Intradermal testing in healthy horses and horses with chronic obstructive pulmonary disease, recurrent urticaria, or allergic dermatitis

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Objective—To compare responses to a variety of intradermally injected allergens among healthy horses and horses with chronic obstructive pulmonary disease (COPD), recurrent urticaria (RU), and atopic dermatitis-insect hypersensitivity (allergic dermatitis [AD]).

Design—Case-control study.

Animals—86 horses.

Procedure—Results of intradermal testing for horses with COPD, RU, or AD were compared with results for healthy horses.

Results—Compared with healthy horses, horses with COPD, RU, and AD were significantly more likely to have positive (≥ 3+) reactions to intradermal allergens (molds, weeds, trees, grasses-crops, and insects) 30 minutes (immediate reaction), 4 hours (late-phase reactions), and 24 hours (delayed-phase reactions) after exposure. In addition, diseased horses reacted to a significantly higher number of allergens in each allergen group than did healthy horses.

Conclusions and Clinical Relevance—Reactions to individual allergens should not be used to determine that horses have hypersensitivity. Overall patterns of reactivity to intradermal allergens may be helpful in management when used in conjunction with a compatible history and evidence of potential exposure to allergens in horses with conditions associated with hypersensitivity to environmental allergens. (*J Am Vet Med Assoc* 2001;62:1115–1121)

Chronic obstructive pulmonary disease (COPD), **recurrent urticaria (RU)**, and atopic dermatitis-insect hypersensitivity (**allergic dermatitis [AD]**) are disorders in which hypersensitivity reactions to environmental allergens may play a role.[1-9,a] Response to **intradermal testing (IDT)** in horses is often used to verify hypersensitivity reactions; however, results may be difficult to interpret, because healthy horses may have reactions to some allergens.[8,10,11] The purpose of the study presented here was to compare patterns of responses to specific allergens and groups of allergens at various evaluation times among healthy and diseased horses. The aim was to establish guidelines to aid in the interpretation of the IDT in horses with diseases associated with hypersensitivity to environmental allergens.

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Supported by la Caixa Foundation fellowships, Barcelona, Spain.

Presented in a poster format at the 18th Annual Veterinary Medical Forum, American College of Veterinary Internal Medicine, Seattle, Wash, 2000.

Materials and Methods

Medical records of all horses with the diagnosis of COPD, RU, or AD for which an IDT was performed between Jan 1, 1989 and Feb 28, 1999 at The Ohio State University Veterinary Teaching Hospital were reviewed. Inclusion criteria for horses with COPD were: history of exercise intolerance, dyspnea, or cough; respiratory rate > 30 breaths/min; abnormal results of thoracic auscultation (presence of expiratory crackles or wheezes) during rebreathing examination; normal rectal temperature (37 to 38 C [98. to 100.5 F]); and bronchoalveolar lavage or transtracheal wash cytologic findings characterized as suppurative nonseptic inflammation. Inclusion criterion for horses with RU was a history of urticaria for at least 2 months. Inclusion criteria for horses with AD were pruritus, scaling, and crusting over the face, mane, neck, thorax, abdomen, or tail; histopathologic findings characterized by eosinophilic perivascular dermatitis; and no improvement after treatment with ivermectin. Healthy horses were selected on the basis of absence of a history of respiratory tract or dermatologic disease, normal results of physical examination, and normal cytologic findings in bronchoalveolar lavage fluid. Ten of the healthy horses were donated to The Ohio State University Veterinary Teaching Hospital for musculoskeletal problems, and 6 healthy horses were selected from the Ohio State University teaching herd.

If corticosteroids had been used prior to allergy testing, drugs were withdrawn for a minimum of 3 weeks. Similarly, antihistamines were withdrawn for a minimum of 7 days. Healthy horses had not received any medications in the month previous to evaluation. None of the horses used in this study had ever received allergen immunotherapy. Data collected from each medical record included age, breed, sex, diagnosis, and response to intradermal injection of a variety of allergens grouped as molds, weeds, trees, grasses-crops, or insects.

