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A commercially available immunoglobulin E-based test for food allergy gives inconsistent results in healthy ponies

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Summary

Reasons for performing study: Commercial immunoglobulin E (IgE)-based tests are available for diagnosis of food allergies and are commonly used in equine practice. However, these tests have been proven unreliable as a screening method in man and other species, but not critically evaluated in equids. Therefore, a commercially available IgE-based test for horses was evaluated.
Objectives: To evaluate the consistency of the results obtained with a commercially available IgE-based test for food allergy diagnosis in ponies (Phase I) and to subject ponies to a provocation trial with the presumed allergens (Phase II).
Study design: Allergen screening followed by experimental food provocation trials in healthy ponies.
Methods: Blood samples of 17 healthy Shetland ponies were taken at 2 different time points, sent blinded to a commercial laboratory for screening of common food allergens and the results were evaluated for consistency (Phase I). Ponies that were positive for food allergens were consecutively challenged orally with each allergen separately for 14 days (Phase II). A washout period of one week was applied in ponies with multiple positive results. Clinical parameters and serum amyloid A were monitored during the provocation trial.
Results: Only 7/17 ponies were negative on the IgE-based test at the 2 time points, 3 had positive results twice but only one tested positive twice for the same food allergen. No abnormalities were noted during the provocation trials.
Conclusions: This study demonstrated that this IgE-based test is not a reliable screening tool for food allergy in healthy equids.

Keywords: horse; food allergy; immunoglobulin E; food provocation test; pony; nutrition

Introduction

Food allergy is characterised by an abnormal immunological response after ingestion of food by a susceptible host, which is reproducible and not dose-dependent [1]. Food intolerance may mimic food allergy, except that it can occur on first exposure since nonimmunological mechanisms are involved [2]. Immune responses linked with allergic reactions can be: 1) IgE-mediated (*type I* hypersensitivity) that lead to immediate or delayed hypersensitivity symptoms or delayed symptoms after mast cell cytokine release; 2) a cytotoxic reaction (*type II*); 3) mediated by immune complexes (*type III*); or 4) cell-mediated (*type IV*) [3].

It has been postulated that a dietary compound could be a cause of idiopathic inflammatory bowel disease (IBD) in horses [4] and some authors have suggested that diets based only on oats, oil and hay to eliminate possible allergens should be used in horses with suspected IBD [5,6]. However, feeding trials have not been performed in these cases and the prognoses were generally poor [5,6]. One case of a possible gluten-dependent enteropathy has been described [7]. However, the elimination diet in this case was not followed by provocation to confirm diagnosis. Gluten has also been implicated in urticaria in sport horses [8,9], the diagnosis was determined by intradermal testing and reintroduction of the food allergen 1–6 months later did not trigger an allergic reaction [8,9]. Urticaria has also been associated with an allergy to garlic in one case, but again this was not confirmed by provocation [10].

Elimination and provocation test protocols are considered the gold standard to diagnose food allergy in man [11], dogs [3,12] and cats [3,13]. Although the occurrence and presentation of food allergy in horses remains unclear [14,15], use of elimination diets for diagnosis is suggested [2,15,16]. Serum immunoglobulin E (IgE)-based tests are commercially available but in other species they are considered unreliable [11–13,17,18]. No IgE-mediated test for food allergy in horses has been clinically validated [15].

The aims of this study were: 1) to evaluate the consistency of the results of a commercially available IgE-based test (Phase I); and 2) to evaluate whether ponies with positive results respond to the presumed food allergen in a provocation test (Phase II).

Materials and methods

A group of 17 healthy mature (age 10.7 ± 3.7 years) Shetland pony geldings were used (bwt 137 ± 33 kg). The study consisted of a 14-day adaptation period followed by 2 phases. In Phase I, 2 blood samples were taken 14 days apart. This was followed by a provocation test in Phase II (Fig 1). During the entire trial, the ponies were fed a basal diet of hay and a commercial vitamin and mineral supplement (Cavalor Nutri Plus)[a] to meet the Nutrient Research Council (NRC) nutrient requirements [19] and had free access to fresh water. Ponies were group-housed in a covered paddock. During the provocation trial in Phase II, the tested ponies were temporarily moved to individual boxes during feeding time in order to feed the suspected allergen.

Phase I

In Phase I, blood samples (16 ml, left jugular vein using a Vacuette needle[b], 20 gauge, 38 mm) were taken on Days -14 and 0. After centrifugation at 3000 ***g*** for 10 min, 5 ml of serum from all samples were sent on dry ice to a commercial laboratory offering screening for food allergens based on detection of immunoglogulins in serum. A general screening (positive/negative) was first performed on samples from both time points, followed by a detailed screening of the positive samples for specific food allergens commonly found in horse diets, including soy, corn, molasses, wheat, sugar beet, oats, alfalfa, rye, carob and barley. The commercial laboratory was not made aware of this study and remained blinded to the samples at all times.

