Comparison of immediate intradermal test reactivity with serum IgE quantitation by use of a radioallergosorbent test and two ELISA in horses with and without atopy

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Objective—To compare a radioallergosorbent test and 2 ELISA with intradermal testing for the determination of environmental allergen hypersensitivity in horses with and without atopic diseases.

Design—Prospective clinical study.

Animals—10 horses with recurrent urticaria, 7 with atopic dermatitis, 16 with chronic obstructive pulmonary disease, and 22 without atopy.

Procedure—History, physical examination, hemogram, serum biochemical analyses, bronchoalveolar lavage, and an intradermal test (used as the criterion standard) with a regional panel of 73 allergens were performed in all horses. Serum was analyzed by use of the 3 in vitro assays of allergen-specific IgE.

Results—An ELISA based on the α chain of the high-affinity IgE receptor, the Fcε receptor immunoglobulin ε chain (FcεRIx) for IgE, had the overall highest kappa statistic (0.238), positive predictive value (49%), and negative predictive value (78%). Overall agreement between the FcεRIx-based ELISA and the intradermal test was fair. The highest kappa statistic was obtained by the FcεRIx-based ELISA in horses with atopic dermatitis (0.330). Kappa statistics for the radioallergosorbent test and a polyclonal antibody-based ELISA agreed slightly with that of the intradermal test at best.

Conclusions and Clinical Relevance—None of the 3 serum allergy tests reliably detected allergen hypersensitivity, compared with the intradermal test. The FcεRIx-based ELISA performed significantly better overall than the other 2 tests. Low sensitivity of all 3 assays indicates the need for continued study to elucidate a more sensitive test for the determination of potentially pathogenic allergens in horses. (J Am Vet Med Assoc 2001;218:1314–1322)

Atopy is defined as a familial hypersensitivity of skin and mucous membranes to environmental allergens, associated with increased IgE production, altered nonspecific reactivity in different organ systems, or both; examples are skin affected by atopic dermatitis (AD) and lung affected by asthma.1 Classical atopy is an immediate or type-I hypersensitivity1 with increased IgE production, T-cell dysregulation, enhanced release of inflammatory mediators, and organ hyperreactivity.

Equine atopy is cutaneous or respiratory in origin and may be a combination of both.2,3 Atopic dermatitis and recurrent urticaria (RU) are 2 cutaneous diseases associated with IgE-mediated hypersensitivity.4,5 The pathogenesis of chronic RU is not completely understood, but IgE-mediated mast cell degranulation is thought to be of central importance in coordinating the urticarial response.6 Both AD and RU have been seen worldwide, but epidemiologic studies to indicate the incidence or prevalence of these cutaneous allergic diseases in horses are lacking. Anecdotal reports suggest that cutaneous allergy is a widespread problem.7,8

Respiratory reactions are commonly referred to as chronic obstructive pulmonary disease (COPD). Although the cause of COPD has often been a source of dispute, hypersensitivity to environmental allergens is believed to play a pathogenic role in many cases.9 An allergic component to the disease has been suggested through documentation of immune responses to environmental allergens by detection of serum antibodies, response to aerosol provocation tests, and reactions to intradermal skin tests.10-13 Chronic obstructive pulmonary disease is a naturally occurring respiratory disease of horses characterized by periods of acute airway obstruction followed by periods of remission.14 The condition is seen worldwide, and epidemiologic studies reveal higher prevalence of the disease in aged horses and in horses and ponies exposed to poor quality hay or bedding and poor ventilation.15

Intradermal testing (IDT) is an accepted method of detecting IgE-mediated allergen hypersensitivity in atopic humans,16 dogs,17 cats,18 and horses.19 Intradermal testing has been regarded as the criterion standard for identification of allergens for inclusion in immunotherapy vaccines in horses.7 The inconveniences associated with IDT such as sedation, clipping of the hair on the neck, purchase of allergens, storage and preparation of allergen dilutions, and injection of multiple intradermal allergens preclude the use of IDT in most general practices. Consequently, practitioners must rely on specialists who are often not in the near vicinity, necessitating extra travel and time for the client. A strong need exists for reliable in vitro serum allergy tests (SAT) that are more accessible and available to equine practitioners. Serum allergy tests offer an alternative means of identifying allergens to which an individual is hypersensitive by measuring concentrations of circulating allergen-specific IgE antibodies in serum.19
Since the first assay to detect IgE antibodies was developed, an increasing number of systems have been proposed for the routine detection of specific IgE. Specificity, sensitivity, predictive value, and clinical importance of detectable specific IgE are used to compare specific IgE reactivity among SAT and IDT. The radioallergosorbent test (RAST) and the ELISA assays are tests that detect relative concentrations of allergen-specific IgE in serum. The sensitivity and specificity of these tests are dependent on the affinity of the IgE-binding reagent to bind all of the IgE present without binding to any other antibody type. The quality of allergen, density of allergen coupled to the solid support, and methodology are factors that influence the sensitivity and specificity of SAT. A high degree of sensitivity and specificity is necessary to ensure that all allergens that induce hypersensitivity are identified for inclusion into an immunotherapeutic vaccine and that only allergen-specific IgE is identified.