Intradermal test—Fifty-eight allergens consisting of individual allergens and allergen-related mixes were used and included 23 molds, 8 weeds, 10 trees, 12 grasses-crops, and 5 insects.[a] Mold allergens included *Alternaria tenuis*, mixed *Aspergillus* spp (*A flavus*, *A fumigatus*, *A glaucus*, *A nidulans*, *A niger*), *Botrytis cinerea*, *Candida albicans*, *Cladosporium herbarum*, *Curvularia spicifera*, *Cephalosporium acremonium*, *Epicoccum purpurascens*, mixed *Fusarium* spp (*F moniliforme*, *F solani*), *Geotrichum candidum*, *Helminthosporium sativum*, *Hormodendrum hordei*, *Monilia sitophila*, mixed *Mucor* spp (*M plumbeus*, *M racemosus*), mixed *Penicillium* spp (*P camemberti*, *P chrysogenum*, *P digitatum*, *P nonatum*, *P roqueforti*), *Phoma betae*, *Pullularia pullulans*, mixed *Rhizopus* spp (*R arrhizus, R nigricans*), *Rhodotorula rubra*, *Saccharomyces cerevisiae*, *Stemphylium solani*, *Trichoderma viride,* and grain smut mix (barley, corn, oat, wheat). Grain

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smut mix was classified as a mold, because airborne spores and other fungal particles occur universally over landscapes, especially field crops, and often form the bulk of suspended biogenic debris typical of a dust.[12] Weed allergens included cocklebur, dock-sorrel, goldenrod, lamb's quarter, marsh elder, pigweed, plantain, and mixed ragweeds (giant ragweed, short ragweed). Tree allergens included mixed ashes (white ash, green ash), beech, mixed birches (black birch, red birch, white birch), black willow, cottonwood, mixed eastern oaks (red oak, black oak, white oak), mixed elms (American elm, Chinese elm), mixed hickories (pignut hickory, shagbark hickory, shellbark hickory, white hickory), mixed maples (red maple, sugar maple, silver maple), and sycamore east. Grass and crop allergens included alfalfa, Bermuda grass, mixed grain mill dusts (barley, corn, oat, wheat), mixed grasses (red top, meadow fescue, sweet vernal, Kentucky blue, orchard, perennial rye, timothy), Kentucky blue, meadow fescue, cultivated oat, red top, cultivated rye, perennial rye, timothy, and cultivated wheat. Insect allergens included black fly, deer fly, flea, horse fly, and mosquito. All allergens were tested at 1,000 **protein nitrogen units (pnu)**/ml concentration, except for grain smut mix, mixed grain mill dusts, mosquito, deer fly, and horse fly that were tested at 500 pnu/ml, 250 pnu/ml, and 125 pnu/ml and black fly that was tested at 500 pnu/ml, 250 pnu/ml, 125 pnu/ml, and 62.5 pnu/ml. For those allergens tested at multiple dilutions, only the response to the 500-pnu/ml dilution was used for analysis of the data.

Horses were sedated with xylazine hydrochloride (0.25 to 0.5 mg/kg [0.1 to 0.25 mg/lb] of body weight, IV) as necessary to ensure compliance during the IDT. The IDT was performed on the lateral aspect of the neck. An area that measured 20 × 25 cm was clipped, wiped with alcohol, and allowed to air dry. A permanent black ink marker was used to indicate the location of allergen injection. The allergens were injected intradermally (0.05 ml) by use of a 26-gauge needle. Phosphate-buffered allergen diluent[b] was injected as a negative control, and histamine[c] (dilution, 1:100,000 [wt/vol]) was injected as a positive control. Thirty minutes (immediate), 4 hours (late phase), and 24 hours (delayed phase)[13,14] after injections, reactions were evaluated subjectively by use of digital palpation and visual inspection and were graded from 0 (no reaction) to 5+ (maximum reaction) according to wheal diameter, depth, and turgor. A positive reaction was defined as a score ≥ 3+. The IDT reactivity was scored by 1 of 2 trained technicians of the Dermatology Service (41/86 and 39/86 horses, respectively) or by 1 resident dermatology clinician (6/86 horses).

To facilitate interpretation of results of IDT, we established cut-off values for the number of allergens within an allergen group to which each horse group responded. Horses that responded to numbers of allergens equal to or greater than the cut-off value were considered to have clinically important hypersensitivity, whereas horses with values below the cut-off value were classified as not clinically hypersensitive. The cut-off values were selected to maximize the specificity and sensitivity of the IDT in predicting which horses had hypersensitivity. The cut-off values were selected so that (sensitivity + specificity)/2 attained its highest value.[15] Sensitivity was defined as the proportion of diseased horses that had a positive result, specificity was defined as the proportion of healthy horses that had a negative result, positive predictive value was defined as the proportion of horses with a positive result that were diseased, and negative predictive value was defined as the proportion of horses with a negative result that were healthy.[15] We assumed that clinically important hypersensitivity is a feature of COPD, RU, and AD, and that clinically relevant hypersensitivity did not affect the healthy horses.

