A TEST OF THE EFFICACY OF IMMUNIZING CATTLE AGAINST ROCKY MOUNTAIN WOOD TICKS

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ABSTRACT

Nine yearling steers were inoculated with an extract of *Dermacentor variabilis* (Say) suspended in aluminum hydroxide adjuvant in an attempt to increase their resistance to the feeding of *Dermacentor andersoni* Stiles. However, when these cattle were infested with *D. andersoni* ticks, there were no significant differences in the proportions of ticks feeding, or mean weights of fed ticks, in comparison with nine cattle treated with adjuvant only, or with two untreated cattle. Five of the cattle in each of the treated groups received additional ticks to test for susceptibility to tick paralysis. Three of the cattle treated with tick extract became paralysed, as did one of the cattle treated with adjuvant only. Serological responses to the immunizing antigen, assayed by indirect haemagglutination, were relatively weak. Some possible reasons for the ineffectiveness of the immunization, in contrast to earlier positive studies with guinea pigs and a few cattle, are discussed. The methods described should contribute to testing the practical efficacy of promising antigens as they are produced.

INTRODUCTION

Serious outbreaks of tick (*Dermacentor andersoni* Stiles) paralysis of livestock occur periodically in British Columbia (Gregson 1966). The recommended preventive treatment consists of spraying acaricides on the backs of cattle before they enter the tick-infested pastures in early April (Costello and Khan 1980, Wilkinson 1981). Although these chemical treatments have provided very useful protection they have several disadvantages, including periodic changes in the regulations on registration and permissible residues, and the possibility of the ticks becoming resistant to pesticides. Sometimes the protection provided (about 3 weeks for a 0.25% lindane spray) is insufficient, necessitating gathering the cattle from extensive rangeland and respraying.

Allen and Humphreys (1979) reported significant reductions in the weights of *D. andersoni* adults fed on ears of calves which had previously been immunized with extracts of partially fed female *D. andersoni* ticks, in comparison with control calves. They speculated that control of tick infestations, following artificial immunizations, might be feasible.

Several authors have reported encouraging results in increasing the resistance of laboratory animals to ticks, by immunization with various extracts and organs of ticks (Wikel and Allen 1982).

In field testing of acaricides, one of us (P.W.) noted that untreated cattle developed skin reactions with serous exudate at the sites of tick attachment, after three weeks exposure to ticks. In the field, these skin reactions appeared to inhibit the feeding and attachment of the ticks (cf. Wikel and Osburn 1982). It was reasoned that an antigen treatment prior to exposure to ticks might have the effect of producing these skin reactions within a few days of tick attachment to the cattle.

A limited quantity of freeze-dried antigen, prepared from *Dermacentor variabilis* (Say) ticks in 1978, was available for use in a pilot experiment. Though this antigen came from a different species of tick, it was used in the knowledge that cross-reactions in immunologically mediated tick-resistance have been demonstrated between these two species (Trager 1939, McTier *et al.* 1981). Because of a shortage of antigen and logistical problems with a field trial, the study described here was carried out with penned cattle, as a preliminary test of the potential of controlling tick paralysis by manipulating the immunological systems of the cattle.

The conclusions are based mainly on the comparison of weights of ticks fed on treated and control steers. A larger dose of ticks was given to some steers to see if differences in susceptibility to paralysis could be observed. Although the immunization failed as a protection against tick feeding and paralysis, the prospects for eventual success are promising, and this paper records test procedures that may be useful to other workers.

METHODS

On March 20, 1982, nine Hereford yearling steers (group T) were each injected with tick-derived antigen and aluminum hydroxide adjuvant, and a similar group of steers (group A) was injected with sterile saline and adjuvant only. Two other steers were left untreated (group U). On March 25, group T had a mean weight of 252 kg (range 240-282 kg), group A had a mean weight of 258 kg (range 240-292 kg), and the two untreated cattle weighed 254 and 276 kg. On April 3, repeat 'booster' injections were administered to all animals in groups T and A.

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The antigen given to animals in group T was a saline extract of internal organs from *D. variabilis* females that had fed on Hereford cattle for 5 days. The extract was prepared in 1978 and lyophilized. Freeze-dried material was reconstituted in sterile saline, and equal volumes of this solution (which contained 20 mg protein/ml, by Lowry assay) and Alhydrogel (Connaught) were allowed to react for 24 hours at room temperature before a total dose of 20 mg protein was injected subcutaneously at four sites on each animal in group T. Group A animals received similar injections of saline and Alhydrogel.

Serum samples were taken from each animal in groups T and A prior to the first injection, at the time of the booster injection, and at the start of the tick infestation. These sera were titrated by indirect haemagglutination, with the same antigen attached to red cells.

On April 19 and 20, after hair was closely clipped from the area of contact, a sleeve cut from a child-size sock was fixed to the withers (Wilkinson 1972) of each of the 20 animals with contact cement. The hair within the sock was left about 1.5 cm in length. Two additional sleeves were fitted to five animals in each of groups T and A to allow for increased tick infestation and to test for susceptibility to paralysis. The sleeves are referred to as sleeve 1, sleeve 2, and sleeve 3, on each animal concerned.

