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Culicoides obsoletus (Diptera: Ceratopogonidae) as a Causal Agent of *Culicoides* Hypersensitivity (Sweet Itch) in British Columbia

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ABSTRACT Six horses severely affected by a seasonal dermatitis similar in both histopathology and epidemiology to *Culicoides* hypersensitivity (CH) and six unaffected or normal horses were inoculated intradermally with an extract of *Culicoides obsoletus* (Meigen), the most common *Culicoides* in southwestern British Columbia. Affected horses developed large welts within 20 min after injection, representing an immediate (type I) reaction; welts were largest 24 h or more after challenge, indicating in addition a delayed (type IV) reaction. This reaction was discernible for >3 wk in some of the affected horses. Normal horses developed small welts which peaked 2–4 h after challenge. Affected horses were irritated by the injections and developed characteristically ridged skin at the injection sites, similar to that seen in natural lesions, whereas the normal horses showed neither discomfort nor clinical signs. *C. obsoletus* is one of the most numerous *Culicoides* species biting horses, feeds on parts of the body where lesions are found, and is, we believe, responsible for dermatitis in the Pacific Northwest. The skin test was repeated 2 yr later in three of the affected horses. In two, the reactions were similar to those in the first test, but in the third horse, the reaction was greatly reduced after the second test. This paralleled a decrease in the severity of its natural clinical signs. Thus, a skin test may be useful in the diagnosis of *Culicoides* hypersensitivity.

KEY WORDS Insecta, *Culicoides* hypersensitivity, *Culicoides obsoletus*

AN IMPORTANT, chronic, recurrent, seasonal dermatitis of horses has been reported worldwide under a variety of names (Kleider & Lees 1984). In Australia, the disease is termed *Queensland itch* and its prevalence ranges from 32 to 60% (Riek 1953). In Japan, under the name of *Kasen*, the prevalence is 4.4% (Nakamura et al. 1956), and in England, as *sweet itch*, its prevalence ranges from 2.0 (British Equine Veterinary Association 1965) to 4.5% (McCaig 1973) depending on region and animals surveyed.

In most cases, the disease appears during the warmer months of the year, affecting only certain animals in a herd, and these animals are affected year after year. The disease usually occurs in horses that are pastured rather than stabled, but some horses also are affected in open or poorly screened stables. The clinical signs and histopathology of the disease are similar worldwide, varying only in the location of the lesions. Initially, the disease is characterized by numerous papules, tufted hair, and skin sensitization. This is followed by intense irritation, scratching, and rubbing which leads to serous effusion, localized hair loss, and the development of secondary lesions (Riek 1953; Robinson 1983, Kleider & Lees 1984). Recurrent attacks lead to thickening of the skin, scaling, and the formation of transverse ridges or ruggae in the skin (Riek 1953, Robinson 1983). The histopathology of a typical lesion shows an eosinophilic perivascular dermatitis (Fadok & Mullowney 1983). An affected horse is irritable and unsightly and has reduced value.

In Australia, Queensland itch is caused by a hypersensitive reaction to the bites of *Culicoides*, especially *Culicoides robertsi* Lee & Reye (Riek 1954). Later studies in Ireland (Baker & Quinn 1978, Quinn et al. 1983), Israel (Braverman et al. 1983), and Hong Kong (Baker & Collins 1984) have indicated a similar etiology. A similar hypersensitivity to *Culicoides variipennis* (Coquillett) has been induced in cattle (Akey et al. 1990). The disease in horses was first described in Canada by Kleider & Lees (1984), and a later survey indicated that up to 26% of horses in British Columbia were affected (Anderson et al. 1988). Most cases were reported from the southwestern region of the Province, and lesions were present from April to October (Anderson et al. 1988). On rare occasions, particularly severe cases have shown signs all year (Kleider & Lees 1984), evidently due to a scratch reflex similar to that seen in flea-sensitive dogs. Scratching renews the pruritus even though fleas are no longer present, and this phenomenon also can occur in humans (Baker & O'Flanagan 1975). Kleider & Lees (1984) proposed that the disease in British Columbia was caused by a hypersensitive reaction to *Culicoides* bites, due to histopathological and

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epidemiological similarities to Queensland itch and sweet itch. Therefore, we have called the disease *Culicoides* hypersensitivity (CH) in British Columbia.