Methods are available to assess agreement among tests of hypersensitivity (SAT and IDT) or the repeatability of a test’s assessment of the same patient. Interpretation agreement evaluating atopic disease in horses has not been reported. Agreement between SAT and IDT can be assessed by use of the kappa statistic (K), which evaluates the proportion of agreement that occurred beyond that expected because of chance. The K can be used to compare multiple levels of agreement (low, normal, high) rather than just 2 levels (abnormal, normal). When comparisons are made between a diagnostic test (SAT) and a criterion standard (IDT), K becomes a measure of agreement beyond chance.

The purpose of the study reported here was to compare the K, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of a RAST and 2 ELISA in vitro SAT in horses with atopic diseases (RU, AD, and COPD) and horses without atopy, using the IDT as the criterion standard.

Materials and Methods

Horses—Horses without atopy were selected for the study from horses donated to The Ohio State University College of Veterinary Medicine. Recurrent urticaria-, AD-, and COPD-affected horses were selected from client-owned horses referred to The Ohio State University Veterinary Teaching Hospital for evaluation of cutaneous or respiratory disease, respectively. The study was performed from July 1998 to August 1999, and skin tests were performed throughout this period of horses without atopy and RU-, AD-, and COPD-affected horses and were not clustered or limited to any particular month. A detailed history was obtained for at least the preceding 12 months for each horse and included specific questions regarding previous or current dermatologic and respiratory disease. Because atopy may be a seasonal or nonseasonal disease, responses to the questions allowed identification and exclusion of horses with seasonal atopy that did not have clinical signs at the time of examination from the group of horses without atopy. All populations of horses were given routine treatment with anthelmintics prior to the study. All horses received a complete physical examination, including auscultation of the thorax during quiet breathing and forced rebreathing. Diagnostic tests performed on all horses included a CBC, serum biochemical profile, bronchoalveolar lavage with cytology, and an IDT. Cytologic examination of the skin surface, bacteriologic culture, fungal culture, skin biopsy for histologic examination, or elimination diet food trial were performed prior to entry into the study only if infectious skin disease or food hypersensitivity was suspected in any individual horse.

If a horse in the RU, AD, or COPD group had IDT reactivity to insect allergens only, the horse was excluded from the study. However, if AD-, RU-, or COPD-affected horses had IDT reactivity to airborne allergens as well as insect allergens, the horses were included in the study. All horses had clinical signs of active disease at the time of entry into the study.

Horses without atopy—Twenty-two horses (14 geldings, 7 mares, and 1 stallion) were selected for inclusion in the study on the basis of absence of historical and clinical evidence suggestive of allergic disease of any organ system. These horses were not considered completely healthy, because they were donated because of osteoarthritis (n = 4), laminitis (3), chronic endomirritis (2), abdominal cryptorchidism (1), navicular disease (4), developmental contracture of the superficial digital flexor tendons (1), sesamoideitis (1), cervical vertebral instability (1), occipito-atlanto-axial malformation (1), arthrogryposis (1), shivers (1), exsanguination (1), and behavior problems (1).

Regarding breeds, this group included 1 Arabian, 1 Bashkir Curly, 1 Oldenburg, 1 Percheron, 6 Quarter Horses, 2 Standardbreds, and 10 Thoroughbreds. Further inclusion criteria for horses without atopy were a respiratory rate < 24 breaths/min, no evidence of crackles or wheezes on rebreathing examination, and absence of cough on physical examination.

Horses with RU—Ten horses (5 geldings, 4 mares, and 1 stallion) with historical and clinical signs compatible with pruritic seasonal and nonseasonal RU and with active clinical disease were entered into the study. This group included 1 Warmblood, 1 grade, 1 Paint, and 7 Quarter Horses.

Horses with AD—Seven horses (3 geldings, 3 mares, and 1 stallion) with historical and clinical signs compatible with AD and with active clinical disease were entered into the study. This group included 2 American Saddle Horses, 2 Arabians, 1 Belgian, 1 Quarter Horse, and 1 Tennessee Walking Horse.

