

Background

Allergy to horse is of clinical significance, especially for those with direct exposure – either occupationally or recreationally, but also for many with indirect exposure.^{1, 2} However, medical literature covering horse allergy is limited compared to other common mammalian allergies, such as dog and cat.

There are several FDA-approved horse allergen extracts available for horse allergy diagnosis and immunotherapy in the US. All are non-standardized and labeled for potency in weight-to-volume (w/v). The AP Horse Hair & Dander product (HollisterStier) undergoes a unique acetone precipitation process to produce a highly-concentrated intermediate and extract.

The major horse allergen Equ c 1 is found in hair, dander and saliva. 76% of horse-allergic patients have serum IgE to Equ c 1.³ There are no published studies comparing the protein or allergen content of the commercially available horse extracts.

There are also no published studies that compare the outcomes of skin prick testing (SPT) using AP Horse extract compared to conventional horse hair and dander extracts. In theory, the more concentrated AP Horse extract may be better at confirming allergy to horse.

Purpose

The study objective was to characterize commercially available horse allergen extracts using qualitative and quantitative methods, and to retrospectively review skin prick test responses from a single allergy clinic.

References:

1. Arseneau et al., Military Medicine 2012
2. Liccardi et al., Respir Med 2009
3. Dandeu et al., J Chromatogr 1993
4. Goubran et al., Eur J Biochem 2001
5. Cabanas, et al., J Investig Allergol Clin Immunol 2000

Methods

Commercial Allergen Extracts

- Obtained from Extract Manufacturers
- AP Horse Hair & Dander, 1:50 w/v (HollisterStier)
- Horse Epithelium, 1:10 w/v (ALK)
- Horse Epithelium, 1:20 w/v (ALK)
- Horse Epithelia, 1:20 w/v (Greer)

Characterization Methods

- Total Protein – commercially available Bradford Assay
- Equ c 1 Allergen sandwich ELISA – developed by InBio
- Protein Profiles using SDS-PAGE with Silver Stain
- Allergen Profiles using Western Blotting
- Mass Spectrometry Compositional Analysis (InBio)

Skin Prick Testing

- Clinic: Allergy & Asthma Care (Missoula, MT)
- 84 patients tested with 3 commercially available Horse Allergen Extracts as part of routine evaluation for allergy.
 - Patients tested included those with a history of reaction after being around horses, currently exposed to horses, or had plans to purchase a horse or take-up riding lessons.
- Tested using Quintip® Skin Test Device (HollisterStier)
- Positive SPT if wheal 3 mm or greater than negative control
- Retrospective review of chart data to compare SPT results.

Results

Fig. 1 – Total Protein (Bradford Assay)

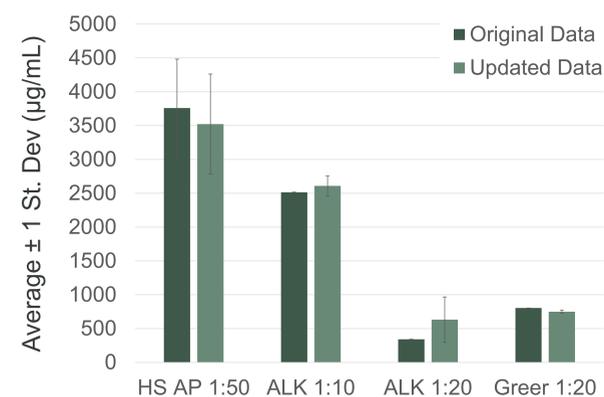
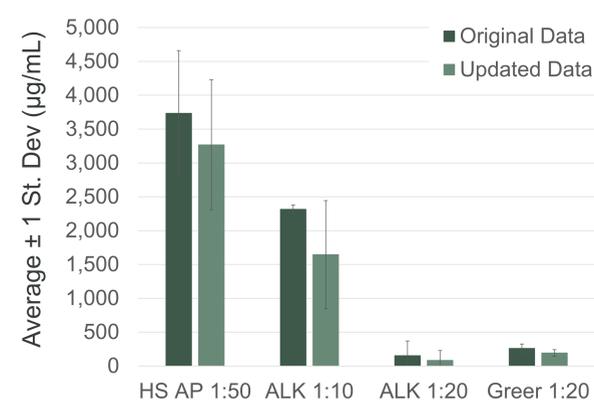


Fig. 2 – Equ c 1 Major Allergen (ELISA)



Mass Spectrometry data not shown – see Discussion section.

Table 1 – Skin Prick Test

	All Extracts	HS AP 1:50 w/v	ALK 1:20 w/v	Greer 1:20 w/v
Positive SPT	35	35	31	32
% Positive	42%	42%	37%	38%
% of All Positive	----	100%	89%	91%

Fig. 3 – SDS-PAGE (Silver Stain)

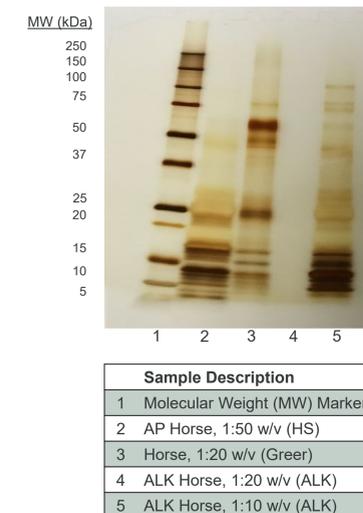
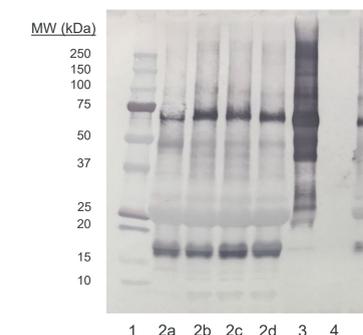


Fig. 4 – Western Blot



Discussion

The World Health Organization, Allergen Nomenclature Sub-Committee recognizes five horse allergens: Equ c 1, Equ c 2, Equ c 3, Equ c 4, and Equ c 6. Mass Spectrometry was able to detect Equ c 1 through 4, but not Equ c 6 in 1:50 w/v AP Horse Hair and Dander and 1:20 w/v Horse Epithelia (Greer). Equ c 1 was the most dominant allergen in terms of percent abundance. Only Equ c 1 was detected in 1:20 w/v Horse Epithelium (ALK).

Development of assays to quantify the other known horse allergens would be useful. Equ c 4 has been shown to have a high IgE binding frequency in horse-sensitized individuals.^{4, 5} Equ c 3 may also be important as it is cross-reactive with other animal albumin allergens.⁵

Of the total number of patients testing positive to all Horse allergen extracts (42%), 100% were positive using HollisterStier 1:50 w/v AP Horse extract, while 89% were positive using ALK 1:20 w/v extract and 91% were positive using Greer 1:20 w/v extract.

Continued extract characterization is recommended. Additional studies are also necessary to determine the therapeutic effective dose range for horse allergen immunotherapy.

Conclusions

There is a wide range of protein and allergen content in commercially available horse extracts. AP Horse contained about 1.6-times more Equ c 1 than 1:10 w/v extract and about 18-times more than 1:20 w/v extracts. AP Horse detected more SPT positive patients, compared to other available extracts. Additional studies may reveal the specific relationship between Equ c 1 and biologic potency and clinical response of the commercial extracts.