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Immunoprophylaxis of rabbits against *Rhipicephalus haemaphysaloides* ticks using immunoaffinity purified 35 kDa midgut antigen

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ABSTRACT

A 35 kDa midgut polypeptide of *Rhipicephalus haemaphysaloides* was isolated for the first time by immunoaffinity chromatography using immunoglobulin ligands from rabbits immunized with 35 kDa midgut polypeptide. An experimental immunization study was conducted on 12 New Zealand white rabbits using affinity purified 35 kDa midgut antigen (Aff - RhGM 35 kDa Ag) with Freund's adjuvant. The humoral immune response was evaluated by ELISA. After tick challenge, reduction in the number, mass and oviposition capacity of engorged females was observed in the tick population that had fed on immunized animals. The results indicated a high efficacy of 83.3%, demonstrating the efficiency of the immune response elicited by 35 kDa midgut antigen to control the tick *Rhipicephalus haemaphysaloides.*

Key words: *Rhipicephalus haemaphysaloides*, 35 kDa midgut antigen, immunoaffinity chromatography, immunization, rabbits

Introduction

Rhipicephalus haemaphysaloides is a three-host tick affecting cattle, sheep and goat directly by consumption of blood and indirectly by transmission of disease. Economic losses due to ticks and tick-borne diseases have been estimated to be from 13.9 to 18.7 billion dollars (DE CASTRO, 1997).

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Conventional tick control measure involves use of acaricides. However, certain intrinsic disadvantages with chemical control, such as geographically widespread resistance to many chemicals (NOLAN et al., 1989) is escalating public concern about chemical residues in food, and difficulties in discovering new acaricides has led to a search for non-chemical technologies such as vaccine in the control of ticks (WILLADSEN, 1997).

Animals can be protected against haematophagous parasites by immunization of hosts using concealed antigens (WILLADSEN and KEMP, 1988). The principle of such immunization is uptake of blood meal, tissue fluid or wound exudates which inevitably carry antibodies, complements and other components of host immune system (WIKEL, 1979). There are many difficulties in the practical exploitation of this idea, the most important of which is the identification of appropriate antigen targets for the immune system.

The concept of a host's ability to produce anti-tick antibodies protecting itself partially or completely against ticks led to the commercialization of a novel midgut vaccine against *Boophilus microplus*, which is the first genetically engineered *Escherichia coli* expressed Bm86 vaccine, TICKGARD plus, in Australia (WILLADSEN et al., 1995). A similar recombinant vaccine GAVAC was commercialized in Cuba (RODRIGUEZ et al., 1995). Immunodominant polypeptides in the range of 43-29 kDa have been identified which may be of an immunoprotective nature (BHASKARAN et al., 2003).

The protective potentiality of affinity purified 39 kDa protein of *Hyalomma anatolicum anatolicum* has been tested against parasite challenge (GHOSH et al., 1999; SHARMA et al., 2000). Nevertheless, no attempts have been directed towards the affinity purification of the midgut antigen of *Rhipicephalus haemaphysaloides*. Therefore, in the present study purification of 35 kDa midgut polypeptide of *Rhipicephalus haemaphysaloides* was attempted and the isolated fraction was used in an immunization protocol for inducing protection against this tick.

Materials and methods

Experimental animals. Tick colony maintenance, immunization and challenge studies were conducted using New Zealand white rabbits aged 3-4 months of both sexes with no previous exposure to ticks. Twelve rabbits were maintained individually and fed with lucerne, bengal gram and were given water *ad libitum*.

Ticks. Laboratory reared *Rhipicephalus haemaphysaloides* ticks were used for the preparation of antigens and for the challenge experiment. The colonies were maintained in a laboratory under controlled temperature of 20 ± 2 °C and 85% relative humidity.

Preparation of midgut antigen. Midgut antigen was prepared as per the method of OPDEBEECK et al. (1988) with minor modifications. Laboratory maintained semi-engorged