Phase II

In Phase II, the ponies that tested positive for one or more food ingredients during the first screening began provocation trials on Day 1. When ponies

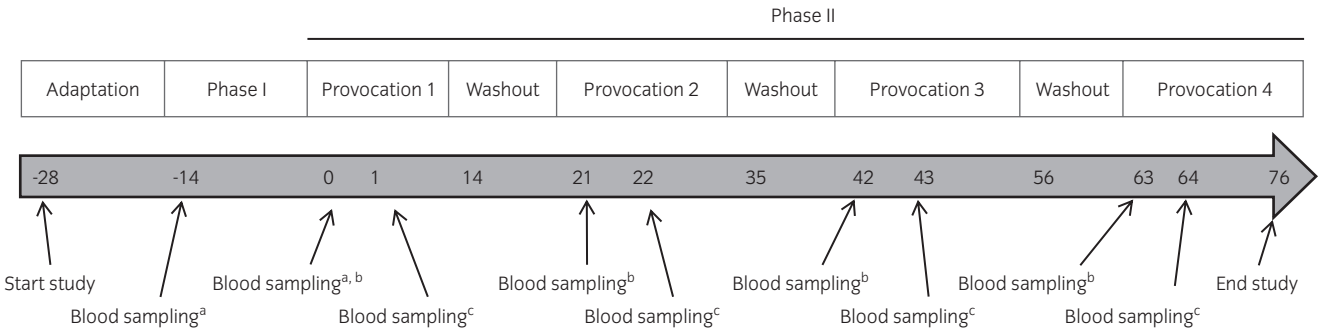

Fig 1: Schematic overview of the experimental design and timeline. Numbers within the arrow represent days. The different experimental phases are indicated above. Small arrows indicate type of interventions and superscripts indicate interventions with different purposes: a) blood sampling for immunoglobulin E test (Phase I); b) blood sampling for packed cell volume (PCV), urea and lactate analysis (Phase II); and c) blood sampling for serum amyloid A (SAA) analysis (Phase II).

tested positive for more than one allergen, each pony was tested for every individual allergen separately in the order shown in Table 1. Each allergen was given separately with a washout period of one week in between consecutive provocation tests, as suggested by Littlewood and Heidmann [20]. The ponies received 100 g of the presumed food allergen twice daily for 2 weeks, in addition to the basal diet of hay and vitamin and mineral supplement. The ponies that were positive for sugar beet were given beet pulp.

Clinical parameters: Before and 30 min after the first feeding, heart rate, respiratory rate and temperature were monitored every hour for 12 h, during the next 36 h, the ponies were checked every 3 h then ponies were monitored 3 times daily for 10 days. At the same times, ponies were checked for urticaria, signs of itching (active scratching, visual inspection for skin lesions), colic and diarrhoea. Urticarial lesions were scored using a previously published system [21] and daily faecal samples were scored for consistency using an arbitrarily chosen 5-point scoring system (1 = severe liquid diarrhoea; 2 = liquid faeces with little structure; 3 = liquid faeces with well-formed parts; 4 = normal consistency; 5 = hard faeces). Half points were used to describe consistencies between the descriptors.

Laboratory parameters: On the first day of the provocation trial, 6 ml of venous blood was drawn from the left jugular vein, preprandial and 6 and 12 h post prandial for analysis of urea (2 ml serum tube, Idexx[c]), packed cell volume (PCV; lithium heparin tube, 2 ml) and lactate (sodium fluoride tube, 2 ml; Accutrend[d]). On Day 2 of the provocation trial, 2 ml of venous blood was drawn 1 h post prandial as described above and centrifuged at 3000 ***g*** for 10 min. Serum was stored at -80°C until analysis of the acute phase protein serum amyloid A (SAA; Eiken SAA TIA[e] on ADVIA1800[f]).

Data analysis: The data obtained at both time points in Phase I were evaluated using the related-samples McNemar test for the general and detailed screening to determine if the consistency of the results was significantly different from one. The correlation between the results of the first and second blood sampling was evaluated using the Spearman rank correlation. Correlations were considered significant when $P<0.05$.

In Phase II, mean and standard deviation were calculated for heart rate, respiratory rate, temperature, PCV, and concentrations of blood lactate and urea measured at one time point preprovocation and at 60 different time points post provocation. Further statistical analysis was not performed.

