

Equine insect hypersensitivity: skin test and biopsy results correlated with clinical data

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Summary

Forty-four seasonally pruritic horses and 21 asymptomatic horses in Florida, USA, were tested for insect, grass and mould hypersensitivity by intradermal injection of allergenic extracts. The affected horses ranged in age from 10 months to over 30 years and included a variety of breeds. Affected horses reacted to varying dilutions of extracts made from *Culicoides*, mosquitoes, horse flies and black flies. Reactions to *Culicoides* were more intense than those caused by injection of antigens from other arthropods. Mild pruritus existed from the end of February until the end of June when the condition worsened and remained severe until November when it improved to an asymptomatic state. Histopathological examination of skin biopsies demonstrated changes compatible with arthropod hypersensitivity. Three clinical syndromes associated with insect hypersensitivity were described as follows: 1) horses with lesions on face, ears, mane, withers, rump and tail; 2) horses with lesions on face, ears, intermandibular space, chest, belly and groin, and 3) those with a combination of dorsal and ventral lesions.

Introduction

INSECT hypersensitivity is a common cause of equine pruritus. The best described is *Culicoides* hypersensitivity, a seasonal pruritic dermatitis identified throughout the world. The classic disease is characterised by pruritus, alopecia and crusting along the dorsal midline of the horse, including the face, ears, mane withers, rump and tail.

Culicoides hypersensitivity (CHS) is a well recognised and well researched entity throughout the world. Studies performed in Europe, Australia, Japan and the Middle East have correlated historical and clinical data with the results of histopathological examination of affected skin, intradermal skin testing with insect extracts and collection of insects feeding on specific areas of the body (Riek 1953; Riek 1954; Nakamura 1956; McCaig 1973; Mellor and McCaig 1974; Hesselholt 1977; Baker and Quinn 1978; Braverman *et al* 1983; Quinn, Baker and Morrow 1983; Townley, Baker and Quinn 1984). Investigators have identified the species of *Culicoides* causing CHS by correlating the temporal acquisition of skin lesions with the species of *Culicoides* actually biting horses. Limited work has been published in the USA on species of *Culicoides* feeding on horses (Jones, Potter and Rhodes 1972; Jones, Hayes, Potter and Franczy 1977; Foil, Stage and Klei 1984; Schmidtmann, Jones and Gollands 1980), or on the correlation of intradermal skin test and biopsy results with the history and

clinical signs of pruritus.

An unpublished study performed in New York by Braun, Raffensperger and Woodworth (1970) demonstrated that horses with dorsally distributed pruritus had positive skin test reactions to *Culicoides* extracts, developed pruritic papular lesions at the site of challenge with live *Culicoides* and had lesions histopathologically compatible with arthropod hypersensitivity. Serum from affected horses could transfer hypersensitivity to normal horses.

Other insect hypersensitivities have also been noted to affect the horse. A hypersensitivity to blackfly species (*Simuliidae*) has been identified as the cause of pruritus, alopecia and crusting of the ears, face, intermandibular space, chest and ventral midline (McMullan 1982). Also, stablefly (*Stomoxys calcitrans*) bites are reported to cause a pruritic papular eruption of the chest, shoulders and limbs (McMullan 1982).

Many horses also have a pruritic, ventrally distributed disease, which has been attributed to hypersensitivity to *Onchocerca microfilariae* (McMullan 1982). In most Florida horses however, these ventral lesions do not resolve after ivermectin treatment, and, therefore cannot be caused by a hypersensitivity to *Onchocerca microfilariae*. The clinical lesions in these horses may be caused by hypersensitivity to the bites of a ventrally feeding *Culicoides* species.

One report suggests that pollen allergies also may contribute to equine pruritic dermatitis and that horses may respond to hyposensitisation (Carr 1981).

There are many seasonally pruritic horses in southeastern USA. Many of these animals are unable to work as riding or show animals because of extreme discomfort and disfiguration. The purpose of this paper was to examine horses with seasonal pruritus in order to correlate their clinical signs and history with results of intradermal skin testing to and histopathological examination of biopsies.

Materials and methods

Horses

Forty four horses with pruritus were examined at the Veterinary Medical Teaching Hospital or on their owners' farms over a two year period.

They ranged in age from 10 months to more than 30 years. The breeds affected included Arabian (10), Quarterhorse (6), Thoroughbred (5), Tennessee Walker (2), Morgan (2), Palomino (2), Pony (2), Saddlebred (1), Standardbred (1), Clydesdale (1) and grade (12).