adult female ticks were dissected individually after surface sterilization with methanol under a Stereozoom microscope (Leica, Germany). The midguts were isolated and placed in 0.15M PBS pH 7.2 and the harvested midguts were mixed with 1 mM disodium EDTA, homogenized at 1500 rpm for 10 m in Potter S Homogenizer (B. Braun Biotech international, Germany) in an ice bath. The homogenates were sonicated at 8 μ amplitude for 10 m, at 4 cycles per m, each cycle lasting for 2 m with an interval of one m each, using a sonicator (B. Braun. Labsonic, Germany) in an ice bath. The sonicated homogenate was centrifuged at 15,000 g for 30 m at 4 °C in a refrigerated centrifuge (Sigma, USA), after which the supernatant was collected. The pellet was mixed with 0.0075 M sodium deoxycholate and the above mentioned steps of homogenization, sonication and centrifugation were followed. Finally, a second supernatant was collected. Both supernatants were dialyzed against PBS (pH 7.2) for 24 h, and then pooled. Phenyl methyl sulfonyl fluoride at 1 mM (PMSF) was added per mL of supernatant antigen to inhibit the proteolysis and stored at -20 °C in aliquots in 1.5 mL cryovials. This supernatant antigen was used as *R. haemaphysaloides* gut membrane antigen (RhGMAg). The protein concentration of antigen was determined as per the method of SMITH et al. (1985) using protein estimation kit based on Bicinchoninic acid (BCA) Kit (Genei, Bangalore).

Raising of anti RhGMAg 35 kDa midgut antibodies. The immunoreactive polypeptide at 35 kDa was extracted from SDS-PAGE gel bands from *Rhipicephalus haemaphysaloides* midgut protein as per the method of AMERO et al. (1994), with minor modifications. The gel bands were lyophilized and used to immunize rabbits. The immunized animals received 3 doses of 35 kDa polypeptide. On 0 day 250 μg of antigen with Freund's Complete Adjuvant (FCA) was given intramuscularly and subsequent doses on 14 and 21 day 125 μg of antigen with Freund's Incomplete Adjuvant (FIA) were given subcutaneously per rabbit. All rabbits were bled intercardially at the 35th day after first injection and the sera were stored at -20 ^{o}C.

Immunoglobulin G (IgG) purification by the caprylic acid method.

Serum was collected from 35 kDa midgut polypeptide immunized animals on 35th day of immunization. This serum IgG was purified by the caprylic acid method described by BHANUSHALI et al. (1994), with minor modifications.

Hyperimmune serum (20 mL) was added with acetate buffer (60 mM) at a ratio of 1:4 and the pH was adjusted to 4.5 with 1 N sodium hydroxide. Twenty-five micro litres of caprylic acid was slowly added per each mL of serum acetate buffer mixture. The solution was stirred for 30 m and insoluble material was removed by centrifugation at 10,000 g for 30 m at 4 °C.

The supernatant was mixed with EDTA buffer (1 l 10 × PBS+ 20 mL 100 mM EDTA) at 10:1 V/V and the pH was adjusted to 7.4 with 5 N sodium hydroxide. IgG was precipitated by addition of 50% saturated ammonium sulphate (0.277 g/mL). The

mixture was stirred for 30 m and centrifuged at 10,000g for 30 m at 4 °C. The pellet was resuspended in 1 mL of 1 × PBS (sodium chloride 0.8 g, potassium chloride 0.02 g, disodium hydrogen phosphate 0.115 g, and potassium dihydrogen phosphate 0.02 g) and dialyzed against PBS for 48 h. The amount of IgG was calculated, measuring absorbance value at 280 nm. Amount of IgG = (Sample OD-Blank OD) × 0.74, where 0.74 is the correction factor for IgG.

Immunoaffinity chromatography. Immunoaffinity chromatography was carried out as per the method described by OPDEBEECK et al. (1989), with minor modifications.

Cyanogen Bromide activated sepharose 4B, 1.5 g in 0.001 N HCl was allowed to swell for 15 m. The gel was washed with deionized water in a sintered glass funnel and collected in 50 mL falcon tube containing 20 mL of coupling buffer, pH 8.4 (0.1 M sodium bicarbonate + 0.5 M sodium chloride) and 5 mL of the purified IgG. Excessive reactive sites were blocked by 0.1M glycine buffer, pH 8.0. Prepared gel was washed with sodium acetate buffer, pH 4 (0.1 M sodium acetate + 0.5 M sodium chloride) to remove unbound IgG. The gel was packed in a 1 cm diameter column and was washed with three bed volume of sodium acetate buffer and 0.1M Tris HCl buffer, pH 8, three times each.