TABLE 1: Results of the first and second immunoglobulin E screening tests for feed allergens in 17 healthy Shetland ponies. Specific putative feed allergens are given within brackets

Pony	First sampling	Second sampling
1	–	–
2	+ (corn)	–
3	–	–
4	–	–
5	–	–
6	–	–
7	–	+ (rye)
8	+ (soy)	+ (wheat)
9	–	+ (wheat)
10	+ (sugar beet, oats, corn, alfalfa)	+ (soy)
11	–	–
12	+ (oats, sugar beet)	–
13	+ (alfalfa, rye, sugar beet)	+ (rye)
14	–	+ (rye)
15	–	+ (molasses)
16	+ (rye)	–
17	–	–

Results

Phase I

Six ponies had positive results at the first blood sampling and 7 had positive results at the second sampling (Table 1). Of the 10 ponies that tested positive, only 3 were positive on both samplings and of those 3, only one pony was positive twice for the same food allergen. Only 7 ponies were negative at both time points. The general ($P<0.2$) and detailed ($P<0.001$) screening were significantly different from one and thus not consistent. No correlation was found between the first and second sampling and the correlation coefficient was low ($P = 0.5$, $r = 0.058$).

Phase II

Six ponies that had positive results at the first sampling were submitted to provocation tests.

Monitoring of clinical parameters: Clinical data are summarised in Table 2. There were no clinically relevant changes in heart rate, respiratory rate or rectal temperature throughout the experimental period. None of the ponies showed any sign of urticaria or itching. All ponies had a normal faecal consistency with a score of 4/5, except for 2 ponies that both had a score of 3.5/5 at one time point on the second day of the first provocation trial.

Monitoring of blood parameters: There were no clinically relevant changes in PCV, concentrations of blood urea and lactate (Table 2). The SAA concentrations (Table 2) of all but one pony (*Pony 13*) were within the reference range for clinically healthy horses (<2.3 mg/l) [22]. *Pony 13* had increased SAA concentration of 93.2 mg/l during its second provocation trial, which was with rye. This pony's SAA concentration returned to normal in a subsequent provocation test with beet pulp.

TABLE 2: Summary of clinical and laboratory data measured at one time point preprovocation and 60 different time points post provocation to cover a 14-day period during provocation trials of putative allergens previously identified with serum immunoglobulin E testing in ponies. Serum amyloid A (SAA) measured once on Day 2 post provocation. Data are presented as mean ± s.d.

	Pony 2 corn	*Pony 8* soy	*Pony 16* rye	*Pony 12* oats	*Pony 12* beet pulp	*Pony 13* alfalfa	*Pony 13* rye	*Pony 13* beet pulp	*Pony 10* beet pulp	*Pony 10* oats	*Pony 10* corn	*Pony 10* alfalfa
Heart rate (beats/min)	42 ± 3.9	48 ± 4.8	40 ± 3.3	42 ± 4.0	43 ± 3.4	41 ± 3.5	46 ± 3.8	45 ± 3.3	39 ± 3.1	41 ± 3.4	39 ± 3.7	38 ± 3.9
Respiratory rate (breaths/min)	20 ± 5.3	23 ± 6.0	20 ± 3.8	21 ± 4.3	26 ± 6.0	19 ± 3.6	25 ± 6.2	27 ± 6.3	19 ± 3.5	24 ± 6.0	28 ± 7.9	24 ± 5.7
Temperature (°C)	37.0 ± 0.34	37.5 ± 0.22	37.0 ± 0.38	37.0 ± 0.30	37.1 ± 0.31	37.2 ± 0.29	37.3 ± 0.40	37.3 ± 0.32	37.1 ± 0.31	37.3 ± 0.28	37.2 ± 0.36	37.1 ± 0.33
Packed cell volume (%)	28 ± 0.0	32 ± 2.0	33 ± 1.1	29 ± 1.1	28 ± 2.0	25 ± 1.1	24 ± 0.0	25 ± 1.1	33 ± 2.3	31 ± 1.1	27 ± 2.3	31 ± 1.1
Lactate (mmol/l)	1.4 ± 0.15	1.1 ± 0.06	1.3 ± 0.32	1.1 ± 0.17	1.4 ± 0.2	1.8 ± 0.06	1.5 ± 0.51	1.8 ± 0.25	1.1 ± 0.10	1.4 ± 0.10	1.6 ± 0.26	1.4 ± 0.20
Urea (mmol/l)	6.1 ± 0.03	3.9 ± 0.38	3.3 ± 0.20	3.8 ± 0.26	3.9 ± 0.23	5.6 ± 0.15	3.8 ± 0.17	3.4 ± 0.15	3.4 ± 0.26	3.0 ± 0.17	2.7 ± 0.0	2.6 ± 0.12
Serum amyloid A (mg/l)	0.25	0.10	0.25	0.25	0.25	0.05	93.2	0.2	0.25	0.35	0.05	0.25

Discussion

This study showed that a commercially available IgE-based test for equine food allergy in equids did not produce consistent results in healthy ponies. Inconsistent responses were seen in both the general screening and the detailed screening when the first and second time points were compared. Moreover, none of the 6 ponies subjected to subsequent provocation tests with the presumed food allergen, according to the gold standard for food allergy diagnosis [3,11–13], displayed adverse reactions. Similar inconsistent observations have been found with an ELISA [23,24] and intradermal IgE-test for skin allergy in horses and other food allergy IgE-tests in cats, dogs and man [3,11,12,17,18,25–27].

