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IMMUNIZATION OF RABBITS AGAINST *SARCOPTES SCABIEI VAR. CUNICULI.*

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SUMMARY

This study was carried out to evaluate the protective role of *Sarcoptes scabiei var. cuniculi* antigen in immunizing rabbits against *Sarcoptes* mites. For this purpose nine rabbits were divided into three groups. The first group (five rabbits) was immunized with Sarcoptes antigen. The second group, (two rabbits) were injected with adjuvant only. The third group included two non-immunized rabbits used as negative control. All rabbits of the three groups were challenged on day 35 with *Sarcoptes cuniculi*. No clinical signs were detected in four rabbits of the immunized group, while the fifth rabbit showed restricted lesion with few numbers of mites. All the immunized rabbits developed higher specific antibody levels by ELISA. Seven polypeptides bands ranging from 142-8.2 KDa were recognized in *Sarcoptes* antigen by SDS-PAGE. Immunoblot revealed that, hyper-immune sera contained antibodies that bound strongly to proteins of 142KDa. So *Sarcoptes scabiei var. cuniculi* antigen can be used as an effective compound to control *Sarcoptes* mange in rabbits.

INTRODUCTION

Sarcoptic mites are tiny burrowing obligate ectoparasites. They cause highly contagious disease known as sarcoptic mange in animals and can also be transmitted to man. Such infestation is considered as the most important parasitic disease of rabbits caused by *Sarcoptes scabiei var. cuniculi*. *Sarcoptes* belong to subclass Acari, class Arachnida, family sarcoptidae (Hughes, 1976). Rabbits are particulary sensitive to such infestation and severe outbreaks of generalized form of crusty lesions have been reported. Sayed (1994) found that 35% of examined rabbits were infested

with *Sarcoptes scabiei* var. cuniculi in Assiut. The most common clinical signs of sarcoptic mange are intense scratching, emaciation and death. Heavy infestations leads to anemia, leukopenia and changes in biochemical level in serum. The lesions usually appear on the sparsely haired parts of the body such as muzzle, nose, around the eyes, base of ears, head and legs. These lesions often become subjected to secondary infection with bacteria. Rabbits are considered an important economical source of meat production in Egypt, FAO (2002) reoprted that, the annual meat production of rabbits in Egypt is about 69000 tonnes carcass weight. The infestation with mange may decrease this production. This is the matter of concern for careful monitoring of the spread and severity of the out breaks as well as possible.

Currently, control methods relied on certain chemical compound such as ivermectin which has not yet authorized by the producer. Organophosphorous compounds have been used but toxic residues, environmental pollution and threat of parasite resistance are all perceived as undesirable features of these applicable methods, providing an impetus for the search for alternatives.

So, developing a vaccine against *Sarcoptes* is thought to be a feasible alternative to chemical control. Some observations suggest that, control by vaccination might eventually be an effective and safe proposition. Morgan and Arlian (1994) revealed that, rabbits immunized with an extract from *Sarcoptes scabiei* var. canis produce antibodies to 20 Sarcoptes proteins using immunoblotting. Arlian et al., (1996) demonstrated that, natural reinfestation of dogs with *S. scabiei var. canis* induced circulating scabies-specific antibody responsible for completely clearing the mites.Vander Heijden et al., (2000) had succeeded to use ELISA to detect *Sarcoptes scabiei var. suis* antibodies in infested pigs.

The potential for the immunological control of *Psoroptes* mites had been investigated for sheep by Smith and Pettit (2004). They immunized sheep with various fractions of soluble extract of *Psoroptes ovis* mites, then sheep were challenged.The rate of growth of lesion in immunized sheep had been reduced to less than third, as well as the numbers of mites to 13 times compared to control ones. Smith et al., (2002) successded to protect sheep against challenge with *P. ovis* infestation. Also Uhlir (1992) recorded that, rabbits immunized with whole body extract of *P. cuniculi induced* cell response and high level of specific serum antibody.