Statistical analyses—Horses were grouped by clinical diagnosis (COPD, RU, AD, and healthy). The χ^2 test and odds ratios were used to assess differences in the proportion of horses within each horse group that responded to any allergen within an allergen group at each evaluation time, as well as the proportion of horses within each group that responded to each specific allergen. The significance level for group differences was set at $P < 0.05$. Odds ratios were considered significant when the 95% confidence interval did not include 1.[d] The mean number of allergens within an allergen group that provoked a positive reaction was calculated for each horse group, and a mixed-effects multiple regression model was used to determine whether significant differences were evident between healthy horses and horses in each disease group.[e] The adjusted value for significance was set at $P < 0.05$.

Results

Eighty-six horses met the inclusion criteria and were classified by diagnosis into 1 of the following groups: COPD (n = 41), RU (20), AD (9), and healthy (16). One horse with RU and 1 horse with AD had IDT performed on 2 occasions, because the horses appeared to have an exaggerated response to almost all allergens. For these horses, only the results of the second skin test, performed 4 and 6 months after the first test, were used for analysis of the data. The first IDT was performed in both instances in late summer when these horses still had overt clinical signs, and the second IDT was performed in late winter when the clinical signs had subsided. Results of the first and second IDT were similar in both horses.

Positive and negative controls—The degree of reaction to intradermal injection of histamine was 4+ for all horses, with the exception of 1 of 16 healthy horses, 3 of 43 horses with COPD, and 3 of 20 horses with RU, which had a 3+ reaction to histamine. All horses had 0+ reactions to intradermal injection of phosphate-buffered allergen diluent.

Immediate reactions—The proportion of horses with COPD, RU, or AD that had 1 or more positive reactions at 30 minutes to allergens within any group of allergens was significantly higher, compared with healthy horses, with the exception of the response of horses with COPD to mold, tree, and insect allergens and the response of horses with RU to mold allergens (**Table 1**). Horses with COPD, RU, and AD were 7.4, 7, and 24.5 times more likely to respond to 1 or more weed allergens, respectively, than were healthy horses. Horses with RU and AD were 27.9 and 52.5 times more likely to respond to 1 or more tree allergens, respectively, than were healthy horses. Horses with COPD and RU were 21.1 and 31.7 times as likely to respond to 1 or more grass-crop allergens, respectively, compared with healthy horses. Horses with RU and AD were 8.8 and 17.6 more likely to respond to 1 or more insect allergens, respectively, than were healthy horses. Mean number of insect allergens that caused a positive reaction was significantly higher in horses with RU and AD, compared with healthy horses (**Table 2**). Horses with AD also had a significantly higher mean number of tree and grass allergens that caused a positive reaction, compared with healthy horses.

Late-phase reactions—The greatest differences between healthy horses and the other groups were iden-

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Table 1—Distribution (No. [%]) of horses with positive reactions (score ≥ 3+) to 1 or more allergens within various allergen groups, determined at various times after intradermal administration in healthy horses (n = 16) and horses with chronic obstructive pulmonary disease (COPD; 41), urticaria (20), or allergic dermatitis (9)

	Healthy			COPD			Urticaria			Allergic dermatitis		
Allergen group	**30 min**	**4 h**	**24 h**	**30 min**	**4 h**	**24 h**	**30 min**	**4 h**	**24 h**	**30 min**	**4 h**	**24 h**
Molds	2 (13)	9 (56)	1 (6)	17 (42)	40 (98)*	21(51)*	8 (40)	20 (100)*	17 (85)*	7 (78)*	9 (100)*	7 (78)*
OR	NA	NA	NA	5	31.1	15.8	4.7	UD	85	24.5	UD	52.5
95% CI	NA	NA	NA	0.9–49	3–1435	1.9–694	0.7–51	UD	7–3776	2–351	UD	3–2513
Weeds	2 (13)	3 (19)	0 (0)	21 (51)*	23 (56)*	4 (10)	10 (50)*	15 (75)*	9 (45)*	7 (78)*	8 (89)*	2 (22)
OR	NA	NA	NA	7.4	5.5	UD	7	13	UD	24.5	34.7	UD
95% CI	NA	NA	NA	1.4–72	1.2–34	UD	1.1–75	2.1–94	UD	2.1–351	2.5–1651	UD
Trees	1 (6)	0 (0)	0 (0)	12 (29)	20 (49)*	2 (5)	13 (65)*	13 (65)*	6 (30)*	7(78)*	7(78)*	1(11)
OR	NA	NA	NA	6.2	UD	UD	27.9	UD	UD	52.5	UD	UD
95% CI	NA	NA	NA	0.8–283	UD	UD	2.8–1260	UD	UD	3–2513	UD	UD
Grasses-crops	6 (38)	9 (56)	0 (0)	38 (93)*	39 (95)*	5 (12)	19 (95)*	20 (100)*	8 (40)*	9 (100)*	9 (100)*	6 (67)*
OR	NA	NA	NA	21.1	15.2	UD	31.7	UD	UD	UD	UD	UD
95% CI	NA	NA	NA	4–143	2.2–162	UD	3–1444	UD	UD	UD	UD	UD
Insects	5 (31)	5 (31)	0 (0)	26 (63)	32 (78)*	6 (15)	16 (80)*	18 (90)*	13(65)*	8 (89)*	9 (100)*	4(44)*
OR	NA	NA	NA	3.8	7.8	UD	8.8	19.8	UD	17.6	UD	UD
95% CI	NA	NA	NA	0.9–16	1.8–35	UD	1.6–54	2.7–216	UD	1.5–850	UD	UD

