

Occupational asthma due to deer dander

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Background: The literature on allergy to deer is limited. The allergenic component remains to be identified.

Methods: We report a case of occupational asthma and rhinitis caused by deer dander. A 44-year-old male farmer had raised three red deer on his farm for 2 years, prior to occurrence of asthmatic symptoms. Dander extract was prepared from the patient's deer.

Results: Skin-prick test elicited positive reactions to dander extracts from goat, sheep, camel, and cow as well as to deer dander extract. Bronchoprovocation test with deer dander extract elicited an early asthmatic response. Serum-specific IgE antibody to the deer dander extract was detected by enzyme-linked immunosorbent assay (ELISA). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analysis showed five IgE binding components (110, 72, 59, 45, and 21 kilodalton) within the deer dander extract.

Conclusions: These results suggest that deer dander can induce occupational asthma through an IgE-mediated mechanism in a farmer raising deer.

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INTRODUCTION

It is well-known that animal secretory proteins can be potentially allergenic to persons inhaling the particles; during the past decade, allergic reactions to proteins of mammalian origin, especially domestic animals, have been studied.¹ There have been only a few reports of allergy to deer dander.²⁻⁴ Here we report a case of occupational asthma due to deer dander in a farmer raising deer, as confirmed by bronchoprovocation test. Specific IgE antibody to deer dander extract was detected in the patient's serum, and their IgE binding components were identified.

CASE REPORT

A 44-year-old male farmer presented with frequent dyspnea and chest discomfort. He had raised three red deer on his farm for three years. He had not experienced any respiratory

symptoms before acquiring the deer, and had had close contact with cows and pigs without any allergic manifestations. He had never been exposed to goats, sheep, or camels, and had not had cats or dogs in his house. Two years after acquiring the deer, he began to experience rhinorrhea, sneezing, coughing, and shortness of breath, which were aggravated by close contact with the deer for example when he groomed them. The patient had these symptoms for one year before coming to our clinic.

On examination, his breath sounds were normal without wheezing, and a chest radiograph was normal. Skin-prick tests with 60 common inhalant allergens (Bencard Allergy Service, Bredford, Middlesex, UK) elicited positive responses to ragweed (2+), *Dermatophagoides farinae* (2+), cat (2+), dog (2+), and most strongly to sheep (4+). His serum total IgE level was 559 IU/mL. Total eosinophil count in peripheral blood was 260/mm³. In a nasal smear, 15% of the total stained cells were eosinophils. An inhalation challenge test with methacholine was positive with PC₂₀, 1.1 mg/mL.

METHODS

Preparation of Deer Dander Extract

Hair and dander were obtained from the deer that the patient had raised. The specimens were finely cut, defatted with diethyl ether, and dried. One gram of defatted deer hair and dander was extracted into 50 mL phosphate buffered saline (PBS) at 4 °C for 72 hours, followed by centrifugation at 10,000 *g* at 4 °C for one hour. The supernatant was dialyzed against distilled water for 48 hours at 4 °C, then lyophilized and stored in a freezer at -20 °C until use.

RESULTS

Skin Prick Test

For the skin prick test, the lyophilized deer dander extract was dissolved in glycerinated Coca's solution at a concentration of 1 mg/mL. Skin prick test elicited a strong reaction ($\geq 3+$) to deer dander extract. Then we tested the patients with other commercial dander extracts from domesticated mammals (cat, dog, sheep from Bencard Co. G.B.; others from Hollister-Stier Co, USA). He had strong reactions ($\geq 3+$) to dander extracts from sheep, goat, cow, and camel, and weaker positive reactions ($< 3+$) to dander extracts from rabbit, cat, dog, and human (Table 1).

Bronchoprovocation Test with Deer Dander Extract

One milliliter of deer dander extract dissolved in phenolized saline was nebulized into a 10-L bag by Pari provocationtest I (Pari Ritzau Pan-Werk GmbH, Germany) and inhaled by the patient at tidal volume. Early bronchoconstriction (38% fall of FEV₁) was observed ten minutes after inhalation of deer dander extract solution at a concentration of 1 mg/mL in 0.4% phenolized saline (Fig 1). There was no delayed response.

