

Intradermal skin tests in equine dermatology: a study of 83 horses

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Summary

Allergic diseases are often diagnosed clinically in the horse without performing diagnostic tests. The purpose of this work was to contribute to the validation of intradermal skin tests in the horse. Eighty-three horses, 14 showing skin or respiratory signs of supposed allergic origin, were subjected to an intradermal skin test using 6 different allergens, positive and negative controls. The tests were read for all animals after 20 min, and for 29 horses after 1 and 4 h. Additionally, 19 horses were tested a few months apart. The comparison after 20 min of the cutaneous reactions to allergens and to positive and negative controls allowed us to propose a threshold of positivity; skin reactions with a diameter above or equal to 15 mm, or with at least a 13 mm diameter and a skin thickness similar to the positive control. There was a marked difference between the healthy group and the allergic group for *Culex pipiens* and *Dermatophagoides farinae*, although positive reactions were not rare in the healthy group. *Tabanus* sp. gave positive reactions in both healthy and allergic animals. There was no significant variation in the reactions observed after 20 min and after 60 min. After 4 h, the progression of the reactions was highly variable and negative controls showed a certain number of positive reactions, which negated their reliability at the point in time, and made it difficult to interpret the other allergens. Finally, the repeatability of intradermal skin tests reactions after 20 min was poor, and probably related to the influence of seasonality for some of the allergens. Further studies are required, notably with others allergens, to interpret intradermal skin tests' responses in clinical practice.

Introduction

Allergic diseases are often diagnosed clinically in the horse without performing diagnostic tests. Intradermal skin tests in man and dog identify allergens responsible for clinical signs and enable hyposensitisation of the patient. In the horse, intradermal skin tests are not standardised. The purpose of this work was to contribute to the validation of intradermal skin tests in the horse by the following steps 1) collect results obtained from a random population of horses; 2) search for variations in selected allergens; 3) to provide a comparative study of results of intradermal skin tests according to

age, breed, colour, sex, health status, husbandry method and period during which animals were tested; 4) to search for immediate and late phase reactions and 5) to investigate the possible repeatability of the tests over time and to evaluate the reaction reading standards.

Materials and methods

The study was performed among horses visited by a veterinary practice located in Southern-Finistère, France. The horses belonged to private owners and to riding schools dealing with both showjumping and trail riding.

Inclusion and exclusion criterias

The owners of horses allowed us to test their horses regardless of factors such as breed, sex or age. We excluded animals which displayed severe signs of disease (excepted allergic disease) at the time of the test, those receiving medical treatment or those treated with medication within the previous 6 months.

Intradermal skin tests

Choice of allergens took into account their commercial availability and the necessity to limit the number of allergens in order to test more horses with each allergen. Priority was given to environmental and storage mites, which represent the highest percentage of sensitisation in man and dog and seem to be involved in hypersensitisation in horses (Evans *et al.* 1992; Petrikowski and Bourdeau 1995; Fadok 1996; Lekeux *et al.* 1996; Littlewood *et al.* 1996; Rosenkrantz *et al.* 1996). We also wanted to include allergens of stinging insects, which seem to be part of the aetiology of allergic dermatitis (Stannard 1994; Fadok 1995; Greiner 1995; Perris 1995; Petrikowski and Bourdeau 1995; van der Hagen *et al.* 2000). One fungus was also chosen to complete the allergic test. The allergens¹ used were the following: *Dermatophagoides farinae* 1000 AU/ml and *Dermatophagoides pteronyssinus* 1000 AU/ml (standardised extracts in biological unities for human allergology), *Tyrophagus putrescentiae* 0.1%, *Alternaria tenuis* 1%, *Tabanus* sp. 0.1%, *Culex pipiens* 0.1%.