Horses with COPD—Sixteen client-owned horses (9 geldings, 6 mares, and 1 stallion) had historical and clinical signs compatible with a diagnosis of COPD. This group included 2 Appaloosas, 2 Arabians, 3 grades, 1 Paint, 6 Quarter Horses, 1 Standardbred, and 1 Thoroughbred. All horses had clinical signs of respiratory tract disease upon entry into the study. Lung sounds were classified on the basis of the terminology of Roudebush. Wheezes were high-pitched continuous sounds, and crackles were low-pitched discontinuous sounds. High-pitched sounds of short duration were termed fine crackles and are synonymous with squeaks. None of the COPD-affected horses had clinical signs or historical evidence of skin disease or food hypersensitivity.

Procedures were performed according to the guidelines established by Institutional Laboratory Animal Care and Use Committee and approved by The Ohio State University College of Veterinary Medicine.

Housing—All horses were housed in individual 4 × 4-m stalls that were designed to allow free airflow and recirculation of air through an air conditioner or ventilation with outside air. All horses affected with COPD were bedded on tan bark and fed pelleted grain and wet hay to reduce the likeli-
hood of an acute exacerbation of clinical signs during hospitalization. All horses without atopy and RU- and AD-affected horses were bedded on straw and fed sweet feed and mixed-grass hay. Nonatopic horses were kept in the stall for a minimum of 36 hours and up to 1 week as a natural (hay and straw) challenge to further eliminate the possibility of undetected respiratory disease. Stalls were cleaned once per day. All groups of horses were allowed water ad libitum.

Hematologic testing—Blood was obtained by jugular venipuncture and collected into sterile glass tubes containing EDTA for CBC and sterile glass serum tubes for serum biochemical profile. The CBC was performed by use of an automated cell counter, and a differential count of 100 WBC was performed manually. Serum biochemical analyses were performed by use of an automated analyzer. Serum was collected for ELISA and RAST before the IDT was performed.

Respiratory tract examination—Physical examination of the respiratory system was performed on all horses by 1 author (GL) before bronchoalveolar lavage was performed. The examination was conducted at rest and consisted of auscultation of the thorax during quiet breathing and forced breathing induced by the use of a rebreathing bag. The location and intensity of wheezes and crackles were recorded. Inclusion criteria for the diagnosis of COPD were expiratory wheezes or crackles on re-breathing examination, high respiratory rate at rest (> 24 breaths/min), or chronic cough on physical examination. Inclusion criteria for horses without atopy and RU- and AD-affected horses were respiratory rate < 24 breaths/min, no evidence of crackles or wheezes on re-breathing examination, and absence of cough on physical examination.

Bronchoalveolar lavage—Horses were sedated with xylazine hydrochloride (0.5 mg/kg [0.23 mg/lb] of body weight, IV). An 8-mm-diameter 2.5-m-long silicone equine bronchoalveolar lavage tube was inserted into the nasal cavity, advanced into the trachea to the level of the carina, and wedged in a secondary bronchus of a caudal lung lobe. Lavage consisted of infusion and immediate aspiration of one 150-ml aliquot of lukewarm sterile saline (0.9% NaCl) solution. Manual aspiration of the lavage fluid was achieved by use of a series of sterile 60-ml syringes. Lavage fluid was transported to the laboratory in a sterile glass tube containing EDTA and was processed immediately after collection. Total concentration of nucleated cells in the fluid was measured by use of an automated cell counter. For each sample, 1 slide was prepared by use of cytocentrifugation. The slide was stained with Wright-Giemsa stain, and 100 consecutive nucleated cells were counted. The cytologist did not know whether the slides were from horses without atopy or RU-, AD-, or COPD-affected horses. Calculation of cell concentrations in lavage fluid was obtained by multiplying the percentage of each cell type by the number of nucleated cells observed.

Serum collection for in vitro SAT—For all horses, 30 ml of blood was collected by jugular venipuncture and placed in sterile glass serum tubes prior to IDT. Samples were centrifuged at 1,000 × g for 10 minutes. Serum was placed in plastic vials supplied by the test companies and sent to the respective laboratories for analysis. All samples were sent on the day of serum collection in packaging and shipping material supplied by the individual companies. Code names for the horses were used so that each laboratory could perform blinded evaluations of the samples. Laboratories were not aware of the IDT results or whether the sample was from a horse without atopy or a clinically affected horse.

