

determine the presence of a parasitic infestation. Large eggs were diagnosed by two successive sieve systems (Fluke finder, Moscow, ID), as described by (Welch *et al*., 1987). We used the collected blood samples for separation of the required serum. We used serum from *Cephalopina*-infested camels that had no other infections in their blood or feces.

RESULTS

We identified seven protein fractions after fractionation of the soluble antigens extracted from the proximal end of third-stage larvae of *Cephalopina titillator* (Table 1). These fractions corresponded to MW standards of 76-85 kD, 58 kD, 47-49 kD, 37-45 kD, 35 kD, 30-32 kD and 23-25 kD. The protein fraction of MW 37-45 kD was not found after fractionation of the larval tegumental antigens. We detected four protein fractions after fractionation of the antigens extracted from posterior end of the larvae, which corresponded to MWs of 76 kD, 58 kD, 35 kD and 23-25 kD. The protein fraction of MW 35 kD disappeared after fractionation of the larval ES antigens. A large amount of protein was extracted from antigenic fractions from the anterior end of the larvae, especially third-stage larvae; the least amount of protein was found in fractionated ES antigens. The protein with a MW of 58 kD had absolute sensitivity (100%). The other fraction (35 kD) achieved sensitivity of 28.6% with a dilution of 1:50 using sera of infested camels and a dilution of 1:100 using sera of experimentally-vaccinated rabbits.

We used a western blot technique (EITB) to identify the specific and sensitive protein fractions, using sera of infested camels and none-infested controls reacted with four fractionated third-stage-larval antigens. We chose these antigens because they were easily available in sufficient quantities. As shown in Table 2 and Figure 1, we observed that six protein fractions, corresponding to MWs of 92kD, 68kD, 58kD, 49kD, 42kD and 35kD, reacted specifically with infested camel sera in fractionated proximal end and tegumental antigens. Only four protein fractions, corresponding to MW of 68 kD, 58 kD, 42 kD and 35 kD, were identified in fractionated posterior end and ES antigens.
The only positive protein fractions that were specific and sensitive corresponded to MWs of 58 kD and 35 kD. These fractions were common in several of the tested antigens and they reacted specifically with the parasite antibodies in sera of infested camels. All the fractions were not reacted against the control sera from non-infested camels. From the size of the reacted fractions observed on the NC strips, the amount of protein was higher in the fraction with a MW of 58 kD in compared with that of 35 kD, as well as the other reacted fractions.

Differentiation between the levels of specific protein fractions in the previous experiment showed that the amounts of 58- and 35- kD fractions were higher in the proximal part antigen extracted from the third-stage larvae in compared with the other three antigens. The next experiment investigated the level of these specific fractions in the proximal part antigens of the first- and second-stage larvae using several NC strips. The results proved that the two specific fractions (58 kD and 35 kD) were present in the fractionated proximal part antigens of the first- and second-stage larvae but smaller quantities than in the third-stage larvae (Figure 2). Based on these results, the two fractions extracted from the proximal part of the third-stage larvae are the only reference antigens available in sufficient quantities to be valuable in diagnosing infestation of living camels using the EITB technique. The final experiment demonstrated that the 58-kD fraction achieved 100% sensitivity for capturing anti-*Cephalopina* antibodies in camels and vaccinated rabbit serum samples up to 1:50 serum dilutions (Table 3; Figure 3). In addition, the 35-kD protein fraction achieved 85.7% sensitivity in serum diluted to 1:40, but it failed to detect anti-Cephalobina antibodies in infested camel sera diluted up to 1:50. The 58-kD fraction detect these antibodies in rabbit sera diluted up to 1:100, but the 35-kD fraction did not (Table 3).

Table 1. Protein fractions identified in *Cephalopina titillator* third-stage larvae using SDS-PAGE and Coomassie stain

Band number	Proximal end antigen (kD)	Excretory-Secretory antigen (kD)	Posterior end antigen (kD)	Tegumental antigen (kD)
1	85-76	76	76	**85-76**
2	58	58	58	**58**
3	49-47	-	-	**49-47**
4	45-37	-	-	-
5	35	-	35	**35**
6	32-30	-	-	**32-30**
7	25-23	25-23	25-23	**25-23**

Table 2. Specific third- stage larvae protein fractions (kD) reacted against infested camel sera using an EITB technique

