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THE MAJOR IMMUNOGENS OF *CEPHALOPINA TITILLATOR* AND *OESTRUS OVIS* THIRD STAGE LARVAE IN EGYPT

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SUMMARY

Salivary glands, midgut and cuticular antigens of *Cephalopina titillator* (nasal bot of camel) and *Oestrus ovis* (nasal bot of sheep) were resolved by gel electrophoresis under denaturating condition. Six groups of rabbits were vaccinated with each antigen type. Resolved polypeptides, were tested by immunoblotting against hyperimmune sera of vaccinated rabbits. 5,3 and 3 specific immunogenic bands were detected for salivary glands, midgut and cuticular antigen of *C. titillator*. 4,4 and 3 immunogenic bands were detected for *O.ovis* larval antigens. Larval salivary glands proved to be the major immunogenes followed by midgut and cuticular antigens.

INTRODUCTION

Cephalopina titillator and *Oestrus ovis* (Diptera, oestridae) are agents of a naso-sinusal myiasis of camels and sheep respectively. The infestations are very frequent and sometime accompanied with purulent nasal discharge that interferes with normal breathing (Lancaster and Meisch, 1986). Both flies torment its host in an attempt to deposit freshly hatched larvae into their nasal cavity, the larvae can crawl up to the nasopharynx and sometime the paranasal sinuses, attached to the mucous membrane (Hussein et al., 1982). Several workers have recommended the use of intranasal insecticide to the infested animals as a mean of control (Sivkov, 1981). The prolonged use of most insecticids results in accumulation of residues in animal flesh, milk and may eventually lead to the appearance of resistant larval strains (Shanahan and Roxburgh, 1974).

The host-parasite relationship has been studied extensively in a number of dipteran species (Allen and Uilenberg, 1994). However, relatively few of these studies have been devoted to the role played by larval contents and its integument in this relationship. The larval salivary glands

and gut tissue interact indirectly with the host through its secretion and excretion, while the integument through its direct contact with host mucous membrane. Thus, the larval tissues and integument may be expected to elicit a specific host immune response (Mclaren, 1984). The presence of antibodies against *O. ovis* has been demonstrated in sera of artificially infested sheep using larval extract as a source of antigen (Bautista Garfias et al., 1988). The origin and nature of the antigenic substances produced by parasitic larvae has been studied by Baron and Colwell, (1991 a). Vaccination against adult or larval fly extracts has been attempted by Pruett et al., (1988); Sandeman, (1990) and Baron and Colwell (1991 b).

This study was achieved to detect the antigenic polypeptides of salivary glands, midgut and integument of *C. titillator* and *O. ovis* third stage larvae. Such polypeptides will lead to realization of serological tests with purified antigens and to the production of effective vaccines.

MATERIAL AND METHODS

Samples:

Larval samples of *C. titillator* were collected from nasal sinuses of slaughtered camels. *O. ovis* larvae were collected from slaughtered sheep through incision in nasal sinuses (Cairo abattoir). Third stage larvae were thoroughly rinsed in PBS at pH 7.2 and transferred to separate glass containers. Salivary glands, midgut and cuticle of both larvae were separately dissected under dissecting microscope. Salivary glands were homogenized in PBS pH 7.2 containing 1 mM Phenylmethylsulphonyl fluorid (PMSF) as proteolytic inhibitor. Larval midgut was prepared as larval salivary glands. Larval cuticle was homogenized and solubilised in 0.05 M Tris-HCI at pH 6.8 containing 5 mM dithiotritol (DTT), 1 mM Phenylthiourea (PTU) and 1 mM PMSF (Stiles and leopold, 1990). The resulting suspension of each tissue type was sonicated five times with one minute interval, then centrifuged at 10,000 rpm for fifteen minutes. The supernatant was collected for further analysis. Protein concentration was determined accoridng to Lowry et al., (1951).

Hyper immune sera:

Hyper immune serum was prepared against each larval antigen by intradermal injection of Boscat white rabbit (one rabbit for each antigen) with 2 mg of antigen emulsified with 0.5 ml of complete Freunds adjuvant, two weeks later the rabbits received a second intradermal dose consisting of the same amount of antigen emulsified with incomplete Freunds adjuvant. After two weeks from the last injection blood was collected from ear vein for sera separation. Sera were kept at 20C° till use.

;el electrophoresis:

'oly acrylamide gel electrophoresis (PAGE) was arried out according to Laemmli (1970), using rotein II maxigels (BIO-Rad) with 10% crylamide gradients. Electrophoresis was run nder denaturing condition at 200 V for 60 inutes. Antigen samples (Three for both *C. itillator* and *O. Ovis)* were boiled for 3 minutes n 0.05 M Tris - Hcl buffer at pH 6.8 containing % SDS and 5 % 2- mercaptoethanol. Molecular weights were determined by omparison with standard protein marker.

