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IMMUNIZATION OF CATTLE AGAINST BOOPHILUS ANNULATUS TICKS USING ADULT FEMALE TICK ANTIGEN

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SUMMARY

Ten crossbred calves (Friesian x Egyptian native) aged between 7-9 months old were used to evaluated the effect of cattle immunization with the whole adult B. annulatus tick antigen. A soluble fraction was obtained from extensively disrupted ticks and this fraction was used to vaccinate calves with 1mg protein antigen. The vaccination protocol for five randomly selected calves involved two immunizations, the first was administered subcutaneously (Plus aluminium hydroxide adjuvant) at the beginning of the experiment and the second was given 4 weeks later. At the same time, calves of the control group were injected with phosphate buffered saline (PBS) plus adjuvant. Ticks were counted on animals and the number of eggs laid per tick was counted and hatching percentage was derermined. The vaccinated and control animals were skin tested with the antigen and calves were injected in three different sites with 50ul of 50, 100 and 200ng of the antigen. At thte same time, control sites were injected with PBS saline and the diameter of the immune response sites was measured using skin caliper. Immunization of calves showed that vaccination resulted in 73% reduction in the mean tick count. At the same time, immunization reduced the oviposition of eggs in vaccinated calves and the reduction percentage in egg laying was 65%. Vaccination induced a good immunity that could protect calves during the tick season (more than 5 months) as indicated from skin hypersensitivity reaction with the tick antigen.

INTRODUCTION

The tick Boophilus annulatus is an important parasite of cattle in tropical and subtropical countries. Death in cattle may be caused by heavy infestations or by tick transmission of babesiosis and anaplasmosis. Tick control can be achieved by application of chemicals but the development of resistance to many acaricides has created problems in this approach (Roulston et al., 1981). Alternative measures using biological control such as pasture spelling and the breeding of tick resistant cattle (Wharton and Norris, 1980) can reduce tick but enhancement of host resistance by immunization would constitute a major advance in control. This situation has created the need to look for alternatives to the current measures of tick control and has stimulated the search for a vaccine.

It has been known for many years that old cattle are less affected by ticks than young animals. It has recently been shown that cattle can be immunized against ticks with a variable degree of success. Vaccination of cattle against the cattle tick Boophilus microplus led to a considerable reduction in tick fecundity (Riek, 1962; Roberts, 1968; Willadsen 1980). Some success with vaccination has been achieved against the ticks Dermacentor andersoni by Allen and Humphries (1979) and Amblyomma americanum by McGowan et al. (1981).

In subsequent observations on vaccinated cattle, it was found that host immunological responses had damaged the female ticks. This was the most important cause of tick rejection, at least during the period immediately following vaccination. Injection of extracts derived from adult female ticks induced partial immunity to B. microplus in both Bos taurus and Bos taurus X Bos indicus breeds of cattle. The immunity induced was still evident after 14 weeks of daily challenge with 1000 larvae and tick populations on vaccinated cattle were reduced by 70% compared to controls during this period (Johnsston et al., 1986). Kemp et al (1986) showed that the loss of attaching adults or larvae of B. microplus ticks on vaccinated cattle on the first 2 days was not greater than on the controls, but there was a continuing loss of females throughout feeding on two out of three vaccinated animals. Unlike the females, there was no continuing loss of larvae. In the resistance acquired by cattle to ticks after repeated tick infestations, the ticks were rejected mainly at the time when each instar was attaching and the resistance was particularly against larval stages. A series of experiments by Australian researchers demonstrated that the inoculation of ticks extracts produced satisfactory resistance, that feeding of ticks on these calves caused lesions in the tick intestine, and that the respective antigen was located in the plasmatic membrane of the ticks gut cells (Kemp et al., 1989).

