

Intradermal skin testing in Icelandic horses in Austria

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Summary

Icelandic horses in Austria are commonly affected by an allergic inflammatory skin disease recurring during the summer seasons, which shares characteristic features with *Culicoides* hypersensitivity. However, the causative agents have not yet been identified. Therefore, intradermal skin testing (IDST) with a standardised extract of *Culicoides variipennis* and 21 other allergens relevant within Austria was performed in 81 Icelandic horses. All horses included into the study were treated regularly with ivermectin and had no history of administration of anti-inflammatory drugs. Forty-three of these horses were affected by summer seasonal recurrent dermatitis (SSRD). No history or signs of any other disease were evident in any horse. Pruritic dermatitis due to ectoparasites, bacteria and dermatophytes were ruled out by means of fungal culture, skin scraping and biopsy.

Culicoides variipennis antigens evoked a positive cutaneous reaction in 1 of 38 normal and 3 of 43 SSRD horses at the proposed dilution of 1:50,000 or 1:25,000, and in 24 of 38 normal and 13 of 43 SSRD horses at a dilution of 1:10,000. Furthermore, no significant differences in onset or intensity of skin reactions to the 21 other allergens, including pollens, moulds, mites and insects, except deerfly and horsefly, were obvious between the 2 groups. Efficiency (percentage of correct results) for the used antigens in the skin test was 0.47–0.60. Maximal sensitivity was 0.51. Altogether, 38 of 43 SSRD horses and 28 of 38 normal horses were positive 4 h after allergen administration. The divergence between IDST results and manifestation of clinical signs found in this study underlines the difficulties associated with establishing a skin test protocol in horses within a geographic area. Whether the outcome of this study would have been influenced significantly by using *Culicoides* spp. present in Austria has to be clarified in future research.

Introduction

Allergic dermatitis is a very common skin disorder in horses worldwide with a prevalence of up to 26% in some geographical areas (Braverman *et al.* 1983; Anderson *et al.* 1988). The temporal distribution of clinical signs during the year varies depending on the

responsible allergens. Allergic skin disorders associated with recurrence during warmer seasons may be attributed to insect bites and aeroallergens and, rarely, to food allergens. Among the best documented allergic skin diseases is the hypersensitive reaction to salivary gland proteins of biting midges of the genus *Culicoides*. *Culicoides* hypersensitivity is known in many parts of the world by different names, including *sweet itch*, *Queensland itch*, *summer seasonal recurrent dermatitis*, *kasen* or *summer eczema* (Baker and Quinn 1978; Braverman 1988; Halldorsdottir and Larsen 1991; Kurotaki *et al.* 1994). Intense pruritus, papules or weals as primary lesions are common to all affected horses, as are the alopecia, scaling and crusting which result from rubbing. The afflicted body regions vary according to the blood-sucking *Culicoides* species prevailing in the geographical area. Prevalence of *Culicoides* hypersensitivity in the Icelandic horse is 15–26%, with an exceptionally high rate among Icelandic horses imported from Iceland (Broström *et al.* 1987; Halldorsdottir and Larsen 1991).

Even though the validity of intradermal skin testing in horses is considered controversial, the published work regarding *Culicoides* allergen demonstrates concordance to a certain degree (Baker and Quinn 1978; Braverman *et al.* 1983; Quinn *et al.* 1983; Baker and Collins 1984; Larsen *et al.* 1988; Halldorsdottir *et al.* 1989; Evans *et al.* 1992; McGorum *et al.* 1993). The relevance of other allergens, either other insects or environmental allergens in seasonal allergic dermatitis of horses, has rarely been co-investigated (Baker and Quinn 1978; Braverman *et al.* 1983; Quinn *et al.* 1983; Fadok and Greiner 1990).

Icelandic horses in Austria are also affected by an allergic inflammatory skin disease recurring during the summer seasons, which shares characteristic features with *Culicoides* hypersensitivity. However, the causative agents have not yet been identified. *Culicoides* species or various insects, as well as environmental allergens, may relevantly be implicated in the pathogenesis of this seasonal allergic skin disease in Icelandic horses in Austria.

The purpose of this study was, therefore, to compare the results of intradermal skin tests performed in Icelandic horses in Austria affected by recurrent seasonal allergic dermatitis with those of nonaffected horses.