The purpose of this study was to evaluate the protective effect of immune responses developed in rabbits after immunization with antigens extracted from the whole body of *Sarcoptes scabiei var. cuniculi.*

MATERIALS AND METHODS

Preparation of *Sarcoptes scabiei* antigen: Different stages of living *Sarcoptes scabiei var.* cuniculi mites of both sexes were collected from the skin scrapings of naturally infested rabbits. Samples were collected in glass Petri-dish, examined microscopically, and warmed to 37°C for 30 minutes. Mites were then picked using a metal probe under dissecting microscope, and placed in phosphate buffered saline pH 7.2. The mites were mechanically homogenized, and then sonicated five times for 2 min each in ice bath. The suspension was centrifugated at 14,000 rpm for 45 min at 4°C. The supernatant was collected and protein concentration was determined using Lowry's method (Lowry et al., 1951). *Sarcoptes* antigen was aliquoted and stored at -70°C until used.

Rabbit immunization: rabbits were immunized according to Smith and pettit (2004). Nine mature Newzealand male white rabbits, each 1.5- 2 Kg were obtained from a special colony proved to be free from *Sarcoptes* mange infestation and any other parasitic infection. They were assigned into three groups. The first group included five rabbits, each one was immunized subcutaneously with 500 ug protein of *Sarcoptes scabiei var. cuniculi* in the following manner. Primary immunization was carried out with 200 ug protein of the antigen in one ml PBS emulsified with complete Freund's adjuvant in equal volume on day zero. First booster dose was carried out with 150 ug protein of the antigen emulsified with incomplete Freund's adjuvant in equal volume on day 14. Second booster dose was done on day 28 as the first booster. The second group, included two rabbits which were injected with adjuvant without antigen and was used as adjuvant control. The third group included two non-immunized rabbits used as negative control.

Challenge with *Sarcoptes scabiei* var. cuniculi mites: all rabbits in the three groups were challenged with skin scrapping harbouring live mites taken from naturally infested rabbits. The scrapping was applied on the fore legs using cloth bag. In addition, a naturally infested rabbit was placed with each group. The challenge was done on day 35 post immunization. The degree of *Sarcoptes* infestation was assessed by body examination, skin scrapping and severity of lesion if present. Skin scrappings were colleced on Parrafin oil and examined under dissecting microscope. Blood samples were taken weekly during a period of 12 weeks (from the beginning of experiment till the termination) from the ear vein of each rabbit in all groups. Sera were collected and stored at -20°C till used. Sera of immunized rabbits were collected on day 35 PI and were used as hyper-immune sera.

Enzyme-linked immunosorbent assay (ELISA): ELISA was applied according to Iacona et al., (1980). ELISA plates with 96 flat bottom wells were coated, each with 100µl of *Sarcoptes*

antigen in carbonate buffer pH 9.6 and incubated overnight at room temperature, The plates were blocked with 0.1% bovine serum albumin. Fifty µl of the collected rabbit sera diluted at 1:100 in PBS were added for 2 hours at 37°C in shacking water bath. After washing the plates, 50µl of alkaline phosphatase labeled anti-rabbit IgG antibodies (Sigma Com.) diluted at 1:5000 in PBS were added for 1 hour at 37°C in shaking water bath. The P-Nnitrophenyl phosphate at 1mg/ml substrate buffer pH 9.8 was added and the absorbance of the coloured reaction was read within 30 minutes at 405 nm using ELISA reader. The positive threshold value was determined as double fold the mean cut off value of negative sera.