*Significant ($P < 0.05$; 2-tailed Fisher exact test) difference, compared with healthy horses.
OR = Odds ratio. NA = Not applicable. UD = Odd ratios undefined because 1 of the cells contains 0. CI = Confidence interval of the OR.

Table 2—Mean No. of allergens that induced a positive reaction in healthy horses and horses with COPD, urticaria, or allergic dermatitis

	Healthy			COPD			Urticaria			Allergic dermatitis		
Allergen group (n)	**30 min**	**4 h**	**24 h**	**30 min**	**4 h**	**24 h**	**30 min**	**4 h**	**24 h**	**30 min**	**4 h**	**24 h**
Molds (22)												
Mean	0.13	0.81	0.13	1.88	3.24*	1.27	1.55	2.65*	2.70*	1.56	2.00	1.33
(SD)	(0.36)	(1.04)	(0.44)	(4.23)	(2.75)	(2.11)	(2.59)	(2.28)	(3.22)	(1.23)	(1.11)	(0.87)
P value	NA	NA	NA	NS	0.003	NS	NS	0.035	0.002	NS	NS	NS
Weeds (8)												
Mean	0.88	0.25	0.00	2.05	2.56*	0.12	1.65	1.65	0.85*	2.89	2.78*	0.22
(SD)	(2.4)	(0.56)	(0)	(2.56)	(2.75)	(0.38)	(2.06)	(1.48)	(1.21)	(2.67)	(2.58)	(0.45)
P value	NA	NA	NA	NS	0.004	NS	NS	NS	0.004	NS	0.037	NS
Trees (10)												
Mean	0.38	0.00	0.00	0.66	1.05*	0.05	1.20	1.30*	0.45*	2.67*	1.56*	0.11
(SD)	(1.52)	(0)	(0)	(1.28)	(1.34)	(0.19)	(1.79)	(1.48)	(0.80)	(3.15)	(1.5)	(0.33)
P value	NA	NA	NA	NS	0.033	NS	NS	0.004	0.02	0.01	0.02	NS
Grasses-crops (13)												
Mean	0.81	0.75	0.00	2.39	2.93*	0.12	3.10	2.55*	0.60	5.56*	3.67*	1.33*
(SD)	(1.44)	(0.84)	(0)	(2.63)	(2.11)	(0.32)	(2.68)	(1.43)	(0.85)	(3.96)	(3.03)	(2.19)
P value	NA	NA	NA	NS	0.001	NS	NS	0.034	NS	< 0.001	0.003	0.001
Insects (5)												
Mean	0.81	0.44	0.00	1.83	2.37*	0.22	2.35*	2.65*	1.20*	3.44*	3.56*	0.78
(SD)	(1.28)	(0.8)	(0)	(1.79)	(1.66)	(0.64)	(1.79)	(1.52)	(1.12)	(1.74)	(1.14)	(1.29)
P value	NA	NA	NA	NS	< 0.001	NS	0.03	< 0.001	< 0.001	0.002	0.0	NS

*Significant ($P < 0.05$; 2-tailed Fisher exact test) difference, compared with healthy horses.
NA = Not applicable. NS = Not significant.

tified in late-phase reactions. The proportion of horses with COPD, RU, and AD that had 1 or more positive reactions to allergens within each allergen group was significantly higher, compared with that of healthy horses (Table 1). Horses with COPD, RU, and AD were 5.5, 13, and 34.7 times more likely to respond to 1 or more weed allergens, respectively, than were healthy horses. Horses with COPD and RU were 7.8 and 19.8 times more likely to respond to 1 or more insect allergens, respectively, than were healthy horses. Almost all horses with RU and AD had 1 or more positive reactions to mold, grass, and insect allergens. Mean number of allergens that caused positive reactions in horses with diseases was significantly higher, compared with healthy horses, with the exception of the response of horses with RU to weed allergens and the response of horses with AD to mold allergens (Table 2).

