

General Articles

Specific immunotherapy in the treatment of *Culicoides* hypersensitive horses: A double-blind study

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Summary

Fourteen privately owned horses completed a six month, controlled, double-blind trial to assess the efficacy of immunotherapy using an aqueous extract of whole, unfed *Culicoides variipennis* in the treatment of *Culicoides* hypersensitivity. Selected horses had a history of a seasonal, pruritic dermatitis, clinical signs and distribution compatible with *Culicoides* hypersensitivity, failed to improve in response to ivermectin therapy, and reacted to several dilutions of *Culicoides* extracts without significant reactivity to other possible allergens including insects, moulds and feeds. One horse from the control group (n=8) and one from the test group (n=6) improved. Statistically, there was no significant difference between horses on *Culicoides* antigen and control horses. In each group, four owners thought that their horses had improved.

Introduction

DERMATITIS in the horse associated with hypersensitivity to bites of *Culicoides* spp has been described in many parts of the world (Riek 1953; Nakamura *et al* 1954; Mellor and McCaig 1974; Braverman *et al* 1983; Fadok 1984; Kleider and Lees, 1984; Baker and Collins 1984; Broström; Larsson and Troedsson 1987; Scott 1988). It is one of the most common allergic diseases in the horse and the incidence is particularly high in Florida.

Successful management of affected horses is time consuming and expensive and, in Florida, the management season may last from late February until November (Fadok and Greiner 1990). Housing in insect-proof stables from dusk to dawn and daily applications of insecticides and repellents are necessary for control. Glucocorticoids, although quite effective initially, become less effective with long-term use (Braun, Raffensperger, and Woodworth 1970) and evidence that their pharmacological actions may contribute to the development of laminitis makes long-term use undesirable (Eyre, Elmes, and Strickland 1979). Clinical improvement with the use of antihistamines is variable. Elimination of breeding grounds for *Culicoides* species or movement of affected

animals to drier, distant pastures is ideal (Braun *et al* 1970, Foil and Foil 1986). However, the abundance of lakes, ponds, and swamps in areas such as Florida makes this task difficult.

The Type I hypersensitivity reaction plays a major role in the pathogenesis of *Culicoides* dermatitis in horses. It has been demonstrated in affected horses using intradermal injections of crude extracts of different *Culicoides* species (Riek 1954, Braverman *et al* 1983, Quinn, Baker, and Morrow 1983, Fadok and Greiner 1990). The presence of skin sensitising antibody in the serum of affected horses has been established using the Prausnitz-Kustner test (Riek 1954; Matthews, Imlah, and McPherson 1983; Quinn *et al* 1983) and further analysis has revealed similarities to human IgE (Matthews *et al* 1983; Morrow, Quinn and Baker 1987). Horses have shown clinical improvement on allergen specific immunotherapy for other conditions involving Type I hypersensitivity mechanisms, such as chronic obstructive pulmonary disease (Halliwell *et al* 1979), insect bite dermatoses (Fadok and Greiner 1990) and urticaria (Rosenkrantz 1987).

Different *Culicoides* species have been identified as causing this disease in different parts of the world (Riek 1953, Yamashita and Kitamura 1957; Mellor and McCaig 1974; Braverman *et al* 1983). Studies have implicated two primary species, *C. insignis* and *C. stellifer*, in the pathogenesis of the equine disease in Florida (Greiner, Fadok, and Rabin 1988). There appears to be cross reactivity between the salivary antigens that different *Culicoides* spp. possess because intradermal injection of extracts of *C. variipennis*, which is not a major species involved in the disease in Florida, elicits strong weal and flare responses in animals with history, physical signs and histopathological lesions compatible with insect hypersensitivity (Fadok and Greiner 1990). Weekly injections of an extract of *C. variipennis* were used successfully to treat one horse in Florida (Fadok and Greiner 1990).