Materials and methods

Recurrent seasonal allergic dermatitis

Clinical signs in Icelandic horses in Austria are observed mainly from the end of February to the end of June, and the end of August

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TABLE 1: Number of control (n = 38) and affected (n = 43) respondents after 30 min

	Skin reaction								Sign 2 x 2 ¹	Sign 2 x 4 ²
	Negative		++		+++		++++			
	Control	Affected	Control	Affected	Control	Affected	Control	Affected		
<i>Culicoides</i> 1:10	37	42	0	0	0	0	0	0	1	1
<i>Culicoides</i> 1:5	37	42	0	0	0	0	0	0	1	1
<i>Culicoides</i> 1:2	26	34	0	0	4	4	0	0	0.3	0.48
Mixed grasses	38	42	0	1	0	0	0	0	1	1
Cultivated rye	38	42	0	1	0	0	0	0	1	1
Birch mix	38	43	0	0	0	0	0	0	NS	NS
Hazelnut pollen	38	42	0	1	0	0	0	0	1	1
Common mugwort	38	43	0	0	0	0	0	0	NS	NS
English plantain	37	43	1	0	0	0	0	0	0.47	0.47
<i>D. farinae</i>	33	37	5	4	0	1	0	4	1	0.56
<i>D. pteron.</i>	33	40	5	2	0	1	0	0	0.46	0.27
<i>Aspergillus fum.</i>	37	43	0	0	1	0	0	0	0.47	0.47
<i>Alternaria ten.</i>	38	43	0	0	0	0	0	0	NS	NS
Cat epithelia	38	43	0	0	0	0	0	0	NS	NS
Nettle	37	42	1	1	0	0	0	0	1	1
Ragweed	38	43	0	0	0	0	0	0	NS	NS
Black ant	31	28	6	11	1	4	0	0	0.13	0.21
Mosquito	36	38	2	5	0	0	0	0	0.44	0.44
Horsefly	35	29	3	14	0	0	0	0	0.007*	0.007*
Deerfly	35	32	3	9	0	2	0	0	0.04*	0.09
Moth	31	39	0	0	1	2	0	0	0.33	0.23
Housefly	34	41	0	0	1	0	0	0	0.41	0.46
Cockroach	33	38	0	0	1	0	0	0	1	0.56
Blackfly	29	32	0	0	0	1	0	0	1	0.64
Total ³	38	43	21	28	6	11	1	1		

¹Level of significance for negative (<2+) and positive (≥ 2+) reactions; ²Level of significance for negative (<2+) and (2+), (3+) and (4+) positive reactions; ³Total based on respondents; NS = No statistical analysis as allergen is constant; *Significant data.

to the end of October, which is paralleled with worsening of signs. During the colder winter season the skin lesions generally disappear, although severely affected horses can remain symptomatic. Affected horses develop skin lesions almost exclusively on both the dorsum, including face, mane, withers and tail, and the ventrum, including intermandibular space, chest and ventral midline. The intense pruritus and skin pathology markedly ameliorates whenever horses are covered with specific blankets and sprayed with high-cis permethrin. Many affected horses on pasture even return spontaneously to the stable when the activity of the midge swarms is extremely high. The only effective medication for these signs is corticosteroids.

Horses

A total of 81 Icelandic horses, age 4–25 years (mean 11 years), were included in the study. All participating horses were vaccinated, treated regularly with ivermectin¹ and were not treated with any anti-inflammatory drugs for at least 12 weeks prior to the study. No history or signs of small airway inflammatory disease, such as tachypnoea, cough, nasal discharge, dyspnoea or abnormal lung sounds, were evident in any horse. Forty-three of these horses were affected by recurrent seasonal dermatitis. Pruritic dermatitis due to parasites, bacteria and dermatophytes were ruled out by means of fungal culture, skin scraping and biopsy.