Thirty nine of the 44 horses had seasonal dermatitis that began at the end of February and remained mild until June. A

TABLE 1: Number of significant^a immediate and delayed^b skin test reactions in twenty-one symptom-free horses (n=21)

	PNU/ml	30 min	4-6 h	24 h		PNU/ml	30 min	4-6 h	24h
Saline ^c		-	-	-	Yellow Jacket ^d	1000	11	3	-
Histamine		21	-	-	Yellow Jacket ^d	500	10	3	-
Mosquito ^d	1000	1	1	-	Yellow Jacket ^d	250	10	2	-
Mosquito ^d	500	-	-	-	Yellow Jacket ^d	125	7	1	-
Mosquito ^d	250	-	-	-	Yellow Jacket ^d	62.5	4	1	-
Mosquito ^d	125	-	-	-	Yellow Jacket ^d	32.5	4	1	-
Mosquito ^d	62.5	-	-	-	Trichoderma	1000	-	-	-
Mosquito ^d	31.5	-	-	-	Alternaria	1000	-	-	-
<i>Culicoides</i> ^d	1000	19	5	-	Helminthosporium	1000	1	-	-
<i>Culicoides</i> ^d	500	12	4	-	Cephalosporium	1000	1	-	-
<i>Culicoides</i> ^d	250	5	2	-	Penicillium	1000	-	-	-
<i>Culicoides</i> ^d	125	1	1	-	Pullularia	1000	-	-	-
<i>Culicoides</i> ^d	62.5	-	-	-	Fusarium	1000	-	-	-
<i>Culicoides</i> ^d	31.5	-	-	-	Epicoecum	1000	1	-	-
Horse Fly ^d	1000	3	-	-	Hormodendrum	1000	1	-	-
Horse Fly ^d	500	1	-	-	Mucor	1000	-	1	-
Horse Fly ^d	250	-	1	-	Aspergillus	1000	1	-	-
Horse Fly ^d	125	-	-	-	Curvularia	1000	-	-	-
Horse Fly ^d	62.5	-	-	-	Alfalfa	1000	1	-	-
Horse Fly ^d	31.5	-	-	-	Johnson ^e	1000	-	-	-
Black Fly ^d	1000	9	1	-	Bahia	1000	-	-	-
Black Fly ^d	500	7	1	-	Timothy	1000	-	-	-
Black Fly ^d	250	1	1	-	Grass Mix	1000	-	-	-
Black Fly ^d	125	1	-	-	Bermuda	1000	-	-	-
Black Fly ^d	62.5	1	-	-					
Black Fly ^d	31.5	-	-	-					

^a: For immediate reactions, significance equals 2 + or greater; ^b: delayed reactions read on 9 horses only, significance equals presence of a weal regardless of size; ^c:phosphate buffered saline; ^d: mosquito = *Aedes taeniorhynchus*, *Culicoides* = *Culicoides variipennis*, Horse fly = *Tabanus* sp., Black fly = *Simulium venustum*, Yellow jacket = *Vespula maculifrons*; ^e=*sorghum halepensis*

sharp increase in severity was noted at the end of June; this intensity was maintained at a constant level until October or November. Horses were mildly pruritic through December. No pruritus was present in January or early February. Five of the horses were pruritic throughout the year, but were worse from mid-summer to fall.

Physical examination of the horses revealed three clinical syndromes. Syndrome I indicates those exhibiting lesions commonly associated with CHS (n=16), displaying excoriation on the face, ears, mane, withers, rump and tail. Syndrome II is composed of those with primarily ventral disease (n=5), consisting of lesions on the face, ears, intermandibular space, chest, belly and groin, but a normal mane and tail. Syndrome III includes those with a combination of the above clinical signs (n=23). For Syndrome I, the ages range from 1 to 23 ($\bar{x}$ =7) years. For Syndrome II, the ages range from 10 months to 8 ($\bar{x}$ =3) years. For Syndrome III, the ages range from 3 to 30 ($\bar{x}$ =13) years.

No ectoparasites or dermatophytes were recovered from any horses.