The objective of this trial was to determine whether or no immunotherapy using an extract of whole, unfed *C. variipennis* was effective in the treatment of *Culicoides* hypersensitive horses.

Materials and methods

Experimental design

The controlled and double-blind design was explained to all owners prior to obtaining their consent to participate in the study. The trial lasted six months (June until December). Owners were asked to manage their horses as they had in

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previous years, avoiding major changes in insect control, diet or housing. Glucocorticoid therapy was to be withheld unless it was needed for humane reasons.

Selection of horses

Horses were selected on the basis of history, clinical signs, exclusion of mange mites and cutaneous onchocerciasis by ivermectin therapy (Herd and Donham 1983; Fadok 1984), and intradermal skin testing using allergenic extracts of six pasture and feed pollens, grain mill dust, 11 common moulds and multiple dilutions of four biting insects including *C. variipennis* and *C. insignis*. Seven of the 28 horses screened were excluded from the study due to strong dermal reactivity to multiple other allergens. Two of the authors (DB and ECG) assigned the horses randomly into two groups (control and test groups) by drawing their names from a hat. Neither the other investigator (JLB), who was evaluating the horses' clinical conditions throughout the trial, nor the owners knew which horses were receiving the control solution and which were receiving the *Culicoides* antigen.

Of the 14 horses completing the study, eight were in the control group and six were in the test group (receiving *C. variipennis* extract). Control horses were aged, on average, 11.5 years and had been affected for a mean of 3.4 years. Six were geldings and two were mares. There were four Quarterhorse crosses, two Thoroughbred crosses, one Arabian, and one Appaloosa. Test group horses had an average age of 15.6 years and had been affected for a mean of 4.2 years. Three were geldings and three were mares. There were two Quarterhorses, two Arabian, one Shetland pony and one grade pony.

Culicoides antigen

Large numbers of nulliparous, female *Culicoides insignis*, from which an antigenic extract was prepared, were obtained by light-trapping and hand sorting. Whole, unfed *C. variipennis* were supplied by Mr. Greg Hunt, USDA Arthropod Borne Animal Disease Research Laboratory, Laramie, WY. Both experimental *Culicoides* antigens were prepared by a commercial laboratory (Greer Laboratories, Lenoir, North Carolina). The resulting *C. insignis* extract (1000 PNU/ml) was used for intradermal skin testing at five concentrations ranging from 500 PNU/ml to 31.25 PNU/ml in serial two-fold dilutions. As *C. variipennis* could be obtained in larger quantities than *C.*

insignis, *C. variipennis* extract (7500 PNU/ml) was used for immunotherapy at the concentrations shown in Table 1 as well as for intradermal skin testing at the same concentrations as for *Culicoides insignis*.

Control solution

Control solution was made from the phosphate buffered isotonic saline provided as diluent for allergenic extracts (Greer Laboratories). For coloration, acid proof #680 caramel food colouring was used (Sethness Products Company, Chicago, Illinois). A darkly coloured solution was prepared and passed through a filter for sterilisation. Drops were added to the isotonic saline until it matched the colour of the same volume of *C. variipennis* extract. Test injections of the control solution were given to normal horses to confirm its safety and lack of side-effects.

Intradermal skin testing

Intradermal skin testing was performed as previously described (Fadok and Greiner (1990). Reactions were graded by JLB at 15 mins. Subsequent responses at 6, 24, and 48 h were recorded by the owners. Grading of the reactions on a reference scale of 0 for the saline control and 4 for the histamine control was performed as previously described (Fadok and Greiner (1990). Xylazine at 0.5 mg/kg bodyweight intravenously (iv) was used to sedate uncooperative horses.

Immunotherapy schedule

The schedule for immunotherapy for horses on *Culicoides* antigen and control horses on isotonic saline is shown in Table 1. It is identical to the schedule used for immunotherapy of atopic dogs at the University of Florida, Veterinary Medical Teaching Hospital.