Serum allergy test procedure—Two ELISA were used. These assays use different IgE detection reagents that have been described. Briefly, the first ELISA is a commercial assay presently used to measure allergen-specific IgE in dog and cat serum. The IgE readout reagent is a recombinant human FcεRIa chain (rHuFcεRIa). The ELISA wells were coated with allergens (100 to 200 protein nitrogen units [PNU]/well), dried, and stored until used. Serum was diluted 1:31, and 100 ml was added to wells and incubated overnight at 4 C. Wells were washed once, biotinylated rHuFcεRIa was added for 2 hours, and wells were washed once again. Streptavidin-alkaline phosphatase was added for 1 hour, and the reaction was stopped with l-cysteine. Color development was read at 405 nm, and background (reading in wells without serum) was subtracted. Optical density was determined at A490 on an automated ELISA plate reader. Optical densities were corrected for background and reported as integers X 1000 ELISA absorbance (EA) units. The signal was normalized against a heterologous 5-point standard curve generated from pooled serum of clinically normal horses and horses with confirmed allergies. The allergen-specific IgE concentration was reported as a class score in EA units. A score of 0 to 150 for an allergen was considered positive.

The general principles of the RAST have been described. In addition, equine serum samples were first pre-treated and absorbed with staphylococcal protein A to remove IgG and subsequently absorbed with helminth antigens to decrease the overall concentration of interfering non-specific IgE. Antigen-impregnated cellulose disks were immersed in equine serum so that allergen-specific IgE bound to the antigen. After washing, disks were immersed in a solution containing radiolabeled anti-IgG globulin. Radioactivity was measured to reflect the amount of allergen-specific IgE for each allergen. Class scores were reported as: 150, negative; 151 to 199, borderline; 200 to 400, positive; and > 400, highly positive. For the purposes of this study, values ≤ 199 were considered negative. Because of the ambiguity of the meaning of a borderline score, values between 150 and 199 were also considered negative.

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The general procedure for the second ELISA was as follows. Polyclonal antiserum to equine IgE were prepared, as described. Briefly, horse serum was incubated in microtiter plates that were coated with a specific stock concentration of tested allergens followed by the addition of rabbit antihorse IgE serum. Plates were incubated with biotinylated goat antiarabbit globulin followed by avidin coupled to phosphatase. The bound enzyme activity was determined by reading color at 450 nm on a spectrophotometer. The allergen-specific IgE concentration was reported as a test score that was the percentage ratio of negative control serum values. Test scores were reported as follows: < 150, negative; 151 to 199, borderline; 200 to 400, positive; and > 400, highly positive. For the purposes of this study, values ≤ 199 were considered negative. Because of the ambiguity of the meaning of a borderline score, values between 150 and 199 were also considered negative.

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A permanent marker was used to indicate injection sites. Injections were made above and below each mark and were spaced at a distance of 5 cm apart. A positive histamine control (0.05 ml) at a concentration of 1:100,000 (w/v) and a negative phosphate-buffered allergen diluent control (0.05 ml) were used on each horse.

Concentrated skin test allergens were purchased from a commercial allergy laboratory, and dilutions were prepared with phosphate-buffered saline allergen diluent, according to standard protocols. Seventy-three specific allergenic extracts consisting of individual species and species-related mixes were tested, including 24 molds, 11 weeds, 16 trees, 15 grasses, and 7 insects. Calcofines extract was available at the beginning of the study and was not tested in 7 of the horses without atopy, 2 of the AD-affected horses, 5 of the RU-affected horses, and 9 of the COPD-affected horses. Grain mill dust 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. All allergens were tested at 1,000 PNU/ml dilution, except insect extracts, grain mill dust, and grain smut mix extracts that were tested at a 500 PNU/ml dilution. Black ant extract was tested at 1,000 PNU/ml injection. A 0.05 ml of each allergen was made ID with a 26-gauge needle. Injection sites were evaluated at 30 minutes for immediate reactions. Reactions were judged subjectively on a scale of 0 to 4, where a + reaction was equal to the histamine-induced reaction, and 0 was equal to the saline solution-induced reaction. Intermediate reactions were graded 1+, 2+, or 3+. Criteria for the subjective score included erythema, induration, and diameter of the wheal. Reactions were considered positive.

Euthanasia, necropsy, and tissue analysis—To ensure that horses without atopy did not have subclinical COPD, they were euthanized and necropsied. After the 24-hour IDT reading, all horses without atopy were sedated with xylazine (0.5 mg/kg, IV). A 14-gauge IV jugular catheter was placed, and euthanasia solution (1.0 ml/4.5 kg, [2.2 ml/10 lb], IV) was injected. At necropsy, the lungs were physically examined, and tissue specimens were collected from the left dorsal cranial region. Specimens were fixed in phosphate-buffered 10% formalin, embedded in paraffin, sectioned at 3.0 to 5.0 µm, and stained with H&E; periodic acid–Schiff, Alcian blue, Masson’s trichrome, and neutral red stains. One pathologist categorized each specimen as normal or abnormal on the basis of a classification scheme that was devised by ranking the known histologic features of COPD on a 2-point scale.