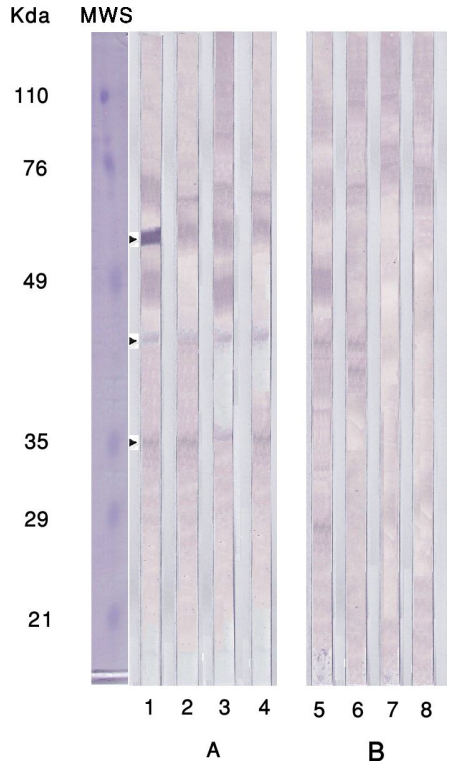

Figure 1. Treatment of nitrocellulose (NC) strip of different third-stag larval antigens reacted against infested and non-infested camel sera using an EITB technique.

(A): NC strips treated with infested camel sera; (1): Proximal end fractionated antigen, (2): Posterior end fractionated antigen, (3): Tegument fractionated antigen, (4): excretory secretory fractionated antigen.
(B): NC strips treated with non-infested camel sera; (5): Proximal end fractionated antigen, (6): Posterior end fractionated antigen, (7): Tegument fractionated antigen, (8): excretory secretory fractionated antigen.
MWS: Molecular weight protein standard

Table 2. Specific third- stage larvae protein fractions (kD) reacted against infested camel sera using an EITB technique

	NC reacted against infested camel sera				NC reacted against non-infested camel sera			
	Proximal end antigen	Posterior end antigen	Tegument antigen	Excretory Secretory antigen	Proximal end antigen	Posterior end antigen	Tegument antigen	Excretory Secretory antigen
1	92	-	92	-	92	-	92	-
2	68	68	68	68	68	68	68	68
3	58	58	58	58	-	-	-	-
4	49	-	49	-	49	-	-	-
5	42	42	42	42	42	42	42	-
6	35	35	35	35	-	-	-	-
7	-	-	-	-	38	-	-	-
8	-	-	-	-	32	-	-	-
9	-	-	-	-	28	-	28	28

NC: nitrocellulose

Table 3. Sensitivity of 58-kD and 35-kD fraction in the diagnosis of anti-*C. titillator* antibodies in infested sera using an EITB technique

Tested sera	Tested serum dilutions							
	1:20		1:40		1:50		1:100	
protein fractions	58 kD	35 kD	58 kD	35 kD	58 kD	35 kD	58 kD	35 kD
Infested Camel 1	+	+	+	+	+	--	--	--
Infested Camel 2	+	+	+	+	+	--	--	--
Infested Camel 3	+	+	+	+	+	--	--	--
Infested Camel 4	+	+	+	+	+	--	--	--
Infested Camel 5	+	+	+	--	+	--	--	--
Vaccinated Rabbit 1	+	+	+	+	+	+	+	--
Vaccinated Rabbit 2	+	+	+	+	+	+	+	--
Sensitivity %	100	100	100	85.7	100	28.6	28.6	0.0

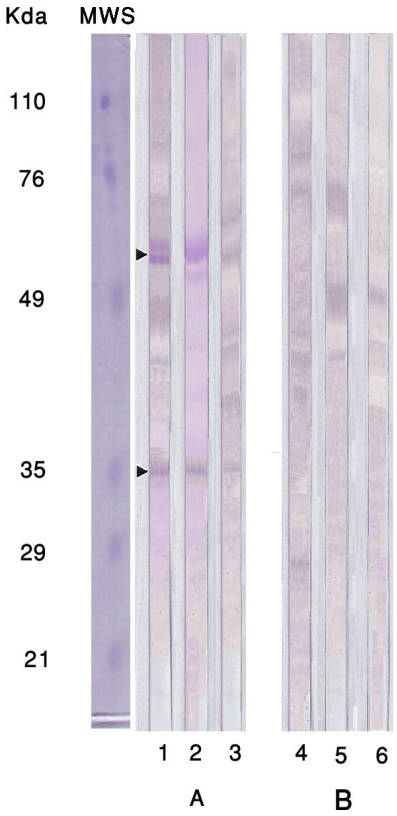

Figure 2. Determination of specific protein fractions in the proximal part antigens of different *C. titillator* larval stages reacted against infested and non-infested camel sera using EITB.

