

Allergens of horse dander: Comparison among breeds and individual animals by immunoblotting

Kumar Felix, PhD, Rafael Ferrándiz, PhD, Roland Einarsson, PhD,
and Sten Dreborg, MD, PhD Linköping, Sweden

Some patients who are allergic to horses have reported that they can tolerate certain breeds, and the presence of breed-specific allergens has been suggested. Breeders and patients with asthma have claimed that Bashkir horses are nonallergenic. We used sodium dodecylsulfate-polyacrylamide gel electrophoresis and immunoblotting to determine IgE-binding profiles of extracts of dander obtained from horses of several breeds. We found considerable inter-breed and within-breed variation but no breed-specific allergens. Danders from all breeds investigated contained the most important allergens, and the allergenic content of dander from Bashkir horses was similar to that of other breeds. Differences in scale production could account for differences in sensitivity to breeds and individual horses. (J Allergy Clin Immunol 1996;98:169-71.)

Key words: Horse dander allergens, horse breeds, allergen extracts, sodium dodecylsulfate-polyacrylamide gel electrophoresis, immunoblotting, IgE

Horses have been recognized as an important source of allergens. The composition of horse dander allergen extracts has been studied, and major allergens have been identified.¹⁻⁴

Some patients allergic to animals, especially those allergic to dogs, report that they tolerate some breeds but not others. The presence of breed-specific allergens has been suggested,⁵ but that study was performed with extracts prepared from mixtures of dander from several animals of each breed. There are no reports on the variation in allergenic composition among individuals of the same breed.

We report on the allergenic composition of extracts prepared from dander from individual horses of several breeds.

METHODS

Horse dander was obtained by grooming 23 horses of seven different breeds and four animals of the Bashkir

breed. The collected dander was sieved (pore size of 300 μ m), defatted with chloroform, and then extracted.⁶

Serum samples were obtained from 20 patients with asthma, rhinoconjunctivitis, or both who were allergic to horse as determined by history, skin prick test, and/or RAST. The patients had not undergone immunotherapy with horse allergen. On the basis of their IgE binding as determined by Magic Lite (ALK, Hørsholm, Denmark) and by immunoblotting, serum samples from each of 11 selected patients were mixed to make a serum pool. Extracts were run in sodium dodecylsulfate-polyacrylamide gel electrophoresis, 8% to 25% under nonreducing conditions, blotted to nitrocellulose membranes by using the Phast System (Pharmacia, Uppsala, Sweden), and then radiostained.⁶ An arbitrary scale of 0 to 5+ was used to grade radiostaining.

RESULTS

The dander extracts showed 19 different allergenic components with molecular weights ranging from 14 to 130 kd. Each horse dander extract contained 6 to 15 well-resolved IgE-binding proteins (Table I). There were no marked differences between breeds regarding the number of allergenic components. No two extracts showed similar IgE-binding profiles. In the molecular weight range between 14 and 33 kd, all extracts showed a high but varying uptake of radioactivity. Regardless of individual horse or breed, all extracts contained the 14, 19, 27, and 33 kd bands.

From the Department of Paediatrics, Faculty of Health Sciences, University of Linköping, Sweden.

Supported in part by the Swedish Institute.

Received for publication June 14, 1995; revised Sept. 19, 1995; accepted for publication Oct. 11, 1995.

Reprint requests: Sten Dreborg, MD, PhD, Department of Paediatrics, Level 14, University Hospital of Linköping, S-581 85, Linköping, Sweden.

Copyright © Mosby-Year Book, Inc.

0091-6749/96 \$5.00 + 0 1/1/69884

TABLE I. Schematic representation of radiostaining of IgE-binding proteins in 24 horse dander extracts with pooled human sera

Horse breed	Animal	Molecular weight (kd)										
	No.	14	19	27	30	33	35	39	43	51	67	74
Swedish Halfbred												
	1	+++	++	+	++	++			+		+	
	2	+++	++	+	+	+++	++	+			+	+
	3	+++	+++++	+++	+	+++++	++	++		++	++	+++
	4	++	+++	++	+	+++		+		++	+++++	+++++
	5	+++	+++++	+++++		+++++		+++	++	++	++	++
Haflinger												
	1	+++++	+++++	+++++	++	+++++		+++++		+		+
	2	+++++	+++++	+++++	++	+++++		+++++		+	+	
	3	++++	+++++	+++++	+++	+++++		+++			+	+
	4	+++++	+++++	+++++	+	++		++	+			+
	5	++	++	++		+++	+	++				+
Arabian thoroughbred												
	1	+++	++++	+++++		++++	+	+++	++		++	+
	2	+++	+++++	++	++	++		+++++			+	++
	3	++++	++++	++	+++	+++++	++	++		+		++
	4	+++	+++	+	++	++		+		+		
	5	++++	+++++	+++++	++	++++	++	++	++	++		++
North Swedish Horse												
	1	++++	+++	+++	++	++++						
	2	+++	+++	+++		+++		+	+		+	+
	3	++	++++	+++		+++		+++		++++		+++
	4	+++	+++++	++++	++	+++++	++	+++		++	++	++
	5	++++	+++++	+++++	++	+++++		++	+	++		
Bashkir Curly												
	1	+++	++	++	++	+++	+	+	+			+
	2	++++	+++++	+++++	++	++++	+	+++	++		++	++
Russian Bashkir												
	Mix	+++	+++	++	++	++++	++	++	+	++		
American Trotting Horse												
	1	++	+++++	+++++		+++++	+	++	+++		+	++++