Allergens were used as they were supplied, without additional dilutions. The positive control was a 1/10,000 dilution of histamine (Larsen *et al.* 1988; Halldordottir and Larsen 1989; Petrikowski and Bourdeau 1995). The negative control was an isotonic phosphate-buffered solution similar to the one used as

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TABLE 1: Characteristics of tested population (age 1–22 years; mean 8.16 years)

Characteristic	n	%	Characteristic	n	%
Breed			Sex		
<i>Riding horses</i>	46	55	Males	52	63
French-bred	13		Stallions	8	
French Trotters	10		Geldings	44	
Anglo-Arabs	6		Females	31	37
Thoroughbred	5		Colour		
Barbe-Arabs	4		Bay	35	43
Quarter Horses	2		Chestnut	26	31
Standardbred	6		Grey	10	12
<i>Heavybred horses</i>	8	10	Black	6	7
Bretons	4		Others	6	7
Frisons	4		Husbandry		
<i>Ponies</i>	29	35	Turned out	78	94
Shetland ponies	14		Stabled	5	6
French ponies	4		Fed CF	71	86
Other breeds	11		Not fed CF	12	14

Turned out = horses kept in fields for at least part of the day; CF = commercial food.

carrier for the allergenic extracts.

The intradermal skin tests were done on the flat surface of the shoulder. This area offers the advantages that the skin is quite thick and the area is easy to inject, even if the horse moves. An adequately large area was shaved, disinfectant applied, injectionsites marked with a permanent pen, located 3 cm apart. A 0.05 ml volume of each suspension was then injected strictly intradermally. No sedation was necessary for any horse.

In each case, one reading was taken after 20 min. When possible, readings after 1 h and 4 h were also performed. The diameter of the skin reaction was measured (mm) with a slide ruler and, when the skin reaction had an irregular shape, a mean diameter was calculated. The thickness of the reaction site was

estimated subjectively and given the following descriptions: 0 = no palpable reaction; +/- = very flat reaction with a badly defined outline; + = reactions with just papable thickness; ++ = reactions with obvious thickness; +++ = reactions with same thickness as histamine control. Every reaction with thickness found at +/- was considered negative, because their outlines were not clear enough to give any validity to the measurement.

Chronology

The animals were assessed July 1996–August 1997. Nineteen were tested a second time after a interval of 4–5 months.

Analytical methods

For statistical analysis, the animals were assigned to group: *allergic group* (if they showed or had shown skin or respiratory signs of supposed allergic origin) or *healthy group* (if they did not have such signs). Other groups were defined by: a) period of the year during which the animals were tested (April to September, hot season/October to March, cold season); b) age (≤ 8 years, >8 years); c) breed (horses/ponies); d) sex; e) type of food received (commercial food/no commercial food) and f) colour of coat.

For each allergen, the means obtained were compared within the tested population and each group mean was compared to the opposite group, using the method of the ‘mean comparison test’ for large sample size ($n > 30$) and Student *t* test for small sample size. Only significant differences with a security coefficient ≥ 0.90 were considered significant.

Results

Population characteristics

Eighty-three animals were tested. The characteristics of this population are summarised in Table 1. In this population 62

TABLE 2: Mean and s.d. of the size (mm) for histamine, negative control, *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* for each group

Group	No.	Histamine		Negative control		<i>D. farinae</i>		<i>D. pteronyssinus</i>	
		Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Total tested population	83	18.06	2.08	4.49	5.11	7.73	5.47	7.87	5.08
‘Healthy’ horses	62	18.02	2.14	4.58	5.14	7.15	5.47	8.00	4.99
Horses with cutaneous allergy	17	18.35	1.75	3.41	4.74	8.88	5.36	6.76	5.66
Horses with respiratory allergy	7	18.29	2.25	4.43	5.12	8.29	6.34	8.43	5.83
‘Allergic’ horses	21	18.19	1.89	4.24	4.99	9.48	5.07	7.48	5.32
Horses tested from April to September	34	18.50	2.00	2.03	4.04	6.44	5.87	7.91	5.08
Horses tested from October to March	49	17.76	2.09	6.20	5.07	9.63	4.98	7.84	5.09
Age ≤ 8 years	45	17.69	2.13	3.78	4.91	6.73	5.43	6.80	5.06
Age > 8 years	38	18.50	1.94	5.34	5.20	8.92	5.27	9.13	4.82
Ponies	29	17.52	1.94	4.48	5.43	5.72	5.65	6.10	5.36
Riding horses	54	18.35	2.10	4.50	4.92	8.81	5.05	8.81	4.66
Males	52	17.81	2.17	4.21	4.99	7.81	5.46	7.58	5.16
Females	31	18.48	1.86	4.97	5.26	7.61	5.49	8.35	4.92
Fed commercial food	71	18.07	2.04	4.61	5.18	7.61	5.46	7.96	5.09
Not fed commercial food	12	18.00	2.35	3.83	4.60	8.50	5.44	7.33	5.04
Black coat	6	17.50	1.26	3.83	5.43	5.50	5.53	5.50	5.53
Grey coat	10	18.80	1.89	4.00	5.00	5.50	5.59	5.50	5.68
Bay coat	35	18.06	2.29	3.77	4.96	7.23	5.40	8.11	4.77
Chestnut coat	26	18.04	2.08	4.88	4.96	9.35	5.02	8.31	5.03