Follow-up and clinical scoring

One investigator (JLB) inspected the horses and graded clinical disease, starting with the initial screening visit and continuing every 6 to 8 weeks throughout the study. Scores ranged from 0 (no signs) to 3 (severe signs) using 1/2 point increments. Scores were based on extent and severity of pruritus and the following lesions: papules, nodules, alopecia, crusts, erosions, erythema, excoriations, draining tracts, lichenification, and pigmentary changes.

Owners were asked to grade the severity of their horses' clinical disease each month using a 0 to 3 scale. Intradermal skin testing was performed at the mid-point of the study and again at the end to assess whether or not the immunotherapy resulted in changes in dermal reactivity to *Culicoides* antigens.

Statistical analysis

Student's *t* test and Chi Square analyses were used to determine whether or not significant differences between the horses on *Culicoides* antigen and horses on control solution could be detected.

Results

Fourteen of the original 21 horses completed the study. One was discontinued because of an apparent anaphylactic reaction 15 mins following subcutaneous administration of the *Culicoides variipennis* antigen, manifested by nervousness, sweating, urticaria and rapid respirations. One horse was sold mid-study and five owners failed to continue the injections

TABLE 1: Immunotherapy schedule and dosages for subcutaneous administration of *Culicoides* extract and control solutions.

Day	Vial No. 1 (75 PNU/ml)	Vial No. 2 (750 PNU/ml)	Vial No. 3 (7500 PNU/ml)
0	0.1 ml		
3	0.2 ml		
6	0.4 ml		
9	0.8 ml		
12		0.1 ml	
15		0.2 ml	
18		0.4 ml	
21		0.8 ml	
24			0.1 ml
27			0.2 ml
30			0.4 ml
33			0.8 ml
36			1.0 ml
46			1.0 ml
66			1.0 ml

1.0 ml of vial No. 3 continued every 20 days during study

after the initiating visit.

Five of the horses in the control group showed the typical pattern of worsening disease through the summer months with improvement by December. One was at its worst in December, one improved dramatically at the beginning of the study when it was moved to a drier location, and another was controlled by stabling, but upon subsequent challenge (one night on pasture), showed a rapid return of clinical disease (Table 2). The mean clinical scores for the control group showed a distinct seasonal worsening during the summer with improvement in December.

Horses receiving *Culicoides* antigen did not show as distinct an increase in clinical signs over the summer as the control group and their mean clinical scores decreased gradually over the course of the study (Table 2). No statistical difference was found ($P=0.32$) between the two groups at their point of maximal difference in September.

Upon final evaluation of clinical scores, one of the investigators (JLB) felt that one control group horse (No. 7) and one test group horse (No. 14) had improved, ie clinical score decreased by at least one whole unit from pre-trial to the September (peak of season) evaluation. The difference between the two groups was not significant. Four owners of control horses (50 per cent) and four owners of test group horses (66.7 per cent) thought that their horses had improved. Thus neither the investigator (JLB) nor the owners could determine which treatment a horse was receiving, control solutions or *Culicoides variipennis* extract, based on the severity of clinical

signs. In addition, there were no observable changes in dermal reactivity to either *Culicoides* extract in any of the horses over the course of the study.

Discussion

This trial demonstrates the need for controlled studies, especially in clinical trials carried out on naturally occurring disease because many variables cannot be controlled. In this case, many owners felt that their horses had improved when compared to previous years. This may have resulted from reduced *Culicoides* populations in an abnormally dry year. The investigator (JLB) was not able to compare the condition of the horses to that of previous years and therefore evaluated the horses by their appearance at the beginning of the trial. The degrees of improvement in the owners' opinions were similar for the two groups, however, demonstrating that the double-blind, controlled nature of the trial separated real response to treatment from improvement due to variables which could not be controlled.

The small number of horses completing the study could be partly responsible for the lack of significant difference between the groups. However, immunotherapy did not provide sufficient improvement in any of the horses for it to be considered useful in practice.