Skin test

Skin testing was performed on unsedated horses using the lateral

aspect of the neck above the jugular furrow. The site for intradermal testing was closely clipped using a No. 40 clipper blade. A permanent marker was used to indicate the individual injection sites, which were placed at least 2.5 cm apart. Histamine and buffered saline solution served as positive and negative controls. Per site, 0.05 ml of each allergenic extract was injected intradermally using disposable fine-dosage syringes with integrated 29 gauge needles². The procedure resulted in a visible and palpable intradermal bleb. The challenged sites were evaluated 30 min and 4 h postinjection. Results were obtained by palpation of injection sites in consideration of weal diameter and weal turgour. The strongest skin reaction evoked by histamine was designated (4+) positive, whereas the site challenged with buffered saline solution was termed negative. Skin reactions provoked by intradermal injection of allergenic extracts were evaluated in comparison to controls. A (1+) or (2+) reaction was one-quarter or one-half the size of the sum of the negative and positive control. A (3+) reaction was half the sum of a (2+) reaction and the positive control. Reactions equal to or greater than (2+) were designated positive. Time of study was in autumn of 1998.

Allergens

In summary, 22 significant allergens present within the Austrian region, including pollens, moulds and insects, were tested at the dilution proposed by the manufacturer³. Standard concentration of most allergens was 1,000 PNU/ml (protein nitrogen units/ml): mixed grasses, cultivated rye, birch mix, hazelnut pollen, common mugwort, English plantain, *Aspergillus fumigatus*, *Alternaria tenuis*, cat epithelia, nettle, mixed ragweed, black ant,

TABLE 2: Number of control (n = 38) and affected (n = 43) respondents after 4 h

	Skin reaction								Sign 2 x 2 ¹	Sign 2 x 4 ²
	Negative		++		+++		++++			
	Control	Affected	Control	Affected	Control	Affected	Control	Affected		
<i>Culicoides</i> 1:10	37	40	1	2	0	1	0	0	0.62	0.56
<i>Culicoides</i> 1:5	37	40	1	2	0	1	0	0	0.62	0.56
<i>Culicoides</i> 1:2	14	30	15	8	5	4	4	1	0.004*	0.022*
Mixed grasses	37	42	1	1	0	0	0	0	1	1
Cultivated rye	38	42	0	1	0	0	0	0	1	1
Birch mix	38	43	0	0	0	0	0	0	NS	NS
Hazelnut pollen	38	42	0	1	0	0	0	0	1	1
Common mugwort	33	38	5	5	0	0	0	0	1	1
English plantain	37	41	1	1	0	1	0	0	1	0.64
<i>D. farinae</i>	28	32	9	5	1	2	0	4	1	0.14
<i>D. pteron.</i>	30	31	5	7	3	5	0	0	0.61	0.76
<i>Aspergillus fum.</i>	35	43	2	0	1	0	0	0	0.1	0.17
<i>Alternaria ten.</i>	38	43	0	0	0	0	0	0	NS	NS
Cat epithelia	37	42	1	1	0	0	0	0	1	1
Nettle	36	38	2	5	0	0	0	0	0.31	0.31
Ragweed	37	43	1	0	0	0	0	0	0.28	0.28
Black ant	25	25	9	10	3	4	1	4	0.5	0.64
Mosquito	30	31	8	10	0	2	0	0	0.6	0.38
Horsefly	27	23	11	18	0	2	0	0	0.12	0.16
Deerfly	29	24	9	14	0	3	0	2	0.064	0.1
Moth	23	22	12	13	2	6	1	2	0.5	0.55
Housefly	23	20	12	19	2	3	1	1	0.27	0.64
Cockroach	25	21	12	19	1	2	0	1	0.18	0.4
Blackfly	22	24	14	18	1	1	1	0	1	0.73
Total ³	38	43	28	36	13	19	6	11		

¹Level of significance for negative (<2+) and positive (≥ 2+) reactions; ²Level of significance for negative (<2+) and (2+), (3+) and (4+) positive reactions; ³Total based on respondents; NS = No statistical analysis as allergen is constant; *Significant data.

mosquito, horsefly, deerfly, moth, housefly, cockroach and blackfly. The house dust mites, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, were tested at dilutions of 1:5,000 w/v (weight to volume) and histamine phosphate at 1:100,000 w/v. The *Culicoides variipennis* extract was used in dilutions of 1:50,000 (1:10), 1:25,000 (1:5) and 1: 10,000 (1:2) w/v; the dilutions of 1:50,000 and 1:25,000 w/v were recommended by the manufacturer.

Prior to the study, 2 healthy Warmblood horses were tested intradermally with the above-mentioned extract concentrations. Neither of them exhibited any skin reaction to the tested allergens.

