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Immunization of rabbits with antigens from *Psoroptes cuniculi*, the rabbit scab mite

J. UHLÍŘ
Institute of Parasitology, Czechoslovak Academy of Sciences, Branišovská 31, 370 05 České Budějovice, Czechoslovakia

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Abstract. Rabbits immunized with the whole body extract of *Psoroptes cuniculi* (Delafond, 1859) developed partial immunity to the infestation with this mite. These rabbits manifested *P. cuniculi* antigen-induced cell response and a high level of specific serum antibody after the immunization. Electrophoretic separation of the mite extract followed by immunostaining with various sera revealed differences between artificially immunized and naturally infested rabbits to most of the *P. cuniculi* antigens. However, the specific antibody pattern, that was developed by the immunized rabbits, was not changed after these rabbits were infested with the mites.

Infestation of mammals with parasitic mites induces immunological reactions. The immune response (humoral and cell-mediated) is a physiological reaction that plays an important role in the host-ectoparasite interaction (Allen 1987). The immunological reactions, in the case of infestations with *Sarcoptes scabiei* (Arlian 1989) and *Demodex canis* (Barta et al. 1983), are associated with deleterious effects on the mites and at least partial protection of the hosts. The same is believed to be true for infestation of cattle and rabbits with *Psoroptes ovis* (Stromberg and Fisher 1986) and *P. cuniculi* (Uhlíř 1991a), respectively. However, the investigation of artificial immunization as a way of controlling parasitic mites have not been reported yet. The possibility of developing a vaccine, based on immunological mechanisms, to control ectoparasite infestation has been intensively studied for tick infestation. A variety of antigen sources have been used to immunize against different tick species (reviewed by Willadsen 1986, Brown 1988). Recently, the midgut antigens, designated as "concealed" antigens, have been shown to protect cattle against *Boophilus microplus* more effectively than the "natural" antigens (Willadsen and Kemp 1989).

In this study, we report for the first time on the immunization of rabbits against *P. cuniculi* with the whole mite body extract.

MATERIALS AND METHODS

Seven rabbits of the Chinchilla breed (Velaz, Prague), both males and females were used in this study. **Group A:** Four rabbits (3–6 months old) were immunized four times (on day 0, 10, 17 and 24) with 1 ml of whole *Psoroptes cuniculi* body extract (total protein content 3 mg per rabbit) emulsified with 1.5 ml of the incomplete adjuvant Al-span-oil (Velaz) (an aqueous suspension of aluminium hydroxide and paraffin oil). The extract was injected subcutaneously in the first three immunizations and intravenously

in the fourth immunization. These rabbits were challenged with *P. cuniculi* (about 500 mites per ear) by the modified technique of Fisher (1983) on day 31.
Group B (control): Three rabbits (3–6 months old) were injected with 1 ml of PBS mixed with 1.5 ml of adjuvant on day 0, 10, 17 and 24 by the same manner. These rabbits were also challenged by *P. cuniculi* on day 31 (about 500 mites per ear, again).
The system of evaluation of the psoroptic scab (ear canker score system) was performed using the method of Guillot and Wright (1981).

Antigen preparation

Psoroptes cuniculi antigen was prepared as was described previously (Uhlíř 1991b). Mites were washed in phosphate buffered saline, repeatedly frozen and thawed and homogenized. The homogenate was then centrifuged at $15.000 \times g$ for 30 min and the supernatant was purified by immunoaffinity chromatography in order to minimize the background of the ELISA technique. Briefly, supernatant was placed on top of a column packed with cyanogen bromide activated Sepharose 4B (Serva) with bound swine serum against rabbit proteins (SwAR–ielfo, Sevac, Prague). The unbound fraction was lyophilized, reconstituted at the required concetration with phosphate-buffered saline and used as the antigen.

ELISA

A modified ELISA technique (Fisher 1983) was used to determine specific serum antibody activity against *P. cuniculi* antigens. The optimal concentration of the antigen was 0.5 µg protein/well. Swine anti-rabbit immunoglobulins conjugate with horse-radish peroxidase (SwAR/Px, SEVAC, Prague) was used as the second antibody. O-phenylenediamine (Sigma) served as a chromogen and ELISA optical density (EOD) was measured at 492 nm. The reference values EOD of the negative sera were obtained from *P. cuniculi* naive rabbits before the experiments. Mean value $\pm$ SD was 0.171 ± 0.044.

