

## Skin hypersensitivity test against nasal bots infestations in donkeys and camels

A.A.Balegh<sup>1</sup>, L.M.El-akabawy<sup>2</sup>, M.Y.Ramadan<sup>2</sup>, S.F.Omar<sup>1</sup> and R.S.El-madawy<sup>2</sup>

<sup>1</sup>Parasitology Dept., Animal Health Research Institute, Dokki, Giza, Egypt

<sup>2</sup>Parasitology Dept., Faculty of Veterinary Medicine, Banha Univ., Banha, Egypt

E-Mail: [aliaabaleg@yahoo.com](mailto:aliaabaleg@yahoo.com)

### Abstract

The objective of this study was assessing the Intradermal test (IDT) in the diagnosis of both cephalopinos and rhinoestrosis. 6 males one-humped dromedary camels (*Camelus dromedarius*) admitted to camel hosting house of El-Basateen slaughter house, beside, 12 female Egyptian donkeys (*Equus asinus*) admitted to Giza zoo abattoir from Bani-suef were subjected to an intradermal injection of 0.5 ml of PBS PH (7.2) at one side of the neck and this served as negative control; on the other side of the neck each animal received 3 intradermal injections of 3 different protein concentrations (0.5mg/ml, 1mg/ml and 1.5 mg/ml) for each antigen (1<sup>st</sup> larval instars crude extract and salivary gland extract of *Cephalopina titillator* larvae, and, Excretory secretory product (ESP), Salivary gland extract, Mid-gut extract and Mixed crude extract of *Rhinoestrus* spp. larvae). The results of the IDT were determined through measuring the diameter of the resulting wheals using a scale bar and by detection of presence or absence of skin reactions. The swelling size in the skin was increased with increasing the concentration of the injected antigen. Most animals showed skin reactions after 30 min. The results signified the critical role of IDT in early diagnosis of cephalopinos and rhinoestrosis.

**Keywords:** Cephalopinos, Rhinoestrosis, Intradermal test, Diagnosis.

### 1. Introduction

*Cephalopina titillator* (Diptera, Oestridae) is an obligate parasite of domesticated camel [16, 2]. It causes cephalopinos that considered as a prevalent parasitic problem in Egypt [1, 16, 5].

Rhinoestrosis is a parasitic disease caused by larvae of *Rhinoestrus* spp. (Diptera, Oestridae), which localize in nasal cavities, sinuses and pharynx of horses, donkeys and zebras [19]. The presence of larvae of *Cephalopina titillator* and *Rhinoestrus* spp. induces local inflammation and the infection is characterized by clinical designations of varying intensity and astringency, ranging from inflammation to dyspnea, sneezing and cough [10]. Moreover, lesions of the upper respiratory tract, lungs and damage of the olfactory nerves were reported [9].

Although *C. titillator* prevails among camel spp., their importance is still underestimation [14]. In contrast to numerous Oestrids *Cephalopina* adult fly don't panic or fright the camels, and great numbers are frequently noticed resting on the camels' heads and around the nostrils. Diagnosis of *C. titillator* in the antecedent studies depended mainly on the post-mortem examinations [3, 13].

Diagnosis of equine rhinoestrosis in live animal is hard even when using endoscope because; the larvae when present are very difficult to be encountered in the pharynx behind the velaum palatinum.

There have been few studies on the use of skin hypersensitivity tests for diagnosis of Myasis producing flies [4, 6-8, 11]. The present work is the first study for assessing the Intradermal test in the diagnosis of both cephalopinos and rhinoestrosis.

### 2. Materials and methods

Intradermal test (IDT) was carried out according to [12] and [4].

#### 2.1 Animals

In June, 6 males one-humped dromedary camels (*Camelus dromedarius*) of 7-8years old, admitted to camel hosting house of El-Basateen slaughter house and showed absence of any clinical signs to cephalopinos. Also, in May, 12 female Egyptian donkeys (*Equus asinus*) of different ages (21-28 years) were admitted to Giza zoo abattoir from Bani-suef. Donkeys were free from any clinical signs of rhinoestrosis. The camels were divided into 2 groups each containing 3 animals. The donkeys were divided to 4 groups each containing 3 animals.