Electrophoretic analysis of *Sarcoptes scabiei* antigen by SDS-PAGE: Ten ug of *Sarcoptes scabiei* antigen were electrophoresed using 10 % SDS-PAGE under reducing conditions (Laemmli, 1970). The fractionated antigen was visualized by silver stain (Pharmacia Com.). The gel was fixed with trichloroacetic acid. The fixed gel was sensitized by ethanol. The gel was stained in silver nitrate solution, then soaked in developing solution till bands became visible. The reaction was stopped by EDTA.Na.H2O

Immunoblot technique: The fractionated *Sarcoptes scabiei* antigen using SDS-PAGE was electrically transferred onto nitrocellulose (NC) membrane. NC sheets were cut into 0.5cm strips (Towbin et al., 1979) followed by blocking in 5% BSA in PBS for 2h on rocker platform. Sera diluted at 1:100 in 5% BSA/PBS-T were reacted with fractionated antigen NC strips for 2h. on rocker platform. Following washing, Horse reddish peroxidase labeled anti-rabbit IgG diluted at 1:1000 (Bio-Rad Com.) in PBS-T was added to NC strips for 1h on rocker platfom. The chromogen 3Amino-9Ethyl Carbazole substrate was added to NC strips and allowed to develop for 30 min. The reaction was visualized by the naked eye.

RESULTS

The immunization effects of *Sarcoptes* antigen on rabbits challenged with *Sarcoptes scabiei var. cuniculi* mites on day 35 post immunization are summarized in table (1). All immunized rabbits appeared clinically healthy (Fig. 1). Four of five immunized rabbits did not exhibit any lesion of Sarcoptic mange, nor any individuals *Sarcptes* mites after scrappings compared to that appeared on all control rabbits manifested with living stages of mites till the termination of experiment (7 weeks post -challenge).

Concerning the 5th immunized rabbit, there were restricted lesions, compared to those appeared on control ones, on the fore legs at 6th week post-challenge. By examining skin scrappings of such lesions, few mites were detected, some of them were found dead.

Rabbits of group II (treated with adjuvant) and group III (negative rabbits) showed typical lesions of sarcoptic mange in 2nd week post-challenge and emaciation (Fig. 1). Control rabbits exhibited widely spread severe crusty lesions on muzzle, eye lids, and legs (Fig. 1). As well, legs suffered from sloughing of nails,and the degree of infestation increased awfully (Fig. 2). Microscopical examination showed huge numbers of different stages of living mites detected in the skin scrappings of both control groups without any difference between them (Table, 1).

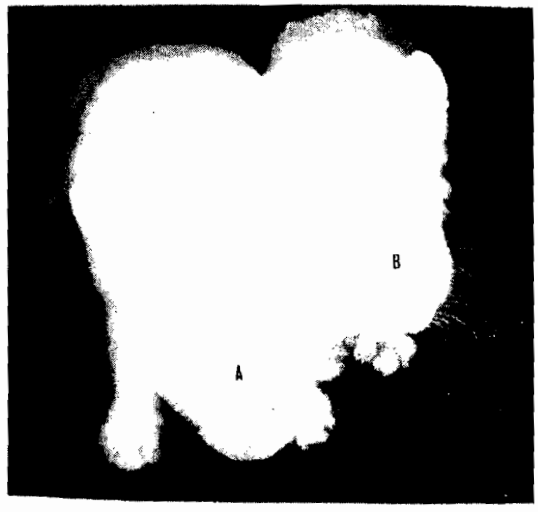


Fig. (1): Results of challenge of rabbits with *Sarcoptes* mites showing clinically healthy immunized rabbit (A) and control rabbit with sarcoptic mange lesions on legs, muzzle, eye and emaciation (B).

Fig. (2): lesions on hind legs of control rabbit.

Table (1): Results of challenge of rabbits with *Sarcoptes scabiei var. cuniculi* mites.