Western immunoblotting:

Polypeptides resolved by SDS-PAGE were ransferred electrophoretically to nitrocellulose heets following the procedure of Towbin et al., (1979) and then tested for their reactivity against abbit antisera. The antisera were diluted 1:800 n 0.1 M PBS at 7.2 pH containing 5 % skim milk ad 0.05 % tween 20, and applied for 2 hours to the nitrocellulose sheet. Anti-rabbit IgG proxidase was 1:2000 in 0.1 M PBS 7.2 pH and applied on the nitrocellulose sheet for 1 hour. Enzyme activity was revealed colorimetrically using O. Phenylenediamine dihydrochloride as substrate.

RESULTS

Polyacrylamide gel electrophoresis (SDS-PAGE) was used under denaturing condition aiming to determine the molecular weight of different polypeptides originated from larval salivary glands, mid-gut and cuticle of both *C. titillator* and *O. ovis.*

Concerning *C. titillator* larval antigen, figure 1 and table 1 showed the electrophoretic patterns of salivary glands as resolved by 10 % polyacrylamide gel. The Major protein fractions (9 bands) around molecular weight 205 to 23 KDa. To determine the tissue specificity of these polypeptides, immunoblotting showed 5 immunogenic bands with molecular weight 205, 192, 136, 112 and 51 KDa (Figure 2).

C. titillator midgut antigen exhibited electrophoretic pattern of polypeptide with molecular weight in the range of 207 to 18KDa (Figure 1 and table 1). Immunoblotting analysis shows that antisera were reacted with 207, 167 and 49 KDa polypeptides. (Figure 2).

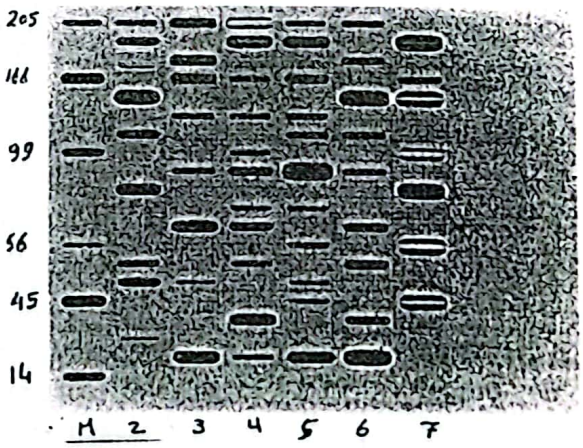

Fig1: Electrophoretic analysis of *C. titillator* and *O. ovis* 3rd instar salivary glands, midgut and cuticular antigen.

Lane 1: Protein marker
Lane 2:*Cititillator salivary* glands
Lane 3:*C. titillator midgut*
Lane 4:*C. titillator cuticle*
Lane 5: *O. ovis salivary glands*
Lane 6: *O. ovis midgut*
Lane 7: *O. ovis cuticle*

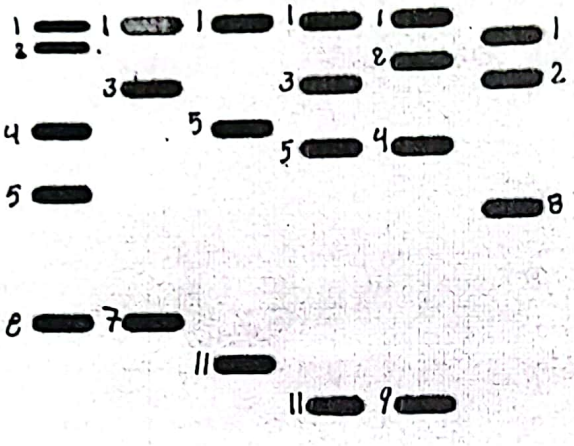

Figure 2: Polypeptides from 3rd larval extract and testing through immunoblotting against immunized rabbit sera

Lane 1: *C.titillator* salivary glands
Lane 2: *C.itititillator* midgut
Lane 3: *C. titillator cuticle*
Lane 4: *O. ovis* salivary glands
Lane 5: *O. ovis* midgut
Lane 6: *O. ovis* cuticle

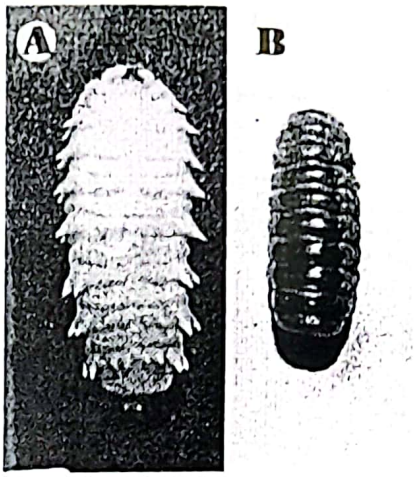

Fig 3: Third larval stage of
A: *Cephalop na titillator*
B: *Oestrus oivis.*

Table 1: Salivary glands, midgut and cuticular antigen Polypeptides from 3rd instar of *C. titillator.*