#### 2.2 Experiment design

An area of 30cm x 15cm at the posterior third of the dorsolateral cervical region of the neck was clipped from both sides and disinfected with ethyl alcohol 70%.

Each animal received an intradermal injection of 0.5 ml of PBS pH (7.2) at one side of the neck and this served as negative control; on the other side of the neck each animal received 3 intradermal injections of 3 different protein concentrations (0.5mg/ml, 1mg/ml and 1.5 mg/ml) for each antigen using 1.5cc syringes and 21gauge needles.

The result of the IDT was determined through measuring the diameter of the resulting wheals using a scale bar and by detection of presence or absence of skin reactions (erythematous swellings or ulcers) at 1/2hr, 1hr, 24hr, 48hr and 72hr post injection. Confirmation of the IDT was done through postmortem examination of slaughtered animals' heads.

#### 2.3 Statistical analysis

The Statistical analysis was carried out using ANOVA with five factors under significance level of 0.05 for the whole results using SPSS (ver. 22). Data were treated as complete randomization design according to [17]. Multiple comparisons were carried out applying LSD.

### 3. Results and discussion

Data recorded in table (1) and (3) indicated that the intradermal injection of different *C. titillator* (LICE and SG) and *Rhinoestrus* spp. larval antigens (ESP, SG, MG, MixCE) produced edematous skin reactions of varying intensity according to the antigen type and the induration's size of the skin increased with increasing the concentration of the injected antigen. Also, there was significant difference in the skin reactions produced by different antigen concentrations ( $p < 0.05$ ) in the control and the tested animals.

Concerning the effect of different *C. titillator* larval antigens on the cellular immunity of camels, data obtained from Table (1, 2) Fig (1) Plate (1, 2) proved that the skin edema and swelling of the skin were significantly enhanced with increasing the concentration of the injected antigen. The skin edematous swelling was significantly increased after 1hr then began to drop off after 24hrs to reach its low size after 72 hr.

Unlike the succeeding results of donkeys, the first larval crude extract of *C. titillator* presented the highest cellular response with a severe increase in the skin edema. Erythematous reaction was difficult to be noticed because of the black color of the camel's skin.

Concerning the effect of different *Rhinoestrus* spp. larval antigens on the cellular immunity of donkeys data obtained from Table (3,4) Fig (2) Plate (3, 4, 5 and 6) revealed that the skin induration was produced after 30 min. of injection of all the used antigens to be more palpable after 1hr. However, after 24hr the skin induration's size began to diminish gradually to reach a low size after 72hr except for the salivary gland antigen and the mid-gut antigen in two animals, where the intense inflammatory skin reaction was clearly visible and reaches its maximum response after 24hr then began to gradually subside. On the other hand, injection of PBS in the control negative cases resulted in relatively small size indurations in all animals that soon completely subsided after 1/2 hrs.

There was a significant effect ( $p < 0.05$ ) to the concentration of 1.5mg of all antigens on the skin

reaction than the other concentrations. There was a significant increase in the induration's size and strong skin reactions after 1hr and 24hr than after 30 min., 48hr and 72 hr. There was significant difference among all antigens, the strongest reaction was to the SG extract antigen followed by ESP antigen. On the other hand, there was no significant difference between MG antigen and MixCE antigen.

Intradermal test was confirmed by careful post-mortem examination of the tested donkeys through detection of *Rhinoestrus* spp. larvae in their heads. The post-mortem examination of the tested donkeys showed that all the injected animal's heads had more than one *Rhinoestrus* spp. 1<sup>st</sup> stage larvae beside one animal had only one L3.

Our results illustrated that the cellular immunity response after intradermal injection of different larval antigens either for *Rhinoestrus* spp. or for *C. titillator* in donkeys and camels respectively, was clearly palpable after 1hr that indicated presence of an immediate type of hypersensitivity reactions. This is the first record of such type of hypersensitivity in donkeys and camels.

These results were parallel to those obtained by other scientists on *Oestrus ovis* of sheep [4, 7] and on *Hypoderma lineatum* in cattle [11, 15].

On the other hand there was late type of hypersensitivity due to involvement of allergen-specific T-cells that presented by salivary gland antigen and to a lesser extent by mid-gut antigens after 24hr and this was analogous to that obtained by [12] where they explained presence of late response by presence of irritant substances in saliva (of Culicoides) that caused non-allergen mediated inflammation and these substances were able to generate edema and erythema in the laboratory animals and humans.