Lymphocyte responsiveness assay

To evaluate the dynamics of *in vitro* response of rabbit blood lymphocytes to *P. cuniculi* antigens, Con A and PHA the lymphocyte blastogenic transformation assay according to Pruett et al. (1986), with a slight modification, was used. Heparinized, sterile blood diluted 1 : 1 with RPMI 1640 culture medium that was supplemented with 20 mM HEPES buffer (SIGMA), 8 mM sodium bicarbonate (SERVA), 2 mM glutamine (SIGMA), 10 µM mercaptoethanol (SIGMA), antibiotics 100 U/ml of penicillin and 100 µg/ml of streptomycin (SPOFA, Prague), were layered onto Ficoll-paque (Pharmacia) and centrifuged at 800 g for 30 min at room temperature. Concentration of collected cells was adjusted to 5×10^5 cells per well with RPMI + 20 % fetal calf serum (SIGMA). The optimum concentrations of *P. cuniculi* antigen (5 µg/well), Con A (1 µg/well) and PHA (1 µl/well) were determined by titration prior to the experiment. Cultures were set up in triplicate and were incubated for three days at 37 °C in a humidified atmosphere (3.5 % CO_2). Eighteen hours before the respective plates were harvested 1 µCi of tritiated thymidine was added to each well. Cells were collected on glass fibre paper disks, using a cell harvester. After cells were air-dried, glass fibre disks were placed in 3 ml of scintillation cocktail and tritiated thymidine incorporation was counted, using a liquid scintillation counter.

Lymphocyte responsiveness to Con A and PHA is expressed as stimulation index, and lymphocyte responsiveness to the *P. cuniculi* antigen is presented as the mean cpm above that of the unstimulated background controls (the system of evaluation was performed according to Pruett et al. 1986).

Electrophoresis and Western blot analyses

The electrophoresis in polyacrylamide gel in the presence of SDS (PAGE) was carried out in a discontinuous system according to Laemmli (1970) under reduced conditions. Electrophoretic separations were carried out on 5–15 % or 10–15 % linear gradient slab gels. Electrophoretically

separated antigens were then transferred to nitrocellulose membrane using Electrophoresis Power Supply (model 3000xi, Bio-Rad, the USA). Antigen components were detected by immunoblotting (Towbin et al. 1979) using various sera.

After washing the blots were incubated with the second antibody, i.e. horseradish peroxidase--conjugated swine anti-rabbit diluted 1 : 2000 with PBS-Tween 20. The product of the peroxidase reaction was stained by incubation of the blot in a substrate solution with 0.6 mM 3.3 diaminobenzidine.

Statistical analysis

Analysis of variance (ANOVA) and Student *t*-test were used to evaluate results obtained by means of the lymphocyte stimulation assay and ELISA. The level of significance for all analyses was $P \leq 0.05$.

RESULTS

The development of serum antibody activity to *Psoroptes cuniculi* antigen, the lymphocyte responsiveness to *P. cuniculi* antigen and the extent and onset of the scab lesions of immunized rabbits (Group A) are demonstrated in Fig. 1. An increase in specific antibody activity was recorded by the third immunization. A further increase of the antibody level was observed also after the mite infestation. The dramatic increase in the lymphocyte responsiveness was observed after the fourth immunization. The small increase trend was recorded after *P. cuniculi* infestation. The lesions caused by the mites were found for the first time 7 days after the infestation. There was a mild increase in the scab lesions until the fifth week post infestation, when they reached the ear score 1.5–2. Then, the level of ear scab was maintained throughout the rest of the infestation.

The development of antigen specific serum antibody activity, lymphocyte responsiveness to *P. cuniculi* antigen and scab lesions of the Group B (control) rabbits are shown in Fig. 2. No changes in the values of antigen specific serum antibody activity and lymphocyte responsiveness were observed before the mite infestation. Mild increase of the observed immunologic parameters was noted after *P. cuniculi* infestation. Scab lesions were not detectable before two weeks after infestation, however, they were more progressive and during three following weeks they reached the ear score 3. This scab formation remained on this level until the rabbits were treated with ivermectin.

The responses of peripheral blood lymphocytes of both A- and B-Groups of rabbits to the T cell mitogens (Con A and PHA) during the experiment are presented in Table 1. Blastogenic responsiveness of the lymphocytes of both groups of animals to PHA was consistent throughout the study except the responsiveness of Group-A rabbits on day 45 and 52, when statistically significant decrease was observed. As regards the lymphocyte responsiveness of both Group-A and -B rabbits to Con A, there was dramatic, statistically significant decrease two weeks after the mite infestation and this low level was maintained until the end of experiment.