Lanes / Bands	Lane 1 Mol. w.	Protein Amount Ug/ml	Lane 2 Mol. w.	Protein Amount Ug/ml	Lane 3 Mol. w.	Protein Amount Ug/ml	Lane 4 Mol. w.	Protein Amount Ug/ml
1	205	11.25	205	7.97	207	11.12	207	3.56
2	166	26.81	192	14.18	179	17.91	200	5.14
3	99	15.61	172	3.60	167	11.10	193	13.06
4	66	8.99	136	22.03	127	8.84	161	6.66
5	45	21.72	112	11.80	91	8.83	124	6.66
6	14	15.61	84	16.19	72	18.68	98	6.62
7			58	7.96	49	4.85	91	11.24
8			51	11.82	18	18.67	77	6.64
9			23	4.44			69	11.27
10							57	6.64
11							33	15.91
12							17	6.59
13								
Sum			100				100	100
In lane			100				100	100

Lane 1: Protein marker
Lane 2: *C. titillator* salivary glands
Lane 3: *C. titillator* midgut
Lane 4: *C. titillator* cuticle

Table 2: Salivary glands, midgut and cuticular antigen Polypeptides from 3rd instar of *O.ovis*.

Lanes / Bands	Lane 5 Mol. w.	Protein Amount Ug/ml	Lane 6 Mol. w.	Protein Amount Ug/ml	Lane 7 Mol. w.	Protein Amount Ug/ml
1	202	6.60	205	7.25	194	10.12
2	193	11.35	178	7.25	184	11.23
3	166	11.15	148	19.88	166	7.71
4	127	7.51	108	7.25	149	8.25
5	112	7.24	91	7.26	136	9.63
6	93	21.14	72	10.69	99	4.86
7	77	6.57	58	10.68	95	5.96
8	66	6.58	33	10.49	85	3.81
9	49	4.09	19	19.24	80	2.48
10	42	6.42			67	7.81
11	18	11.35			62	8.96
12					45	9.03
13					37	10.14
Sum		100		100		100
In lane		100		100		100

Lane 5: *O.ovis* salivary glands antigen Lane 6: *O.ovis* midgut antigen
Lane 7: *O.ovis* cuticular antigen

titillator cuticular antigen demonstrated 12 polypeptides with molecular weight ranged from 207 to 17 KDa (Figure 1 and table 1). The highly immunogenic band were 207, 124 and 33 KDa (Figure 2).

O. ovis salivary glands, midgut and cuticular antigen exhibited electrophoretic pattern of polypeptides in the range of 262 to 18 KDa (11 bands), 205 to 19 KDa (9 bands) and 194 to 37 KDa (13 bands) respectively (table 2 and figure 1). Immunoblotting analysis revealed 4, 4 and 3 immunogenic bands for *O. ovis* salivary glands, migdut and cuticlar antigen respectively. (figure 2).

DISCUSSION

C. titillator and *O. ovis* third stage larvae were investigated to detect the most immunogenic antigens to infested camel and sheep. To accomplish these goals, larval salivary glands, midgut and cuticle were extracted and tested against sera from immunized rabbits.

Salivary glands polypeptides of *C. titillator* and *O. ovis* were most reacative against rabbit antisera in the range of 202 to 51 and 202 to 18 KDa respectively. Data reported in is study allow the conclusion that polypeptides originating from

the two larval tissues of both parasite species are immunogenic to camel and sheep. This conclusion stems from several lines of evidence. First of all, antisera tested in this study reacted positively with its related antigen, second, the protein content detectable by gel electrophoresis was nearly the same in the two antigen type, third, Innocenti et al (1995) mentioned that salivary glands of *O. ovis* larvae contains the major immunogenic antigens, but polypeptides from larval cuticle were less. This conclusion agrees with the earlier evidence by Skelly and Howells (1987), which showed that salivary glands of *Lucilia cuprina* larvae maintain a high immunogenic activity even dentaured by sodium dodysyl sulfate (SDS).

Dealing with *C. titillator* and *O. ovis* midgut antigen, our results showed that, polypeptides in the range of 207 to 49 and 205 to 19 KDa respectively were most reactive with rabbit immune sera. Innocenti et al (1995) mentioned that sheep sera does not reacted against haemolymph of *O. ovis* larvae. This is in contrast with his finding that haemolymph it self proves to be highly immunogenic when administrated intradermally to experimented rabbits. This constitutes a clear indication that no direct contact was established between the larval content (midgut) and the animal immune system. Our results showed that larval cuticle proved to be immunogenic. Polypeptides of *C.tililator* and *O. ovis* were reactive against rabbit immune sera. Innocenti et al (1997) mentioned that in spite of direct contact between larval parasite and sheep nasal mucosa, cuticular antigen proved to be less immunogenic than salivary glands. The reason is unclear, one possibility could be that extraction procedure of antigen could perhaps cause degradation of some immunogenic epitopes in the cuticle or the integral cuticle polypeptides are not directly exposed to the host humoral environment. Leid et al., (1987) mentioned that the parasite has a capability to undergo antigenic variation on its surface coat.

Finally, the present study has demonstrated the immunogenic polypeptides of *C. titillator* and *O. ovis* third stage larvae. We can conclude that the three larval tissues are immunjogenic and protective against infestation, specially the salivary glands polypeptides which gives it the priority to be effective vaccine.

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