TABLE 3: Mean and s.d. of the size (mm) for *Tyrophagus* sp., *Alternaria* sp., *Tabanus* sp. and *Culex* sp. for each group

Group	No.	<i>Tyrophagus</i>		<i>Alternaria</i>		<i>Tabanus</i>		<i>Culex</i>	
		Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Total tested population	83	9.37	4.37	5.76	5.08	9.11	5.31	8.47	4.90
'Healthy' horses	62	9.21	4.57	5.58	5.19	8.74	5.27	7.83	4.99
Horses with cutaneous allergy	17	9.65	3.82	6.06	4.81	9.59	5.61	10.4	4.32
Horses with respiratory allergy	7	9.29	4.37	5.71	4.98	9.29	6.06	9.57	5.07
'Allergic' horses	21	9.86	3.68	6.29	4.72	10.2	5.26	10.3	4.07
Horses tested from April to September	34	9.50	4.41	3.79	4.66	8.47	5.31	7.18	5.15
Horses tested from October to March	49	9.29	4.34	7.12	4.92	9.55	5.26	9.37	4.50
Age ≤8 years	45	8.91	4.70	4.91	5.00	8.33	5.64	8.38	4.72
Age >8 years	38	9.92	3.87	6.76	5.00	10.0	4.72	8.58	5.11
Ponies	29	7.28	5.03	5.86	5.46	8.97	5.43	8.34	4.59
Riding horses	54	10.5	3.48	5.70	4.87	9.19	5.24	8.54	5.06
Males	52	9.31	4.28	5.88	5.04	9.81	4.60	9.06	4.56
Females	31	9.48	4.52	5.55	5.15	7.94	6.14	7.48	5.28
Fed commercial food	71	9.49	4.40	5.49	5.24	8.69	5.38	8.24	4.99
Not fed commercial food	12	8.67	4.13	7.33	3.68	11.6	4.03	9.83	4.04
Black coat	6	9.83	4.78	2.17	4.84	9.00	6.48	8.67	3.99
Grey coat	10	9.90	3.78	6.30	5.29	9.20	4.83	8.60	4.43
Bay coat	35	8.83	4.63	5.80	4.93	8.40	5.56	7.66	5.41
Chestnut coat	26	9.23	4.33	5.62	4.88	9.62	4.64	9.38	4.50

horses (75%) had no history or sign of allergic disease; 10 horses (13%) were suspected to have an allergic skin disease (7 horses had ‘summer itch’, one showed head lymphadenopathy and recurrent bilateral conjunctivitis, 2 had tail pruritis of unknown origin, one had recurrent head and thorax pruritus); 3 horses (4%) showed respiratory disease with presumed allergic aetiology (signs of heaves, bronchitis, emphysema or rhinitis associated with conjunctivitis); one horse (1%) showed signs of both skin and respiratory diseases of presumed allergic origin (summer itching and bronchitis); 6 horses (7%) were healthy, but previously had signs of a skin and/or respiratory disease with a suspected allergic component.

Reading at 20 min

Means ± s.d. of reaction diameters for each group by control or allergens are summarised in Tables 2 and 3. Figure 1 shows the distribution of the reaction diameters according to their thickness (negative control and positive histamine controls are excluded). The ordinate scale is the number of each group. Note that reactions 10–14 mm had a very variable thickness, but generally the larger the diameter, the thicker the reaction.