The packed column was washed with PBS pH 7.2 to ensure OD value at 0.00. The crude midgut antigen was filtered through a 0.2 μm filter, over equilibrated with equilibrating buffer pH 7.2 (10 mM sodium phosphate + 5 mM disodium EDTA). The antigen was loaded into the column in batches. Care was taken to avoid air bubbles. The column was washed with equilibrating buffer to remove unbound protein until UV absorbance returned to base line. The bound antigen fractions were eluted by 0.1 M glycine buffer pH 2.8 into Tris base, pH 8.5 containing vial to increase pH to 7.3. The eluted fractions were dialyzed extensively against PBS, pH 7.2 for 48 h and concentrated with polyethylene glycol. The purified 35 kDa midgut antigen (Aff-RhGM 35 kDa Ag) was used in immunization studies in New Zealand white rabbits.

Immunization trial. All rabbits in Group I were immunized with Aff-RhGM 35 kDa Ag. Rabbits in Group II were kept as control. Each group contained 6 rabbits. The immunization schedule is presented in Table 1.

Challenge infestations. Immunized and control rabbits were challenged with 10 pairs of hungry unfed adults of *R. haemaphysaloides* on the 35th day post-immunization. The female ticks which naturally detached were collected, counted, weighed and incubated in laboratory conditions at room temperature. On completion of oviposition, the eggs were weighed and allowed to hatch. The larvae per g of eggs were weighed.

Table 1. Immunization schedule of rabbits with Aff-RhGM 35 kDa Ag

Immunization (Group I)	Day of injection	Adjuvant used	Dose (µg)	Route	Challenge protocol
Primary immunization	0	FCA	200	IM/SC	Challenged with 10 pairs of unfed adult *R. haemaphysaloides* on 35 DPI
First booster	14	FIA	100	SC	
Second booster	21	FIA	100	SC	

Group II: Control animals received only adjuvant with PBS on respective days. FCA: Freund's Complete Adjuvant; FIA: Freund's Incomplete Adjuvant; IM: Intramuscular; SC: Subcutaneus; PBS: Phosphate Buffered Saline

Assessment of biological parameters. Following challenge infestations the ticks were monitored daily to assess feeding and fertility parameters, such as:

Percentage attachment, Feeding Period, Feeding Efficiency Index, Preoviposition Period, Oviposition Period, Fertility Efficiency Index, Egg Rate Conversion Efficiency (ERCE), Incubation Period.

The results of the biological parameters were analysed using the following formulae (DE LA FUENTE et al., 1995):

1. DT (%) = 100 (1-NTV/NTC)

   DT (%) = Percentage reduction of adult females

   NTV = Number of adult females in the immunized group

   NTC = Number of adult females in the control group

2. DO (%) = 100 (1-PATV/PATC)

   DO (%) = Percentage reduction of mean mass of eggs

   PATV = Average mass of eggs of the immunized group

   PATC = Average mass of eggs of the Control group

3. DR (%) = 100 (1-PMTV/PMTC)

   DR (%) = Percentage reduction of mean mass of adult females

   PMTV = Mean mass of adult females in the immunized group

   PMTC = Mean mass of adult females in the control group

4. DF (%) = 100 (1-PPLOV/PPLOC)

   DF (%) = Percentage reduction of fertility

   PPLOV = Mean mass of larvae per g of eggs in the immunized group

   PPLOC = Mean mass of larvae per gram of eggs in the control group

5. E (%) = 100 [1 - (CRT × CRO × CRF)]

E (%) = Efficacy of immunogen

CRT = Reduction in the number of adult females (NTV/NTC)

CRO = Reduction in egg laying capacity (PATV/PATC)

CRF = Reduction in fertility (PPLOV/PPLOC)

Values obtained from immunized and control groups were compared to assess the efficacy of immunization. Statistical analysis was carried out by Student *t*-test.

Assessment of humoral immune response. Blood samples were collected from all the animals on days 0, 7, 14, 21, 28, 35, 42, 49 and 56. Sera were separated and diluted at l in 100 and anti-Aff-RhGM Ag antibodies were detected by ELISA using the antigen at a coating level of 1 μg/100 μl in Carbonate bicarbonate buffer, pH 9.6. Peroxidase conjugated anti-rabbit IgG was used and OD values were measured at 405 nm.

Results

Concentration of protein was estimated to be in the range of 2.5 to 4 mg per mL of antigen. Amount of IgG was estimated at 280 nm to be 2.4 mg per mL of dialysed sample.

A total of 83 mg of RhGMAg was loaded in batches and about 3.6 mg (4.3%) affinity purified 35 kDa gut membrane antigen (Aff-35 kDa GM Ag) was eluted.

Effect of immunization with purified 35 kDa midgut antigen was assessed by the changes in feeding and fertility parameters of challenge ticks (Table 2).