### 5. Conclusion

The results recorded in our study showed that the highest response to intradermal test was after 30min-1hr so it magnified the critical role of intradermal test in early diagnosis of rhinoestrosis and cephalopinos.

### 6. Acknowledgements

I would like to express my immense appreciations to all veterinarians and workers of El-Basateen hosting house and Giza zoo for their help, encouragement and good hospitality.

**Table (1)** Skin reaction measurements (Inch) during different periods by different *Cephalopina titillator* larval antigens concentrations

Time(P.I)	Animal number	Antigen type							
		First larval crude extract				Salivary gland extract			
		Control negative	0.5 mg	1 mg	1.5 mg	Control negative	0.5 mg	1 mg	1.5 mg
30 min.	1	0	6	6.4	7.1	0	4	4.5	4.9
	2	0	3.2	4	6	0	3.5	4.6	5.5
	3	0	6.5	7.8	9.5	0	5	5.9	6
1 hr	1	0	7.4	6.9	7.6	0	5.1	6	6.5
	2	0	5	5.6	7.3	0	3.6	4.8	5.9
	3	0	6.6	8.1	10	0	6.9	7.3	7.8
24 hr	1	0	4.6	5.5	6	0	3.1	4.3	4.8
	2	0	3.1	3.8	5.9	0	3.2	3.4	3.5
	3	0	5.5	5.9	6	0	3.8	4.5	5.2
48 hr	1	0	4.2	4.5	5	0	3.1	4.2	4.5
	2	0	3	3.7	5.9	0	2.8	2.9	3
	3	0	4.6	5.5	6	0	3.5	3.9	4.5
72 hr	1	0	4	4.3	4.9	0	3	3.7	4
	2	0	2.6	3.5	4.8	0	2.5	2.9	3
	3	0	3	3.5	4.3	0	3.4	3.8	3.9

-P.I.: post injection

**Table (2)** Mean size of skin reaction (Inch) using different *C. titillator* larval antigen concentrations at different period of time

Antigen type	Concentration (mg)	Mean size of observed reaction					Mean
		0.5hr	1hr	24hr	48hr	72hr	
L1 crude extract (LICE)	0 (control)	0±0	0±0	0±0	0±0	0±0	0±0
	0.5	5.23±1.03	6.33±0.71	4.4±0.7	3.93±0.48	3.2±0.42	4.62±0.39
	1.0	6.07±1.11	6.87±0.72	5.07±0.64	4.57±0.52	3.77±0.27	5.27±0.4
	1.5	7.53±1.03	8.3±0.85	5.97±0.03	5.63±0.32	4.67±0.19	6.42±0.42
	Mean	4.71±0.94	5.38±1	3.86±0.72	3.53±0.66	2.91±0.54	-
Salivary gland extract (SG)	0 (control)	0±0	0±0	0±0	0±0	0±0	0±0
	0.5	4.17±0.44	5.2±0.95	3.37±0.22	3.13±0.2	2.97±0.26	3.77±0.29
	1.0	5±0.45	6.03±0.72	4.07±0.34	3.67±0.39	3.47±0.28	4.45±0.31
	1.5	5.47±0.32	6.73±0.56	4.5±0.51	4±0.5	3.63±0.32	4.87±0.34
	Mean	3.66±0.67	4.49±0.85	2.98±0.55	2.7±0.5	2.52±0.46	-
Mean of antigen type		LICE	SG	-	-	-	-
		4.08±0.36 <sup>A</sup>	3.27±0.28 <sup>B</sup>	-	-	-	-
Mean of concentration		0 (control)	0.5 mg	1.0 mg	1.5 mg	-	-
		0.0±0.0 <sup>D</sup>	4.19±0.25 <sup>C</sup>	4.86±0.26 <sup>B</sup>	5.64±0.30 <sup>A</sup>	-	-
Mean of time		0.5 hr	1 hr	24 hr	48 hr	72 hr	-
		4.18±0.58 <sup>B</sup>	4.93±0.65 <sup>A</sup>	3.42±0.45 <sup>C</sup>	3.12±0.42 <sup>CD</sup>	2.71±0.35 <sup>D</sup>	-

a, b & c: Superscripts to be compared statistically within the same column. Values with different letter superscripts are significantly different (P<0.05).