Delayed-phase reactions—The proportion of horses with RU that had 1 or more positive reaction at 24 hours to allergens within an allergen group was significantly greater than that of healthy horses (Table 1). Horses with COPD were 15.8 times more likely to respond to 1 or more mold allergens, compared with healthy horses. The proportion of horses with AD that had 1 or more positive reactions to mold, grass, and

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Table 3—No. of horses (OR, compared with healthy horses) that had a positive reaction to various allergens

	Healthy			COPD			Urticaria			Allergic dermatitis		
Allergen	**30 min**	**4 h**	**24 h**	**30 min**	**4 h**	**24 h**	**30 min**	**4 h**	**24 h**	**30 min**	**4 h**	**24 h**
Rhizopus mix	0	1	0	7	11	22*	1	5	14*	3	2	6*
Candida albicans	0	1	1	5	13	24 (21)*	1	4	18 (90)*	0	1	4
Grain smut mix	2	9	0	8	36 (5.6)*	8	5	16	6	4	9*	3
Dock-sorrel	2	2	0	15	18 (5.5)*	1	6	10 (7)*	5	3	4	0
Goldenrod	2	1	0	13	16 (9.6)*	0	4	6	3	4	4*	0
Lamb's quarter	2	0	0	11	13*	0	6	2	3	6 (14)*	5*	0
Marsh elder	2	0	0	14	14*	0	4	3	1	5	3	0
Pigweed	2	0	0	10	10*	2	2	3	2	4	4*	1
Maple mix	0	0	0	8	9*	0	6*	5	1	4*	2	1
Black willow	1	0	0	6	15*	1	2	6*	0	4*	4*	0
Alfalfa	2	0	0	8	19*	1	1	7*	0	6 (14)*	7*	1
Grass mix	1	1	0	5	6	0	6	3	0	4*	2	1
Kentucky bluegrass	0	0	0	6	4	0	6*	3	1	5*	1	1
Meadow fescue	0	0	0	4	6	0	3	3	2	4*	2	1
Red top	1	0	0	4	3	0	3	3	0	4*	3	1
Rye, cultivated	2	0	0	7	9*	0	4	1	0	4	1	0
Wheat, cultivated	0	0	0	9*	10*	0	4	5	3	5*	5*	3
Grain mill dust mix	5	8	0	36 (16)*	38 (13)*	4	19 (42)*	20*	3	9*	9*	3
Mosquito	3	1	0	14	21 (16)*	2	5	9 (12)*	3	7 (15)*	7 (53)*	0
Black fly	5	1	0	14	18 (12)*	1	12	11 (18)*	4	6	8 (120)*	1
Deer fly	2	0	0	20 (6.7)*	20	3	12 (11)*	12*	7*	7 (25)*	8*	1
Horse fly	2	4	0	18 (5.5)*	25(4.7)	2	11 (8.6)*	14 (7)*	7*	8 (56)*	7 (11)*	3

Values are listed only for those allergens that induced a positive reaction in at least 40% of the horses within at least 1 group of horses. *Significant ($P < 0.05$; 2-tailed Fisher exact test) difference, compared with healthy horses.

Table 4—Allergen groups that induced a positive reaction in horses with various diseases, compared with healthy horses