Vaccination of calves with a recombinant tick antigen of 86KDa molecular weight caused resistance manifested as an increase in the mortality of the ticks that fed on these animals and a reduction of the fertility of ticks that survived (Tellam et al., 1992). Panda et al. (1993) immunized calves with extracts derived from adult B. microplus ticks and noticed that calves rejected more adult ticks and the ticks had poor reproductive performance. Many adult female ticks recovered from calves showed red coloration in their legs and failed to reproduce or laid smaller egg masses with low hatching percentage.

Recently, Rodriguez et al. (1995) developed a vaccine against Boophilus microplus employing a recombinant Bm86 (rBm86) antigen preparation and it was shown to induce a protective response in vaccinated cattle.

The aim of this work was to investigate the effect of immunization of cattle with the whole extract of adult female B. annulatus tick antigen on induction of protective immunity in vaccinated calves.

MATERIAL AND METHODS .

Animals:

The crossbred calves (Friesian X Egyptian native) about 7-9 months old with no previous exposure to B. annulatus ticks were used in this study. Animals were housed in their pens till the onset of vaccine injection, after which they were set to the pasture to acquire natural tick infestation. Animals were allocated into vaccinated and control groups each consist of 5 calves.Vaccinated animals were immunized using extracts deriving from adult female B. annulatus ticks.

Antigen preparation:

Adult female ticks of more than 4. 5mm in length were collected from naturally infested calves. A soluble fraction was obtained from adult B. annulatus ticks extensively disrupted in a Virtis blender in an ice bath. They were filtered throug a Buchner funnel to remove the tick cuticles. The filtrate was suspended in phosphate buffered saline (PBS) ph 7.4 containg 60 μg sodium penicillin plus 100 μg neomycin sulphate per ml. The filtrate was centrifuged at 5000 g/hr at 4C and the protein concentration of the supernatant was estimated with the method of Miller 1959. The supernatant fluid was stored at - 20C till used.

Tick fecundity:

The number of female ticks of more than 4.5mm in length attached to the right side of both vaccinated and control calves were counted before immunization and every week thereafter and multiplied by two to get the total number of ticks (Nolan et al., 1981). Tow hundred ticks from vaccinated calved along with an equiavalent number of ticks from control animals were collected and weighted. They were then incubated at 29C and 95% relative humidity to monitor egg laying and hatching of larvae. After about 14 days when egg laying was completed individual egg masses were placed in test tubes with a gauze top to monitor egg hatching.

Experimental design:

The vaccination protocol for five randomly selected calves involved two immunizations, both were administered subcutaneously plus aluminium hydroxide adjuvant using 1mg of the soluble protein antigen of adult female ticks per vaccination. The second vaccination was given 4 weeks later plus adjuvant. The control group received PBS saline plus adjuvant.

Skin testing:

The vaccinated and control calves were skin tested with the antigen using 1ml 25 gauge tuberculin syringes. Calves were skin tested at three and five months post-vaccination. Animals were sheared and injected in three different sites with 50 μl of 50, 100 and 200 ng of the antigen. At the same time, control sites were injected with PBS. The diameter of the immune response sites was measured at 24 and 48 hours (delayed hypersensitivity) after skin testing using skin caliper.

RESULTS

Data displayed in Table (1) show that vaccination of calves resulted in 73.15% reduction in the mean tick burdens. The vaccinated calves had a lower mean tick count than those of the control group and the difference in tick number in vaccinated and control groups was significant ($P < .05$). The overall tick count in vaccinated calves was 327, while that of controls was 1221.39. The mean tick weight in vaccinated calves (162.53mg) was significantly ($P < 0.05$) lower than that in control animals (257.46 mg) with a 36.87 % reduction in the mean tick weight.

At the time of vaccination, the mean tick count was (0) in both vaccinated and control calves, four weeks post-vaccination the mean tick count was 265 and 329.5 ticks per animal in vaccinated and control animals, respectively. Eight weeks post-vaccination, the mean tick count began to decrease, it was 410 in vaccinated animals, while it was fluctuating in control animals throughout the period after immunization. Sixteen weeks after vaccination, the mean tick count in vaccinated calves was 245.4 and the count was 1523.8 in controls. At the end of the experiment (24 weeks after immunization), the mean tick count was 176.2 and 1259.6 ticks per animal in vaccinated and control calves, respectively (Fig. 1).

