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SOME SEROLOGICAL AND IMMUNOLOGICAL STUDIES ON *OESTRUS OVIS* INFESTING SHEEP

(With 4 Tables and One Figure)

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بعض الدراسات السيرولوجية والمناعية على الدودة النغفية فى الأغنام

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تم فحص عدد ٤٢٠ من الاغنام خلال ستة أشهر لتشخيص الإصابة بيرقات ذبابة النغف عن طـريق استكشف وجود اليرقات فى رأس الحيوانات بالعين المجردة كما تم تجميع عينات دم مـن تلك الحيوانات مع ملاحظة ترقيم العينات التي جمعت من الحيوانات المصابة باليرقات لفحصـها بأسـتخدام اختـبار الاليزا. كما تم معمليا استخلاص المواد الإفرازية والإخراجية لـيرقات الطـور الأول والثاني للذبابة النغفية باستخدام الوسط الغذائي ار بى ام اى ١٦٤٠ (تحـت ظروف معملية محددة) والتي استخدمت كأنتجين ولقاح تجريبي. أظهرت النتائج ان معـدل إصـابة الأغـنام وصل الى ١٩,٥ % و ٢٤,٣ % بطريقة العين المجردة و اختبار الالـيزا على الترتيب وقد وصلت حساسية اختبار الاليزا الى ٩٧,٦ %. تم حقن عدد عشرة أغـنام بجرعتين من اللقاح المحضر من المواد الإفرازية والإخراجية كما تم استخدام عشر حيوانات أخرى كضابط وجمعت عينات دم لفصل المصل لمدة عشرة أسابيع من بداية الحقن واجـري اختـبار التحدي لجميع الحيوانات بعد ١٥ و٢٨ يوم من حقن جرعة اللقاح الثانية وذلـك باسـتخدام يرقات الطور الأول لذبابة النغف و ذبحت جميع الحيوانات بعد الأسبوع الخـامس من اختبار التحدي الثاني. تبين وجود اليرقات فى الحيوانات الضابطة والمحصنة بنسـبة ١٥ % و ٣٣,٥ % على الترتيب واظهر اختبار الاليزا وجود أجسام مناعية مرتفعة فـى الحـيوانات المحصنة وصلت الى أعلى معدل لها فى الأسبوع الرابع من جرعة اللقاح الثانـية (التاسع من بداية التجربة) كما ظهرت بعض الأجسام المناعية فى عدد ٢ حيوان من المجموعة الضابطة.

SUMMARY

Four hundred and twenty sheep were examined for *Oestrus ovis* larval infestation by naked eye and serological test (Direct ELISA) during six months (from June to November, 2005). Excretory - secretory product (ESP) from first (L1) and second (L2) instar of *O.ovis* larvae were used as a coating antigen and a vaccine. The naked eye examination showed a positive prevalence of 19.5 % while the ELISA test showed 24.3 %.

The obtained data proved that the sensitivity of ELISA test was 97.6 %. Twenty female lambs of three months old were allocated into two groups. The first one received two IM injections of ESP in the neck, 4 weeks apart, initially in Freunds complete adjuvant and then in Freunds incomplete adjuvant. In control group, the animals received two injections of PBS with Freunds complete and incomplete adjuvants. Challenge test was carried out twice, 15 (15 larvae per animal) and 28 days (25 larvae per animal) after the second immunization. Sera samples were collected for ten weeks. On the 5th week post the second challenge test, all animals were slaughtered, all larvae were collected, identified and counted. ELISA data showed that *O.ovis* antibody began to increase one week after the first immunization and reached a peak on the 4th week post the second dose. Two animals among control group showed a moderate level of antibody, one on the 5th week and other on the 8th week. The results of challenge test showed that the establishment rate of *O.ovis* larvae were 33.5 % (134 out of 400) and 15 % (60 out of 400) in the control and vaccinated group respectively. It was concluded that diagnosis of *O.ovis* by ELISA using L1 and L2 ESP as a coating antigen is considered effective and the results obtained with *O.ovis* ESP L1 and L2 immunization are encouraging.