Skin sensitivity to insect bites in certain animal species is known to go through several stages (Larrivee et al. 1964). The first stage is induction, during which there is no change in response to bites. In the second stage, delayed skin reactions appear. In the third stage, both immediate and delayed reactions are present, and by stage four, only immediate reactions occur. Eventually, in stage five, no skin reaction is observed and the animal is considered desensitized. Such a sequence occurs in guinea pigs (Benjamini et al. 1961) and also could occur in horses.

The present study was undertaken to determine whether *Culicoides obsoletus* Meigen can cause CH disease in horses in British Columbia. *Culicoides* seemed the most likely suspect as their seasonal occurrence and geographical distribution corresponds with the appearance of clinical signs of CH in British Columbia (G.S.A., unpublished data; Costello 1982). Hornflies, *Haematobia irritans* (L.), cause a ventral midline dermatitis, but the lesions usually are found only in the umbilical region (Fadok & Mullowney 1983). Also, hornflies are obligate parasites of cattle, and the adult fly usually remains on the host. Therefore, only horses pastured with cattle are likely to be attacked (Foil et al. 1990). No animals used in this study were pastured with cattle. Other insects that bite horses include tabanids, blackflies, and mosquitoes. Tabanids bite most commonly on the chest, flanks, and limbs (Foil & Foil 1986), where CH signs rarely occur. Blackflies feed most commonly on the ears of horses (Foil & Foil 1990) and are not common in southwestern British Columbia. Although mosquitoes are known to bite horses, no specific dermatology has been attributed to their feeding (Foil & Foil 1986). Riek (1954) compared skin tests using extracts of *Culicoides*, mosquitoes, and stable flies in horses affected by Queensland itch and obtained consistently positive responses only with the *Culicoides* extract. This was later confirmed by similar tests in England (Baker & Quinn 1978).

Culicoides obsoletus makes up 99.3% of the *Culicoides* caught in light traps in southwestern British Columbia (Costello 1982). *C. obsoletus* was one of the three most common *Culicoides* captured on horses (Schmidtmann et al. 1980) in New York and was the most common species captured on horses in Ireland (Townley et al. 1984) and England (Mellor & McCaig 1974). In England and Israel, lesions were found dorsally; this was related to the site on the horse where the *Culicoides* species feed (Mellor & McCaig 1974, Braverman 1988). However, in British Columbia, the most commonly affected area was the ventral midline, although the mane and tail also were affected frequently (Anderson et al. 1988). *C. obsoletus* bites horses most frequently along the ventral midline in North America (Schmidtmann et al. 1980) and England (Mellor & McCaig 1974) and bites cattle most frequently in this region in Denmark (Nielson 1971). In England, *C. obsoletus* is not considered to be a causal agent of CH disease (Mellor & McCaig 1974). However, *C. obsoletus* is actually a complex of several species (*avaritia* group), and the species in England may be different.

Materials and Methods

Six clinically normal horses (N1–N6) and six horses affected with severe and typical lesions (A1–A6) of CH disease were evaluated. The affected horses had the disease for several years severely enough to warrant veterinary attention. Each normal horse was kept in the same pasture under the same conditions as an affected horse both before and during the tests and both normal and affected horses were exposed equally to *C. obsoletus* under natural conditions. We have shown previously that there were no differences in susceptibility to *Culicoides* bites due to the sex, color, breed, or height of horses (Anderson et al. 1988). Therefore, we selected the affected horses solely on the basis of their clinical signs, and the normal horses because of their proximity to the affected horses. The affected horses ranged from 7 to 22 yr old, from grey to bay in color, and were mostly crossbred thoroughbreds, quarterhorses, Arabians, and Appaloosas. All were affected severely and had clinical signs in the ventral midline region and tail. Most of them also had lesions in the mane, withers, face, rump, chest, and genital region. All of the affected horses were known to have developed lesions yearly for a minimum of 5 yr before this study was undertaken. In some cases, the time of first onset of lesions was unknown because previous owners could not be contacted. The normal horses included the same range of ages, colors, and breeds.