It is evident from Table (2) that immunization of calves had a noticeable effect on oviposition of ticks. The mean number of eggs laid was 525 eggs/tick in vaccinated calves and 1503 in control animals with a 65% reduction in egg laying. The mean number of eggs hatched from ticks collected

Table(1): Number and weight of female *Boophilus annulatus* ticks recovered from vaccinated and control calves

Treatment	Mean tick number ±SE	Reduction % in tick number	Mean tick weight(mg) ± SE	Reduction % in tick weight
Vaccinated	327.97[a] ± 36.45	73.15	162.53[a] ± 29.63	36.87
Control	1221.39[b] ± 80.71		257.46[b] ± 13.69	

Columns with unlike superscripts are significantly ($p<0.05$) different.

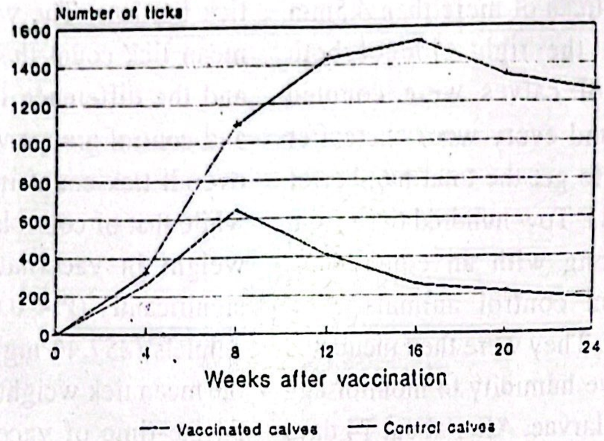

Fig.(1): Mean number of ticks in vaccinated and control calves

The original data of figure (1): Mean number of ticks in vaccinated and control calves

Weeks after vaccination	Number of ticks	
	Vaccinated calves	Control calves
0	0	0
4	265	329.5
8	647.4	1093.2
12	410	1432.2
16	254.4	1523.8
20	215.8	1336.2
24	176.2	1259.6

Table (2): Number and hatchability of eggs laid from ticks fed on vaccinated and control calves

Treatment	Mean Number. of eggs laid ± SE	Reduction % in egg laying	Mean Number. of eggs hatched ± SE	Reduction % in hatchability
Vaccinated	525.02[a] ± 84.93	65.07	380.07[a] ± 55.52	74.65
Control	1502.91[b] ± 31.86		1499.04[b] ± 37.94	

Columns with unlike superscripts are significantly ($p<0.05$) different

Table (3): Skin delayed hypersensitivity reaction at 3 months post-vaccination in vaccinated and control calves

Treatment	Diameter of reaction sites (cm) ± SE			
	Antigen dilution			
	50 ng	100 ng	200 ng	Control
Vaccinated	1.73[a] ± 0.29	1.99[a] ± 0.18	2.18[a] ± 0.16	0.00
Control	0.00[b] ± 0.00	0.38[b] ± 0.08	0.42[b] ± 0.05	0.00

Columns with unlike superscripts are significantly ($p<0.05$) different

Table (4): Skin delayed hypersensitivity reaction at 5 months post-vaccination in vaccinated and control calves

Treatment	Diameter of reaction sites (cm) ± SE			
	Antigen dilution			
	50 ng	100 ng	200 ng	Control
Vaccinated	1.44[a] ± 0.12	1.80[a] ± 0.22	1.99[a] ± 0.24	0.00
Control	0.21[b] ± 0.04	0.32[b] ± 0.08	0.36[b] ± 0.05	0.00

Columns with unlike superscripts are significantly ($p<0.05$) different

from vaccinated calves was 380 larvae and 1499 larvae in control animals and the reduction % in egg hatchability was 74.65%.