Comparison of controls and allergens: Comparison between mean reactions to histamine control and other mean reactions, by calculating the interval of confidence of large cross-sections, shows that the reaction to histamine was significantly higher than the reaction in other tests (security coefficient (SC) 0.99999 for all the comparisons). Comparison between the mean reactions to negative control and other mean reactions show that the reaction to the negative control was significantly lower than other tests (SC of 0.99999 for histamine, *Tyrophagus*, *Tabanus* and *Culex*; 0.9999 for *D. pteronyssinus* and 0.9998 for *D. farinae*) except *Alternaria* (SC 0.88). The results showing significant differences between subgroups of the population are shown in Table 4.

Late-phase reactions

Following the reading after 20 min, readings after 1 h and 4 h were taken in 29 horses. This group was composed of 21 horses presumed exempt from allergic disease and 8 horses showing, or which had shown signs of, a supposed allergic disease.

Controls: In the positive histamine control group, after the first hour, the reaction size was quite stable and the thickness of skin diminished progressively. After 4 h, 71% of the reactions to histamine had resolved. Those still present had a constant diameter but their thickness was clearly less. There was no significant difference between ‘healthy’ and, allergic, horses.

For the negative control and the ‘healthy’ group, between 20 min and 1 h after injection, diameters of skin reactions were 0–13 mm, with a thickness score from 0 to ++. After 4 h, there were 2 groups; 52% had reactions with low thickness scores (0 to +/-) and nonmeasurable reaction surfaces, while 48% showed an increase of the reaction which could reach 16 mm in diameter and a score of ++ in thickness (mean 13.3 mm with thickness + to ++). There was no significant difference between ‘healthy’ and, allergic, horses.

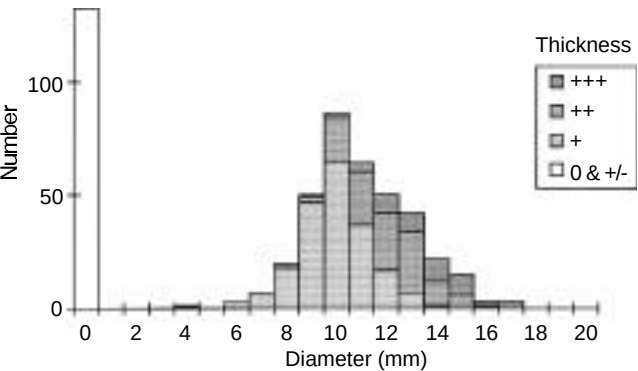


Fig 1: Diameter distribution according to reaction thickness to allergens at 20 min.

TABLE 4: Summary of reaction comparisons at 20 min

Compared populations		Calculated significant positive and negative differences with security coefficient ≥0.90						
		Negative		<i>D. farinae</i>	<i>D. pteronyssinus</i>	<i>Tyroph.</i>	<i>Alternaria</i>	<i>Tabanus</i>
		Histamine	control					
Healthy group	Tested population							
Skin allergic	Healthy group							+ 0.93
Respiratory allergic	Healthy group							
Allergic group	Healthy group			+ 0.92				+ 0.95
Tested Oct–March	Tested Apr–Sept	– 0.90	+ 0.999				+ 0.995	+ 0.95
Age >8 years	Age <9 years	+ 0.93		+ 0.93	+ 0.96		+ 0.90	
Horses	Ponies	+ 0.93		+ 0.99	+ 0.98	+ 0.998		
Males	Females							
No commercial food	Commercial food							+ 0.91
Chestnut horses	Grey horses			+ 0.92				

– = negative; + = positive.

Allergens: For all the studied allergens or the tested groups of horses, changes through time of intradermal skin tests were highly variable and unpredictable; 26% of the horses showed identical diameters at each reading of the skin reaction and a quarter of this group showed no skin reaction, and 25% showed a diameter of the skin reaction which was stable between 20 min and 1 h, after which it decreased significantly and had almost resolved after 4 h. Thirty six percent of skin reactions increased significantly until after 4 h, 10% had a decreasing diameter between 20 min and 1 h after injection, followed by an increase after 4 h, and only 3% had an increased diameter between 20 min and 1 h after injection, but the thickness never increased to +++.