(A) Nitrocellulose (NC) strips treated with infested camel sera containing fractions of third- (1), second- (2) and first-stage larvae (3).
(B) NC strips treated strips treated with non-infested camel sera containing fractions of third- (4), second- (5) and first-stage larvae (6). MWS= Molecular weight protein standard

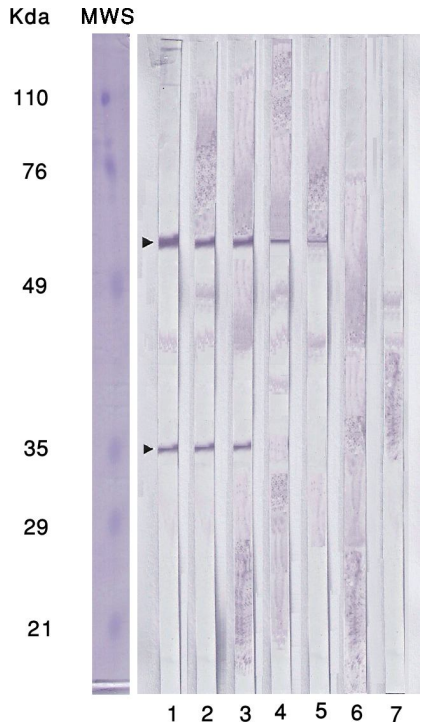

Figure 3. Diagnostic efficacy of the third-stage larvae proximal part antigen fractions in capturing anti-*Cephalopina* anti-bodies in different sera using an EITB technique.

(1) Nitrocellulose (NC) strip treated by rabbit sera vaccinated with the same antigen (1:50); **(2)** NC strip treated with natural infested camel sera (1:20); **(3)** NC strip treated with natural infested camel sera (1:40); **(4)** NC strip treated with natural infested camel sera (1:50); **(5)** NC strip treated with rabbit sera vaccinated by the same antigen (1:100); **(6)** NC strip treated with control non-infested camel sera (1:40); **(7)** NC strip treated with control negative rabbit sera (1:40).
MWS: Molecular weight protein standard

DISCUSSION

Camel nasal myiasis is a serious veterinary problem worldwide. Several factors contribute to parasitic infestation, including the free movement of camels between different localities due to the lack of closed-farm systems for camel breeding, the absence of strict controlling methods on imported animals, and the absence of specific and sensitive techniques for routine diagnoses of infestation in living camels. Most previous reports of camel nasal myiasis in Saudi Arabia are based on data from slaughterhouses that identified infestation during post-mortem examination of camel heads (Alahmad, 2002; Amin, 2005). No data is available regarding serological diagnosis or antigenic structures of *Cephalopina titillator* in Saudi Arabia. The present study is the first to evaluate such properties. We used third-stage larvae as the antigenic source, since they are present in the camel for a long time, they are easily collected and differentiated, and they contains a large amount of protein. We identified six protein fractions, corresponding to MWs of 92 kD, 68 kD, 58 kD, 49 kD, 42 kD and 35 kD, that reacted specifically against infested camel sera in fractionated proximal end and tegumental antigens of third-stage larvae.

Only four fractions, corresponding to MWs of 68 kD, 58 kD, 42 kD and 35 kD, were observed when the same test was applied to fractionated posterior end and ES antigens. Similarly, (Roelfstra *et al*., 2009) previously described a few specific protein fractions from fractionated *Gastrophilus equi* larvae. After EITB test, we identified the 58- and 35-kD protein fractions as the only specific and sensitive proteins among the various positive protein fractions. These fractions were also present in large amounts in the proximal part antigens. The EITB test proved that the amount of protein was higher in the 58-kD fraction than the 35-kD fractions, as well as other reacted fractions. This finding agree with (Innocenti, 1979), who reported that the proximal part of the *Oestrus ovis* larvae contained immunogenic polypeptides antigens that were more immunogenic than the other tegumental antigens of the larvae. This differs from the findings of (Alcaide, 2005), who described the ES antigens as the most specific for diagnosis of nasal myiases in sheep using an ELISA technique.

The present study also investigated the presence of specific antigenic fractions in the different larval stages of the parasite. We proved the presence of these antigens in all of the larval stages, but in differing quantities. In addition, we tested the diagnostic value of these fractions using sera of camels with a known infestation and laboratory-prepared rabbit sera containing anti-*Cephalopina* antibodies. The data showed that the 58-kD had absolute sensitivity in capturing the anti-*Cephalopina* antibodies at high serum dilutions, compared with the 35-kD fraction. This agrees with (Otranto, 2001), who reported that EITB could be useful as an immunological test for the specific diagnoses of myiasis. This study is the first to characterize the antigenic fractions of *Cephalopina titillator* in camels in Saudi Arabia. The 58-kD protein, which was extracted from the anterior end of third-stage larvae is a specific and sensitive protein fraction that can be used for diagnosis of *Cephalopina* infestation in the sera of living camels.