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Table 1. Skin Prick Test Results with Dander Extracts of Domesticated Mammals

Order and Family	Domesticated Stocks	Skin Prick Test, mm*
Rodentia		
Leporidae	Rabbit	3 x 3/36 x 28
Muridae	Rat	Negative
	Mouse	Negative
	Guinea pig	Not done
Cavidae		
Carnivora		
Canidae	Dog	2 x 2/38 x 33
Felidae	Cat	2 x 2/20 x 30
Mustelidae	Mink	Not done
Perissodactyla		
Equidae	Horse	13 x 6/45 x 45
Artiodactyla		
Suidae	Pig	Not done
Camelidae	Camel	4 x 4.5/54 x 38
Cervidae	Deer†	15.5 x 7.5/63 x 45
Bovidae	Cow	8 x 4.5/48 x 42
	Sheep	14 x 7/68 x 42
	Goat	10.5 x 8/48 x 53
Human hair		2 x 2/37 x 23
Histamine		4 x 3/41 x 31

* Largest x smallest diameters of wheal/erythema.

† Deer dander extract (1 mg/mL) was prepared from the patient's deer. Domesticated mammals were categorized according to zoologic classification.⁵

Detection of Specific IgE Antibody by ELISA

Serum-specific IgE antibody to deer dander extract was detected by

ELISA, using a previously described procedure.⁶ Briefly, each well of a microplate (Flat bottom, Costar corporation, Cambridge, MA) was

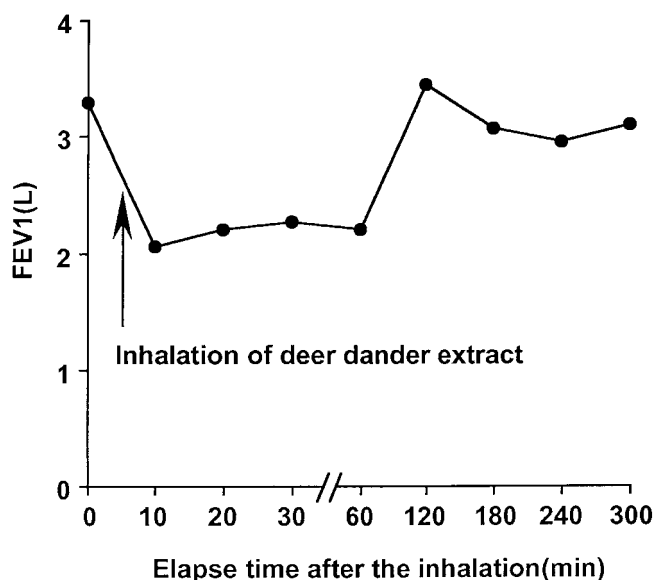


Figure 1. The result of bronchoprovocation test with deer dander extract.

coated with 2 μ g of the deer dander extract in 50 μ L of carbonate buffer (pH 9.6). After incubation overnight at 4 °C, each well was washed 3 times with phosphate buffered saline with 0.05% Tween (PBST), and the remaining binding sites blocked by incubation with 100 μ L of 1% bovine serum albumin in PBST for one hour at room temperature. The wells were then incubated for one hour at room temperature with 50 μ L of the patient's serum. After washing three times with PBST, biotinylated goat anti-IgE antibody (1:1000 wt/vol) (Vector Laboratories, Inc, Burlingame, CA) was added to the wells and incubated for one hour at room temperature. The wells were then washed three times with PBST and then incubated with 50 μ L of streptavidin-peroxidase (1:1000 wt/vol) (Sigma Chemical Co, St. Louis, MO) was incubated for 30 minutes before another washing step followed by incubation with 100 μ L ABTS (2,2-azino-di-3-ethylbenzthiazolin-6-sulfonic acid in a citrate phosphate buffer) for ten minutes at room temperature. The reaction was stopped by addition of 100 μ L 2 mM sodium azide and the absorbance was measured at 410 nm by an automated microplate reader. For ELISA inhibition, each 100 μ L portion of the patient's serum was incubated with 4 μ L (40 μ g) of inhibitor (Dermatophagoides farinae, cat dander, and deer dander extract) for one hour at room temperature. Specific IgE antibody to deer dander extract was measured in the same way. Complete inhibition (99%) was noted with deer dander extract, and partial inhibition (23.6%) with cat dander extract (Table 2).