Results from Tables (3 & 4) show that the diameter of the reaction wheels in vaccinated calves was larger than that in control animals and the difference in diameter was significant ($P < 0.05$) in vaccinated versus control animals at all antigen dilutions at 3 and 5 months after vaccination. Three months postvaccination, the diameter of the reaction wheels was 1.73 cm in vaccinated calves and (0) in controls at 50 ng antigen dilution. At 200 ng antigen dilution the diameter of the reaction wheels was 2.18 and 0.42 cm in vaccinated and control animals, respectively. Five months post-vaccination, the diameter of the reaction wheels was 1.44 cm in vaccinated animals and 0.21 cm in control animals at 50 ng antigen dilution. At 200 ng antigen dilution, the diameter of the reaction wheels was 1.99 cm in vaccinated animals and 0.36 cm in control animals.

DISCUSSION

The results reported in this paper show for t first time that immunization against B. annulat ticks is feasible. The degree of immuni produced was reasonable and the immuni induced by vaccination was effective again challenge infestation by larvae for more than months. These data confirm the results of Kem et al., (1986) and Panda et al., (1993) wh immunized cattle with extracts derived fro female adult ticks and demonstrated tha vaccination of animals was protective.

Similar results were also obtained by Johnston e al. (1986) who mentioned that injection of cattl with crude adult B. microplus ticks reduced th tick populations on vaccinated cattle by 70% a compared to that of controls.

Not only did immunization of calves with adul B. annulatus ticks reduce the number of ticks which developed on vaccinated calves, but it also significantly reduced the reproductive capacity of female ticks as demonstrated by the reduction of the average number of eggs laid and its lower hatchability. These results were in accordance with those mentioned by Panda et al., (1993) who found that calves immunized with adult tick antigen rejected more adult ticks, and the ticks had poor reproductive performance. Rodriguez et al., (1994) demonstrated the effect of the vaccination on the fertility of female ticks in experiments under controlled conditions. Vaccination might lower tick fertility which could reduce the number of developed ticks, and the tick population in vaccinated animals could be effectively controlled.

Ticks collected from vaccinated cattle were red in colour whereas ticks dropped from control cattle were dark grey. This indicated some degree of damage of the gut of ticks which is possibly induced by the immune reactions against gut cells. This gut damage results in the leakage of bovine erythrocytes through the damaged gut cells into the hemolymph. The gut damage could also affect the oviposition in females by stopping digestion or blocking synthesis of vitellogenins by gut cells. Dead males were found on vaccinated calves as early as 24 h after infestation but some of the survivors showed gut damage and large numbers of host leucocytes in the gut lumen. Host leucocytes which had escaped into the haemolymph of these males had invaded and destroyed part of the accessory reproductive gland (Rodriguez et al. 1994).

Ticks collected from vaccinated cattle were lighter in weight than those collected from controls. This indicated some immune reactions developed by the host which cause damage to the tick's gut, and this damage prevents them from feeding properly. This is an important consideration because inhibition of feeding would prevent transmision of pathogens during blood sucking.

In our field experiment, vaccination of calves with soluble extracts from adult ticks induced a reasonable immunity that could protect calves from reinfestation during the tick season. This was obviously indicated from the skin hypersensitivity reaction elicited at the 3rd month post - immunization and still so till the 5th month after immunization.

In summary, the results presented in this study demonstrate that adult B. annulatus ticks contain potent protective immunogens capable of inducing protective immunity when used in an appropriate vaccination protocol. The expected effect of the vaccination in this field trial, is not principally in the direct killing effect of ticks within a single generation but rather in progressive control of tick numbers in successive generations through a reduction in reproductive capacity. The results of the experiment described here have demonstrated that this expectation is a real possibility and that this vaccine against B. annulatus can be used, alone or in combination with other control methods, to control tick populations in the fields.

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