Keywords: *Oestrus ovis, excretory- secretory product, antigencity, ELISA, Immunization, challenge test and necropsy.*

INTRODUCTION

Oestrosis is a cosmopolitan myiasis of the nasal and sinusal cavities of sheep and goat caused by the obligatory parasites, *Oestrus ovis* (Hall and Wall, 1995). The infestation is associated with considerable economic losses (Steelman, 1976). Nasal and sinusal inflammation (Dorchies, *et al.*, 1998) with a mucopurulent and sometimes haemorrhagic discharge, lung abscesses, interstitial pneumonia (Dorchies *et al.*, 1993) and human ophthalmomyiasis were recorded (Dar *et al.*, 1980). The serological immune response of sheep to *O.ovis* had been studied by using larval extract as antigen source by intradermal test (Ilchmann and Hiepe, 1985), indirect haemagglutination test (Bautista *et al.*, 1988); direct enzyme-linked immunosorbent assay (Marchenko and Marchenko, 1989; Yilma, 1992 and Deconinck *et al.* 1995) and dot ELISA (Duranton *et al.*, 1995). Alcaide *et al.* (2005) showed that excretory – secretory products (ESP) from the *O.ovis* L1 in

winter and L2 during summer were the most sensitive coating antigen for serodiagonsis of *O.ovis* infestation. The current methods of oestrosis control is chemotherapy (Dorchies *et al.*, 1996; Dorchies *et al.*, 1997; Lucientes *et al.*, 1998). Because no alternative approach is yet available (no baits, no traps and no vaccines) nevertheless, epidemiological studies have shown that under field conditions, the intensities of infestation are less important in ewes than in lambs (Frugere *et al.*, 2000). Moreover, the survival of *O.ovis* larvae after artificial infestation was higher in immunodepressed animals than in immunostimulated one. Marchenko and Marchenko, (1989) suggested that immunological control of larval populations could occur in the field. Several trials of vaccination with ESP were carried out against common sheep parasites such as *Fasciola hepatica* (Spithill and Dalton, 1998); *Haemonchus contortus* (Schallig *et al.* 1997) and *Lucilia cuprina* (Tellam *et al.*, 1994 & Tellam and Bowles, 1997).

The aims of this study were (I) Evaluation the use of *O .ovis* L1 and L2 ESP as a coating antigen for serodiagnosis of *O.ovis* infestation. (II) Immunization of lambs by *O.ovis* L1 and L2 ESP.

MATERIALS and METHODS

Animals and Sera samples

A total of 420 sheep heads and the corresponding serum samples were collected during 6 months (from June and November 2005) from Riyadh slaughter house. Sera samples were stored at – 20 °C until use. The heads of slaughtered sheep were separated from the body and then they were cut sagittaly to examine the septum, the turbinates, the ethmoid and sinusal cavities. The larvae found were collected and identified according to entomological classification keys described by Zumpt (1965).

Preparation of The excretory- secretory product of *O. ovis* larval stages (ESP antigens)

First and second instar (L1 and L2) *O.ovis* larvae which were obtained from heads of naturally infested sheep in slaughter house were sorted according to larval stage, washed six times in phosphate-buffered saline (PBS) with 100 U/mL of penicillin and 100 ug/mL of streptomycin. The viability of larvae was checked under a stereomicroscope. The excretory- secretory products (ESP) were obtained from culture in vitro of the different larval stages. 100 live L1 larvae were gathered in a cell culture flask (NUNCLON – 50 mL

capacity) containing 8 mL RPMI – 1640 medium (MP formerly ICN, Australia; Cat No 1460054) with penicillin and streptomycin. Five L2 in 10 mL medium were used. All culture flasks were incubated in darkness for 24 h in a 5 % CO2 atmosphere at 37 °C. Larvae were removed and the remaining liquid was collected, centrifuged at 2000 xg for 20 min at 4 °C, the supernatants was filtered through 0.22 *um* filters (ICN, CAT NO. 64-001-04) and protein concentration was measured according to Bradford, (1976). The filtrate was stored at – 80 °C until use (Alcaide *et al.,* 2005). These solutions were termed the excretory – secretory products of L1 and L2.

Testing the antigencity of ESP by ELISA (Goddard *et al.,* 1999)

Five positive control serum samples (obtained from *O.ovis* infested sheep), five negative control serum samples (obtained from newly born ewes) and 420 sera samples collected from the slaughter house were tested in ELISA assay with special reference to sera samples collected from *O.ovis* infested sheep. ESP antigen was diluted in carbonate buffer (pH 9.6) to a final concentration of 2 *ug*/mL, distributed in 96 well plates (NUNCLON, DELTA) and incubated for 1 h at 37 °C then overnight at 4 °C. The wells were washed three times with phosphate buffer saline tween 20 (PBST: 0.01 M phosphate, 0.15 M sodium chloride, pH 7.2 and 0.1 Tween 20).100 *ul* of duplicate serum samples diluted (1:200) in PBST containing 2 % normal horse serum (GIBCO BRL Cat No 26050-039) were incubated 60 min at 37 °C. The plates were washed three times with PBST before addition of 100 *ul* / well horseradish peroxides conjugated rabbit anti-sheep IgG (MP, Australia. Cat No 654671) diluted (1: 2000) in PBST containing 2 % normal horse serum. Three final washes and then incubation at 37 °C of 100 *ul* / well of the Substrate, Tetramethyl benzidine (TMB), the reaction was stopped after 1 h with 10 % H2SO4 and the optical densities (OD) determined at 450 nm. Positive prevalence of infested sheep was recorded and the sensitivity of the ELISA assay was calculated using the formulae of Bautista *et al.,* (1988)