Statistical analyses—Data analysis for sensitivity, specificity, PPV, and NPV was conducted by use of software for calculation of 2 × 2 tables. Sensitivity was defined as the proportion of horses with the target disorder that had a positive test result, represented as the true positive results divided by the sum of the true positives and false negatives. Specificity was defined as the proportion of horses without the target disease that had a negative test result, represented as true negative results divided by the sum of the false positives and true negatives. The PPV was the proportion of horses with a positive test result that had disease, represented as true positives divided by the sum of the true positives and false positives. The NPV was the proportion of horses with a negative test result that were free of disease, represented as true negatives divided by the sum of the false negatives and true negatives. Predicative values are relative, varying with the prevalence of disease in the population.

The level of agreement between the IDT and SAT was expressed as the K, which evaluated the proportion of agreement that occurred beyond that expected because of chance. The agreement beyond chance was calculated as the proportion of observed agreement (Pₒ) minus the proportion of expected agreement (Pₑ). The maximum possible excess was 1 – Pₑ. The K is a ratio of these 2 differences:

\[ K = Pₒ - Pₑ / 1 - Pₑ \]

Generally, K values of –1.0 indicate perfect disagreement, ± 0.0 to 0.2 indicates slight agreement, ± 0.2 to 0.4 indicates fair agreement, ± 0.4 to 0.6 indicates moderate agreement, ± 0.6 to 0.8 indicates substantial agreement, and ± 0.8 to 1.0 indicates almost perfect agreement between tests.

Analysis of bronchoalveolar lavage data was conducted by use of a mixed-effects multiple regression model to determine whether significant differences were present in percentage of neutrophils, total WBC count, and age among the 4 groups. Differences were considered significant at P ≤ 0.05 for all tests.

Results

Age, CBC, biochemical profile, and respiratory tract examination—The COPD-affected horses were significantly (P < 0.001) older than the horses without atopy (mean ± SD, 14 ± 4 and 6 ± 5 years, respectively). There were no significant differences in age among the horses without atopy and AD- (11 ± 6 years) and RU-affected (8 ± 5 years) horses. Values for CBC and biochemical profiles of all horses were within reference ranges for our laboratory. Results of respiratory tract examinations were normal in the horses without atopy and RU- and AD-affected horses. In COPD-affected horses, crackles or fine crackles were audible in at least 1 peripheral lung field in 16 of 16 horses, diffuse wheezes were auscultated in 11 of 16 horses, and 9 of 16 horses had a cough at rest. Mean resting respiratory rate for the COPD-affected horses was 35 ± 12 breaths/min. None of the horses without atopy or COPD-affected horses had evidence of skin disease.

Bronchoalveolar lavage—The proportion of neutrophils observed in cytologic preparations of the bronchoalveolar lavage fluid was ≥ 17% in 14 of 16 COPD-affected horses. In 2 of 16 COPD-affected horses, the cells were too degenerate to identify, and percentage and absolute numbers of neutrophils could not be determined. The COPD-affected horses had a significantly (P = 0.001) greater percentage of neutrophils in the lavage fluid (mean, 53 ± 36%), compared with the horses without atopy (mean, 9 ± 8%) and the AD- (6 ± 5%) and RU-affected (8 ± 6%) horses. For the horses without atopy and RU- and AD-affected horses, the proportion of neutrophils was ≤ 17%, which was within the reference range reported for healthy horses. There were no significant differences in percentages of neutrophils among the horses without atopy and AD- and RU-affected horses.

Serum allergy tests—Overall, the percentage of false-positive reactions (positive reactions to an allergen that did not elicit a positive IDT reaction) was low for the polyclonal-based ELISA (11%) and the FcεRI-based ELISA.
The FcεRIα-based ELISA had the overall highest percentage of false-positive reactions (41%). The lowest false-negative reactions (negative reactions to an allergen that elicited a positive IDT reaction) were obtained by the RAST (58%). The FcεRIα-based ELISA and polyclonal-based ELISA had false-negative reactions to 63 and 89% of antigens that elicited a positive IDT reaction, respectively (Table 1).