Culicoides obsoletus were collected from the sites of five of the affected horses using New Jersey light traps equipped with 100-watt light bulbs. To test the reactions of the horses to *Culicoides*, sterile extracts were prepared from entire insects. *C. obsoletus* were identified, freeze-dried, and stored at −18°C until they were used. Approximately 3,500 whole, freeze-dried insects weighing 0.17 g were crushed in 0.5 ml of physiological saline (0.9% NaCl). The extract was diluted with saline to 1:100, then sterilized by filtering through decreasing sizes of sterile millipore filters, the smallest having a pore diameter of 0.22 μm. The extract was measured into aliquots and kept frozen at −18°C until just before use. Aliquots of physiological saline also were autoclaved and frozen until they were used.

Each horse was clipped along the side of the neck, using size 40 clipper blades. The clipped area was cleaned and sterilized with 70% alcohol immediately before injection. The skinfold thickness of the injection site was measured immediately before the injection with vernier calipers.

Table 1. Reaction of affected and normal horses 24 h after injection with *Culicoides* extract or saline

Horse[a]	Welt width increase, mm		Skinfold thickness increase, mm	
	Extract	Saline	Extract	Saline
A1	49.6	0.0	6.6	1.7
A2	80.2	0.0	7.5	1.0
A3	128.8	17.0	14.6	0.1
A4	213.7	0.0	10.0	0.0
A5	235.8	0.0	28.8	0.0
A6	64.1	0.0	3.0	0.1
Mean ± CL	128.7 ± 84.8	2.8 ± 7.5	11.8 ± 9.8	0.5 ± 0.7
N1	17.2	0.0	3.7	0.0
N2	0.0	0.0	1.0	0.9
N3	23.6	0.0	1.8	0.0
N4	40.0	0.0	3.0	0.0
N5	0.0	0.0	2.0	0.0
N6	0.0	0.0	1.4	0.0
Mean ± CL	13.5 ± 17.7	0.0	2.2 ± 1.0	0.2 ± 0.5

[a] A, affected horse; N, normal horse.

Each affected horse received four intradermal injections: three of 0.1 ml 1% *C. obsoletus* extract and one of 0.1 ml physiological saline as a control. Each normal horse received two injections: one of 0.1 ml 1% *C. obsoletus* extract and one of 0.1 ml saline. Tuberculin syringes with 26-gauge needles were used to minimize skin trauma.

The increase in skinfold thickness (difference between pre- and postinoculation measurements) and width of the welt at its widest point were measured with vernier calipers at 20 min and 1, 2, 3, 4, 24, 48, and 72 h after injection. In cases where the reaction had not subsided completely after 72 h, readings also were made up to 3 wk later. In horse A3, both skinfold thickness and welt width measurements were repeated three times at each of the first five reading times to estimate the error associated with each measurement technique.

Curves were plotted of both the welt widths and the skin thickness increases over time from 0 to 72 h for each normal and affected horse, and the areas under these curves were measured using an Apple 2 Plus graphic tablet. The areas under the curve for affected horses were compared with those for normal horses using a *t* test. The welt width and increase in thickness of the skinfold in normal and affected horses attained 24 h after injection also were compared using a *t* test. The mean of the reactions to three inoculations in the affected horses was used at each time interval as the experimental unit and compared with the reactions of the normal horses to the inoculation of extract.

Three of the affected horses (A1, A3, and A5) were tested again ≈2 yr after the first challenge. Measurements were made as before, and the housing and management of these horses were unchanged. Each horse was given one 0.1-ml intradermal injection of 1% *C. obsoletus*, freshly made. The results of these tests were compared with those of the earlier tests, but statistical comparisons were not made because only three horses were involved.