SDS-PAGE and IgE Immunoblot Analysis

Sodium dodecyl sulfate-polyacrylamide sulfate was performed according to published procedure,⁷ with 12% homogeneous gel. A 90 x 40-mm mini-gel apparatus (Hoeffer Scientific Instruments, San Francisco) was used. When the gel was

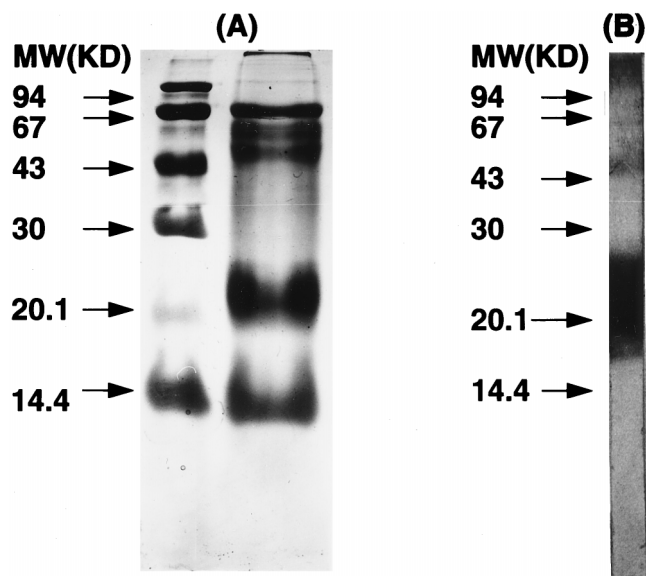


Figure 2. Protein staining of SDS-PAGE (12%) of deer dander extract (A) and IgE binding components on immunoblot analysis with the patient's serum (B).

fixed and stained with Coomassie brilliant blue, at least five protein bands ranging from 14 to 67 kilodaltons (kD) could be observed. The gel was electroblotted onto a sheet of nitrocellulose membrane (Hoeffer Scientific Instruments, San Francisco), which was blocked for one hour in PBST with 1% bovine serum albumin. Then the membrane was incubated with undiluted serum for 18 hours at room temperature. After being washed with PBST, the membrane was incubated with biotinylated anti-IgE antibody (1:1000 wt/vol) (Vector Laboratories, Inc.

Burlingame, CA) for three hours at room temperature. The membrane was washed again with PBST, incubated for one hour with streptavidin-peroxidase (1:1000 wt/vol) (Sigma Chemical Co, St Louis, MO). The activity of bound peroxidase was then identified using 4-chloro-1-naphthol. At least five IgE binding protein bands (110, 72, 59, 45, and 21 kD) were noted within deer dander extract (Fig 2).

Follow-Up

The patient was advised to avoid further exposure to deer. After 1 year's avoidance, he did not experience asthmatic symptoms other than occasional intermittent coughing, which could be controlled by sodium cromoglycate only.

DISCUSSION

A few reports of deer-dander-induced asthma and rhinitis exist in hunters exposed to wild deer.^{2,3} As deer horns continue to be used as an important material in traditional herbal medication in Asia, an appre-

ciable number of farmers raise deer. This particular patient had never experienced respiratory symptoms before raising deer, but began to experience asthmatic symptoms after 2 years' exposure. Skin-prick test and ELISA revealed presence of specific IgE antibody to deer dander extract in the patient's serum. Broncho-provocation test with deer dander extract elicited early asthmatic response. Furthermore, immunoblot analysis disclosed five IgE binding components (110, 72, 59, 45, 21 kD) within deer dander extract. These findings suggest that deer dander can induce occupational asthma through an IgE-mediated mechanism in a farmer raising deer.

Atopic predisposition may play an important role in the development of IgE sensitization to occupational allergens with high molecular weight.⁸ Previous reports have indicated most patients with deer allergy had atopy.²⁻⁴ In this study, the patient had atopy and had immediate skin reactions to house dust mite and ragweed.

Previous studies have indicated that deer dander had cross-reactivity with dander extracts from cat³ and horse.⁴ In this study, the patient had strong skin reactions to dander extracts from mammals of the order Artiodactylia (deer, sheep, goat, cow, and camel) and Perissodactyla (horse), even though he had never been exposed to sheep, goats or camels. He showed relatively weak reaction to the order Rodentia (rabbit, mouse, rat) and Carnivora (dog, cat). Furthermore, the result of ELISA inhibition suggested a partial cross-reactivity between deer and cat allergens. These findings suggest the possibility of cross-allergenicity between the dander allergen of deer and that of closely-related species.

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Table 2. ELISA Inhibition of Deer Dander-Specific IgE with Other Inhalant Allergens

Inhibitor* Used	Absorbance Value at 410 nm	% Inhibition
None	0.144	0
<i>D. farinae</i>	0.131	9
Cat dander	0.110	23.6
Deer dander	0.002	99

* 40 µg of each allergen extracts was added to 100 µL of serum.

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