The FcεRIα-based ELISA had the overall highest K, PPV, and NPV when considering all allergens tested in all horses (Table 2). Compared with the IDT, overall sensitivity was similar for the RAST and the FcεRIα-based ELISA; the polyclonal-based ELISA had a notably lower sensitivity. The SAT with the overall greatest specificity, compared with the IDT, was the polyclonal antibody-based ELISA; the FcεRIα-based ELISA also had good specificity overall.

Results of the 3 SAT were evaluated for agreement beyond chance with the IDT results in each group of horses (Table 3). The greatest K, PPV, and NPV for every diagnosis category were obtained by the FcεRIα-based ELISA, with the exception of the NPV for RU-affected horses in which the RAST had the highest value. A K of 0.330 represented the highest value for any diagnosis category and revealed that there was fair agreement in AD-affected horses between the FcεRIα-based ELISA and IDT results. For the RAST and the polyclonal-based ELISA, the K consistently indicated slight disagreement to slight agreement with the IDT results in all diagnosis categories. For COPD-affected horses, the agreement beyond chance between SAT and IDT results was notably lower than the overall K for each SAT (Tables 2 and 3).

Results of the 3 SAT were evaluated for agreement beyond chance with the IDT results in each allergen group (grasses, insects, molds, weeds, and trees; Table 4). The FcεRIα-based ELISA had the highest K, PPV, and NPV for all allergen groups when compared with the IDT. The FcεRIα-based ELISA and polyclonal-based ELISA had false-negative reactions to 63 and 89% of antigens that elicited a positive IDT reaction, respectively (Table 1).

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were observed with insect and weed allergens. The K for the FcεRIα-based ELISA for mold allergens was substantially lower than any other K with this assay in large part because of low sensitivity of this assay in this allergen group. The PPV for mold allergens was the lowest for any allergen group in all SAT as well as lower than the overall PPV. The IDT results of the horses without atopy and RU-, AD-, and COPD-affected horses have been reported.29,30

Necropsy and histologic examination of lungs—Physical examination of lung tissue in all horses without atopy revealed no gross abnormalities for horses without atopy. Parenchymal histologic scores of the lung specimens from the left dorsal cranial region were all well below values described for COPD-affected horses, thus, excluding the possibility of subclinical COPD.29,30

Discussion

To our knowledge, this is the first study to compare IDT results with results of SAT in horses with or without atopic disease. Although it is difficult to define the absolute diagnostic sensitivity and specificity of serum IgE antibody assays, because there is no completely reliable reference method for defining sensitivity or specificity to an allergen, most clinical studies compare results of SAT with results of in vivo diagnostic tests (skin tests or inhalation challenge tests) and with case histories of atopic patients.7

All 3 SAT evaluated in the study reported here had poor sensitivity. We are not aware of any published data regarding the specificity or sensitivity for equine IgE of the allergen-specific anti-IgE detection reagents that were used. The low sensitivity may be attributable to lack of ability of these anti-IgE reagents to detect equine IgE. Alternatively, IgE heterogeneity may be present in horses, as it is in humans38 and dogs,39 and may be physiochemical and functional. Peng et al39 reported the existence of IgE subclasses in dogs; monoclonal antibodies could detect 1 subset of IgE but not the other.39

Low sensitivity in SAT could result from differences in concentrations of allergen-specific IgE in the serum and the skin. Performing SAT at the peak of the allergen season should optimize the assays' performance, because serum allergen-specific IgE increases as a result of exposure to pollen and insects.40 In humans, concentrations of specific IgE typically peak approximately 4 weeks after a seasonal pollen exposure and then gradually fall to a nadir before the next pollen season.40,41 In dogs, serum allergen-specific IgE titers may decrease quickly after exposure to allergens is avoided, whereas skin hypersensitivity may persist for many months, even after results of serologic allergen-specific IgE assays become negative.42 Because all horses in the AD-, RU-, and COPD-affected groups had active disease upon entry into the study, it is highly likely that they all had current or recent allergen exposure. Thus, it would seem unlikely that the low sensitivity in our study was caused by low serum IgE concentrations attributable to lack of recent allergen exposure.

Table 4—Performance of 3 serum allergy tests for groups of allergens in 33 horses with atopic diseases and 22 horses without atopy, with the intradermal test as the criterion standard

<table>
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<tr>
<th>Allergen group</th>
<th>Variable</th>
<th>FcεRIα-based ELISA</th>
<th>Polyclonal antibody-based ELISA</th>
<th>RAST</th>
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<tr>
<td>Grasses</td>
<td>K</td>
<td>0.183</td>
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<td></td>
<td>Sensitivity (%)</td>
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<td></td>
<td>Specificity (%)</td>
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<td></td>
<td>NPV (%)</td>
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<td>Insects</td>
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<td>9.1</td>
<td>16.3</td>
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See Table 2 for key.
Other considerations regarding the low sensitivity of the SAT in our study include the source of allergens, quality of allergens, density of the allergens coupled to the solid support, the material used for the solid support, and the degree of nonspecific IgE binding by the solid support.\textsuperscript{41,47} Details regarding allergens and solid support used in 2 of the assays\textsuperscript{89} could not be obtained (proprietary information). Results of SAT and IDT usually correlate best when relatively pure and potent allergens are used.\textsuperscript{13,43} The absence of allergens standardized for biopotency is potentially a major problem in equine allergy diagnosis.