Statistical analysis

The null hypothesis of association between intradermal skin test results and the manifestation of allergic skin disorders was evaluated at 30 min and 4 h using a Chi-squared test. If the expected frequency in any one of the 4 cells was less than 5, the Fisher's exact test was employed. The total number of positive reactions was compared using a Student's *t* test. A probability value of $P < 0.05$ was considered significant. Efficiency, specificity, sensitivity and predictive values were explored for each allergen 30 min and 4 h after allergen administration.

Results

Culicoides variipennis

Three horses with SSRD and 1 normal horse had positive skin

responses to *C. variipennis* at a dilution of 1:50,000 or 1:25,000 (Tables 1,2). Challenging with a weaker dilution 1:10,000 increased the positive skin reactions not only in horses with SSRD (13 of 43) but also in normal horses (24 of 38). The efficiency for *Culicoides* was not more than 51%, and decreased with further concentration. The percentage of affected horses with positive intradermal skin test results to *Culicoides* amounted to between 2 and 7% at the proposed dilutions of 1:50,000 and 1:25,000, and increased with the stronger concentration. The specificity for *Culicoides* was 97% for the proposed dilutions, but decreased with the weaker dilution. The probability of disease in cases of positive intradermal skin test results to *Culicoides* was calculated at 75% at the proposed dilutions, but only about 40% with the weaker dilution. The predictive values for a negative test result in the case of *Culicoides* was less than 50% for the stronger dilutions, and concentrating further decreased the value at 4 h.

Other allergens

No significant differences in skin reactions to other allergens, except deerfly and horsefly, were detected between control horses and affected horses at 30 min or at 4 h (Tables 1,2). However, the significance regarding reactivity on deerfly and horsefly antigens was not associated with a high sensitivity (26% and 33%, respectively). At 4 h, 28 of 38 normal horses and 38 of 43 horses with SSRD reacted to insects, and 16 of 38 normal horses and 27 of 43 horses with SSRD had cutaneous responses to pollens in addition to insects. Only one normal horse and 2 horses with SSRD reacted exclusively to the house dust mite.

Complete negative test results were visible in 10 of 38 normal horses and 5 of 43 horses with SSRD. Efficiency did not exceed 60% for any allergen. The sensitivity varied between 0 and 54%. The percentage of nonaffected horses with negative intradermal skin test results for the other allergens was between 58 and 100%.

The predictive values for a positive test result regarding the other allergens showed a broad variation between 0% and 100%. The probability of not being affected in the case of negative results to any other allergen was about 50%.

Nevertheless, significant differences in the mean number of total positive cutaneous responses were observed between the 2 groups at 30 min and 4 h. Additionally, the mean number of (2+), (3+) and (4+) positive reactions was significantly different between control and affected horses at 4 h. At 30 min, approximately 40% of the overall reaction at 4 h was evident in normal as well as in affected horses.

Discussion

In the present study, there is a striking number of positive skin reactions to many types of allergens apart from *Culicoides* in SSRD-affected horses as well as in normal horses. Indeed, several responses to diverse allergens in addition to *Culicoides* were obvious in SSRD horses in a study performed by Fadok and Greiner (1990). In that study, titration series showed that the allergen extracts at a dilution of 1,000 PNU/ml were acceptable in horses, concerning mould, grass and most insect extracts. Only yellowjacket and blackfly elicited positive reactions in 9 of 21 normal horses at 1000 PNU/ml and in 7 normal horses at 500 PNU/ml, indicating that the concentration of the remaining allergens were found suitable for intradermal skin testing in horses. In the present study, according to previous studies and to manufacturer's instructions, the allergen extracts were also used at a dilution of 1,000 PNU/ml for pollens, moulds and insects. Nevertheless, the tested concentrations might have been too strong, and titration series might perhaps have revealed less respondents.

