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A TRIAL TO PROTECT RABBITS AGAINST *PSOROPTES CUNICULI* MITES BY IMMUNIZATION WITH *PSOROPTES* EXTRACT.

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SUMMARY

This study based on the evaluation and characterization of the immune response induced by *Psoroptes cuniculi* mite extract. For this purpose, eight rabbits were used and divided into two groups. The first group composed of five rabbits was immunized subcutaneously with *Psoroptes cuniculi* extract emulsified with freund's adjuvant on day 0, 14 and 28. The second group composed of three rabbits considered as control. All rabbits were challenged on day 35 post-immunization by placing live mites in external auditory canal. No clinical signs of *Psoroptes* mange were observed among immunized rabbits. Although few eggs were detected at third week post-challenge in immunized rabbits, but no developmental stages of mites were observed until the termination of the experiment. Analysis of *Psoroptes cuniculi* antigen by SDS-PAGE revealed that there were 3 polypeptide bands with molecular weights 154.5, 54 and 32 KDa. Immunoblot using hyper-immune sera from immunized rabbits recognized a polypeptide band with molecular weight 54 KDa.

INTRODUCTION

The ear mite *Psoroptes cuniculi* is a worldwide, obligate ectoparasite of rabbits. It is responsible for a highly contagious skin disease in the ear (Soulsby, 1986). *Psoroptes cuniculi* mites are mainly found inside the rabbit ear pinnae. If they are not treated properly, the mites may spread and infest head, neck, legs, ventral abdomen and perianal region. The mucus and fecal material of mites induce an inflammatory reaction that leads the rabbit to scratch its ear. The blood that comes out of the scratched lesions serves as a source of nutrition for the mite beside the serous exudate

and skin secretions. The clinical signs of ear mange are characterized by itching ears, frequent shaking of the head and formation of crusts and scabs, which, in case of untreated animals, can completely fill the external ear canal (Bates, 1999). Also, the infested animals can lose condition and finally die. Often, secondary bacterial infections will develop, which may damage the inner ear and reach the central nervous systems causing torticollis. So, ear damage is an important cause of economic loss to rabbits industry.

Generally, the control of animals mange is based mainly on the use of acaricides. Till now, several problems are associated with the use of such acaricides including toxicity for animal and human, environmental damage (Halley et al., 1993) and development of mite drug-resistance (Currie et al., 2004).

For these reasons, immunologically based control methods must be required. Some investigations suggested that vaccination might be possible, as infested rabbits with *P. caniculi* developed a high level of specific serum antibody and induced T cell (Uhlir, 1991). The same author in 1992 immunized rabbits with whole body extract of *P. cuniculi*, they developed partial immunity against the infestation with this species of mite. Further more, rabbit immunoglobulins (IgG) were found immuno-localised on the surface or in the cytoplasm of gut cells of *P. cuniculi* (Pettit et al., 2000). Also Smith and Pettit (2004) immunized sheep with various fractions of soluble extract of *P. ovis* mites, the rate of growth lesion had been reduced to less than third in immunized sheep, as well as, the numbers of mites were reduced to 13 times when compared to control sheep.

Therefore, the aim of the present study was to evaluate the efficacy of *P. cuniculi* extract for control and complete eradication of ear mites in rabbits.

MATERIALS AND METHODS

I Collection of *Psoroptes* mites: different stages of live *Psoroptes cuniculi* were collected from the ears of naturally infested rabbits. Mites were separated from crusts by putting them in an incubator at 28°c for 2 hours then, mites were picked up using a metal probe under dissecting microscope and collected in phosphate buffered saline pH 7.2 (Boyce and Brown,1991).

II Preparation of antigens: The collected mites were homogenized and then sonicated five times for 2 min in ice bath. The suspension was centrifugated at 14000 rpm for 45 min at 4°C. The supernatant was collected and protein content was determined using Lowry's method (Lowry et al., 1951). *Psoroptes* antigen was stored at – 40°C until used.

III Rabbits immunization: Eight adult male white NewZealand rabbits 6 months old, each

1.5-2 Kg were used. All rabbits were obtained from a colony proved to be free from *Psoroptes* and *Sarcoptes* mange infestation. Rabbits were divided into two groups. First group (group I) was composed of five rabbits, each one was immunized subcutaneously with 200ug of Psoroptes antigen in one ml PBS emulsified with equal volume of complete freund's adjuvant on day 0 (Uhlir, 1992). Two booster doses were carried out with 150ug protein of the antigen for each emulsified with equal volume of incomplete freund's adjuvant on day 14 and 28. The second group (group II) included three non-immunized rabbits, which was considered as control.

IV Challenge with *Psoroptes* cuniculi mites: All rabbits in both immunized and non-immunized control groups were challenged on day 35 post-immunization (Rossi et al., 2007). Infestation was done by placing crusts harboring live mites, taken from naturally infested rabbits, deeply in external auditory canal and then closed with cotton and ear bag. All rabbits were observed daily to evaluate the presence of crusts, animal behavior and skin irritations. Ear swabs were collected from auricular area and external ear canal weekly for six weeks post-challenge. Swabs were examined for live mites and their eggs using a stereomicroscope. Detected mites were transferred to microscopic slides for morphological differentiation of larvae, nymphs and adults.