Skin testing procedure

The horses were prepared for intradermal skin testing by clipping an area 30 x 15 cm on the lateral neck. The site was swabbed with alcohol if gross contamination was present. The skin test sites were marked with a felt tipped pen to facilitate reading. Fractious animals were sedated with intravenous xylazine. Skin test antigens had been purchased from a commercial allergy laboratory (Greer Laboratories, North Carolina) and diluted with

phosphate buffered saline. Insect extracts in multiple dilutions, mould extracts, and grass pollen extracts were selected for testing. The *Culicoides* extract, not commercially available was prepared by the allergy laboratory (Greer Laboratories) from a colony of *C. variipennis*. The dilutions (Table 1) used were selected after multiple test dilutions were performed in 21 symptom-free horses. Those dilutions causing few or no reactions in normal horses were selected for use in the test population (Table 1). Injections were approximately 20 to 25 mm apart. A negative control, consisting of phosphate buffered saline, and a positive control of histamine 1:100,000 were used.

Injection of 0.05 ml of each antigen was made intradermally with a 26 gauge needle. Reactions as manifested by a weal were read at 20 to 30 mins for the immediate hypersensitivity component of the reaction. A 4+ reaction was a weal equal in size to the histamine; 0 was a weal equal in size to the saline injection. Intermediate reactions of 1+, 2+ and 3+ were recorded. Reactions of 2+ or greater to a given antigen dilution was considered significant.

Nine horses in the test population were evaluated for delayed reactions at 4 to 6 h and 24 h after injection. Skin test sites were evaluated for presence or absence of weal.

Horses with active lesions were biopsied. The site was clipped, anaesthetised with 2 per cent lidocaine, (Lignocaine, Vedco Inc, Missouri) and a 6 mm punch biopsy taken. Samples were preserved in 10 per cent buffered formalin, and processed routinely for staining with haematoxylin and eosin.

Two horses were injected with a series of *C. variipennis* extracts in an attempt to hyposensitise them. The inoculation schedule was five injections of 50 PNU of extract every 3

days, followed by five inoculations of 500 PNU of extract every 5 days and finally five injections of 5000 PNU of extract every 20 days. The volume of all inoculations in this series was 1 ml.

Results

All horses received repeated doses of ivermectin (Merck and Co. New Jersey) without response. Horses showed variable responses to fly spray. Some owners noted a 50 per cent

TABLE 2: Immediate hypersensitivity skin test results of horses with syndrome I (classic CHS)^c

Antigen	PNU/ml ^a	Syndrome I (n=16) #Responders ^b	Syndrome II (n=5) #Responders ^b	Syndrome III (n=23) #Responders
Saline ^d		0	0	0
Histamine		16 100%	5 100%	23 100%
Mosquito	500	8 50%	2 40%	9 39%
Mosquito	250	3 19%	2 40%	4 17%
Mosquito	125	5 31%	1 20%	4 17%
Culicoides	250	13 81%	4 80%	20 87%
Culicoides	125	13 81%	3 60%	17 74%
Culicoides	62.5	10 63%	2 40%	14 61%
Culicoides	31.5	6 38%	2 40%	10 43%
Horse Fly	500	14 38%	2 40%	16 70%
Horse Fly	250	9 56%	2 40%	8 35%
Horse Fly	125	5 31%	2 40%	6 26%
Black Fly	500	12 75%	2 40%	19 83%
Black Fly	250	8 50%	2 40%	10 43%
Black Fly	125	7 44%	2 40%	8 35%
Black Fly	62.5	3 19%	1 20%	4 17%
Black Fly	31.5	2 12%	0	2 9%
Yellow Jacket	1000	11 69%	3 60%	16 70%

a: protein nitrogen units/ml; b: significant response equals 2+ or greater 15-20 mins after injection of antigen; c: trichoderma, alternaria, helminthosporium, cephalosporium, penicillium, pullularia, fusarium, epicoccum, hormodendrum, mucor, aspergillus, curvularia, alfalfa, johnson, bahia, timothy, grass mix, bermuda. Only five plant antigens had over 30 per cent of the horses reacting in a single group of horses and none of these elevated reaction rates occurred in more than two groups. The highest rate of reaction was against alfalfa at 44 per cent; d: phosphate buffered saline

decrease in signs; however, most owners noted no improvement. Those owners who were able to stable their horses from dusk to dawn in a barn that had at least three walls noted partial to complete improvement in pruritus. Stabling horses during the day had no benefit. Corticosteroids had been given to 12 of the horses, 10 had received injections of triamcinolone acetonide (ER Squibb and Sons, New Jersey) intramuscularly every 2 to 4 weeks. These horses improved significantly following treatment. Two horses had received oral prednisone (Goldline Laboratories, Florida) at a dosage of 1.0 mg/kg daily initially. One horse experienced relief and was maintained comfortably on 500 mg every other day. The other horse had no response even when the dosage was increased to 2 mg/kg daily.