The use of IgE in horses for diagnostic purposes is questionable because high IgE serum concentrations are found in healthy horses [15]. We have been unable to obtain more detailed information on the exact mechanism of this commercial IgE-based test. It is therefore unclear which IgE threshold was used, how the analysis was performed, and which source of detection of antibodies or positive standard was used. Thus, we cannot comment on the exact reason for the inconsistent results of this test but the results of this study confirm a previous assertion that there is no blood test available with published data on validation to use as a method to diagnose food allergy [27].

Food allergy in horses has not been clearly described and skin allergy due to insect bite hypersensitivity remains the only recognised IgE-mediated allergy in horses. However, sensitisation to allergen-specific IgE is not equal to an allergy where clinical signs need to be taken into account for the diagnosis [11,27]. These discrepancies may explain the lack of positive provocation tests in the current study, but not the inconsistency between samples taken at 2 different time points.

As there are no protocols described in literature, expected clinical signs monitored during these provocation tests were extrapolated from other species and monitoring for urticaria, itching and faecal score, were based on clinical signs described in dogs and cats [3]. Colic was included because this is a common sign of equine gastrointestinal problems. The parameters tested in the blood were based on commonly used parameters for evaluating shock in horses [28] and these were selected so that appropriate treatment could be instituted had anaphylactic shock occurred. Flaring of the skin as a possible sign of food allergy was not monitored in the present study. Nevertheless, it was concluded that the provocation tests were negative since none of the ponies showed adverse clinical signs except mild changes in faecal consistency of short duration in 2 cases.

The acute phase protein SAA was used to monitor subclinical and clinical inflammation and tissue damage [29,30]. SAA has been used as a marker for horses with heaves [31], an asthma-like disease with an allergic component [32]. In human asthma patients, SAA is a useful marker [33,34]. However, SAA has not been tested yet in horses with food allergy [35–37]. Samples were taken on Day 2 of the provocation trial for SAA analysis as a rapid and high increase in SAA concentration would be expected after only a few hours, reaching its peak after 24–48 h [22,30] with a more than 10-fold increase and short half-life [37]. Based on these values, a one-week washout period between provocation trials was considered sufficient to prevent overlap of reactions.

Pony 13 had a SAA concentration above the reference range for healthy horses [22,35] during its second provocation trial with rye. We considered that this increase was not clinically relevant as typically horses with inflammation have much higher concentrations [37]. However, normal SAA concentrations in healthy horses are very low, so this moderate increase could indicate that an inflammatory stimulus was present [36]. Interestingly, this increase was only seen in the pony that tested positive for the same food allergen, rye, twice. It is, however, unclear if the increase in SAA indicated hypersensitivity to rye, or if there was, for example, a small wound present that was not detected.

None of these ponies had a history of allergy, thus sensitisation without clinical signs might have been present. However, this does not explain the lack of correlation between samples. The study design would have benefited from the inclusion of a positive control group of horses with proven food allergy; however, such a group has not been identified yet.

Conclusions

An IgE-based food allergy test led to inconsistent results in healthy ponies. Misleading results from this commercially available test could lead to unnecessary or inappropriate changes in food management when eliminating the presumed food allergens from the daily ration.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine of Ghent University, Belgium (EC 2012/64).

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Authorship

S. Dupont contributed to data collection and study execution and was responsible for data analysis and interpretation, and preparation of the manuscript. A. De Spiegeleer was responsible for the study design and execution, and data collection, and contributed to data analyses and interpretation. D.J.X. Liu contributed to data collection and preparation of the manuscript. L. Lefère contributed to the study design, data analyses and interpretation. D.A. van Doorn contributed to the study design and preparation of the manuscript. M. Hesta (supervisor of S. Dupont, A. De Spiegeleer and D.J.X. Liu) contributed to the study design, data collection, study execution, data analyses and interpretation, and preparation of the manuscript. All authors contributed to and approved the manuscript.

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[c]Catalyst, Hoofddorp, The Netherlands.
[d]Hoffmann-LaRoche, Bazel, Switzerland.
[e]Eiken, Shanghai, China.
[f]Siemens, Munich, Germany.

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