The profile of *P. cuniculi* antigens recognized by sera derived from all rabbits is illustrated in Table 2. Some qualitative and quantitative differences were found between the Group-A and Group-B rabbits sera. The sera from the Group-A

Table 1. Lymphocyte blastogenic responses to Con A and PHA stimulation in cells from the Group A and the Group B rabbits

	mitogens	DAYS								
		10	17	24	31	38	45	52	59	66
immunized rabbits N = 4	Con A	15.6±8.7*	18.3±4.4	19.7±7.3	18.4±9.3	16.3±3.7	11.5±3.0	11.3±4.7	11.7±3.7	12.6±5.1
	PHA	4.7±1.2	5.1±2.3	3.9±0.7	4.8±1.1	3.3±1.1	2.8±2.0	2.9±1.1	5.1±1.4	4.0±0.9
control rabbits n = 3	Con A	17.4±4.3	16.2±3.8	15.1±4.4	14.3±7.1	15.2±3.1	10.7±3.3	10.9±2.8	9.9±3.1	9.7±2.6
	PHA	5.3±2.1	5.8±1.9	4.9±2.3	5.3±1.1	4.8±2.2	4.6±2.3	5.1±3.0	5.3±2.1	5.1±1.0

* – mean ± SD
data reported by means Si index (SD)
Con A – Concanavalin A
PHA – Phytohemagglutinin

Table 2. Immunoblotting reactivity of sera from rabbits artificially immunized with the whole-body *Psoroptes cuniculi* extract (Group A, rabbits 1–4, sera obtained 7 days before mite infestation) and rabbits of the Group B (rabbits 5–7, sera obtained 14 days after the mite infestation)

RABBIT						
Group A				Group B		
1	2	3	4	5	6	7
48* 36 22.5 16.5 14 10	48 22.5 16.5 14 10	48 36 22.5 14 10	48 36 16.5 12 10	59 48 25 18	59 48 40 25 18	48 25

* Molecular weight in kDa.

Sera of the Group A rabbits obtained on day 14 after *P. cuniculi* infestation recognized the same antigens which were recognized by the sera obtained on day 7 before the mite infestation. Sera of the Group B rabbits 7 days before *P. cuniculi* infestation reacted with antigen component of 30 kDa molecular weight.

