

Studies on Micropolyspora faeni and Chronic Obstructive Pulmonary Disease (COPD)

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Identification of horses with respiratory allergy was attempted by intradermal skin testing, Oucherlony's double gel diffusion test and counterelectrophoresis.

Materials, Methods and Results

Skin testing was performed as an intracutaneous skin test. In order to identify the sites of injection the hair was removed by shaving 2 × 2 cm areas on the neck. With a 25-gauge needle, 0.1 ml allergen was injected. Negative and positive controls were isotonic NaCl and histamine (0.1 ml), respectively. Reading was done after 15, and 30 minutes for type I allergy and after 5–6 hours for type III allergy. The skin reaction was evaluated as 0, +, ++ or +++ by comparison with the positive (+++) and negative (0)

control. Two series of experiments were performed with different allergen concentrations (Tables 1 and 2). The allergens were obtained from ALK (Allergologisk Laboratorium). Pollen allergens were standardized in SQ-units whereas mold, bacterial and mite allergens were crude extracts.

Skin tests with strong allergens (Table 1) in 50 horses with anamnesis of obstructive respiratory disease gave very few +++ immediate reactions. Considerably more +++ reactions were seen after 6 hours and therefore it was concluded that the allergen concentration had been too high. The results with *Micropolyspora faeni* differed from those of the other allergens in this series as 64 per cent of the horses showed a 6-hour reaction which may indicate a type III allergy to that bacteria.

In the next series 29 horses were skin-tested with weak allergens (Table 2). This reduced the number of +++ reactions both after 30 minutes and 6 hours, again with the exception of *Micropolyspora faeni*. The forage mite allergens gave 53 per cent +++ reactions at 6 hours which may be explained as a non-specific reaction, as mite allergens are known to give immediate reactions. Sera from 74 horses admitted to the clinic with various kinds of medical diseases were examined using Oucherlony's gel diffusion test against *Micropolyspora faeni* (Table 3), *Thermoactinomyces vulgaris* and *Aspergillus fumigatus*. Precipitating antibodies against *Micropolyspora faeni* could be demonstrated in the serum of only one Icelandic horse with a chronic gastrointestinal disorder.

In order to get better sensitivity, 119 sera were examined by counterelectrophoresis. The results are shown in Table

Table 1: Skin tests with strong allergens (50 horses)

Allergen	Per cent reactions after 30 min				Per cent reactions after 6 hours				
	0	+	++	+++	0	+	++	+++	
Pollen*	Fescue grass	44	50	6		38	32	18	12
	Timothy grass	47	47	6		53	37	4	6
	Rye grass	36	46	18		24	36	26	14
	Cock's foot grass	56	34	10		54	30	12	4
	Onion couch	55	25	18	2	59	23	12	6
	Bird grass	51	39	10		43	41	8	8
	Mugwort	37	47	14	2	33	31	18	18
	Fungi**	<i>Alternaria iridis</i>	48	38	12	2	30	42	20
<i>Cladosporidium herbarum</i>		52	40	8		52	30	14	4
<i>Penicillium expansum</i>		51	37	12		35	33	24	8
<i>Aspergillus fumigatus</i>		50	33	17		40	38	20	2
Bacteria**	<i>Micropolyspora faeni</i>	28	39	33		8	5	23	64
	Negative control (NaCl)	98	2			94	6		
	Positive control (histamine)			2	98	22	36	8	34

*) 10⁶ SQ-units/ml ALK (Allergologisk Laboratorium)

***) 1:10 extracts ALK (Allergologisk Laboratorium)

Table 2: Skin tests with weak allergens (29 horses)

Allergen	Per cent reactions after 30 min				Per cent reactions after 6 hours				
	o	+	++	+++	o	+	++	+++	
Pollen*	Fescue grass	29	50	21	54	25	17	4	
	Timothy grass	36	54	10	74	22	4		
	Rye grass	38	55	7	57	32	7	4	
	Cock's foot grass	31	62	7	61	32	7		
Fungi**	Mucor pusillus	48	45	7	82	18			
	Penicillium expansum	55	41	4	82	14	4		
	Scopulariopsis brevicaulis	62	28	10	75	21	4		
	Aspergillus fumigatus	41	45	14	68	21	7	4	
Bacteria**	Micropolyspora faeni	31	41	17	11	18	11	39	32
	Thermoactinomyces vulgaris	55	35	10	71	25	4		
Mites***	Forage mite	36	39	18	7	0	26	11	53
	Hay mite	21	43	29	7	22	30	30	18
Negative control (NaCl)		93	7			96	4		
	Positive control (histamine)				100	71	29		

*) 10⁵ SQ-units/ml ALK (Allergologisk Laboratorium)

**) 1:10 extract ALK (Allergologisk Laboratorium)

***) 1:1000 extract ALK (Allergologisk Laboratorium)

3. Antibodies against *Micropolyspora faeni* were demonstrated in 11 sera and among those, COPD was confirmed in 4 horses of which none had a +++ 6-hour reaction in the skin test against *Micropolyspora faeni*. Despite this low frequency of antibody detection, type III reactions against *Micropolyspora faeni* using the skin test were more frequent in horses with COPD than in a group of horses where COPD could not be verified by clinical examination or with a work-performance test.