There was a significant reduction in mean mass of engorged tick ($P \leq 0.01$) in immunized group (216.44 ± 17.72) when compared to control group (344.65 ± 11.83). The ticks collected from immunized animals were dark red in colour, shrunken and smaller in size compared to control group.

Mean egg mass weight decreased significantly ($P \leq 0.01$) in immunized group (63.44 ± 9.58) compared to control group (241.04 ± 11.79). Egg mass from immunized group was darker and smaller in quantity than the control group, whose eggs had a glistening appearance.

A highly significant ($P \leq 0.01$) reduction in larval mass weight was observed in immunized group (18.02 ± 0.12) compared to control group (119.07 ± 0.14).

Table 2. Effect of immunization with purified 35 kDa midgut antigen in feeding and fertility parameters of *Rhipicephalus haemaphysaloides* ticks and its assessment (mean ± S.E.)

Sl. N°	Variables	Control group	Immunized group
1.	Percent attachment	90	85
2.	Feeding period (in days)	5.42 ± 0.09	7.28 ± 0.03a
3.	Engorged tick mass (in mg)	344.65 ± 11.83	216.44 ± 17.72 a
4.	Feed efficiency index	60.19 ± 0.14	29.67 ± 0.10 a
5.	Preoviposition period (in days)	3.46 ± 0.07	3.64± 0.10b
6.	Oviposition period (in days)	23.22 ± 0.08	18.72 ± 0.18 a
7.	Egg mass (in mg)	241.04 ± 11.79	63.44 ± 9.58 a
8.	Egg rate conversion efficiency	67.96 ± 0.03	22.07 ± 0.01 a
9.	Incubation period (in days)	29.04 ± 0.02	30.02 ± 0.03 a
10.	Larval mass (in mg)	119.07 ± 0.14	18.02 ± 0.12 a
11.	DT% (Percentage reduction of adult females)	-	15.0
12.	DR% (Percentage reduction of mean mass of adult females)	-	37.3
13.	DO% (Percentage reduction of mean egg mass)	-	73.7
14.	DF% (Percentage reduction of fertility)	-	25.0
15.	E% (Efficacy)	-	83.3

[a] = statistically highly significant (P≤0.01)
[b] = statistically not significant (P≥0.05)

The efficacy (E%) of affinity purified 35 kDa midgut antigen was 83.3% in immunized group.

ELISA absorbance value increased from day 7 onwards in immunized animals, which reached a peak on 35 DPI. Thereafter, significant antibody titre was observed until 56 DPI. Absorbance value of ELISA is presented in Table 3.

Table 3. Humoral immune response of rabbits immunized with purified midgut antigen of *Rhipicephalus haemaphysaloides* (Mean ELISA absorbance value ± SE)

Days (Post immunization)	Immunized group	Control group
0	0.742 ± 0.005	0.753 ± 0.003
7	0.972 ± 0.004	0.741 ± 0.004
14	1.152 ± 0.006	0.748 ± 0.008
21	1.216 ± 0.008	0.736 ± 0.005
28	1.512 ± 0.013	0.743 ± 0.004
35	1.770 ± 0.007	0.739 ± 0.002
42	1.761 ± 0.020	0.750 ± 0.000
49	1.734 ± 0.003	0.734 ± 0.003
56	1.702 ± 0.008	0.749 ± 0.005

Discussion

Attempts have been made earlier to immunize rabbits (ARULJOTHI, 2003) against *Rhipicephalus haemaphysaloides*, and cattle (ESSUMAN et al., 1991) against *Rhipicephalus appendiculatus* using semi-purified midgut antigens, with some encouraging results. However, crude homogenates contain a mixture of antigens, most of which may not be responsible for protective immunity. Identification of the particular antigens responsible for protective immunity to *Rhipicephalus haemaphysaloides* can only be confirmed by purification of antigens to homogeneity.

Although some immunodominant antigens had been identified in midgut antigens of three common small ruminant ticks, *Haemaphysalis bispinosa*, *Hyalomma marginatum isaaci* and *Rhipicephalus haemaphysaloides,* probing with homologous and heterologous antisera, polypeptides in the range of 43 - 29 kDa were found to be common for all three antigens (BHASKARAN et al., 2003). In the present experiment, midgut antigens of *Rhipicephalus haemaphysaloides* have been purified for the first time by immunoaffinity chromatography using immunoglobulin ligands from rabbits immunized with 35 kDa midgut polypeptide and 83.3% protection against adult tick challenge. The phenomenon of engorged ticks turning dark red or black, and not ovipositing and dying as recorded in the current study, was reported by KEMP et al. (1986). *R. sanguineus* which fed on immunized hosts were dark, distended, did not oviposit, and after death turned black and hard in consistency (SZABO and BECHARA, 1997).