It is possible that the low sensitivity of the SAT may reflect the presence of false-positive IDT reactions. False-positive irritant reactions may occur with molds and insect allergens.\textsuperscript{33,34} However, only 5 allergens (4 of the molds [grain smut mix, grain mill dust, Candida albicans, and Rhizopus mix] and 1 insect [black ant]) consistently had high prevalence of positive 30-minute IDT reactions in all groups of horses. Therefore, the low sensitivity of these SAT is unlikely attributable to false-positive irritant reactions to these 5 allergens, which accounted for < 10% of all allergens tested.

Reviews on the use of RAST and ELISA in humans indicate that each is capable of differentiating clinically normal individuals from highly reactive patients but not from less allergic individuals.\textsuperscript{44,46} In our study, we did not consider the strengths of the reactions to each allergen with either IDT or SAT. However, in considering the notion that SAT may perform better than the IDT in distinguishing horses with atopic disease from horses without atopy, we note that the performance of each SAT was similar in horses without atopy and atopic horses, with the exception of the FceRIa-based ELISA in AD-affected horses. Thus, we consider it unlikely that SAT have superior ability to distinguish between clinically affected and nonatopic horses.

Although a test's sensitivity and specificity are important properties, clinicians should be concerned with a test's predictive value (the probability that a test result reflects the true disease status) and K (agree-ment). Results of the study reported here indicate that the FceRIa-based ELISA provides the best overall correlation with IDT allergen reactions when the K, PPV, and NPV are considered. The better statistical concordance with the IDT, compared with other SAT, may be a result of using the high-affinity mast cell receptor for IgE (FceRIa), rather than anti-IgE antibodies, to measure concentrations of allergen-specific IgE in serum. In humans, the FceRIa receptor has absolute specificity for IgE, thus, eliminating binding of IgG.\textsuperscript{27} The FcεRIa-based ELISA retained good specificity in all groups of horses.

The polyclonal-based ELISA had the overall highest specificity (89%) but the lowest sensitivity and K among the 3 SAT. This assay had poor sensitivity, with 89% false negatives. Thus, approximately 9 of 10 allergen hypersensitivities would not have been identified, and these allergens would not have been considered for inclusion in an immunotherapy vaccine. The RAST had the highest specificity (43%), but specificity was markedly lower than the other assays. The RAST had the lowest specificity (59%) and, subsequently, the highest false-positive percentage (41%) of all the assays. This was surprising, because the laboratory that performs the RAST pretreats serum samples with staphylococcal protein A to remove circulating allergen-specific IgG and absorbs serum with helminth antigens to remove nonallergen-specific IgE. Both of these procedures should decrease the number of false-positive reactions and improve specificity. The lack of specificity with the RAST could conceivably be attributable to the presence of N-linked carbohydrate groups of glycoproteins that induce IgE, leading to cross-reactivity between several foods and grass allergens.\textsuperscript{67,68} These so-called cross-reactive carbohydrate determinants (CCD) are present on glycoproteins of vegetables.\textsuperscript{67,68} Cross-reactive carbohydrate determinants from several allergenic sources induce specific IgE, and these epitopes are shared by taxonomically distant allergenic sources such as pollens from unrelated botanical families, foods, and arthropods.\textsuperscript{46,49,50} Positive RAST results in humans, induced by the presence of anti-CCD IgE, are estimated to occur in 10 to 15% of patients sensitized to grass pollens.\textsuperscript{49} Our population of horses without atopy and atopic horses had been sensitized to many airborne pollen allergens as well as to arthropod allergens. The possibility of the occurrence of CCD specific IgE, although not investigated, cannot be discounted.