	COPD			Urticaria			Allergic dermatitis		
Allergen group (n)	**30 min**	**4 h**	**24 h**	**30 min**	**4 h**	**24 h**	**30 min**	**4 h**	**24 h**
Molds (22)									
Cut-off No.	≥ 1	≥ 2	≥ 1	≥ 1	≥ 2	≥ 1	≥ 1	≥ 2	≥ 1
Sensitivity	41	76	29	40	60	40	78	56	56
Specificity	88	88	100	88	88	100	88	88	100
PPV	89	94	100	80	86	100	78	71	100
NPV	37	58	36	54	64	57	88	78	80
Weeds (8)									
Cut-off No.	≥ 1	≥ 2	≥ 1	≥ 1	≥ 2	≥ 1	≥ 1	≥ 2	≥ 1
Sensitivity	51	51	10	50	50	45	78	67	22
Specificity	88	94	100	88	94	100	88	94	100
PPV	91	95	100	83	91	100	78	86	100
NPV	41	43	30	58	60	59	88	83	70
Trees (10)									
Cut-off No.	≥ 1	≥ 1	≥ 1	≥ 1	≥ 1	≥ 1	≥ 1	≥ 1	≥ 1
Sensitivity	29	49	5	65	65	30	78	78	11
Specificity	94	100	100	94	100	100	94	100	100
PPV	92	100	100	93	100	100	88	100	100
NPV	34	43	29	68	70	53	88	89	67
Grasses-crops (17)									
Cut-off No.	≥ 2	≥ 2	≥ 1	≥ 2	≥ 2	≥ 1	≥ 2	≥ 2	≥ 1
Sensitivity	39	76	12	55	75	40	89	78	67
Specificity	81	88	100	81	88	100	81	88	100
PPV	84	94	100	79	88	100	73	78	100
NPV	34	58	31	59	74	57	93	88	84
Insects (14)									
Cut-off No.	≥ 3	≥ 2	≥ 1	≥ 3	≥ 2	≥ 1	≥ 3	≥ 2	≥ 1
Sensitivity	37	66	15	45	75	65	67	100	44
Specificity	81	94	100	81	94	100	81	94	100
PPV	83	96	100	75	94	100	67	90	100
NPV	33	52	31	54	75	70	81	100	76

Cut-off No. = No. of allergens that induced a positive reaction, within each group of allergens. Sensitivity = Proportion (%) of horses that had a positive reaction to ≥ the cutoff No. of allergens. Specificity = Proportion (%) of healthy horses that had a positive reaction to < the cutoff No. of allergens. PPV= Positive predictive value (proportion [%] of horses with diseases that had a positive reaction to ≥ the cutoff No. of allergens). NPV = Negative predictive value (proportion [%] of healthy horses that had a positive reaction to < the cutoff No. of allergens).

insect allergens was significantly higher than the proportion of healthy horses responding to these allergens.

Horses with RU had a significantly higher mean number of allergens that caused positive reactions in every

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allergen group (except grass allergens), compared with healthy horses (Table 2). Horses with AD had a significantly higher mean number of grass and crop allergens that caused positive reactions, compared with healthy horses. Few allergens induced a positive reaction at 24 hours without previous positive reactions at 30 minutes, 4 hours, or both. *Candida albicans* and mixed *Rhizopus* spp induced positive reactions at 24 hours without previous reactions in 20 to 30% of horses with COPD, RU, and AD; for the rest of the allergens, only 0 to 10% of these horses had positive reactions.

For the following allergens, 3 or more of the 16 healthy horses had a positive reaction at 1 or multiple evaluation times: grain smut mix, mixed grain mill dusts, blackfly, and horse fly. Allergens that induced a positive reaction in < 10% of the horses within any group of horses included *C spicifera*, *H sativum*, *H hordei*, mixed *Penicillium* spp, mixed *Aspergillus* spp, *E purpurascens*, *P betae*, and *S solani*. Allergens that induced a positive reaction in > 50% of the horses within at least 1 group of horses included mixed *Rhizopus* spp, *C albicans*, grain smut mix, lamb's quarter, marsh elder, alfalfa, Kentucky bluegrass, cultivated wheat, mixed grain mill dusts, mosquito, blackfly, deer fly, and horse fly (**Table 3**).

Cut-off values for number of allergens within an allergen group for each horse group ranged from ≥ 1 to ≥ 3, and corresponding specificities ranged from 81 to 100% among all observation times (**Table 4**).

Discussion

Healthy horses as well as horses with COPD, RU, or AD had positive reactions to a variety of intradermal allergens. However, for most allergen groups and at most observation times a greater proportion of horses with COPD, RU, or AD reacted to a greater number of allergens, compared with healthy horses. These findings are similar to those reported in previous studies[10,11] with smaller sample sizes. In general, horses with COPD, RU, or AD were at least 6 times (and as much as 85 times) as likely to respond to 1 or more allergens within a group of allergens as healthy horses were, and the mean number of allergens that induced a positive reaction in a group of horses with these diseases was approximately 2 to 10 times greater than in healthy horses. These findings suggest an association between these diseases and a clinically important component of hypersensitivity to environmental allergens.