Thus, the present observation is probably due to gut damage in ticks fed on immunized animals resulting from the action of antibodies and complements, which can be visualized

through leakage of ingested rabbit erythrocytes into the haemocoel of ticks fed on immunized animals. The reduction in engorgement mass in ticks feeding on immunized animals has resulted in reduction in egg mass weight. PATARROYO et al. (2002) have reported that a vaccine against ticks would not be able to completely suppress a tick population in single generation, but would rather progressively control successive generations. Thus, vaccination with affinity purified antigen in the present study affected the number of eggs laid and thereby the number of larvae passing to the next generation were decreased.

Previously, anti-gut IgG was used to isolate concealed immunoprotective antigen of *Boophilus microplus* (OPDEBEECK et al., 1988), and concealed antigens of *Hyalomma anatolicum anatolicum* were purified for the first time using anti-gut IgG as ligands (DAS et al., 2003). GHOSH et al. (1999) who had immunized crossbred animals by a 39 kDa larval antigen purified by immunoaffinity chromatography, achieving a more than 16.94 and 28.48% reduction in the number of resultant nymphs and adults respectively fed on immunized animals. This may be due to the presence of common immunoprotective antigen of 39 kDa in both larvae and nymphs (SHARMA et al., 2000).

The study demonstrated for the first time that a 35 kDa immunoaffinity purified protein antigen was responsible for the induction of immunity to *Rhipicephalus haemaphysaloides.*

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References

AMERO, S., T. C. JAMES, C. R. ELGIN (1994): Production of antibodies using proteins in Gel Bands. In: Methods in Molecular Biology, Basic Protein and Peptide Protocols. (J. M. Walker, Ed.). Humana Press Inc., Totowa, New Jersey. 32, 401-406.

ARULJOTHI, A. (2003): Immunological control of *Rhipicephalus haemaphysaloides.* M.V.Sc. Thesis submitted to Tamilnadu Veterinary and Animal Sciences University.

BHANUSHALI, J. K., J. M. GILBERT, L. R. MCDOUGALD (1994): Simple method to purify chicken immunoglobulin G. Poultry Sci. 73, 1158-1161.

BHASKARAN, R. L., D. RAJESWARI, S. C. AIYASAMY, R. SARITA, C. VASANTHI, G. PATTABIRAMAN, G. RAJAVELU (2003): Western blot analysis for detecting midgut antigens cross reactivity of ixodid ticks in small ruminants. Ind. J. Small Rum. 9, 122-126.

DAS, G., S. GHOSH, J. K. SHARMA, M. H. KHAN, S. C. GUPTA (2003): Attempted immunization of crossbred (*Bos taurus* × *Bos indicus*) calves by affinity purified concealed antigens of *Hyalomma anatolicum anatolicum*. Ind. J. Anim. Sci. 73, 713-717.

DE CASTRO, J. J. (1997): Sustainable tick and tick borne disease control in livestock improvement in developing countries. Vet. Parasitol. 7, 77-97.

DE LA FUENTE, J., M. RODRIGUEZ, H. FRAGOSO, M. ORTIZ, C. L. MASSARD, O. GARCIA, R. LLEONAR (1995): Efficacy of vaccination with Gavac in the control of *Boophilus microplus* infestations. In: Recombinant Vaccines for the control of cattle tick. Dela Fuente, J. (Ed). Elfos Scientae, La Habana, 177-185.

ESSUMAN, S., O. O. DIPEOLU, T. R. ODHIAMBO (1991): Immunization of cattle with a semi-purified fraction of solubilized membrane - bound antigens extracted from the midgut of the tick *Rhipicephalus appendiculatus*. Exp. Appl. Acarol. 13, 65-73.

GHOSH, S., M. H. KHAN, N. AHMED (1999): Cross-bred cattle protected against *Hyalomma anatolicum anatolicum* by larval antigen purified by immunoaffinity chromatography. Trop. Anim. Hlth. Prod. 31, 263-273.

KEMP, D. H., R. I. S. AGBEDE, L. A. Y. JOHNSTON, J. M. GOUGH (1986): Immunization of cattle against *Boophilus microplus* using extracts derived from adult female ticks: Feeding and survival of the parasite on vaccinated cattle. Int. J. Parasitol. 16, 115-120.