A, B & C: Superscripts to be compared statistically within the same row. Values with different letter superscripts are significantly different (P<0.05).

**Table (3)** Skin reaction measurements (Inch) during different periods by different *Rhinoestrus* spp. larval antigens concentrations

Time (P.I)	Animal number	Antigen type															
		Excretory-secretory product(ESP)			Salivary glands extract			Mid-gut extract			Mixed crude extract						
		Control negative	0.5 mg	1 mg	1.5 mg	Control negative	0.5 mg	1 mg	1.5 mg	Control negative	0.5 mg	1 mg	1.5 mg	Control negative	0.5 mg	1 mg	1.5 mg
30 min.	1	0	2	4	4.5	0	3	3.8	3.9	0	2.5	2.9	4	0	2	2.1	3
	2	0	1.7	3	3.5	0	2.5	3	3.6	0	1.8	2.2	2.4	0	1.5	3	4
	3	0	2.7	4	4.5	0	2.4	2.5	2.6	0	1.5	1.7	2.1	0	2.5	3	3.3
1hr	1	0	3.5	4.5	5.7	0	5.3	5.5	6	0	4.5	4.6	5.8	0	2.8	5	5.5
	2	0	3.6	4.6	4.8	0	3.6	4.6	4.7	0	1.8	2.5	3.2	0	3.5	3.9	4
	3	0	4.8	5.1	5.6	0	4	4.5	5	0	2.9	3.1	3.4	0	3	3.5	3.5
24hr	1	0	3.4	4.2	5.5	0	6	6.4	6.5	0	2.8	3.5	4.5	0	2.8	4	4.8
	2	0	3.6	4.1	4.5	0	3.8	4.9	5	0	3	3.5	3.5	0	1.5	2.5	4
	3	0	4.5	4.7	5	0	4	4.5	5	0	3	3.3	3.6	0	1.3	1.5	2
48hr	1	0	1.9	2	2.5	0	5.1	6.1	6.5	0	2.5	2.7	4.1	0	3.6	3.7	4.5
	2	0	2.6	3	3.2	0	2	3	3.4	0	2.7	3	0	0	1.5	2.5	4
	3	0	2.5	3.4	3.8	0	3.5	3.7	4.3	0	2.2	3.3	3.6	0	1.3	1.5	1.9
72hr	1	0	1.9	2	2.4	0	4	4.8	5.9	0	2	2.7	4.1	0	2	2.4	3.5
	2	0	2.2	2.7	3.2	0	1.4	2	3.4	0	2.4	2.7	0	0	1.5	2.5	4
	3	0	1.7	2.5	3.6	0	2.7	3	4.2	0	2.2	2.7	3.4	0	1.3	1.5	1.9

-P.I.: post injection.

**Table (4)** Mean size of skin reaction (Inch) using different *Rhinoestrus* spp. larval antigen concentrations at different periods of time