Results

When measurements of the reactions of affected and normal horses made 24 h after inoculation were compared, the affected horses had a significantly greater increase in welt width ($t = 3.48$, df = 10, $P < 0.05$) and skinfold thickness ($t = 2.54$, df = 10, $P < 0.05$) than did normal horses (Table 1). Total area under the curve of a graph showing the reaction of affected horses showed a greater total increase in welt width ($t = 3.03$, df = 10, $P < 0.05$) and skin thickness ($t = 3.47$, df = 10, $P < 0.05$) than normal horses over 72 h (Fig. 1 A and B).

In affected horses, the reaction to *Culicoides* extract did not peak, in most cases, for at least 24 h (Table 2). Horse A6 peaked 4 h after challenge, but the welt width decreased only slightly by 24 h, whereas skinfold thickness had decreased greatly, indicating a large shallow welt. Horse A1 reached peak welt width at 24 h but skinfold thickness peaked at 4 h, again indicating a change in the shape of the welt. The duration of a measurable welt in affected horses ranged from 72 to 529 h (≈3 wk). During the reaction, the welts on the affected horses changed appearance. For the first 4 h, they were firm, hard to the touch, and well defined, whereas after 24 h, they became less indurated, larger, and poorly defined.

In contrast, the reaction in the unaffected horses peaked 2–4 h after challenge. Welt width returned to zero between 4 and 48 h and skinfold thickness to normal after 72 h except in horse N4, which still showed a very minor skinfold thickness increase for up to 120 h after inoculation. All reactions were greatly reduced after 4 h.

Several days after injection, the affected horses showed small vertical ridges in the injection site similar to the ruggae seen in natural cases of the disease at the lesion sites. The ridges were not present before the test, and we have never seen the