Positive intradermal reaction to an allergen may indicate clinically important hypersensitivity, subclinical hypersensitivity, or a false-positive reaction. An allergen that acts as an irritant and causes mast cell degranulation could induce a positive reaction in healthy horses and those with allergy-related diseases. In our study, grain smut mix, mixed grain mill dusts, blackfly, and horse fly allergens induced a positive reaction in 3 or more of 16 healthy horses. Similarly, in a previous study of IDT in horses with COPD, urticaria, chronic head-shaking syndrome, and reactive airway disease, allergens that were reported to induce the most substantial reactions included insect (mosquito and blackfly), weed (ragweed, lamb's quarter, rough pigweed, yellow dock, English plantain, cocklebur, wormwood, kochia, and red sheep sorrel), dust (grain mill dust), smut (grass smut mix and grain smut mix), and miscellaneous (sheep wool and mixed feathers) categories of allergens.[9] We, therefore, recommend that if a positive result is obtained by use of these allergens (grain smut mix, mixed grain mill dusts, blackfly, and horse fly), results should be interpreted cautiously. We propose that the greater reactivity to these allergens in healthy horses may reflect a true immunologic reaction attributable to subclinical hypersensitivity to environmental allergens to which horses are commonly exposed. The only method to prove that these allergens have induced an immunologic reaction is to perform a passive cutaneous anaphylaxis test (Prausnitz-Küstner test).[16] This procedure was outside the scope of our study and is not commonly used in association with the IDT in clinical practice. Certain allergens (*C spicifera*, *H sativum*, *H hordei*, mixed *Penicillium* spp, mixed *Aspergillus* spp, *E purpurascens*, *P betae*, and *S solani*) caused a positive reaction in a few horses of each group in our study population; these allergens may not be useful when designing an IDT for horses.

Assessment of the delayed response to intradermal injection of allergens (24 hours) provided little additional information, because few allergens induced a positive reaction at 24 hours without previous positive reactions at 30 minutes or 4 hours. Only 2 of 58 allergens (*C albicans* and mixed *Rhizopus* spp) induced positive reactions at 24 hours without previous reactions (in 20 to 30% of horses with allergy-related diseases); for all other allergens, this occurred in only 0 to 10% of these horses. Similarly, in a study of IDT in horses with COPD, urticaria, reactive airway disease, and chronic headshaking syndrome, few positive reactions were observed at 24 hours.[9] Therefore, our recommendation is to evaluate reactions 30 minutes and 4 hours after the intradermal injection, which will simplify this diagnostic procedure without substantially reducing its usefulness.

To facilitate interpretation of the IDT, cut-off values were established for the number of positive intradermal reactions in each allergen group for each horse group at each observation time. The cut-off values were selected to maximize specificity and sensitivity of the IDT. Specificity values (81 to 100%) and positive predictive values (67 to 100%) were high for reported cut-off values. We interpret these results to mean that for horses with a history and clinical signs compatible with the diseases we studied, observation of numbers of positive intradermal reactions at or above the cut-off values indicated that, 67 to 100% of the time, clinically important hypersensitivity was a component of the disease. The corresponding sensitivities for these cut-off values were often quite low; for example, for horses with COPD and AD, sensitivity was 5 and 11%, respectively, for reactions to ≥ 1 tree allergen at 24 hours. We interpret these low sensitivity values to indicate that our cut-off values excluded some horses for which hypersensitivity was a component of their disease. However, our goal was to devise a method of identifying horses likely to have clinically apparent hypersensitivity in a clinical setting in which many potential false-positive IDT results are obtained. Where possible, cut-off values were selected to maximize sen-

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sitivity and specificity (for example, ≥ 2 positive reactions to grass-crop allergens in horses with COPD at 4 hours or ≥ 2 positive reactions to insects in horses with AD at 4 hours). For such cut-off values, fewer horses that presumably have clinically important hypersensitivity disease are falsely identified as being outside the positive responder category.