Comparison test of means: This test was to compare the histamine control and negative control to allergens at the 3 reading times. Histamine gave a higher reaction after 20 min and 1 h (SC = 0.99999). There was no significant difference between histamine and the other tests after 4 h.

The negative control had a thin reaction with a significantly smaller diameter than any other test after 20 min (SC = 0.99998 for all allergens except for *Alternaria* SC = 0.97) and also after 1 h (SC = 0.95 for *Alternaria*, 0.995 for *Tyrophagus*, 0.99999 for the others) but the diameter was much less after 4 h; the diameter was significantly smaller than only *Culex* (SC = 0.98), *Tabanus* (0.96), *D. Pter* and *nyssinus* (0.94).

Repeatability of intradermal skin test

Intradermal skin tests were repeated twice, 4–5 months apart, on 19 horses of which 7 were from the ‘allergic’ group. Considering the previous results, we considered all the responses with a skin thickness below ++ to be negative.

Repeatability of the reaction to histamine and to the negative control was excellent for all animals (19/19). The results for the other tests were follows: 10/19 for *Dermatophagoides farinae*, 6/19 for *Dermatophagoides pteronyssinus*, 9/19 for *Tyrophagus*, 15/19 for *Alternaria* (negative reactions on the 2 tests), 8/19 for *Tabanus* and 6/19 for *Culex*. On the other hand, repeatability was quite good in the ‘allergic’ group (4 horses/7) and bad in the ‘healthy’ group (1 horse/12). A probability comparison test showed that repeatability of intradermal skin tests was better for ‘allergic’ horses than for ‘healthy’ horses (SC>0.95).

Discussion

Results at 20 min

Comparison of mean reactions at 20 min showed that reaction to histamine was always 14–23 mm. Scott (1988) considered that the test is interpretable only if this reaction reaches for at least a 15 mm diameter. We considered that all horses showed an interpretable test. Also, reactions to histamine differed very significantly from other reactions. Reaction to the negative control was very significantly less than the reaction to histamine and to all allergens except *Alternaria*. The difference observed with the reaction to histamine is a validation element of the test. The responses to the negative control and to *Alternaria* were similar. Several explanations can be proposed; no horses were sensitive to *Alternaria* in the tested population (in fact, a few positive reactions were observed), concentration of the used extract was inadequate to trigger an allergic reaction, or other allergens had irritant properties, at least in some horses. This last hypothesis can be supported by the fact that the reactions of these allergens were very significantly different to reactions at negative control and histamine. They then progressed (on average) to an intermediate size reaction and did not behave generally as the negative control. They resulted, much more often than the negative control, in a skin reaction of sufficient thickness, the diameter of which was measurable. Sloet Van Oldruitenborgh-Oosterban *et al.* (1996) proposed the use of these allergens at lower dilution. Lorch *et al.* (2001c) considered that allergens with a positive test result in >25% of normal horses were irritants.

Statistical differences appear between groups of ‘healthy’ and ‘allergic’ horses, similar to those reported previously (Larsen *et al.* 1988; Fadok and Greiner 1990; Evans *et al.* 1992; Petrikowski and Bourdeau 1995; Rosenkrantz *et al.* 1996; Lorch *et al.* 2001c). The allergens resulting in the most marked reactions were *Culex* and *D. farinae*. These observations are in accordance with observations by Petrikowsky and Bourdeau (1995) and Rosenkrantz *et al.* (1996). We did not show any statistical difference in the reaction to histamine between the 2 groups of horses, in accordance with Halldorsdottir and Larsen (1989), but unlike Foster *et al.* (1995).