Ticks

On April 21, 30 male and 30 female ticks from a laboratory culture were placed in each sleeve 1. Thirty female ticks collected from vegetation during March and April and stored at 5 °C 95% RH were placed in each sleeve 2. On four animals, in each of groups T and A, sleeve 2 ticks were collected within 64 km of Kamloops, B.C., and on the remaining animal in each of these groups ticks were an Alberta strain bred on rabbits kept outdoors in a 'rodentarium' (Wilkinson 1968). In each sleeve 3 the infestation was 60 females from the same culture used in sleeve 1. This culture was reared from larvae and nymphs fed on laboratory rabbits indoors. The larvae originated from ticks collected near Kamloops in 1981. The engorged nymphs were kept in unlit incubators at 25 °C 75% RH until ecdysis was complete on January 12 and the resulting adults at 5 °C 95% RH until April 20. On April 20, they were exposed to fluorescent room lighting from 0845 to 1645 hrs, while still in glass tubes in a glass humidifier, with similar treatment the next day until placement on the cattle at 1330 hrs. Room temperatures commenced at 8 °C, rose to 15 °C, then dropped to 7 °C at night, and rose again to 15 °C. These temperatures and light changes were intended to assist in breaking the diapause (Wilkinson 1973).

On April 28 the sleeves were opened, dead ticks were removed and the progress of feeding was checked qualitatively. On April 30 and May 1 ticks were removed from animals that were paralysed to the stage of sternal recumbency. All remaining ticks from all animals were removed on May 3. The ticks from each sleeve were stored at 5 °C 95% RH in separate tubes, then counted and weighed. To obtain mean weights of fed female ticks, male ticks and females with no appreciable enlargement of the opisthosoma (red colored ticks) were discarded and the number of fed females with tan and grey colored opisthosomata was divided into their total weight, for each sleeve.

RESULTS

Examination on April 28

The total number of dead ticks in sleeves 1 was similar for groups T, A and U, allowing for numbers of sleeves. There were 22 male and 43 females from group T, 17 males and 38 females from group A, and 7 males and 8 females from group U. Since the sleeves were left in place, some dead ticks may have been missed. Qualitatively,

TABLE 1. Weights of fed female ticks removed on day of paralysis, in relation to weights of cattle on May 6, 1982. Treatment code: T = cattle treated with tick antigen, A = cattle that received adjuvant only. The ticks were placed on the cattle on 21 April 1982.

Animal No.	Weight (kg)	Treatment	Date paralysed	Total weight (g) female ticks in sleeve 1	2	3	Parasite ratio+
316	267	T	Apr 30	4.19	5.89	3.67	51.5:1
319	285	T	Apr 30	2.05	3.91	1.40	25.8:1
318	276	T	May 1	4.83	5.88	1.71	45.0:1
330	256	A	May 3	2.24	8.34	3.42	54.7:1

+Weight of all fed ticks in mg: weight of steer in kg.

TABLE 2. Mean weights of fed female ticks (FFT) in sleeves 1, 2 and 3 on paralyzed animals on day of sternal recumbency and sleeves 2 and 3 on unparalysed animals on May 3, 1982. See text for weights of ticks in sleeve 1 on May 3, on unparalyzed animals.

Sleeve class	Treatment of cattle[+]	No. replicates	P=paralysed NP=not paralysed	Mean wt. FFT (mg)
1	T	3	P	167
1	A	1	P	448
2	T	3	P	190
2	A	1	P	298
3	T	3	P	81
3	A	1	P	155
2	T	2	NP	342
2	A	4	NP	281
3	T	2	NP	215
3	A	4	NP	137

[+]T = treated with tick antigen, A = treated with adjuvant only.

degree of engorgement of live ticks appeared to be similar among groups. Mortality of ticks in sleeves 2 was low, while in sleeves 3 the number of dead females per sleeve was higher than in sleeves 1, i.e., a total of 41 in 5 sleeves in group T and 48 in 4 sleeves counted in group A.

Occurrence of paralysis

Table 1 shows the treatment groups of paralysed animals. On April 30, animal 319 was in sternal recumbency at 0800 hrs. All ticks were removed, commencing at 0830 hrs. The animal was still unable to rise at 1130 hrs, but was unsteadily on its feet at 1415 hours. At 1415 hours, animal 316 was unsteady; it was sternally recumbent at 1945 hrs when all ticks were removed from it.

On May 1, animals 319 and 316 had recovered, but 318 was sternally recumbent at 0815 and the ticks were removed. No animals were paralysed on May 2. On May 3 at 0815, animal 330 was in sternal recumbency, but regained its feet and was able to walk to the examination chute in the afternoon. However, at 1600 hrs it collapsed while entering the chute and the ticks were removed while it was in sternal recumbency. This animal was normal the next day. The weights of the fed ticks in relation to animal weight are shown in Table 1, for comparison with other records of paralysis (Wilkinson 1982). Paralysis did not occur in the two animals carrying Alberta 'rodentarium' ticks in sleeve 2.