Number of groups	Number of rabbits in each group	Number of rabbits infested with mange lesions post challenge			
		2 weeks	4weeks	6 weeks	8 weeks
Group I (immunized)	5	-	-	1*	1*
GroupII (treated with adjuvant)	2	2*	2**	2***	2***
(GroupIII (non-immunized)	2	2*	2**	2***	2***

* restricted lesions
** moderate lesions
*** severe lesions

The result of ELISA for sera obtained serially from all immunized rabbits showed that, specific antibodies were detected on day 14 post-immunization and increased gradually up to 35 days PI. The antibody level started to decline after one week from the challenge with mites then slightly increased again on 2nd week post- challenge. The antibody level remained at constant levels for about 2 weeks then decreased gradualy. There was no reaction observed with the sera obtained from the control rabbits (groupII& groupIII) up to 35 days post- immunization. However, antibodies which were detected on 2nd week post-challenge had lower level than those detected in the immunized rabbit sera till the termination of experiment (Fig. 3).

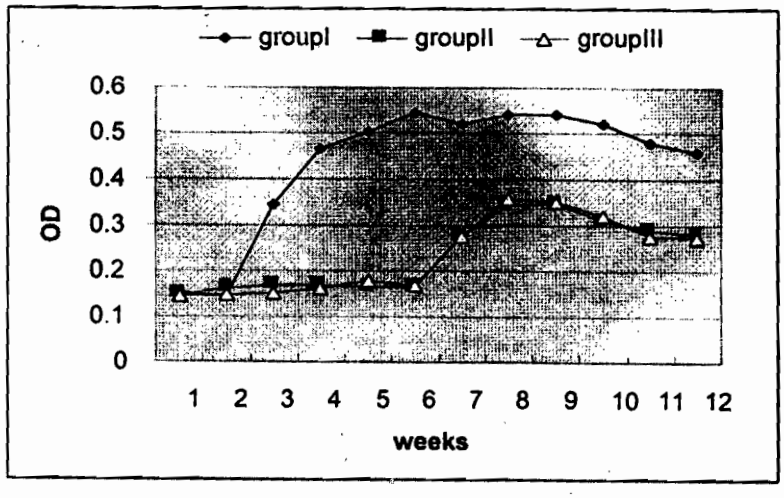
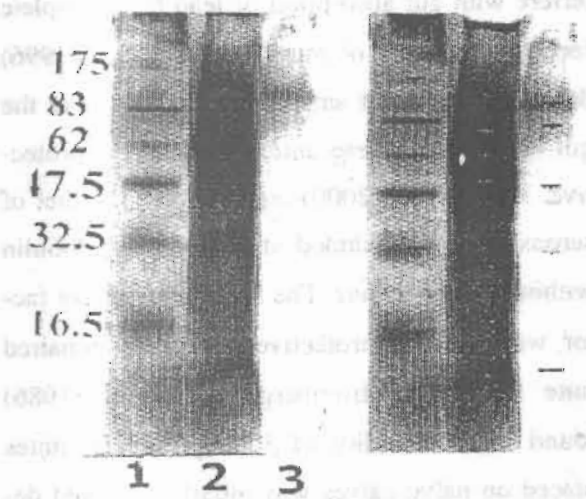


Fig.(3): Serum antibody levels in both immunized and control rabbit groups detected by ELISA.

Sarcoptes scabiei antigen was analyzed by SDS-PAGE and stained with silver nitrate revealed that, there were seven polypeptides ranging in their molecular weight from 142 to 8.2 KDa. Immunoblot analysis using hyperimmune sera from immunized rabbits was recognized 6 polypeptides. The polypeptide with molecular weight 142 KDa was reacted strongly with the specific antibodies. These findings suggested that 142 KDa protein is a specific immunogenic epitope in *Sarcoptes scabiei* antigen (Plate 1, Table 2, Figs. 4, 5 and 6).