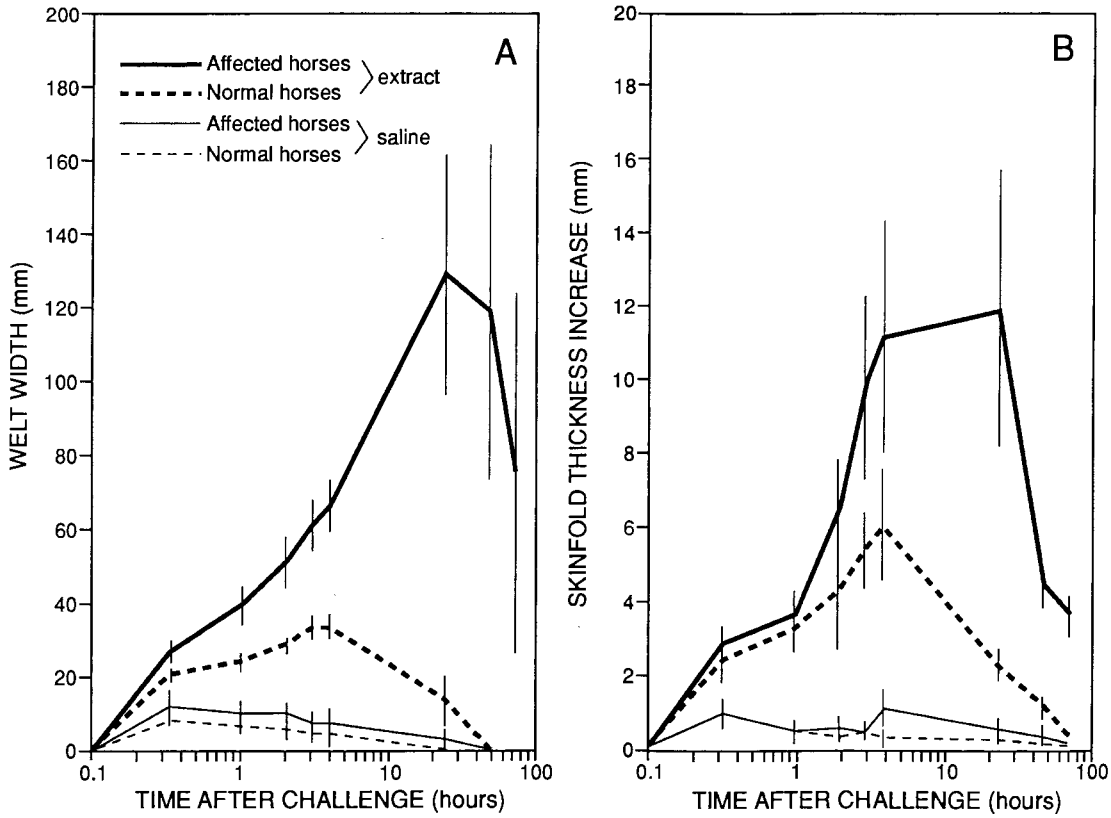

Fig. 1. Welt width (A) and skinfold thickness increase (B) (±SEM) of six affected and six normal horses over time after challenge with *C. obsoletus* extract and saline control.

side of the neck affected by natural lesions. These ruggae disappeared within a few weeks. Normal horses never showed such ridging and did not appear at all discomforted by the injections. However, all the affected horses showed some irritation at the injection site. Most attempted to rub the area, and in horse A3, the entire injection site was extremely sensitive during the first 24 h despite the fact that the reaction had not yet reached its peak. During this period, the horse reacted violently to being handled in the region of the injection site. The following day, however, it showed little sign of discomfort. Several of the affected horses were irritable and difficult to handle at the injection site during the first 24 h after challenge.

After 2 h, horse A4 developed a number of extraneous small welts around the injected area, and after 4 h, five extra welts appeared. These welts were separated from the injection sites by several centimeters of unaffected skin and were not a result of insect bites (the area was monitored closely). By 72 h, eight small scabs had appeared and some were pustular. Three weeks (498 h) after challenge, four of the extra welts were still present. Two of the other affected horses showed small welts near the injection site, but none was as extreme as in horse A4. The normal horses did not display these extra welts, so they were not attributed to clipping. In later tests, unclipped horses have shown similar extra welts (unpublished data).

Each horse's reaction to the insect extract and saline controls was compared at 24 h postinoculation using a t test. The affected horses exhibited significantly greater welt width ($t = 13.87$, df = 10, $P < 0.01$) and skinfold thickness ($t = 2.99$, df = 10, $P < 0.05$) to extract than to saline inoculation. Normal horses inoculated with extract did not have a significantly larger welt width 24 h after inoculation, but they still showed a significantly increased skinfold thickness ($t = 4.54$, df = 10, $P < 0.01$) when compared with saline controls. There was no significant difference between the reaction of affected and unaffected horses to saline, indicating that differences were due to the solution injected rather than to the trauma of inoculation. In the affected horses, peak welt width from the saline injection ranged from 0 to 27.8 mm, which occurred 20 min to 4 h after injection. The peak width in unaffected horses ranged from 10.4 to 20.0 mm, and it also occurred 20 min to 4 h after injection. The welt following saline injection disappeared between 0 and 48 h in affected horses and 20 min to 24 h in unaffected horses. The peak skinfold increase in response to saline in affected

Table 2. Peak and duration of reaction of affected and normal horses after challenge with *Culicoides* extract or saline[a]