However, a similarly high incidence of positive intradermal skin test results in normal horses has been reported by other authors, even though much stronger dilutions were tested (Evans *et al.* 1992; Rosenkrantz *et al.* 1998). In the present study, a particularly high incidence of positive reactions to insect extracts was visible, independent of whether the Icelandic horses tested had SSRD. Deerfly and horsefly were the only extracts which evoked stronger skin responses significantly more often in the SSRD-affected horses, albeit only at 30 min. Therefore, our findings may indicate that many horses which appear normal are sensitised to most of the insects present in the specific geographical area. Schütz-Kiss *et al.* (1995) reported that it is common to find positive skin reactions in skin prick tests in apparently asymptomatic human subjects. They found positive reactions in 39% of cases and elevated serum levels of total IgE or specific IgE in 60% of cases. Therefore, we believe that the studied horses could be apparently asymptomatic cases of insect bite hypersensitivity, small airway inflammatory disease or atopy. It must be taken into account that this study was undertaken in a genetically predisposed breed with an exceptionally high prevalence for allergic dermatitis of up to 26%. Further, even though no horse in this study had any clinical signs of respiratory disorders, more subtle investigations such as bronchoalveolar lavage or lung function testing could perhaps have exposed latent

small airway inflammatory disease (SAID) in horses with positive skin reactions. It has been shown that, in 50% of horses without a history of lung disease, COPD was diagnosed on clinical examination, tracheoscopy, tracheobronchial lavage cytology and arterial pO₂ levels (Bracher *et al.* 1991). Furthermore, a high incidence of positive skin reactions in COPD horses has been shown in a previous study, in which 66 of 99 horses with COPD had positive skin reactions to moulds or feedstuff, and 62 responded positively to immunotherapy without any other changes in feed or environment (Beech and Merryman 1986). Similarly good responses of horses with heaves and SAID to hyposensitisation were reported by Tallarico and Tallarico (1998). On the other hand, intradermal end-point titres of *Micropolyspora faeni*, *Thermoactinomyces* and *Aspergillus fumigatus* did not correlate with bronchoalveolar neutrophilia in COPD horses in a study undertaken by McGorum *et al.* (1993). The diversity of positive intradermal skin test results in association with the manifestation of allergic diseases on the one hand and good responses of positive horses to immunotherapy on the other, may be based on the fact that allergen-specific IgE and, perhaps, IgG can be produced without the expression of clinical signs (Schütz-Kiss *et al.* 1995). The Icelandic horses tested in this study were kept on pasture most of the time and, therefore, had a high allergen exposure, particularly regarding insect allergens. The manifestation of clinical signs requires a level of exposure to allergens that is unique to each patient. This level, referred to as the allergic threshold, may vary depending on nonallergenic factors such as psychological stress, bacterial infections or contact with irritants. Whenever the allergen load, or the accumulation of different allergens, exceeds the allergic threshold, clinical signs are present.

In contrast, we observed very few responses to the concentration recommended by the manufacturer regarding *Culicoides variipennis* extract in the Icelandic horses tested. As this concentration was not able to elicit positive skin reactions in affected horses, we tested all horses with weaker dilutions. Whereas doubling did not show any modification in the reaction pattern in either group, further concentrating up to 1:10,000 increased the positive skin reactions not only in horses affected by SSRD but also in normal horses. Whether these skin reactions were merely irritant or really positive in subclinical horses remains unclear.

In previous studies, intradermally-injected *Culicoides* extracts gave consistently positive reactions in horses affected by sweet itch and only minimal reactions in nonaffected horses in Ireland and Hong Kong (Baker and Quinn 1978; Baker and Collins 1984). These findings were corroborated by studies performed in northern Europe. In Sweden, 12 of 14 affected horses responded well to *Culicoides* antigen, whereas 8 of 9 nonaffected horses failed to respond (Halldorsdottir *et al.* 1989). Similarly, in Norway all of the 12 affected horses but only one of 33 nonaffected horses responded to *Culicoides* extract (Larsen *et al.* 1988). Furthermore, intradermal skin tests performed in Iceland yielded only a weak reaction in one horse out of 110 (Larsen *et al.* 1988). Consistent positive skin reactions to *Culicoides* antigen in horses affected by seasonal allergic dermatitis was further demonstrated by Braverman *et al.* (1983), although no control horses were included in the study to produce evidence of failing reactions in nonaffected animals. All of the 7 affected horses and also 3 of the 6 control horses showed strong reactions to *Culicoides* extracts in a study undertaken by Quinn *et al.* (1983). In two studies, *Stomoxys calcitrans* in addition

to *Culicoides* produced strong reactions in some affected horses (Baker and Quinn 1978; Braverman *et al.* 1983), even though the response to *Stomoxys* at the sites of passive sensitisation was consistently weaker than to *Culicoides* (Quinn *et al.* 1983). An intradermal test study using a wide range of various allergens including *Culicoides variipennis* was conducted in seasonally pruritic horses in Florida by Fadok and Greiner (1990). Based on the test results, they concluded that most of the reactions in affected horses were to *Culicoides* solely or in addition to other insects.