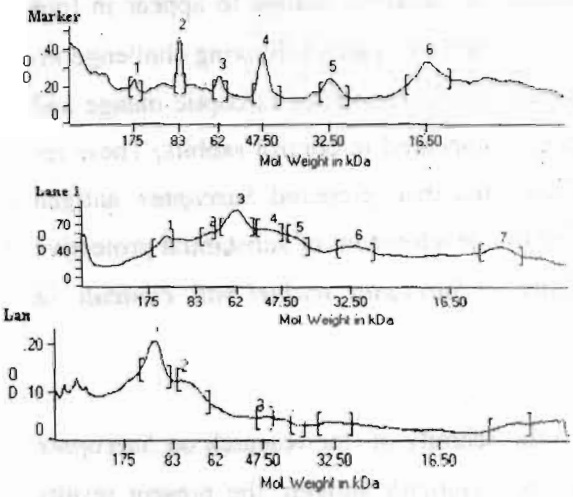


Plate (1): Characterization of *sarcoptes* antigen by SDS-PAGE and Immunoblot .
Lane 1. prestained wide range molecular weight markers (Bio-lab.comp.).
Lane 2. analysis of *sarcoptes* antigen by SDS-PAGE.
Lane 3. Immunoblot analysis of *Sarcoptes* antigen using hyper-immune sera.

Fig. (4): Electrophoretic analysis of the molecular weight markers.

Fig. (5): Electrophoretic analysis of *sarcoptes* antigen

Fig .(6): Immunoblot analysis of *sarcoptes* antigen using hyper-immune sera

Table (2): Results of analysis of *Sarcoptes* antigen using SDS-PAGE and Immunoblot

SDS-PAGE analysis of Sarcoptes antigen	Immunoblot analysis of Sarcoptes antigen
142	142
75.8	75.8
61.7	-
51.3	51.3
44.5	44.4
31.6	31.6
8.2	8.2

DISCUSSION

The present study aimed to induce host resistance against *Sarcoptes scabiei* mites by immunization of rabbits with a prepared *Sarcoptes* antigen.

The resistance was relised through prevention of the lesions of sarcoptic mange to appear in four of five immunized rabbits following challenge infestation. Typical lesions of sarcoptic mange and emaciation appeared in control rabbits. These results indicated that, prepared *Sarcoptes* antigen induced the development of substantial protective immunity to *Sarcoptes scabiei var. cuniculi* in rabbits.

Due to the scarcity of the research on *Sarcoptes scabiei var. cuniculi* antigen, the present results can be discussed in the view of very close results obtained in case of other species of *Sarcoptes* and *Psoroptes* mites.

The previous results were in agreement with Uhlir in 1992 found that immunized rabbits with the whole body extract of *Psoroptes cuniculi* developed partial immunity to the infestation with this mite. Arlian et al., (1995) who showed that, rabbits immunized with house dust mite extract were resistant to infestation by *Sarcoptes scabiei var. canis.* The resistance was evidenced by a marked reduction in parasite load. Smith and Pettit (2004) reported that, sheep immunized with *Psoroptes ovis* antigens and challenged with these mites showed reduced lesion growth to less than a third and mite numbers by more than 13 times compared to control.

The mechanism of protection may be attributed to different factors, first of all mites may ingest rabbit antibodies during feeding, which will interfere with gut absorption or lead to incomplete feeding and death of mites. Arlian et al., (1996) detected that rabbit antibodies are present in the gut of mite and these antibodies may be protective. Pettit et al., (2000) reported that the diet of serous exudate included sheep immunoglobulin within *Psoroptes ovis.* The second important factor was that the protective immunity impaired mite fecundity. Stromberg and Fisher (1986) found that, fecundity of *Psoroptes ovis* mites placed on naîve calves was intially high and declined after challenge infestation. So, the appearance of restricted lesion and low numbers of dead mites on the 5th immunized rabbit could be attributed to such previous factors.. Also, vaccination with specific scabies tissue antigens could enhance the cell-mediated response to protect the hosts against natural infestation.