Horse	Peak reaction								Duration of reaction			
	Welt width, mm		Time of occurrence, h		Skinfold thickness increase, mm		Time of occurrence, h		Welt width, h		Skinfold thickness increase, h	
	Ext.	Sal.	Ext.	Sal.	Ext.	Sal.	Ext.	Sal.	Ext.	Sal.	Ext.	Sal.
A1[b]	49.6	0.0	24	0	9.1	2.4	4	4	>72	0.0	>72	48–72
A2	80.2	27.8	24	0.3	7.5	2.6	24	0.3	283–529	2–3	283–529	0.3–1
A3	128.8	19.5	24	1	14.6	0.8	24	0.3	114–165	24–48	114–165	1–2
A4	315.0	14.0	72	4	13.7	0.8	3	0.3	120–214	4–24	242–357	2–3
A5	235.8	0.0	24	0	28.8	0.2	24	0.3	72–169	0.0	169–266	0.3–1
A6	68.8	18.1	4	1	13.7	2.7	3	4	48–72	4–24	72–189	4–24
Mean ± CL	146.4 ± 113.9	13.2 ± 12.1	—	—	14.5 ± 8.0	1.6 ± 1.3	—	—	—	—	—	—
N1	27.8	10.4	2	0.3	4.5	2.0	4	0.3	24–48	0.3–1	48–72	1–2
N2	38.4	11.5	3	0.3	4.0	2.0	4	0.3	4–24	4–24	48–72	24–48
N3	38.8	11.6	3	1	4.4	1.0	3	0.3	24–48	1–2	48–72	2–3
N4	46.2	11.9	4	0.3	12.4	0.6	4	0.3	24–48	3–4	48–72[c]	0.3–1
N5	36.0	11.6	4	0.3	4.0	0.1	4	0.3	4–24	1–2	48–72	1–2
N6	35.0	20.0	4	4	8.2	1.4	4	3	4–24	4–24	48–72	4–24
Mean ± CL	37.0 ± 6.4	12.8 ± 3.9	—	—	6.3 ± 3.6	1.2 ± 0.8	—	—	—	—	—	—

[a] Ext., 1% *Culicoides* extract; Sal., saline.
[b] Horse A1 was monitored for only 72 h.
[c] Horse N4 had a very minor skinfold thickness increase of 0.6 mm up to 120 h after inoculation, but the increase was very minor by 72 h after inoculation.

horses ranged from 0.2 to 2.7 mm between 20 min and 4 h after inoculation, and the peak in unaffected horses ranged from 0.1 to 2.0 mm after 20 min to 3 h. The skin returned to its normal thickness between 1 and 72 h in the affected horses and between 2 and 72 h in the normal horses.

Each measurement for horse A3 was made in triplicate up to 4 h after challenge. The error involved in each measuring technique was 0.0–0.3 mm for skinfold thickness increase and 0.1–5.5 mm for welt width. These represent 0–2% of the maximum skinfold thickness increase and 0.1–4.3% of the maximum welt width for this horse.

Three of the affected horses (A1, A3, and A5) were tested again after 2 yr; their reactions are shown in Fig. 2. Horse A1 had a larger welt after the second test, but the increase in skin thickness was similar to that in the first test. In contrast, horses A3 and A5 had smaller reactions the second time. In particular, horse A5 had an extremely severe reaction to the first challenge but reacted only slightly to the second. The disease, which previously had been very severe in this horse in 1986, did not recur in 1987 and has since been very mild.

Discussion

Affected horses reacted more severely to the extract of *C. obsoletus* than did the unaffected horses. All the affected horses showed reactions characteristic of immediate hypersensitivity or type I reactions (Coombs & Gell 1975). A typical type I reaction is a sharply defined, indurated welt, appearing a few minutes after the challenge, reaching peak size in a few hours and then subsiding (Pepys 1975). The welt is, in most cases, followed by a peripheral reddening of the skin, the "wheal and flare reaction." However, in most of the affected horses, the reactions did not peak until 24 h after challenge and were more consistent with a delayed type IV hypersensitivity reaction. A typical delayed reaction is characterized by a less well-defined welt which appears after 6 h, reaches a maximum 24–48 h after challenge, and thereafter subsides (Katz 1978). The reaction may take weeks to subside entirely. Both immediate wheal and flare reactions and delayed-type skin lesions frequently occur following the bite of one insect (Humphrey & White 1970, Roitt et al. 1985), as observed in our experiments.

Tests in Australia showed that the intensity of the reaction of sensitized horses to *C. robertsi*, the causal agent in Queensland, did not vary between tests using extracts of entire male or female specimens or among extracts of head, thorax, and abdomen (Riek 1954). A similar response also has been shown to certain mosquito species (Rockwell & Johnson 1952, McKiel 1959). We concluded that, although the allergen is evidently present in the salivary glands, it or a very similar molecule also is present throughout the insect.