On the basis of our cut-off values, we offer some guidelines for clinicians in evaluating the responses to IDT in an individual horse: the observation time point with optimal sensitivity and specificity is usually at 4 hours, and in horses with COPD, RU, or AD, the specificity (negativity in health) of the cut-off values at 4 hours varies from 88 to 100%, and the positive predictive value (proportion of horses with a positive reaction that have the target disorder) varies from 71 to 100%. Because the diagnostic test has a sufficiently high specificity, a positive result supports diagnosis of the target disorder.[15] Therefore, horses in which COPD, RU, or AD has already been diagnosed and in which the IDT at 4 hours reveals ≥ 1 positive reaction to tree allergens or ≥ 2 positive reactions to mold, weed, grasses-crops, or insect allergens are likely to have clinically important hypersensitivity. We speculate that such horses would be more likely to have a positive response to appropriately formulated allergen immunotherapy vaccines. When formulating allergen immunotherapy vaccines, we believe one must consider the history, clinical signs, and environment to determine the nature of the disease and the likelihood of exposure to allergens in order to assess the clinical relevance of hypersensitivity reactions to individual allergens in each individual horse. Allergen immunotherapy vaccination based on IDT results has been suggested by some as an effective alternative to conventional treatments for COPD, urticaria, and reactive airway disease, on the basis of clinical observations.[9] However, to our knowledge, there is no double-blind placebo-controlled study in horses with COPD, RU, or AD that evaluates the efficacy of allergen immunotherapy vaccination and management changes to decrease allergen exposure versus management changes alone.

The selection of subjective scores that are regarded as a positive IDT reaction is somewhat controversial. We based our assessments of positive or negative IDT reaction on the wheal size relative to the size of the positive control wheal. The maximum wheal size in response to the positive control (histamine) was defined as a 4+ reaction. A few horses developed wheals after injection of selected allergens that were larger than the positive control and were graded 5+. We selected a wheal size of 3+ as a positive reaction. This value was greater than the mean difference between the positive and negative controls for all horses (2+).

Some investigators suggest that a 2+ reaction on a 4-point scale should be considered a positive indication of a hypersensitivity reaction to an allergen,[17] and a previous study[9] of IDT in horses with a variety of allergic conditions considered reactions of ≥ 1.5+ to be positive. Reactions of 2+ were commonly seen in healthy horses in our study, and positive reactions to IDT in healthy humans have been commonly reported.[18] Such reactions could represent nonspecific irritant reactions, previous sensitization to the allergen, or subclinical hypersensitivity.[18] Healthy humans with positive skin test reactions clearly are more likely to develop clinical hypersensitivity-related diseases such as asthma than are persons with negative results of IDT.[13,19-21] Humans with allergic rhinitis and horses with *Culicoides* hypersensitivity have a greater number of IDT reactions than healthy individuals do.[10,22] In addition, horses with clinical signs of *Culicoides* hypersensitivity have stronger IDT reactions than healthy horses do.[10] Because our purpose was to identify clinically relevant hypersensitivity in horses with clinical signs, we chose to consider the stronger 3+ IDT reaction as positive in order to exclude detection of healthy horses that may have subclinical hypersensitivity. The most accurate evaluation of the diagnostic value of the IDT, and the choice of 2+ or 3+ as positive, may be to compare these tests with the gold standard of induction of clinical disease with a provocative challenge; this comparison was outside the scope of our study.

After compiling the data of this study on the basis of 3+ as a positive reaction, all data were recompiled by use of the 2+ value. When a 2+ reaction was considered positive, 50 to 90% of healthy horses had substantial immediate and late-phase positive reactions to 1 or more allergens within a group of allergens, and the mean number of allergens within a group of allergens that induced a positive reaction doubled, compared with results obtained with 3+ as a positive reaction (data not shown). We concluded from this comparison that considering 2+ as a positive reaction likely identifies subclinical hypersensitivities that do not warrant treatment in many horses.

It should be noted that false-negative IDT reactions may be observed. McGorum et al[3] detected a lack of correlation between dermal and bronchial reactivity to *M faeni* (now named *F rectivirgula*), *A fumigatus*, and *T vulgaris* in horses and noted that such divergence between pulmonary and dermal reactivity has also been reported in humans. It is likely that responses to IDT do not provide a comprehensive assessment of bronchial hypersensitivity.

Healthy horses in our study had 1 or more positive reactions to allergens in each allergen group, and the clinical relevance of positive reactions to individual allergens is therefore difficult to interpret. We recommend that reactions to individual allergens not be used to determine that horses have hypersensitivity. However, a pattern of positive reactions to multiple allergens within an allergen group, particularly at 4 hours, when observed in the context of a compatible history and physical examination, suggests that clinically relevant hypersensitivity is present.

[a]Littlewood JD, Patterson S, Shaw SC. Atopy-like skin disease in the horse, in *Proceedings* (abstr). 3rd World Congr Vet Dermatol 1996;94.
[b]Greer Laboratories, Lenoir, NC.
[c]Center Laboratories, Port Washington, NY.
[d]STATCALC, EpiInfo, version 6, Centers for Disease Control and Prevention, Atlanta, Ga.
[e]PROC MIXED, SAS, version 6.1.2, SAS Institute Inc, Cary, NC.

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