A possible cause for the few responses in the present study might be the lacking of cross-reactivity, as *Culicoides variipennis* is a species not prevailing in Austria. The allergens of *Culicoides* species have not yet been characterised well. Skin tests using fractionated *Culicoides* extract, with *C. pulicaris* as the predominant species, indicated that the allergic activity was associated with allergens showing considerable variation in molecular weight and isoelectric point (Morrow *et al.* 1986; Ungar-Waron *et al.* 1990). One of the salivary proteins present in *C. variipennis* was shown to have apyrase activity and this apyrase may act as antigen in the development of SSRD (Perez de Leon and Tabachnik 1996). Furthermore, a salivary vasodilator in *C. variipennis* has been identified as having a relative molecular weight of 22.45 kD (Perez de Leon *et al.* 1997).

Nevertheless, in previous studies, SSRD-affected horses also responded consistently to nonindigenous *Culicoides* species upon intradermal skin testing. These observations have been made by using extracts of Irish and Israeli *Culicoides* species in horses in Hong Kong and Ireland, respectively (Braverman *et al.* 1983; Baker and Collins 1984). The findings were further confirmed by Anderson *et al.* (1993), who evaluated the skin reactions to different exotic extracts in affected horses in British Columbia. However, although marked positive responses in SSRD-affected horses were evident at 20 min and 4 h, divergences in onset of the greatest reactions were seen among several *Culicoides* species. Similarly, there was a certain variability in the reaction pattern to various midge species in an Irish horse affected by SSRD (Braverman *et al.* 1983). The maximal reaction to Irish *Culicoides* spp. and *C. circumscriptus* from Israel, which is actually a bird feeder, was at 24 h, in contrast to Israeli *C. puncticollis* which was at 1 h (Braverman *et al.* 1983). Furthermore, the maximal reaction to *C. puncticollis* was only half the size of the response to the Irish *Culicoides* spp. Similarly, the Israeli bird feeder *C. distinctipennis* evoked a much weaker skin reaction compared to that seen with the Israeli *C. imicola* in a horse affected by SSRD in Israel. Likewise, Halldorsdottir *et al.* (1989) reported that 10 of 14 SSRD-affected Icelandic horses in Norway showed cutaneous responses to at least 3 of 4 species using *C. pulicaris*, *C. chiopterus*, *C. obsoletus* and *C. impunctatus*, whereas 2 horses did not exhibit any reaction. Thereby, various reaction patterns to the several species were observed in the individual horses.

However, the relevance of *Culicoides* species for the allergic dermatitis seen in Icelandic horses in Austria cannot be determined until *Culicoides* species prevailing in this geographical area have been tested intradermally in these horses. Nevertheless, in consideration of the literature, we suppose that *Culicoides* species play a role in the pathogenesis of the seasonal recurrent dermatitis affecting Icelandic horses in Austria. This would be in concordance with the good response to insect prophylaxis of the Icelandic horses studied, as well as with the observation that increasing activity of *Culicoides* is paralleled by worsening of symptoms in autumn. Additionally, allergens other than *Culicoides* did not produce

significantly different skin reactions in horses affected by SSRD at 4 h either; the relevance of the allergens tested in this case within the Austrian region has been demonstrated in other studies (D'Amato *et al.* 1998; Schütz-Kiss *et al.* 1995).

In conclusion, we observed a high incidence of positive skin reactions, particularly to insect allergens, in clinically normal Icelandic horses in Austria. Apart from the skin responses to horsefly and deerfly at 30 min, there were no significant differences in the response pattern to any of the tested allergens, including *C. variipennis*, various other insects and environmental allergens, between affected and nonaffected Icelandic horses in the present study.

Manufacturers' addresses

¹Merck Sharp & Dohme, Haarlem, The Netherlands.

²Braun Medical AG, Sempach, Switzerland.

³Greer Laboratories, Inc, Lenoir, North Carolina, USA.

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