In Australia, the reactions of horses challenged with *Culicoides* extract were measured only 1 h after challenge, so delayed reactions were not observed. However, when monitored for 72 h, horses in Ireland challenged with *Culicoides* extract exhibited both immediate and delayed reactions, although the duration of the delayed response was not measured (Quinn et al. 1983). Immediate and delayed responses also were seen in horses in Hong Kong (Baker & Collins 1984). In the present study, a reaction was still discernible in some of the affected horses for up to 3 wk. Even when a defined welt was no longer visible, an increase in skinfold thickness indicated the persistence of a slight edema.

The normal horses reacted to the extract, but their reactions were less severe and less prolonged than the affected horses. Some reaction is expected to the injection of any foreign protein, which probably accounts for this observation. A certain percentage of normal animals often respond to intradermal challenge with antigens (Kieffer & Kristenson 1979, Moriello & McMurchy 1989). Horse N4 showed a larger reaction than most of the normal horses, although it had no clinical signs of CH. It is possible that this horse had a subclinical sensitivity to *Culicoides*. All the normal horses had been exposed constantly to *Culicoides* bites, so some may have been sensitized slightly. However, in most of the present cases, a clear distinction was seen between affected and unaffected horses. Our previous survey in British Columbia (Anderson et al. 1988) showed that in all cases where the ancestry of an affected horse could be traced, an affected relative also was identified. This supported other studies (Riek 1953, Ishihara & Ueno 1957, McCaig 1975) that indicated there is a genetic predisposition for this disease.

Our study clearly indicated that the disease in British Columbia is a hypersensitivity to *Culicoides* species and that *C. obsoletus* is one of the causal or sensitizing agents. This is supported by the significantly larger and more prolonged reactions in affected horses, the irritation experienced by the affected but not the unaffected horses after challenge, and the appearance of ruggae similar to those seen in natural lesions in the skin of the affected horses. *C. obsoletus* is probably the most important sensitizing agent in this area because it is by far the most common species (Costello 1982). However, other *Culicoides* species also may be involved. We have skin-tested affected horses with extracts from other species of *Culicoides* from this and other regions of the World, and preliminary results show that affected horses crossreact to many horse biting species, even though the species may be uncommon in, or absent from, British Columbia (unpublished data).

Our skin tests indicated that all the affected horses were in stage three of the five stages of skin reactivity, showing both immediate and delayed reactions (Benjamini et al. 1961). Therefore, it may