Horses tested during the cold season had a weaker reaction to histamine, but a stronger one to the negative control, *Alternaria* and *Culex* than those tested during the hot season. *Alternaria* reactions were again similar to negative control. These results

TABLE 5: Summary of reactions comparisons at 20 min using suggested criterions of reading

		Calculated significant positive and negative differences with a security coefficient ≥0.90							
		Negative		<i>D. farinae</i>	<i>D. pteronys.</i>	<i>Tyroph.</i>	<i>Alternaria</i>	<i>Tabanus</i>	<i>Culex</i>
Compared populations		Histamine	control						
Healthy group	Tested population								
Skin allergic	Healthy group			+ 0.90					+ 0.99
Respiratory allergic	Healthy group			+ 0.999					+ 0.99
Allergic group	Healthy group			+ 0.99					+ 0.999
Tested Oct–March	Tested Apr–Sept								
> 8 years	< 9 years				+ 0.95				
Horses	Ponies						– 0.95		
Males	Females								
No commercial food	Commercial food							+ 0.95	+ 0.95
Black horses	Bay horses						+ 0.98		
Black horses	Chestnut horses						+ 0.95		

were unusual, as a stronger skin reaction to *Culex* during the hot season, when the insect is active, would be more probable than during the cold season. Very significant differences in the reaction to histamine and negative control are surprising. The majority of the horses were tested outdoors; therefore, the temperature could have had an influence on histamine reaction. Reactions to insects could be dependent on their presence in the environment and response to that antigen would fluctuate with the seasons.

Older horses reacted considerably more than younger horses to histamine, *D. farinae*, *D. pteronyssinus* and *Alternaria*. These results are identical to those of Scott (1988). The intensity of the responses increased with age.

Horses’ reactions were considerably larger in all the tests compared to ponies, except to *Alternaria* and the negative control. It is possible that pony skin is less reactive than horse skin. The difference is even clearer for the acarids’ allergens; this could have been due to a partially irritating effect of these extracts, or the fact that the ponies, being born and living outside, have less chance to become sensitised to acarids. Comparison between males (geldings and stallions) and females (pregnant mares or not) did not reveal any statistical difference.

Comparison between the horses receiving or not receiving a commercial food showed a significant difference in the reactions to *Tabanus*. The horses not receiving a commercial food were raised permanently outside while some of the others were brought in at night to receive their feed or did not go outside at all. It would be expected that those horses were more exposed to horsefly bites (the former group) would have developed a greater reaction than the horses less exposed. Finally, comparison of allergen reactions according to coat colour show that chestnut horses reacted more to *D. farinae* than grey, black or bay horses. Other studies should be undertaken to clarify this observation and to determine its importance.

Reading criteria after 20 minutes

Positive and negative controls are reliable reference marks. The difficulty is to consider as positive a reaction to an allergen. Figure 2 shows percentage distribution of diameter for each skin reaction after 20 min according to skin thickness, as well as diameters to histamine reactions and negative controls, each taken separately. The negative control gave a reaction with a diameter up to 14 mm, whereas the histamine reaction started with a diameter of 14 mm. Therefore, interpretation of intradermal reaction cannot be based upon size only. This was also the conclusion of Evans *et al.* (1992).

A reaction can be considered positive only if it is clearly different from that to the negative control. Based on our data, we hypothesise that it must be larger than 14 mm or have a thickness close to that of the histamine reaction. We then considered positive every reaction with a diameter larger than 12 mm (the 12 mm class is excluded) and a skin thickness identical to one observed with histamine. We also considered positive every reaction with a skin diameter larger than 14 mm. Horses showing allergic dermatosis and/or ‘allergic’ respiratory disease, when compared to healthy horses, showed a significantly positive difference (Table 5). They are comparable to previous results, but the security coefficient is much higher than previously. Note the significant positive difference between healthy horses and horses with allergic respiratory disease to *Culex* and *Dermatophagoides farinae*, which is different from that observed by Lorch *et al.* (2001c). A great part of the differences found previously according to age, season or breed have disappeared. These findings support the method of estimating the result according to the mean of positive reaction.

The difference between horses receiving compared to those not receiving commercial food is significant for *Culex* and *Tabanus*. It underlines the hypothesis that the second population of horses had potentially more contact with the outside than the first population. Only black ponies had positive reactions to *Alternaria* and we have no explanation for this observation.