Antigen type	Concentration (mg)	Mean size of observed reaction					Mean
		0.5 hr	1 hr	24 hr	48 hr	72 hr	
Excretory secretory product (ESP)	0 (control)	0±0 <sup>dA</sup>	0±0 <sup>dA</sup>	0±0 <sup>dA</sup>	0±0 <sup>dA</sup>	0±0 <sup>dA</sup>	0±0 <sup>d</sup>
	0.5	2.13±0.3 <sup>cBC</sup>	3.97±0.42 <sup>cA</sup>	3.83±0.34 <sup>cA</sup>	2.33±0.22 <sup>cB</sup>	1.93±0.15 <sup>cC</sup>	2.84±0.26 <sup>c</sup>
	1.0	3.67±0.33 <sup>bc</sup>	4.73±0.19 <sup>bA</sup>	4.33±0.19 <sup>bB</sup>	2.80±0.42 <sup>bd</sup>	2.40±0.21 <sup>bE</sup>	3.59±0.26 <sup>b</sup>
	1.5	4.17±0.33 <sup>aC</sup>	5.37±0.28 <sup>aA</sup>	5.00±0.29 <sup>aB</sup>	3.17±0.38 <sup>ad</sup>	3.07±0.35 <sup>ad</sup>	4.15±0.28 <sup>a</sup>
	Mean	2.49±0.50 <sup>B</sup>	3.52±0.64 <sup>A</sup>	3.29±0.60 <sup>A</sup>	2.08±0.39 <sup>C</sup>	1.85±0.36 <sup>C</sup>	-
Salivary gland extract (SG)	0 (control)	0±0 <sup>cA</sup>	0±0 <sup>dA</sup>	0±0 <sup>cA</sup>	0±0 <sup>dA</sup>	0±0 <sup>dA</sup>	0±0 <sup>d</sup>
	0.5	2.63±0.19 <sup>bc</sup>	4.30±0.51 <sup>cA</sup>	4.60±0.70 <sup>bA</sup>	3.53±0.9 <sup>bB</sup>	2.70±0.75 <sup>cC</sup>	3.55±0.33 <sup>c</sup>
	1.0	3.10±0.38 <sup>ad</sup>	4.87±0.32 <sup>bB</sup>	5.27±0.58 <sup>aA</sup>	4.27±0.94 <sup>bc</sup>	3.27±0.82 <sup>bd</sup>	4.15±0.34 <sup>b</sup>
	1.5	3.37±0.39 <sup>aC</sup>	5.23±0.39 <sup>aA</sup>	5.50±0.50 <sup>aA</sup>	4.73±0.92 <sup>aB</sup>	4.50±0.74 <sup>aB</sup>	4.67±0.31 <sup>a</sup>
	Mean	2.28±0.42 <sup>D</sup>	3.60±0.65 <sup>A</sup>	3.84±0.71 <sup>A</sup>	3.13±0.66 <sup>B</sup>	2.62±0.57 <sup>C</sup>	-
Midgut extract (MG)	0 (control)	0±0 <sup>dA</sup>	0±0 <sup>dA</sup>	0±0 <sup>dA</sup>	0±0 <sup>cA</sup>	0±0 <sup>cA</sup>	0±0 <sup>c</sup>
	0.5	1.93±0.30 <sup>cC</sup>	3.07±0.78 <sup>cA</sup>	2.93±0.07 <sup>cA</sup>	2.47±0.15 <sup>bB</sup>	2.20±0.12 <sup>bBC</sup>	2.52±0.19 <sup>b</sup>
	1.0	2.27±0.35 <sup>bc</sup>	3.40±0.62 <sup>bA</sup>	3.43±0.07 <sup>bA</sup>	3.00±0.17 <sup>aB</sup>	2.70±0.10 <sup>aB</sup>	2.96±0.17 <sup>a</sup>
	1.5	2.83±0.59 <sup>aB</sup>	4.13±0.84 <sup>aA</sup>	3.87±0.32 <sup>aA</sup>	2.57±1.29 <sup>bBC</sup>	2.50±1.27 <sup>abC</sup>	3.18±0.40 <sup>a</sup>
	Mean	1.76±0.36 <sup>B</sup>	2.65±0.55 <sup>A</sup>	2.56±0.46 <sup>A</sup>	2.01±0.45 <sup>B</sup>	1.85±0.42 <sup>B</sup>	-
Mixed crude extract (MixCE)	0 (control)	1.50±0.12 <sup>dA</sup>	0.47±0.03 <sup>cB</sup>	0±0 <sup>cC</sup>	0±0 <sup>dC</sup>	0±0 <sup>dC</sup>	0.39±0.16 <sup>d</sup>
	0.5	2.00±0.29 <sup>cB</sup>	3.10±0.21 <sup>bA</sup>	1.87±0.47 <sup>bB</sup>	2.13±0.74 <sup>cB</sup>	1.60±0.21 <sup>cC</sup>	2.14±0.21 <sup>c</sup>
	1.0	2.70±0.30 <sup>bB</sup>	4.13±0.45 <sup>aA</sup>	2.67±0.73 <sup>aB</sup>	2.57±0.64 <sup>bB</sup>	2.13±0.32 <sup>bC</sup>	2.84±0.27 <sup>b</sup>
	1.5	3.43±0.30 <sup>aBC</sup>	4.33±0.60 <sup>aA</sup>	3.60±0.83 <sup>aB</sup>	3.47±0.80 <sup>aB</sup>	3.13±0.63 <sup>aC</sup>	3.59±0.27 <sup>a</sup>
	Mean	2.41±0.25 <sup>B</sup>	3.01±0.49 <sup>A</sup>	2.03±0.47 <sup>C</sup>	2.04±0.47 <sup>C</sup>	1.72±0.38 <sup>C</sup>	-
Mean of antigen type	ESP	SG	MG	MixCE	-	-	
Mean of concentration	0 (control)	0.5 mg	1.0 mg	1.5 mg	-	-	
Mean of time	0.5 hr	1 hr	24 hr	48 hr	72 hr	-	
		2.23±0.20 <sup>B</sup>	3.19±0.29 <sup>A</sup>	2.93±0.22 <sup>A</sup>	2.32±0.29 <sup>B</sup>	2.01±0.24 <sup>B</sup>	-