Concerning the measurement of antibody levels in the sera of all immunized and control rabbits, ELISA showed that, high antibody levels were detected along the period extended from 2nd week post-immunization to the termination of the experiment (12 week). The increase of antibody levels in the immunized rabbits had been con-

firmed by the complete disappearance of mange lesions. This might be attributed to rabbit immunoglobulin which prevented feeding of mites. These results were nearly similar to those of Arlian et al., (1995) who recorded that, all immunized rabbits with dust mite extract developed specific antibody. Also, Uhlir (1992) obtained the same previous results with *Psoroptes cuniculi* antigen. The same author (1991) detected high levels of specific antibody in rabbits infested with *Psoroptes cuniculi* mites. Although antibody levels in both control groups increased after challenge, it was not enough to overcome the appearance of typical lesions of sarcoptic mange. The decrease of antibody levels at the termination of experiment indicated that, rabbits need booster dose of antigen every 3 months or increase the dose of antigen used.

Electrophoretic analysis of *Sarcoptes* antigen revealed that, seven polypeptides ranging in their molecular weight from 142 to 8.2 Kda were obtained. Six of them were recognized by hyperimmune sera using immunoblot technique. A polypeptide 142 KDa was strongly recognized and, the other polypeptides appeared faintly. These observations are similar to those of Arlian and Morgan (2000) who found that, all infested dogs with *Sarcoptes scabiei var canis* developed antibodies against house dust mite antigens at 142 KDa. Lee et al., (2002) recognized three high molecular weight 100- 180 KDa in whole *Psoroptes ovis* extract. In contrast, Morgan and Arlian (1994) showed that, rabbits immunized with an extract made from *Sarcoptes scabiei var canis* produced antibody to 20 *Sarcoptes* protein but bound strongly to proteins of 25 and 39-52 KDa. These differences could be due to the methods of employment and mite strain.

Vaccination with *Sarcoptes scabiei var cuniculi* might be used for control sarcoptic mange in rabbits, other susceptible animals and even man. Arlian et al., (1996) and Morgan et al., (1997) recorded that, antigens from one variety of mites may induce protection against infestation of mites of different varieties. These results support the view that control of *Sarcoptes* by vaccination may be possible but the protective effect can be improved by further fractionation to concentrate the active components.

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تطعيم الأرانب ضد الساركوبتس إسكابى

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أجريت هذه الدراسة لتقييم الدور المناعى لمولد الضد المحضر من الساركوبتيس إسكابى الخاص بالأرانب وذلك بتطعيم الأرانب ضد هذه العثة، ولهذا الغرض تم إستخدام تسعة أرانب قسمت إلى ثلاث مجموعات، المجموعة الأولى وتضم خمسة أرانب تم تطعيمها بمولد الضد الساركوبتى والمجموعة الثانية تضم أرنبين تم حقنها بمساعد الدواء أما الأرنبان الآخران بالمجموعة الثالثة فلم يحصنا وتم إستخدامها كمجموعة ضابطة سالبة. تم إجراء تحديد عند اليوم الخامس والثلاثون بعثة الجرب الساركوبتى وقد تبين عدم ظهور أى أعراض للجرب الساركوبتى على أربعة أرانب من المجموعة المحصنة أما الأرنب الخامس فقد أظهر أعراض طفيفة مع أعداد طفيفة مع وجود أعداد قليلة من عثة الجرب. وبإستخدام الإمتصاص الإنزيمى المناعى تم الإستدلال على الأجسام المناعية المضادة فى جميع الأرانب المحصنة. وبتحليل مولد الضد الساركوبتى بإستخدام الإستقطاب الكهربى أظهر وجود ١ حلقات ببتيدية بأوزان من ١٤٢ إلى ٨.٢ كيلو دالتون وبإستخدام الطبع المناعى لتحديد الحلقات المناعية أظهر المصل العالى المناعى إحتوائه على أجسام مناعية إرتبطت بقوة بالحلقة الببتيدية ١٤٢ كيلو دالتون وبذلك يمكن إستخدام مولد الضد المحضر من الساركوبتس إسكابى للسيطرة على الجرب الساركوبتى فى الأرانب.