V Electrophoretic analysis of *Psoroptes cuniculi* antigen by SDS-PAGE: Ten ug of *Psoroptes cuniculi* antigen were electrophoresed using 10 % SDS-PAGE under reducing conditions (Laemmli, 1970). The fractionated antigen was visualized by Coomassie blue stain. The gel was soaked overnight in the Coomassie blue R (0.25% Coomassie blue powder dissolved in destaining solution). The gel was then destained with destaining solution (45% methanol, 5% glacial acetic acid and 50% distilled water) with several changes till the bands became clear.

VI Immunoblot technique: The fractionated *Psoroptes cuniculi* 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. Hyperimmune 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 raddish 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

Results of challenge with *P. cuniculi* mites have been summarized in table 1. Abnormal behaviour as restlessness, shacking of head and ears were observed in all rabbits in both groups for two weeks post-challenge. No clinical signs of *Psoroptes* mange were observed among immunized rabbits from the third week till the termination of the experiment. Rabbits in control group, showed characteristically extensive exudative dermatitis and dense crusts present in the external auditory canal from the 3rd week post-challenge, this increased gradually till the termination of experiment. All immunized rabbits were completely free from any crusts. Ear swabs taken from all rabbits in the first two weeks post-challenge contained few live adult mites. Few dead mites were isolated from immunized rabbits only. All developmental stages of mites (eggs, larvae, nymphs and adults) were detected from the 3rd week post-challenge and increased gradually to become numerous at the termination of the experiment in control rabbits. Although few eggs were detected at third week post-challenge in immunized rabbits no developmental stages of mites were observed until the termination of the experiment.

Psoroptes cuniculi antigen was analyzed by SDS-PAGE and stained with Coomassie stain, this revealed that there were 3 polypeptide bands their molecular weight were154.5, 54 and 32 KDa. Immunoblot using hyper-immune sera from immunized rabbits recognized a polypeptide band with molecular weight 54 KDa (Fig. 1).

Table (1): Results of challenge with *P. cuniculi* mites in immunized and control rabbits

Weeks	1		2		3		4		5		6	
groups	I	II	I	II	I	II	I	II	I	II	I	II
Live mites	+	+	+	+	-	++	-	++	-	+++	-	+++
Dead mites	+	-	+	-	+	-	-	-	-	-	-	-
Eggs	-	-	-	-	+	++	+	++	-	+++	-	+++
Crust	-	-	-	-	-	+	-	++	-	+++	-	+++
Developmental stages	-	-	-	-	-	+	-	++	-	+++	-	+++

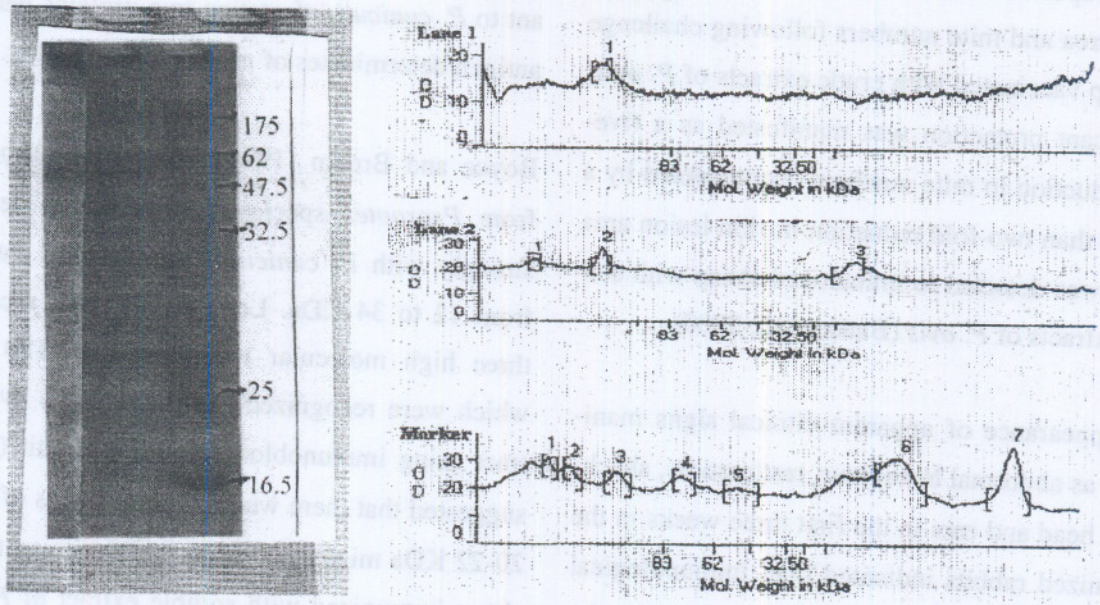

Fig.(1): Characterization of *Psoroptes cuniculi* antigen by SDS-PAGE and Immunoblot .

Marker: prestained wide range molecular weight markers (Bio-lab.comp.).