a, b & c: Superscripts to be compared statistically within the same column. Values with different letter superscripts are significantly different (P<0.05).

A, B & C: Superscripts to be compared statistically within the same row. Values with different letter superscripts are significantly different (P<0.05)

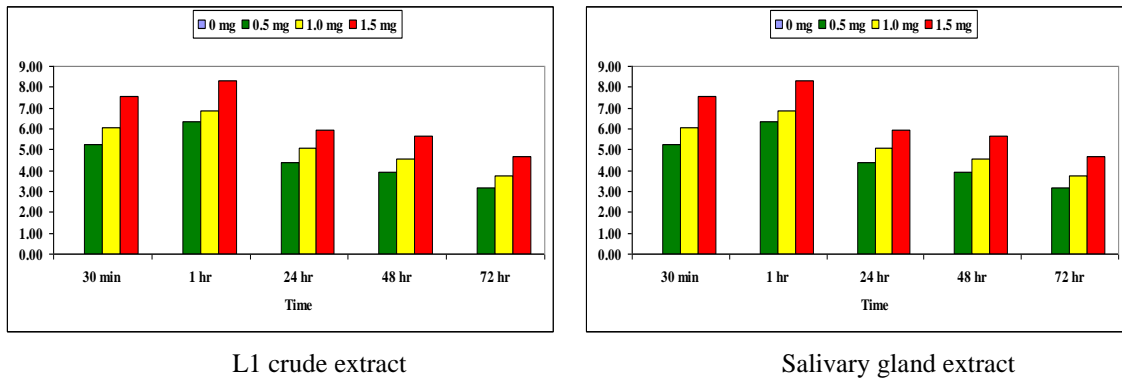


Fig (1) Mean size of skin reaction using different *C. titillator* antigen concentrations at different periods of time