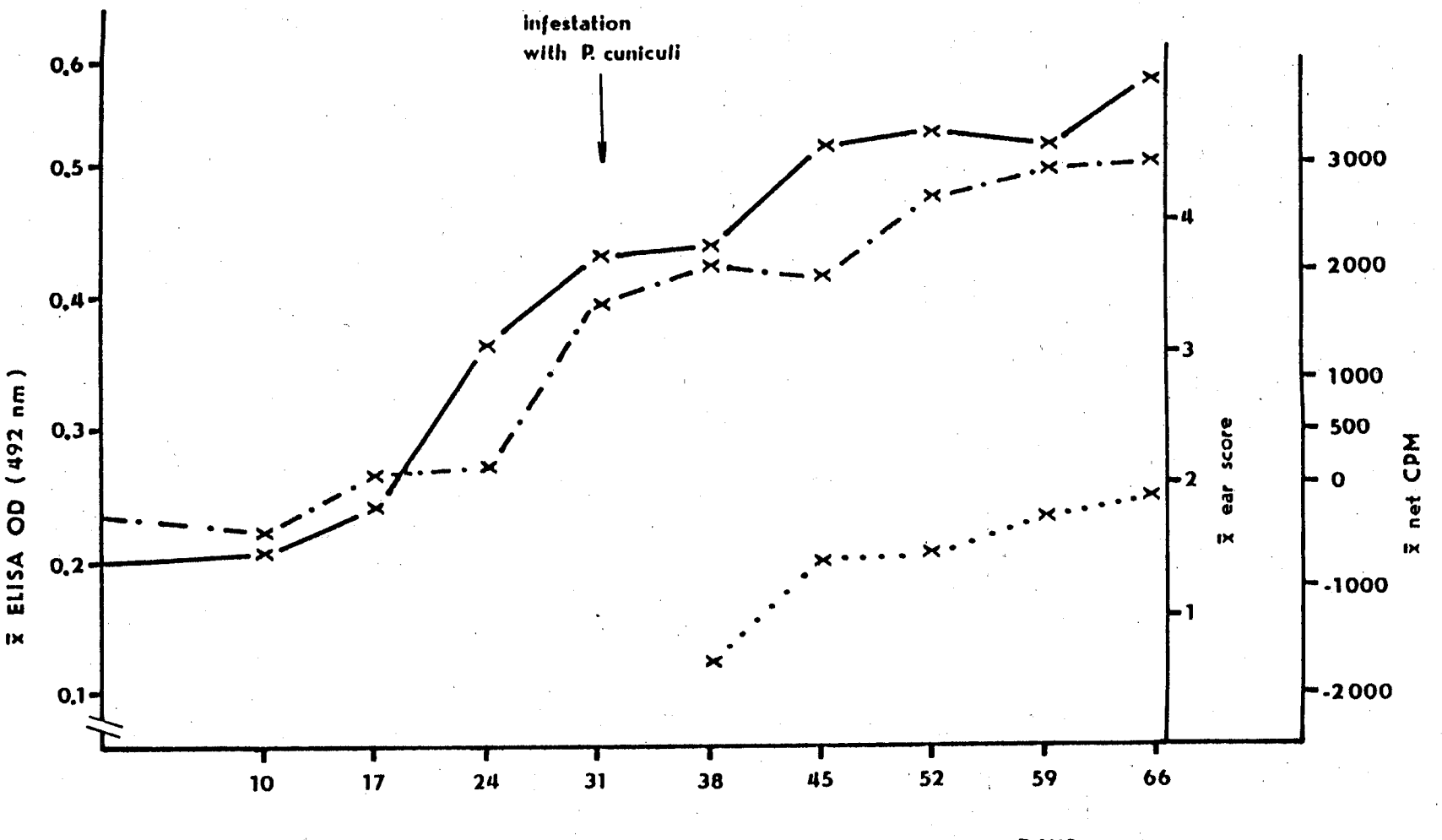

Fig. 1. The development of circulating anti-*Psoroptes cuniculi* antibodies, *P. cuniculi* antigen-induced lymphocyte responsiveness, and the mite-induced lesions in the Group A rabbits. Immunization with *P. cuniculi* extract on days 0, 10, 17, 24.