Intradermal skin test results

Intradermal skin test results in normal horses showed that less than 25 per cent of normal horses reacted to *Culicoides* extract at 250 PNU/ml, and 30 per cent reacted to black fly extract at 500 PNU/ml (Table 1). These dilutions were selected as the highest dilutions to be used for these extracts with multiple dilutions selected in an attempt to evaluate the sensitivity of the horses in the test population to these antigens. The relative lack of reactivity to the mould and grass extracts suggested that 1000 PNU/ml, as is used in small animal skin testing, would be acceptable for horses.

Skin test results for affected horses are summarised in Tables 2 and 3. Approximately 80 per cent of all the affected horses showed positive reactions to at least the highest dilution of *Culicoides* extract. Approximately seventy per cent of all horses reacted to the second highest dilution. Horses exhibiting signs of Syndrome I and Syndrome III seemed to have greater numbers of reactions to the lower dilutions of *Culicoides* than did horses with Syndrome II. Eighty per cent of horses with Syndromes I and III had positive reactions to black fly at the highest dilution. Less than half reacted to the next highest dilution. Sixty to 70 per cent of horses in all categories had positive reactions to yellow jacket (*Vespa maculifrons*). Less than half the horses responded to mosquito or horse fly antigen. There were some positive reactions in each group of horses to other extracts, particularly the moulds and alfalfa.

Most antigens elicited delayed hypersensitivity reactions at

TABLE 3: Delayed hypersensitivity responses (n=9)

Antigen	PNU/ml ^a	#Responders ^b				Antigen	PNU/ml ^a	#Responders ^b			
		6 hrs	%	24 hrs	%			6 hrs	%	24 hrs	%
Saline		0	0	0	0	Trichoderma	1000	5	56%	1	4%
Histamine		0	0	0	0	Alternaria	1000	1	11%	0	0
Mosquito	500	3	33%	0	0	Helminthosporium	1000	1	11%	0	0
Mosquito	250	1	11%	0	0	Cephalosporium	1000	3	33%	0	0
Mosquito	125	0	0	0	0	Penicillium	1000	1	11%	0	0
<i>Culicoides</i>	250	7	78%	2	22%	Pullularia	1000	3	33%	0	0
<i>Culicoides</i>	125	7	78%	2	22%	Fusarium	1000	0	0	0	0
<i>Culicoides</i>	62.5	4	44%	0	0	Epicoccum	1000	0	0	0	0
<i>Culicoides</i>	31.5	3	33%	0	0	Hormodendrum	1000	2	22%	0	0
Horse Fly	500	4	44%	0	0	Mucor	1000	2	22%	0	0
Horse Fly	250	4	44%	0	0	Aspergillus	1000	0	0	0	0
Horse Fly	125	3	33%	0	0	Curvularia	1000	3	33%	0	0
Black Fly	500	3	33%	0	0	Alfalfa	1000	0	0	0	0
Black Fly	250	3	33%	0	0	Johnson	1000	1	11%	0	0
Black Fly	125	2	22%	0	0	Bahia	1000	1	11%	0	0
Black Fly	62.5	1	11%	0	0	Timothy	1000	0	0	0	0
Black Fly ^d	31.5	1	11%	0	0	Grass Mix ^c	1000	0	0	0	0
Yellow Jacket	1000	2	22%	1	11	Bermuda	1000	0	0	0	0

a: protein nitrogen units per ml; b: results recorded as presence or absence of a weal at time indicated; c: red top (*Agrostis alba*), meadow fescue (*Festuca elatior*), sweet vernal (*Anthraxanthum odoratum*); Kentucky bluegrass (*Poa pratensis*), orchard (*Dactylis glomerata*), perennial rye (*Lolium perenne*) and timothy (*Phleum pratense*)

6 h post inoculation, but only the two highest concentrations of *Culicoides*, the yellow jacket and the *Trichoderma* antigens remained reactive at 24 h in a small percentage of the horses examined (Table 3).