Lane 1. analysis of *Psoroptes* extract by SDS-PAGE.

Lane 2. Immunoblot analysis of *Psoroptes* antigen using hyper-immune sera.

DISCUSSION

The present study demonstrated that, *P. cuniculi* extract could produce antibodies to acquire a resistant status against the infestation with *P. cuniculi mites*. The resistance was detected through a reduction in number of mites and prevention of the formation of different developmental stages in the immunized rabbits with *Psoroptes* antigen. These results indicated that, the immune response interfered with the feeding of mites and impaired the reproduction of mites. Similar results were recorded by Uhlir(1992) who proved that *P. cuniculi* mites were not survived and not reproduced on the vaccinated rabbits with *P. cuniculi* antigen but caused only transient disease in ear then mites died off. The obtained results of challenge were coincide with data of Rossi et al., (2007) who demonstrated that, the sera of chronically infested and resistant rabbits reacted strongly with the digestive system of *P. cuniculi* using immunocytochemistry. Also, Pettit et al., (2000) found that, *P. cuniculi* mites ingested host immunoglobulin.

As well, concerning *Psoroptes* ovis, Stella et al., (1997) reported that, there were reduction in both lesion area and mite numbers following challenge of sheep vaccinated with crude extracts of *P. ovis*. Significant protection was manifested as a five-fold reduction in mite numbers accompanied by a greater than two-fold reduction in skin lesion area which was detected in immunized sheep with soluble extracts of *P. ovis* (Bates et al., 2000).

The appearance of apparent clinical signs manifested as abnormal behaviour, restlessness, shacking of head and ears in the first three weeks in the immunized rabbits indicated that, immunological reactions to *P. cuniculi* antigen may influence the development of ear lesions and specific antibody reactions after challenge infestation with *P. cuniculi* mites.

Concerning the results of analysis of *P. cuniculi* antigen by SDS-PAGE there were 3 bands ranging between 154.5 to 32 KDa and The polypeptide 54 KDa was strongly recognized with hyperimmune sera of immunized rabbits using immunoblot. These findings were more or less different from those, which were recorded by many authors (Uhlir (1993); Boyce and Brown (1991); Lee et al., (2002); Smith and Pettit (2004).

Uhlir (1993), in immunoblot the antisera from rabbits heavily and mildy infested with *P. cuniculi,* recognized the immunogenic antigen with a molecular mass of 48 KDa. Donadio et al., (2002) recorded in polyclonal antiserum of rabbit resistant to *P. cuniculi* infestation two proteins bearing antigen determinates of mites in immunoblot.

Boyce and Brown (1991) found that antibodies from *Psoroptes* species infested sheep reacted strongly with *P. cuniculi* mite antigens ranging from 12 to 34 KDa. Lee et al., (2002) recorded three high molecular weights of 100-180 KDa which were recognized in the whole *P. ovis* extract using immunoblot. Smith and Pettit (2004) suggested that there were a pair of bands of about 20-22 KDa might be responsible for protection of sheep immunized with soluble extract of *P. ovis* after challenge infestation. These differences could be due to the methods employed and the strain of mites.

Preliminary results obtained in the present study suggested that, *Psoroptes* antigen might play a successful possible role in host resistance against *P. cuniculi* mites. Also, these results may encourage further studies on large scale and identify the specific epitopes that may have an immunogenic role to vaccinate rabbits and other animals subjected to be infested with mites as *P. cuniculi* proved to have antigenic similarities with other species of *Psoroptes* (Rafferty and Gray, 1987).

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محاولة لحماية الأرانب ضد عثة الجرب السوربتيسى بالتطعيم بمولد الضد السوربتيسى

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هذه الدراسة ميزت الاستجابة المناعية المحدثة بواسطة مولد الضد المحضر من عثة السوربتس كانيكيولاي ولهذا الغرض تم استخدام ثمانية أرانب قسمت إلى مجموعتين. المجموعة الأولى تكونت من خمس أرانب حصنت تحت الجلد بمولد الضد المحضر من السوربتس كانيكيولاي مع مساعد الدواء عند اليوم 0 و 14 و 28. .أما المجموعة الثانية والتي تتألف من ثلاثة أرانب فقد اعتبرت مجموعة ضابطة وتم إجراء التحدي لجميع الأرانب عند اليوم 35 من التحصين بوضع عثة السوربتس الحية في القناة السمعية الخارجية..وقد لوحظ عدم وجود أي أعراض للجرب السوربتيسي في الأرانب المحصنة..وعلى الرغم من ظهور عدد قليل من البيض عند الأسبوع الثالث من التحدي بالأرانب المحصنة إلا انه لم تشاهد أي مراحل لحياة العثة حتى انتهاء التجربة..وبتحليل مستضد السوربتس باستخدام الاستقطاب الكهربي ظهرت 3 حلقات ببتيدية أوزانها الجزيئية 154.5 و 54 و 32 كيلو دالتون..بينما الطبع المناعي فقد ميز حلقة ببتيدية واحدة وزنها الجزيئي 54 كيلو دالتون باستخدام المصل العالي المناعي من الأرانب المحصنة.