x̄ ELISA OD ————————
x̄ ear score .....
x̄ net CPM — · — · — · —

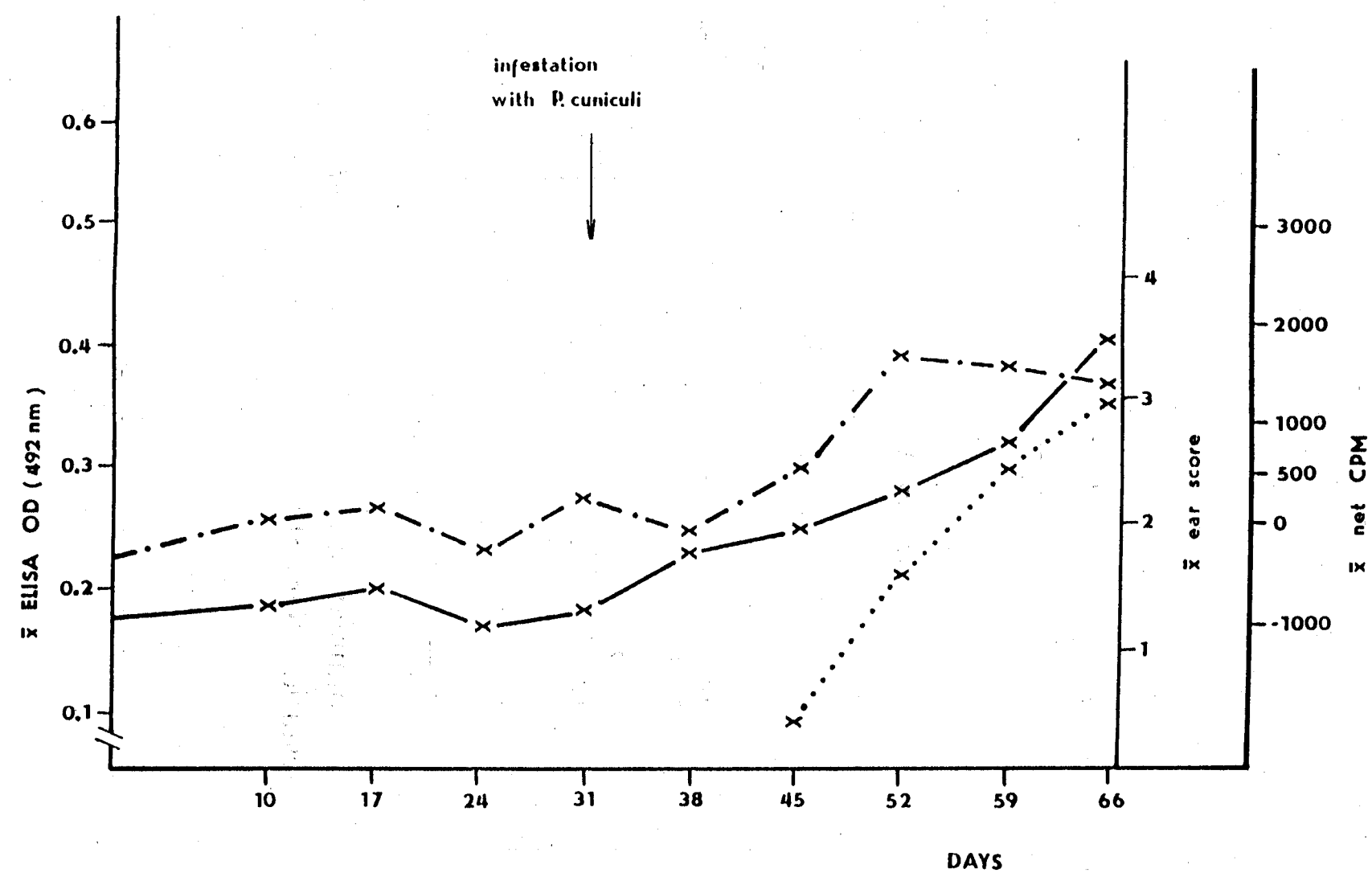

Fig. 2. The development of circulating anti-*Psoroptes cuniculi* antibodies, *P. cuniculi* antigen-induced lymphocyte responsiveness, and the mite-induced lesions in the Group B rabbits.

x̄ ELISA OD ————————
x̄ ear score .....
x̄ net CPM — · — · — · —

animals reacted with 5 or 6 protein components, while no differences were observed using the sera obtained from the same rabbits 7 days before and 14 days after the mite infestation. Sera from the Group-B rabbits obtained 14 days after the mite infestation recognized from 2 to 5 proteins, but sera obtained from the same rabbits immediately before *P. cuniculi* infestation recognized one non-specific band of 30 kDa.

DISCUSSION

Information about attempts to immunize host artificially against parasitic mites have not been published yet. It is rather surprising owing to the fact that parasitic mites belong to very important parasites, and compared to the number of studies dealing with artificial immunization of hosts using tick antigens.

In our previous study (Uhlíř 1991a) we have found that rabbits exhibit some degree of resistance, manifested by the smaller extent of the mite-caused lesions, after challenged with *Psoroptes cuniculi*, and this resistance is immune-mediated. A very similar degree of resistance was also elicited by the immunization with the whole-mite body extract. The mite-caused lesions were less progressive in the artificially immunized rabbits after the mite infestation compared to those in rabbits infested with the mites only. The antigen-specific serum antibody activity and the antigen-specific lymphocyte responsiveness in artificially immunized animals were higher compared to those of control rabbits. Both the tested immunologic parameters reached very similar values before the mite infestation as they were manifested by the resistant animals closely after the challenge infestation with *P. cuniculi* (Uhlíř 1991a). The loss of cellular responsiveness to Con A occurred at the time when the mite-caused lesions started to appear. Perhaps, some factors associated with the lesions were responsible for this effect.

The profile of specific serum antibody, tested by immunoblotting, was different in artificially immunized rabbits compared to this profile in control rabbits after the mite infestation. This finding is not surprising. Antigens, which are recognized by the host immune system during the natural infestation may not be the same as those elicited during the artifical immunization or vaccination, when a different immune mechanisms can be stimulated just by different antigens (Willadsen and Kemp 1989).

However, the specific serum antibodies directed against *P. cuniculi*-body component 48 kDa were found in sera of artificially immunized rabbits as well in the sera of control rabbits 14 days after the mite infestation. This protein component is probably localized in the mite hemolymph, as was found by the indirect immunofluorescence studies (Uhlíř, unpublished data). We speculate that this protein represents the main antigen that plays the key role in the immune-mediated resistance of rabbits against *P. cuniculi*. This speculation could be supported by the following observation. The rabbits which were artificially immunized and manifested some degree of resistance after the subsequent mite infestation developed the

specific pattern of serum antibodies after the immunization, and this pattern was not changed after the consequent mite infestation. Furthermore, the antibodies directed against 48 kDa protein were found also in rabbits that manifested resistance against *P. cuniculi* after the natural mite infestation (Uhlíř 1992).

In conclusion, the degree of resistance against *P. cuniculi* elicited by the artificial immunization with whole-mite body extract partially protected the rabbits against the mite infestation and this resistance was comparable with that exhibited after the natural infestation.

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