Histopathology of skin biopsies

Histopathological examination was performed on skin biopsies from 25 horses. The biopsies of 22 horses had superficial and deep perivascular dermatitis, with infiltration of eosinophils, lymphocytes and histiocytes. This pattern is compatible with arthropod hypersensitivity (Ackerman 1978). In association with this pattern, superficial perivascular neutrophils in four horses (three had an ulcerated epidermis), subepidermal vesiculation in two horses, intraepidermal vesiculation in one horse, focal dyskeratosis in two horses and leukocytoclastic vasculitis in one horse were seen.

One horse had a superficial eosinophilic and lymphohistiocytic perivascular dermatitis that could be compatible with insect hypersensitivity (Ackerman 1978). Two horses had lymphohistiocytic superficial and deep perivascular dermatitis, compatible with chronic insect hypersensitivity.

Discussion

The results of this study suggest that insect hypersensitivity is an important cause of pruritus in horses in Florida. There are several features of interest that distinguish the insect hypersensitivity in Florida horses from that occurring in other parts of the world. The climate of this southeastern state supports a varied and large community of biting insects. There are at least 122 species of *Culicoides* identified in the North American continent; of these approximately 50 have been recognised in Florida (Blanton and Wirth 1979). At least 65 species of mosquitoes (King, Bradley, Smith and McDuffie 1960), 18 species of black flies (Pinkovsky and Butler 1978) and 44 species of tabanids (Jones and Anthony 1964) are established in Florida. The vast amount of diverse aquatic habitats and warm weather throughout the year provide an almost year round breeding season for these insects. Some species of *Culicoides* feed diurnally, others in the crepuscular (transition periods between day and night) periods and still others are nocturnal (Blanton and Wirth 1979). Most horseflies (*Tabanidae*) feed during the daylight hours, but a few species continue feeding into the early nocturnal hours (Jones and Anthony 1964; Muirhead-Thomson 1968). Mosquitoes (*Culicidae*) as a group feed any time of the day or night and many will enter buildings to obtain a blood meal (King *et al* 1960). Black flies (*Simuliidae*) are primarily diurnal feeding species (Muirhead-Thomson 1968). Humans and livestock consequently are attacked with a vengeance throughout the year.

The horses had only one month during which they were free of pruritus, approximately 5 months of mild to moderate pruritus, and 6 months of moderate to severe pruritus. Compared to horses in other parts of the world, horses in Florida have the potential to develop insect hypersensitivity at an early age, even as yearlings. This may be related to intensity of exposure throughout the year from a very early age. In this study, several breeds of horses were affected, Quarterhorses and Arabians comprising 39 per cent of those afflicted. This possibly reflects the likelihood of owners of these breeds seeking help for dermatological problems, rather than a true predisposition to the disease. Information about relatives of affected horses was collected for some of the horses in this study.

Another interesting feature of the horses examined in this study was the occurrence of three clinical syndromes.

Syndrome I, approximately 1/3 (16/44) of the horses, was identical to that reported for classic *Culicoides* hypersensitivity, a dorsally distributed disease. A small number (5/44) were afflicted with pruritus and dermatitis that was distributed primarily ventrally: face, ears, chest, belly and groin, with a normal mane and tail (Syndrome II). These horses had an average age of 3 years, younger than those in the other groups. The largest number of horses (23/44) had generalised disease affecting both the dorsum and ventrum (Syndrome III). It is important to note that ivermectin was not effective in ameliorating the skin disease in any of these horses, which ruled out onchocerciasis as the cause of the ventral midline dermatitis.

Skin reactions to insect extracts must be interpreted carefully. The solutions used are extracts of the whole insect body. Although the ideal extract would be made from salivary glands, the labour required to isolate insect salivary glands is prohibitive. There are many substances in insect extracts potentially involved in the development of a positive skin test (Bariga 1981; Nelsen *et al* 1977). Several proteins occur which may be irritating as well as allergenic. Without the knowledge of which is the most important in the development of the allergic response, the clinician must accept the results of skin testing with currently available extracts as an approximation. In spite of these obstacles, intradermal skin testing can be a useful tool in the pruritic horse.

Our skin test results, supported by histopathological examination of skin biopsies, suggest that insect hypersensitivity is a major cause of equine pruritus and that multiple insects may be involved in the pathogenesis. There were several positive reactions in the test horses to moulds and a few to grasses. It is not unusual for normal horses to manifest positive reactions to these antigens (Halliwell *et al* 1979). Due to the nature of their environment, horses are continually exposed to moulds and grasses and it would not be unusual for individuals to produce antibodies to some of these antigens without the development of clinical disease. Practical experience suggests horses with large numbers of positive reactions to mould and pollen extracts manifest respiratory, not dermatological disease. The histopathological pattern of eosinophilic lymphocytic superficial and deep perivascular dermatitis supports the aetiology of insect-induced disease.