$$\text{Sensitivity \%} = \frac{\text{Number of } O.ovis \text{ infested sheep positive to the test X 100}}{\text{Total number of } O.ovis \text{ infested sheep}}$$

Immunization of lambs with ESP and experimental challenge

Ten lambs of three months old were received two IM injections of ESP in the neck, 4 weeks apart, initially in Freunds complete adjuvant

and then in Freunds incomplete adjuvant. The total amount of ESP injected into each sheep was 1.5 mg of protein (1 mg in the first injection and 0.5 mg in the second). In control group (Ten lambs), the animals received two injections of PBS with Freunds complete and incomplete adjuvants. Challenge test was carried out twice, 15 (15 larvae per animal) and 28 days after the second immunization (25 larvae per animal) using Pasteur pipette. Sera samples were collected for ten weeks.

ELISA for detection of *O.ovis* antibody in serum of vaccinated and control sheep

Serum samples of ten vaccinated and ten control sheep were diluted to 1:200 in PBST containing 2 % normal horse serum and then were used. The remaining procedure were Completed as before.

Postmortem examinations

On the 5th week post the second challeng test, all animals were slaughtered. After separating of the heads, all larvae were collected, identified and counted.

RESULTS

In Table (1), examination of a total of 420 sheep skulls for *O.ovis* infestation during Six months extended from June to November 2005 resulting in a positive prevalence of 19.5 %. The ELISA test showed 24.3 % of sheep were positive for *O.ovis* antibody. The protein content for ESP of L1 and L2 was 2 mg/ml.

In Table (2), out of 82 sera samples obtained from *O. ovis* larvae infested sheep (infestation was determined by the direct observation of *O.ovis* larvae in head of slaughtered sheep) 80 samples were positive by direct ELISA. The sensitivity of test was 97.6 %.

ELISA data (Table 3 and Figure 1) showed that *O.ovis* antibody began to increase one week after the first immunization with ESP of L1 and L2 and reached a peak four weeks post the second dose. Two animals among control group showed a high level of antibody, one on the 5th week and other on the 8th week post first immunization.

In Table (4), the results of challenge test showed that the establishment rate of *O.ovis* larvae were (134 out of 400) 33.5 % and 15 % (60 out of 400) in the control and vaccinated group respectively.

and vaccinated groups respectively after experimental challenge test with L1 larvae recovered from slaughtered sheep. Frugere *et al.*, (2000) used ESP L3 *O.ovis* and recorded very similar establishment rates in control and vaccinated groups (39 % and 35 %). Cepeda-Palacios *et al.*, (2000) showed that 38 % reduction in adult populations of *O.ovis* might achieved by 40 % reduction of mature larval weight. Frugere *et al.* (2000) demonstrated that the percentage of developing stages was lower in lambs immunized with L3 ESP than in control lambs. The lack of larval establishment rate despite high antibodies response may related to the short time of necropsy after immunization and experimental challenges since the duration required for the effect of antibody on larval stages was unknown. Also some ESP can not interfere with the parasite establishment but interfere with the fecundity of the parasite (Frugere *et al.*, 2000).

Since the development of *O.ovis* larvae occurs in contact with the nasal and sinusal mucosa, it could be better in the future to choose the intranasal route for immunization other than intradermal or subcutaneous one. Bowles *et al.* (1987) demonstrated that intranasal immunization of sheep with a second stage ESP of *Lucilia cuprina* resulted in a significant reduction in larval numbers after experimental challenge whereas intradermal immunization does not protect the animals.

Two animals among control group were recorded in the present study to have a high level of *O.ovis* antibody, one on the 5th week and other on the 8th week post first immunization which might resulted from exposure of the control to natural infestation during the experiment.

In conclusion, diagnosis of *O.ovis* by ELISA using L1 and L2 ESP as antigen is considered effective and the results obtained with *O.ovis* ESP L1 and L2 immunization are encouraging.

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