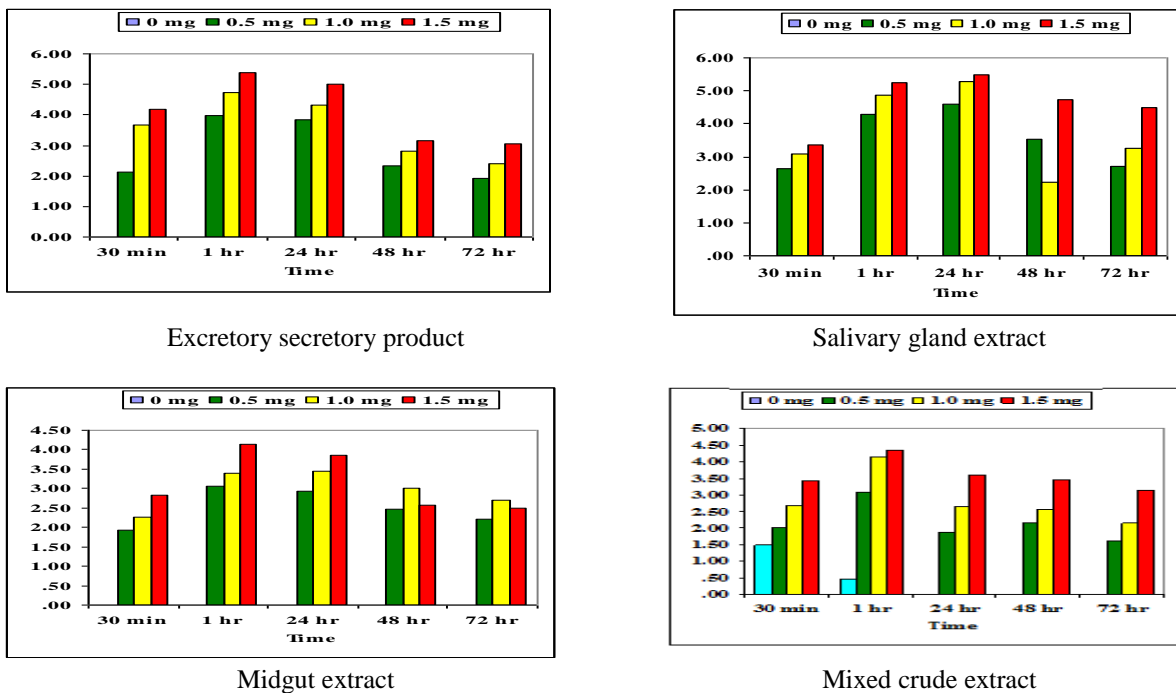


Fig (2) Mean size of skin reaction using different *Rhinoestrus* spp. antigen concentrations at different periods of

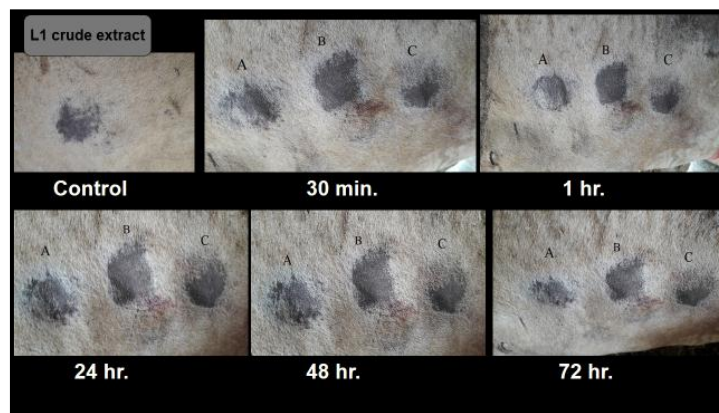
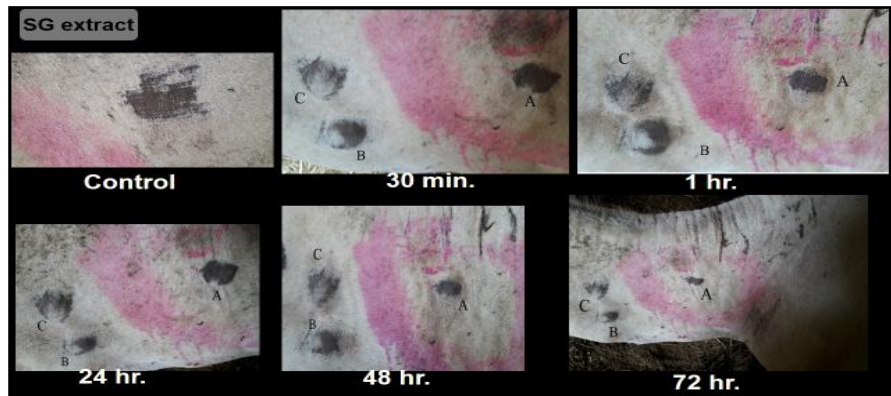
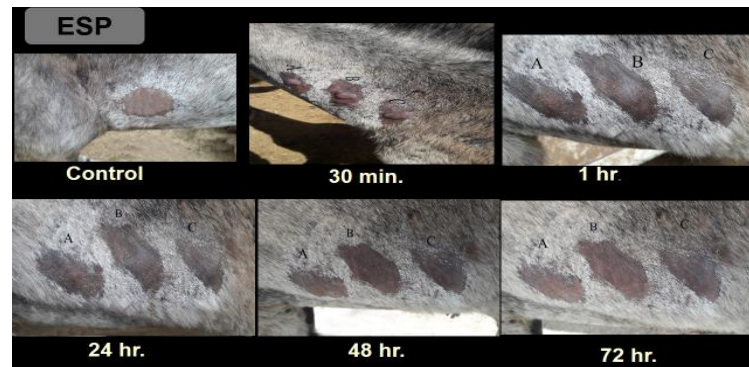