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REFERENCES

Alahmed, A. 2002. Seasonal prevalence of *Cephalopina titillator* larvae in Camels in Riyadh region of Saudi Arabia. Arab Gulf. J. Scient Res., 20:161-164.

Alcaide, M., Reina, D., Frontera, E., Navarrete, I. 2005. Analysis of larval antigens of *Oestrus ovis* for the diagnosis of oestrosis by enzyme-linked immunosorbent assay. Med Vet Entomol., 19: 151-157.

Amin, A. 2005. Seasonal prevalence of *Cephalopina titillator* myiasis in camels. Arab Gulf. J. Scient Res, 24: 346-367.

Cheng, R., Ko, R. 1991. Cross-reaction between crude antigens of larval Taenia solium (*Cysticercus cellulosae*) and other helminthes of pigs. Vet. Parasitol., 39: 161-170.

El-Bahy, M. 2002. Diagnosis of Fasciola infection by Dot ELISA technique using SDS-PAGE eluted excretory secretary (ES) protein fractions. 2nd. Veterinary Cong., Fac. Vet. Med., Minufiya, Egypt. 43- 57 .

Escartin-Pena, M., Bautista-Garfias, C. 1993. Comparison of five tests for the serologic diagnosis of myiasis by *Gasterophilus* spp. larvae (Diptera: Gasterophilidae) in horses and donkeys: a preliminary study. Med Vet Entomol., 7: 233-237.

Gegory, A., Schatz, S., Laubach, H. 2004. Ophthalmo myiasis caused by the sheep bot fly *Oestrus ovis* in Northern Iraq. Optom Vis Sci., 81: 586-590.

Goddard, P., Bates, P., Webster, k. 1999. Evaluation of a direct ELISA for the serodiagnosis of *Oestrus ovis* infections in sheep. Vet. Rec., 1: 497–501.

Innocenti, L., Lucchesi P., Giorgi F. 1979. Integument ultrastructure of *Oestrus ovis* (Diptera: Oestridae) larvae: host immune response to various cuticular components. Int. J. Parasitol., 27: 495–506.

Laemmli, U. 1970. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature., 15: 680-685.

Langely, R., Hillyer, G. 1989. Detection of circulating parasite antigens in murine fascioliasis by two-sites enzyme linked immunosorbent assay. Am. J. Trop. Med. Hyg., 41: 472-478.

Lowry, O., Rosenbrough, N., Farr, A., Randall, R. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chemis., 193: 265-275.

Oryan, A., Valinezhad, A., Moraveji, M. 2008. Prevalence and pathology of camel nasal myiasis eastern area on Iran . Trop Bio Med., 25: 30-36.

Otranto, D. 2001. The immunology of myiasis: Parasite survival and host defense strategies. Trends Parasitol., 17: 176-182.

Roelfstra, L., Deeg, C., Hauck, S., Buse, C., Membrez, M., Betschart, B., Pfister, K. 2009. Protein expression profile of *Gasterophilus intestinalis* larvae causing horse gastric myiasis and characterization of horse immune reaction. Parasites & Vectors., 8: 614-621.

Rokni, M., Samani, A., Massoud, J., Babaei M. 2006. Evaluation of Dot-ELISA Method Using Excretory–Secretory Antigens of Fasciola hepatica in Laboratory Diagnosis of Human Fascioliasis. Iranian J. Parasitol., 1: 26-30.

Soulsby, E. 1986. Helminths, Arthropods and Protozoa of domesticated animals (text book). Williams and Wilkins Company. Baltimore. United Kingdom.

Towbin, H., Stachelin, T., Gordon, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Procedures and some applications. Proc. Nat. Acad. Sci., 76: 4350-4354.

Wattal, C., Khan, I., Agarwal, S., McManus, P. 1986. Diagnostic methods, treatment and control of echinococcosis. Acta Trop. 36–71.

Welch, S., Malone, J., Geaghan, H. 1987. Herd evaluation of Fasciola hepatica infection in Louisiana cattle by an ELISA. Am. J. Vet. Res., 48: 345-347.

World Health Organization [WHO] 1983. Diagnostic techniques in schistosomiasis control. Report of the schistosomiasis unit of the WHO parasitic diseases program (PDP). WHO Tech. Rep. Ser., 386.

Zumpt, F. 1965. Myiasis in man and animals in the old world. Butterworths, London, United Kingdom.

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