An attempt was made to determine if reactions to a particular insect could be correlated with a specific clinical syndrome. This was not possible. There were horses in all three categories, including 2/5 with ventral disease which showed reactions to *Culicoides* extracts only. There were a few horses in both Syndrome I and II with reactions to black fly only. The observation was made that in general, horses showed reactions to *Culicoides* alone, or to *Culicoides* in combination with other insects.

This study originally was designed to examine immediate skin test reactions in horses; however, nine of nine horses examined for delayed reactions demonstrated this type of response. Seven of these nine horses showed positive reaction at 6 h to the two highest dilutions of *Culicoides*, with two horses having reactions to these extracts at 24 h. There were lower numbers of positive reactions to other insect extracts at 6 h, and only one (yellow jacket) at 24 h (Table 3). The presence of delayed reactions to these insect extracts supports insect hypersensitivity as a cause of the skin disease. The higher number of delayed reactions to *Culicoides* may support the theory that this insect is the major cause of hypersensitivity in the Florida horse. Insect hypersensitivity in man, the dog and laboratory animals is known to have immediate and delayed components. Contributing reactions can be Type I hypersensitivity, associated with IgE or other reagents, or Type IV hypersensitivity, the classic delayed reaction mediated by

lymphocytes, and cutaneous basophil hypersensitivity (Barriga 1981; Nelson *et al* 1977; Mahapatro and Mahapatro 1984). There were a few horses in the group of nine with no immediate hypersensitivity reactions, but positive delayed reactions, suggesting that, as in flea allergy dermatitis in dogs, a subpopulation of insect allergic horses exists which may exhibit delayed reactions only.

Treatment of equine insect hypersensitivity in Southeastern USA is frustrating. Very few farms have barns that have three or four solid walls, making stabling horses from dusk through dawn impossible or ineffective. This would primarily give relief to only *Culicoides* induced problems as most black flies and horse flies feed during the day and most nocturnal mosquitoes will enter stables to feed. A high percentage of Florida is covered by standing water, making it almost impossible to remove horses from the potential breeding sites of *Culicoides*, mosquitoes and horseflies. Many of the commercially available insecticides are ineffective repellents, accounting for the poor response to insecticide application seen in the horses in this study. The most effective products seem to be those containing pyrethrins or synthetic pyrethroids and repellents; however, the required frequency of application discourages many owners. Response to corticosteroid therapy is variable; often the dosages required to abrogate the itch are so high as to be a potential threat to the horse's health.

This pruritic dermatosis due to insect hypersensitivity is a major annoyance to both horse and owner, and a cause of economic loss. Many horses in Florida are sold or euthanased because of this problem. The ideal therapy would be directed at modulating the immune response, through hyposensitisation or the production of tolerance. Such immunotherapy will require much more knowledge about the pathogenesis of insect hypersensitivity in the equid. Two of the horses in this study were hyposensitised successfully with insect extracts. One mare, manifesting signs of Syndrome III, had a dramatic decrease in pruritus after four weekly injections of *C. variipennis* extract. Another mare, with ventrally distributed lesions, and skin test reactions to mosquito, blackfly and horsefly only, responded well to a vaccine composed of these extracts. Hyposensitisation with insect extracts in the horse requires critical evaluation, as it has not been successful in flea allergy in the dog or cat (Halliwell 1981; Kunkle and Milcarsky 1985).

In summary, insect hypersensitivity appears to be an important cause of equine pruritus. Based on intensity of the equine responses to multiple dilutions of insect extracts, *Culicoides* spp. would appear to be the most significant cause in Florida.

Acknowledgements

The authors wish to acknowledge the American Academy of Veterinary Dermatology and the Academy of Veterinary Allergy for their support of this work. We thank Ed Rabin and Michelle Greenberg for assisting in skin testing. Dr Mary Pichler contributed data on nine normal horses. We also thank Drs Gail Kunkle, Sandy Merchant, Karin Beale and Joy Barbet for constructive criticism of the manuscript. Published as Florida Agricultural Station Journal Series No. 9074.

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Received for publication: 22.6.88

Accepted: 20.1.89