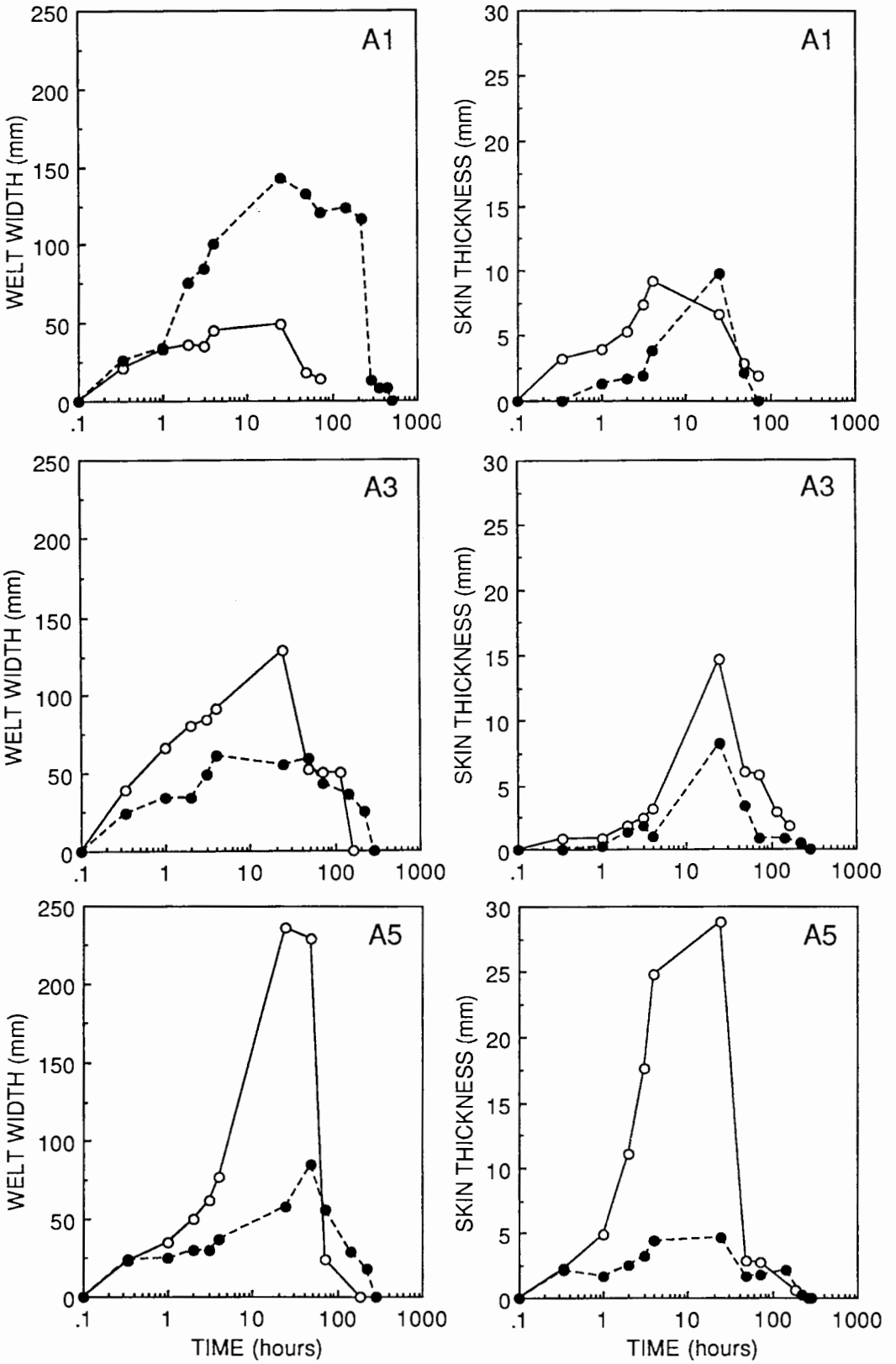

Fig. 2. Welt width and skinfold thickness increase in three horses affected by *Culicoides* hypersensitivity after two separate injections of *C. obsoletus* extract administered in 1986 (open circles) and 1988 (closed circles).

be possible to desensitize, or hyposensitize, affected horses by repeatedly injecting them with small but increasing doses of the allergen over a period of time, similar to procedures used for humans with Hymenoptera venom allergy and dogs with flea dermatitis. We currently are evaluating hyposensitization of horses in British Columbia.

The subsequent test on three of the affected horses indicated that the reaction may have been different after a second injection of extract. As only three horses were retested, no statistical analysis was made, but it is probable that the reactions after the two sets of injections in horses A1 and A3 were not significantly different. The severity of their natural clinical signs did not change during this period. Quinn et al. (1983) reported challenging a single horse with *Culicoides* extract twice in 2 yr and observing no difference in the reaction, although the reaction to other extracts did change. However, our horse A5 showed a dramatic decrease in sensitivity similar to the decrease observed in clinical signs. When first injected, this horse was already very severely affected by the disease, but 2 yr later it did not develop clinical signs of CH. The clinical signs seen in horses A1 and A3 did not change, and the normal horses did not develop clinical signs over this time. If the skin test varies with the severity of the clinical signs and, indirectly, with the hypersensitivity of the horse, it could be a useful diagnostic test. The present method of diagnosis is by the elimination of other potential causes, such as onchocerciasis by using a punch biopsy.

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