Discussion

Presently, the use of an allergic skin test as a supplementary diagnostic test in COPD horses is at a preliminary stage. Our investigation shows that a great proportion of horses reacting to the skin test are diagnosed as having COPD. Also *Halliwell et al.* (1979) demonstrated significantly more positive reactions in skin tests in horses with diseases in the lower respiratory tract than in normal horses. *Asmundsson et al.* (1983) frequently demonstrated antibodies against *Micropolyspora faeni* in Icelandic horses, and they related the finding to the poor quality of hay. *Lawson et al.* (1979) increased the sensitivity of the gel diffusion test by concentrating the horse sera 5 times. In our investigation, counter-electrophoresis increased the sensitivity which is in accordance with *Møller et al.* (1976).

In conclusion, allergens in hay and straw are abundant in the environment of horses during the stabling period, and a high concentration of allergens is inhaled into their airways. COPD is a slowly developing syndrome in which al-

Number of horse sera examined in gel diffusion test	74
Number of positive reactions	1
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Number of horse sera examined in counter-electrophoresis	119
Number of horse sera with questionable reaction	29
Number of horses with COPD diagnosed	6
Number of horses with other diagnoses	23
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Number of horses with positive reaction	11
Number of horses with COPD diagnosed	4
Number of horses with other diagnoses	7

Table 3: Examination for antibodies against *Micropolyspora faeni* in horse sera

lergy may play a role in the pathogenesis. A positive skin test indicating a type I allergy should always be confirmed by a positive provocation test or response to treatment with bronchodilating drugs. A positive skin test indicating type III allergy should be followed by an attempt to demonstrate circulating antibodies.

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Group	Number of horses	Number of horses with COPD	Number of horses with other diagnoses	Number of horses with positive reactions	Number of horses with negative reactions
Group 1	12	11	1	11	1
Group 2	12	11	1	11	1
Group 3	12	11	1	11	1
Group 4	12	11	1	11	1
Group 5	12	11	1	11	1
Group 6	12	11	1	11	1
Group 7	12	11	1	11	1
Group 8	12	11	1	11	1
Group 9	12	11	1	11	1
Group 10	12	11	1	11	1
Group 11	12	11	1	11	1
Group 12	12	11	1	11	1

In our study, the use of the skin test as a diagnostic tool for the identification of horses with COPD is supported by the high percentage of positive reactions in horses with COPD and the low percentage of positive reactions in horses with other diagnoses.

Group	Number of horses	Number of horses with COPD	Number of horses with other diagnoses	Number of horses with positive reactions	Number of horses with negative reactions
Group 1	12	11	1	11	1
Group 2	12	11	1	11	1
Group 3	12	11	1	11	1
Group 4	12	11	1	11	1
Group 5	12	11	1	11	1
Group 6	12	11	1	11	1
Group 7	12	11	1	11	1
Group 8	12	11	1	11	1
Group 9	12	11	1	11	1
Group 10	12	11	1	11	1
Group 11	12	11	1	11	1
Group 12	12	11	1	11	1

Table 2. Comparison of antibodies against *Micropolyspora phaeni* in horses.

type III allergy should be followed by an attempt to de-monstrate circulating antibodies. A positive skin test indicating a type I allergy should still be confirmed by a positive provocation test or response to treatment with bronchodilating drugs. A positive skin test indicating type III allergy should be followed by an attempt to de-

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Discussion

Presently, the use of an allergic skin test as a supplementary diagnostic tool in COPD horses is in a preliminary stage. Our investigation shows that a great proportion of horses reacting to the skin test are diagnosed as having COPD. As to Wallstedt et al. (1979) demonstrated significantly more positive reactions in skin tests in horses with disease in the lower respiratory tract than in normal horses. Subsequently, (1983) tentatively demonstrated antibodies against *Micropolyspora phaeni* in Icelandic horses, and their related the finding to the poor quality of hay (Lawson et al. 1979). Increased the sensitivity of the gel-diffusion test by comparing the horse sera 2 times. In our investigation, however, elutriophoresis increased the sensitivity which is in accordance with Møller et al. (1976).