NOLAN, J., J. T. WILSON, P. E. GREEN, P. E. BIRD (1989): Synthetic pyrethroid resistance in field samples in cattle tick (*Boophilus microplus*). Aust. Vet. J. 66, 179-182.

OPDEBEECK, J. P., J. Y. M. WONG, L. A. JACKSON, C. DOBSON (1988): Hereford cattle immunized and protected against *Boophilus microplus* with soluble and membrane associated antigens from the midgut of ticks. Parasite Immunol. 10, 405-410.

OPDEBEECK, J. P., J. Y. M. WONG, L. A. JACKSON, C. DOBSON (1989): Vaccines to protect hereford cattle against the cattle tick, *Boophilus microplus*. Immunology 673, 363-367.

PATARROYO, J. H., R. W. PORTELA, R. O. DE CASTRO, J. COUTO PIMENTEL, F. GUZMAN, M. E. PATARROYO, M. I. VARGAS, A. A. PRATES, M. A. DIAS MENDES (2002): Immunization of cattle with synthetic peptides derived from the *Boophilus microplus* gut protein (Bm 86). Vet. Immunol. Immunopathol. 88, 163-172.

RODRIGUEZ, M., M. L. PENICHET, A. E. MOURIS, V. LABARTA, L. LORENZO LUACES, R. RUBIERA, C. CORDOVES, P. A. SANCHEZ, E. RAMOS, A. SOTO, M. CANALES, D. PALENZUELA, A. TRIGUERO, R. LLEONART, L. HERRERA, DE LA FUENTE (1995): Control of *Boophilus microplus* populations in grazing cattle vaccinated with a recombinant Bm86 antigen preparation. Vet. Parasitol. 57, 339-349.

SHARMA, J. K., S. GHOSH, M. H. KHAN, G. DAS (2000): Immunoprotective efficacy of purified 39 kDa nymphal antigen of *Hyalomma anatolicum anatolicum*. Trop. Anim. Hlth. Prod. 33, 103-116.

SMITH, P. K., R. I. KROHN, G. T. HERMANSON, A. K. MALLIA, F. H. GARTNER, M. D. PROVENZANO, E. K. FUJIMOTO, N. M. GOEKE, B. J. OLSON, D.C. KLENK (1985): Measurement of protein using bicinchoninic acid. Anal. Biochem. 150, 76-85.

SZABO, M. P. J., G. H. BECHARA (1997): Immunization of dogs and guinea pigs against *Rhipicephalus sanguineus* ticks using gut extract. Vet. Parasitol. 68, 283-294.

WIKEL, S. E. (1979): Acquired resistance to ticks. Expression of resistance by C4 deficient guinea pigs. Am. J. Trop. Med. Hyg. 28, 586-590.

WILLADSEN, P., D. H. KEMP (1988): Vaccination with concealed antigen for tick control. Parasitol. Today 4, 196-198.

WILLADSEN, P., P. BIRD, G. S. COBON, J. HUNGERFORD, F. M. TOMLEY, D. W. TAYLOR (1995): Commercialisation of a recombinant vaccine against *Boophilus microplus*. Parasitol. 110, 543-550.

WILLIADSEN, P. (1997): Novel vaccines for ectoparasites. Vet. Parasitol. 71, 209-222.

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SAŽETAK

Polipeptid od 35 kDa podrijetlom iz srednjega crijeva krpelja *Rhipicephalus haemaphysaloides* prvi put je izdvojen postupkom imunoafinitetne kromatografije rabeći imunoglobulinske ligande dobivene imunizacijom kunića. Ukupno 12 bijelih novozelandskih kunića bilo je pokusno imunizirano ubrizgavanjem pročišćenoga antigena pomiješanog s Freundovim adjuvansom. Humoralni odgovor bio je određen imunoenzimnim testom. Nakon izazivačke infestacije imuniziranih kunića uočene su značajne razlike u odnosu na kontrolnu skupinu. U imuniziranih kunića ustanovljen je manji broj krpelja, njihova manja težina te smanjena plodnost nasisanih ženki. Rezultati su pokazali učinkovitost od 83,3% što pokazuje da je imunosni odgovor na antigen srednjega crijeva od 35 kDa potaknuo dovoljan imunosni odgovor za kontrolu infestacije krpeljom *Rhipicephalus haemaphysaloides*.

Ključne riječi: *Rhipicephalus haemaphysaloides*, antigen srednjega crijeva, imunoafinitetna kromatografija, imunizacija, kunići