Individual study of positive reactions: Twenty-five out of 83 horses (30%) had a positive response; among them, 10 were horses with suspected allergy (47% of the allergic group) and 15 horses (24%) belonged to the healthy group. A comparison test of the

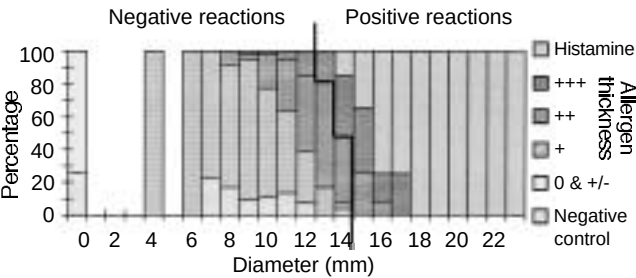


Fig 2: Reading at 20 min: thickness distribution according to size reactions for allergens compared with size of histamine and negative control reactions. The black line divides negative reactions from positive reactions with suggested criterions of reading.

percentages did not confirm that they are significantly different, all allergens taken into account. As found by authors previously, many healthy horses have positive reactions (Quinn *et al.* 1983; Fadok and Greiner 1990; Evans *et al.* 1992; Lorch *et al.* 2001a,c).

Reaction evolution over time

Histamine was the positive control up to 1 h. After that, the skin reaction diminished both in size and thickness. The negative control was also reliable after 1 h but showed a limited number of positive reactions after 4 h. Reading after 4 h appeared difficult to interpret, in contrast to findings observed by other authors (Quinn *et al.* 1983; Scott 1988; Evans *et al.* 1992; McGorum *et al.* 1993; Lorch *et al.* 2001a,c).

Only a few skin reactions reach their maximum thickness after 1 h. We believe that reading the skin reaction after 1 h is to be recommended (Quinn *et al.* 1983; Halldordottir and Larsen 1989; McGorum *et al.* 1993). No positive reaction was observed after 4 h.

Repeatability of the tests

Results repeatability tests after 20 min are disconcerting. The reactions to histamine and control were repeatable, as was the absence of response to *Alternaria*. Repeatability of the reaction to other allergens was variable and often mediocre. The seasonal factor has been mentioned previously to explain results observed with *Tabanus* and *Culex*. The partially irritant nature of acarid extracts might also have affected the results.

In conclusion, this study draws the following conclusions: a) using commercial allergens, negative and positive controls allow good reading of intradermal skin tests up to 1 h but, after that, reactions are not reliable; b) a reaction between 20 min and 1 h after injection can be considered positive if its diameter is at least 15 mm, regardless of skin thickness, or if the diameter is at least 13 mm and the skin thickness is similar to that of the positive control; c) positive reactions are frequent in healthy horses; d) *Culex pipiens* and *Dermatophagoides farinae* allergens give more noticeable reactions in animals showing cutaneous and/or respiratory allergic diseases; e) *Tabanus* allergen gives positive reactions too often to aid diagnosis. Furthermore, the reactions to this allergen vary according to seasonal factors; f) repeatability of the test towards the selected allergens, although slightly better in allergic horses, is poor and probably due to seasonal factors for some of the allergens; g) the commercial acarid extracts appear to be partially irritant and should be used at a lower dilution than in our study.

Similar experimentations with other allergens, pollens and fungi may increase our knowledge. However, it is clear that those tests will be truly significant only when they include the *Culicoides* allergen, which is highly incriminated in horse's hypersensitivity (Larsen *et al.* 1988; Scott 1988; Stannard 1994; Bourdeau and Petrikowski 1995; Marti *et al.* 1995; Perris 1995; Rosenkrantz *et al.* 1996; Foster *et al.* 1998) and not available on the market at the time of this work. Some work has been done with this allergen (Quinn *et al.* 1983; Larsen *et al.* 1988; Evans *et al.* 1992; Foster *et al.* 1998). The correct way of using intradermal skin tests for diagnostic purposes in horses needs to be known, because there is no other test available, as serum IgE quantification tests seems to be less reliable (Lorch *et al.* 2001b).

Manufacturer's address

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