The 39 kd component was represented in 22 extracts (92%), and the 30 and 74 kd bands were found in 18 extracts (75%); whereas the other allergens were less often represented (30% to 54%). In almost all of the extracts, the 19 kd allergen showed a higher uptake of specific IgE than the other bands, as indicated by both the intensity and width of the band. No particular allergen was unique for any breed. The 125 kd allergen was represented in all the extracts from Hafinger horses, but in only two other extracts and only at low concentrations.

The two extracts of dander from Bashkir horses,

the breed claimed to be nonallergenic, had allergenic composition similar to that of the other breeds.

In the selection of sera for the serum pool, 80% of the patients showed specific IgE binding against the 19 kd allergen. The majority of patients (i.e., more than 50%) also had specific IgE antibodies directed against the 14, 27, 39, and 74 kd allergens (data not shown).

DISCUSSION

The number of allergens found was similar to that reported earlier.¹⁻³ Three major horse aller-

Molecular weight (kd)							
80	88	94	100	110	115	125	130
+	++	+		+	+		
+++		++					
++++		++		+	+		++
++		++		+	+	+	++
	++		++			++	
+	+	+		+	++	++	
	++			+	++	++	
	+		+			++	++
++	+		++	+		++	
+				+	++		
++		++	++		++		
	+		+		++		
++		+	+			+	
			+				
+		+					
++	+++			++			++
	++				+		++
	+	+		+	+		
	++						+
		+	+				++
++	++	+		+	++		

gens have been reported: Equ c 1 (Ag 6, 19 kd), Equ c 2 (Ag 9, 51 kd), and Equ c 3 (Ag 11, 31 kd).^{2,4,7} We found four components (14, 19, 27, and 39 kd), which could be considered major allergens according to the degree and frequency of IgE binding.

The allergen Equ c 1 is probably identical to the 19 kd allergen found in our study and in the study by Løwenstein et al.²; Equ c 3 corresponds to our 27 kd allergen, and the 31 kd allergen corresponds to the major allergen, Ag 11, described by Løwenstein et al.² Our data indicate that two other allergens fulfill the criteria for major allergens, the

14 kd and 39 kd proteins. We could not confirm frequent binding to Equ c 2.

Breeders and patients with asthma have claimed that Bashkir horses are nonallergenic. Our study showed that the allergenic content of dander from Bashkir horses is similar to that of other breeds. Because less dander is released by these long-haired animals, the amount of dander rather than its allergenic content could be the explanation for the perceived low "allergenicity." Variations in allergenic composition between individual danders have not been studied among other animal species. Because horses deliver much more dander than dogs, we obtained enough material to study the variation between individuals of the same breed. We found considerable inter-breed and within-breed variation, both qualitatively and quantitatively. No breed-specific allergens were found.

We conclude that there is pronounced variation in the allergenic composition of dander extracts among individual horses. Danders from all horse breeds investigated contained the most important allergens. We hypothesize that the differences in scale production could account for the differences in sensitivity to breeds and individuals noted by patients.

We thank Ing-Marie Sandberg for collecting patient sera and Kerstin Hagerstén for valuable support in the laboratory.

REFERENCES

1. Franke D, Maasch HJ, Wahl R, Schultze-Werninghaus, Bretting H. Allergens of horse epithelium. I. Physicochemical and immunochemical characterisation of five different horse epithelium raw materials used for allergen extract preparation. *Int Arch Allergy Appl Immunol* 1990;92:309-17.
2. Løwenstein H, Markussen B, Weeke B. Isolation and partial characterisation of three major allergens of horse hair and dandruff. *Int Arch Allergy Appl Immunol* 1976; 51:48-67.
3. Fjeldsgaard BE, Smedstad Paulsen B. Comparison of IgE-binding antigens in horse dander and a mixture of horse dander and skin scrapings. *Allergy* 1993;48:535-41.
4. Dandeu JP, Rabillon A, Divanovic A, Carmi-Leroy, David B. Hydrophobic interaction chromatography for isolation and purification of Equ.c1, the horse major allergen. *J Chromatogr* 1993;621:23-31.
5. Lindgren S, Belin L, Dreborg S, Einarsson R, Pålman I. Breed specific dog-dandruff allergens. *J Allergy Clin Immunol* 1988;82:196-204.
6. Ferrándiz R, Casas R, Dreborg S, Einarsson R, Bonachea I, Chapman MD. Characterization of allergens from house dust mite *Dermatophagoides siboney*. Purification of Der s 1 and Der s 2 allergens. *Clin Exp Allergy* 1995;25:922-8.
7. King TP, Hoffman D, Løwenstein H, Marsh DG, Platts-Mills TAE. Allergen nomenclature. *Bull WHO* 1994;72: 797-806.