Evaluation of the 3 SAT for agreement with the IDT results in each group of horses revealed that the FcεRIa-based ELISA had the highest K in each group, with fair agreement in the horses without atopy (0.238) and AD-affected horses (0.330). The K for the polyclonal-based ELISA and RAST were poor; results of the polyclonal-based ELISA were in only slight agreement with the IDT in RU-affected horses, and results of the RAST were only in slight agreement with the IDT in COPD- and RU-affected horses. The poor performance of all 3 SAT in RU- and COPD-affected horses may indicate that circulating IgE concentrations are not as important as local or tissue-bound IgE in the pathogenesis of these allergic diseases. Support for a central role of local pulmonary IgE antibody versus a systemic antigen-specific antibody production in the immunopathogenesis of COPD has been reported by Halliwell et al.\textsuperscript{16} Local IgE and IgA antibody concentrations, detected by ELISA, to Micropolyspora faeni and Aspergillus fumigatus were increased in bronchoalveolar lavage fluid but not in sera of COPD-affected horses.

A third of humans with chronic urticaria have circulating autoantibodies directed against the high-affinity FcεRIa IgE receptor or against IgE.\textsuperscript{32,39} Although these autoantibodies have not been reported in horses, binding of autoantibodies to allergen-specific IgE as well as to FcεRIa detection reagent could cause false-negative results.

The polyclonal-based ELISA and RAST had the lowest K, PPV, and NPV of the 3 SAT, compared with results of the IDT for each allergen group (grasses, insects, molds, weeds, and trees). The FcεRIa-based ELISA had the highest K as well as the greatest PPV and NPV for all allergen groups. For the FcεRIa-based ELISA, the mold allergen K was markedly lower than the K obtained from the other allergen groups. This
low value may further support the suggestion that positive mold IDT reactions are irritant reactions, because the standardization of these extracts is lacking in veterinary medicine. Standards for reagent preparation of molds must address demonstrated differences among strains (ie, different isolate forms of 1 species) and batch-to-batch variations that develop during serial culture of single strains. Evidence exists that extracts of spores alone, spore-free hyphae, and separated metabolite products of single species differ allergenically, which emphasizes the need to define preparation methods when comparing IDT and SAT. Likewise, mold hypersensitivity reactions may not be IgE-mediated. The mold Candida albicans has been used in humans to evaluate the overall capacity of an individual to generate cell-mediated delayed-type hypersensitivity responses.

It is apparent that the performance of the SAT evaluated in the study reported here was poor. It is tempting to speculate that the high specificity of the FcεRIα-based ELISA (85% overall) and the polyclonal-based ELISA (80% overall) may be useful to clinicians. Generally speaking, if a test has high specificity, there are few false positives and a positive result is, therefore, highly likely to be a true positive. However, for the population of horses in our study, this was not true.

First, this generalization only holds true where the prevalence of positive results (in our study, the prevalence of positive IDT reactions) is fairly high. In the study reported here, the prevalence of positive IDT reactions with allergens used in the FcεRIα-based ELISA was 27.8%, and with allergens used in the polyclonal-based ELISA it was 27.1%. Thus, the majority (73%) of IDT reactions were negative. Consequently, although these assays had good specificity, there were still many false positives with the FcεRIα-based ELISA and the polyclonal-based ELISA. This effect is clearly indicated by the lower than expected positive predictive value (48.5% with the FcεRIα-based ELISA and 25.8% with the polyclonal-based ELISA) than is generally seen in tests with high specificity, which usually have high positive predictive values.

Second, if pretest probability (prevalence) of a positive reaction could be increased (for example, careful selection of an atopic patient with disease confined to the spring, which would increase the pretest probability of positive reactions to trees), the value of the SAT would be in its negative test results. However, the low overall sensitivity of the assays would be problematic, because the FcεRIα-based ELISA has 37% sensitivity, whereas the polyclonal-based ELISA has 11% sensitivity. Thus, although allergens to which the patient was truly hypersensitive to would be detected by these assays, most allergens would be excluded from an immunotherapeutic vaccine, and there would be lower probability that such a vaccine would be efficacious.

Therefore, we believe that none of the 3 assays should be used as a screening test for allergen hypersensitivity, and they cannot be used in place of IDT. It is possible that with the careful selection of patients through good history taking, physical examination, rule-out of differential diagnoses, and well-defined disease that is highly suggestive of specific allergen-group hypersensitivity the good specificity of the FcεRIα-based ELISA and polyclonal-based ELISA maybe useful in documenting the lack of allergen hypersensitivity for select allergen groups. The higher sensitivity of the FcεRIα-based ELISA suggests that this assay is more likely to be helpful in these circumstances. However, this possibility remains speculative.

References


43. Tretter V, Altmann F, Kubelka V, et al. Fucose alpha 1,3-linked to the core region of glycoprotein N-glycans creates an important epitope for IgE from honeybee venom allergic individuals. *Int Arch Allergy Appl Immunol* 1993;102:259–266.