There are a number of possible reasons why immunotherapy was not successful. First, the injection intervals and timing may not be optimal for horses. Weekly injections were used in reports of successful immunotherapy for other Type I hypersensitivity diseases in horses (Carr 1982; Rosenkrantz 1987; and Fadok and Greiner 1990). The study began well after horses showing clinical signs of the condition. Perhaps the greatest advantage would be gained by starting immunotherapy earlier in the season. The trial may have been too short for the horses to achieve a clinical response, but it is reported that allergic horses usually require only four to eight weeks of therapy before improvement occurs (Rosenkrantz 1987; Fadok and Greiner 1990).

Secondly, the aqueous extract of whole, unfed *Culicoides variipennis* may not have contained the appropriate antigens or adequate amounts of appropriate antigens. Intradermal skin testing revealed no instances when *Culicoides insignis* reactions were positive without *Culicoides variipennis* reactions being positive as well, suggesting that species differences were not great. However, five different fractions of *Culicoides pulicaris* have been shown to have dermal reactivity in affected horses (Morris, Quinn, and Baker 1986), suggesting that a number of allergenic moieties are present in the extracts.

Finally, it is possible that *Culicoides* hypersensitivity in horses is not entirely IgE mediated and that Type IV hypersensitivity mechanisms are involved. In such a situation one would not expect immunotherapy to be successful. Evidence for delayed type hypersensitivity is seen in some horses that have delayed intradermal reactions at 6h post injection, without gross evidence of a preceeding immediate hypersensitivity reaction (Fadok and Greiner 1990). Histopathology from clinical lesions of *Culicoides* hypersensitivity in horses is also suggestive of delayed type hypersensitivity because a significant percentage of horses have dermal lymphohistiocytic perivascular infiltrates as well as, or instead of, the dermal perivascular eosinophilic infiltrates classically associated with arthropod hypersensitivity reactions (Ackerman 1978; Fadok and Greiner 1990). Lymphohistiocytic perivascular infiltrates are not exclusive to delayed type hypersensitivity reactions, however, and may be associated with chronic allergic skin disease or with IgE mediated late-phase reactions (Kaliner 1984; Fadok and Greiner 1990). It is evident then that the nature of the hypersensitivity reaction and

TABLE 2: Individual clinical sign scores* and group means during different months throughout the trial.

Horse	June/ pre-trial	August	September	December
Control group				
1	2.0	2.0	1.5	2.5
2	0.5	0.5	1.5	0.5
3	1.0	1.5	2.0	2.0
4 ^a	0.0	0.0	0.5**	0.0
5	3.0	3.0	2.5	2.0
6	1.0	2.0	2.5	1.0
7 ^b	2.5	1.0	1.0	0.5
8	0.5	1.0	1.0	0.5
Mean	1.3	1.4	1.6'	1.3
Test group				
9	2.0	3.0	2.5	2.0
10 ^c		1.0	1.0	1.0
11	1.0	1.5	1.0	0.5
12 ^d	1.0	0.5	1.0	1.5
13 ^e	1.5	1.0	1.0	0.5
14	1.5	1.0	0.5	1.0
Mean	1.4	1.3	1.2'	1.1

* - Clinical scores were based on extent and degree of pruritus and the following lesions: papules, nodules, alopecia, crusts, erosions, erythema, excoriations, tracts, lichenification, and pigmentary changes. A scale using 1/2 point increments from 0 to 3 was employed, with 0 indicating no clinical disease and 3 indicating severe clinical disease

^a - stabled daily dusk to 11:00 am ** = score 2 weeks post challenge (on pasture one night)

^b - moved to drier location a few miles distant at start of study

^c - started study in August

^d - swelling noted at injection sites on full strength *Culicoides* extract

^e - received steroid injections in September and October

' - Values compared by t-test reveal no significant difference ($P=0.32$)

the nature of the antigens involved remain unclear.

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