Weights and numbers of fed ticks removed on May 3, 1982

A comparison of the effects of immunization on degree of engorgement of the cultured ticks can be based on the mean weights of fed ticks in sleeves 1 on May 3. Ticks from animals paralysed before that date are not comparable, since the ticks were removed after a shorter feeding period. Weights of ticks in sleeves 2 and 3 are less significant, since only two of five animals with three sleeves each remained unparalysed in group T until May 3.

Mean weight of fed female ticks in the tan and grey stages, removed from sleeves 1 in group A on May 3, was 494 ± 41.72 mg ($P = 0.05$) (includes animal 330). In group T the mean weight of fed female ticks from the six 'not paralysed' animals was 477 ± 54.81 mg. In group U the mean was 455 mg, $N = 2$. The differences between the means were not significant.

Some other mean values, for which significance of between-group differences was not tested, because of the small number of replicates, are shown in Table 2. On the paralysed animals, mean weights of ticks from the T group animals were lower than those from the A group animal 330, because the ticks were removed from the latter at a later date. On the 'not paralysed' animals mean weights of ticks in sleeves 2 and 3 were slightly greater in group T than in group A.

One sleeve 2 on an unparalysed animal in group T contained a male tick, resulting in apparent fertilization and increased weight of 7 out of 31 females. These seven females, and eight females from a sleeve 1 of an unparalysed T group animal, were transferred to 25 °C. and high humidity where they produced normal egg masses and numbers of larvae, except that percentage hatch was below normal for one egg mass. Thus, the antigen treatment did not affect the fertility of the engorged females as reported by Brossard and Girardin (1979) and C.S.I.R.O. (1983). There were also two sleeves 2 in group A containing 31 females instead of the correct number, 30. These errors had a negligible effect on the conclusions from the experiment.

The numbers of ticks per sleeve that fed appreciably in sleeves 1 were group T 19.56, group A

15.33, and group U 12.5. For sleeves 2, the mean numbers of fed females in groups T and A were 28.0 and 29.4 and for sleeves 3, 28.0 and 20.8, respectively. The differences in the mean numbers of fed ticks in sleeves 1 in groups T and A were not significant. There were insufficient replicates of the other sleeves to warrant testing of significance of the small differences of means.

Results from serological work

Titres of 1:20 were recorded occasionally from animals in group A (Table 3). Such titres could be considered as false positives, or at best insignificant. Titres of 1:40 were obtained from two animals in group T at the time of the booster injection, and titres to 1:640 were found in immunized animals at the time of tick infestation.

DISCUSSION

The inoculation did not produce any significant difference in mean weight of fed ticks compared with the adjuvant-only treatment. The mean weight per fed tick was used to eliminate variability in the number of ticks that fed in each sleeve. The numbers of ticks that fed in sleeves 1, 2 and 3 were not significantly different between groups T and A. Hence, the treatment did not significantly affect the attachment and feeding of the ticks.

Further work is necessary to determine whether the antigen produced increased susceptibility to paralysis, since the number of cases was too low for statistical inference.

Serological results indicated relatively poor responses to the *D. variabilis* antigen in immunized animals. Previous work (Allen, unpublished) showed that titres of at least 1:1280 were associated with effects on tick feeding. The low titres could have been due to several causes, including low doses of antigen and deterioration of antigen in storage. The lack of effects on tick feeding, in contrast to the results of Allen & Humphreys (1979), could have been due to differences in total antigen dose (which was less than $^1/_5$ of that used in their experiment), the dosage regimen or insufficient cross-reactivity between *D. variabilis* and *D. andersoni* antigens.

Further purification of *D. andersoni* antigens, which is now being done, and further immunization trials with higher doses of antigens will be required before an immunological approach to the control of tick paralysis can be recommended.

The laboratory-bred ticks displayed a lower percentage of successful attachment than did the wild ticks, and a higher preattachment mortality. Mean weights of fed ticks were generally less in sleeves 3 than in sleeves 2 on May 3. These effects were probably due to difficulty of terminating diapause in laboratory cultures (Wilkinson, 1973).

TABLE 3. Serological results: IHA titres from animals in groups T and A.

Animal no.	Group	IHA titres		
		Pre-treatment	At time of booster	At start of infestation
311	T	0	0	1:160
312	T	0	1:20	1:160
313	T	0	0	1:640
314	T	0	1:20	1:320
316	T	0	0	1:160
317	T	0	1:40	1:320
318	T	0	0	1:320
319	T	0	1:40	1:40*
320	T	0	1:20	1:40
321	A	0	0	0
322	A	0	1:20	1:20
323	A	0	0	0
324	A	0	0	0
325	A	0	0	0
326	A	1:20	1:20	0
327	A	0	0	0
328	A	0	0	1:20

*Animal 319 suffered from pneumonia one week before the last serum sample was taken.

Despite this, the test was useful as a demonstration of methods available for testing the efficacy of potential vaccines, using penned cattle and both wild and cultured ticks.

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