Plate (1) Skin reaction using different *Cephalopina titillator* L1 crude antigen concentrations at different periods of time  
 A. 0.5mg/ml  
 B. 1mg/ml  
 C. 1.5mg/ml



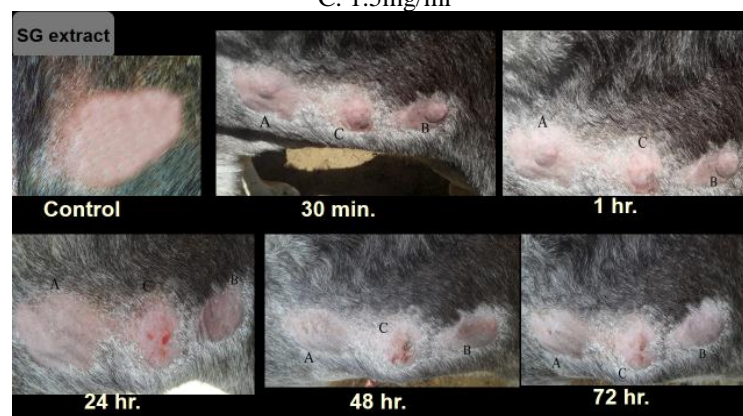
**Plate (2)** Skin reaction using different *Cephalopina titillator* Salivary gland antigen concentrations at different periods of time

- A. 0.5mg/ml
- B. 1mg/ml
- C. 1.5mg/ml



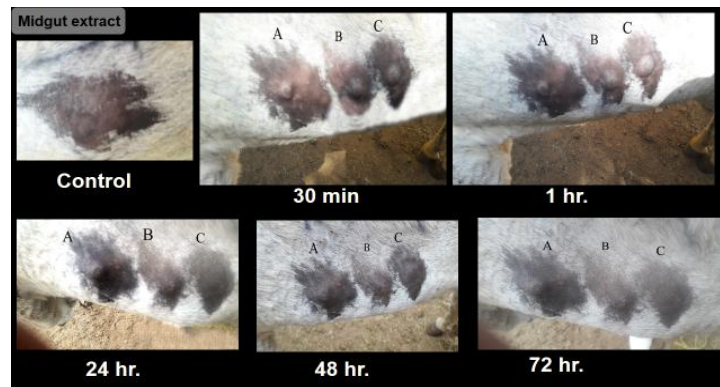
**Plate (3)** Skin reaction using different *Rhinoestrus* spp. ESP antigen concentrations at different periods of time

- A. 0.5mg/ml
- B. 1mg/ml
- C. 1.5mg/ml

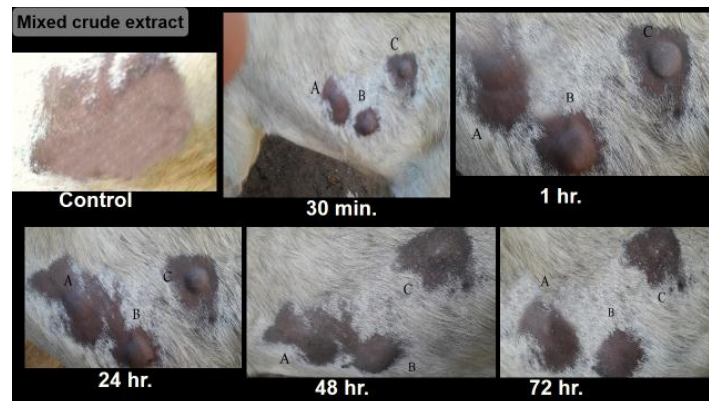


**Plate (4)** Skin reaction using different *Rhinoestrus* spp. Salivary gland antigen concentrations at different periods of time

- A. 0.5mg/ml
- B. 1mg/ml
- C. 1.5mg/ml



**Plate (5)** Skin reaction using different *Rhinoestrus* spp. Midgut antigen concentrations at different periods of time  
 A. 0.5mg/ml  
 B. 1mg/ml  
 C. 1.5mg/ml



**Plate (6)** Skin reaction using different *Rhinoestrus* spp. Mixed crude antigen concentrations at different periods of time  
 A. 0.5mg/ml  
 B. 1mg/ml  
 C. 1.5mg/ml

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