
Occupational respiratory allergy to roe deer

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Background: Although scattered reports have been published on roe deer allergenicity, no systematic studies of allergenicity or possible cross-reactions have appeared.

Objectives: To describe 2 patients with occupational roe deer allergy, demonstrated by positive skin and conjunctival provocation test results, and to note cross-reactions to other animal (mainly cow) allergens.

Methods: Two workers at animal rehabilitation centers were sensitized to roe deer. One patient had a history of rhinoconjunctivitis and the other a history of rhinoconjunctivitis and probable asthma. Both patients underwent skin testing with a standard battery of inhaled and epithelial allergens and with roe deer hair and dander extract and conjunctival provocation tests with roe deer hair extract. Immunodetection for IgE (both patients) and IgE immunoblot inhibition tests to determine inhibitory effect (1 patient) were also performed.

Results: The results of skin tests and conjunctival provocation tests showed that both patients were sensitized to roe deer allergens. In one patient specific IgE to roe deer extract was detected, and this extract completely inhibited IgE binding to cow hair and dander extract in immunoblotting tests. Specific IgE to roe deer proteins could not be demonstrated in the other patient.

Conclusions: Our results suggest that roe deer epidermal derivatives can cause occupational respiratory disease in exposed workers and that allergy to this species should be considered in individuals who present with similar symptoms and exposure histories. Immunoblot inhibition studies suggested the possibility of cross-reaction between roe deer proteins and cow proteins.

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INTRODUCTION

The European roe deer (*Capreolus capreolus*), the smallest species of deer in Spain, is characterized by its grayish brown coat in winter and richer reddish brown coat in summer. It inhabits a variety of native and repopulated forest habitats and is the only species of deer native to the region of Galicia in northwestern Spain. There have been scattered reports of allergy to roe deer,^{1–3} manifested as cutaneous (contact urticaria) and respiratory symptoms (rhinitis and asthma). We report 2 cases of allergy in workers exposed and sensitized to this species during employment at animal recovery centers run by the Galician regional government environmental council. One patient had a history of rhinoconjunctivitis and the other had rhinoconjunctivitis and probable bronchial asthma.

METHODS

Patient 1

Patient 1 was a 44-year-old man with a history of perennial daytime rhinoconjunctivitis since the age of 29 years, with no seasonal changes. He related episodes of symptoms on contact or exposure to different animals, particularly cows. He had no clinical symptoms of asthma. As an environmental engineer employed by the regional environmental council, he was stationed in 1995 at the Ancares Game Reserve, where he handled different species of animals, including roe deer. In 1997 he related his rhinoconjunctivitis to contact with roe

deer. He had no dermatitis or urticaria, possibly because he wore gloves during work. Throughout the years, he had been exposed to roe deer approximately 50 times. In 2000, he stopped working at the game reserve and since then has had no further contact with roe deer.

Patient 2

Patient 2 was a 46-year-old man with a history of perennial rhinitis (mite allergy) since childhood, for which he had received no specific treatment but had obtained adequate relief with medication to alleviate his symptoms (topical corticosteroids and antihistamines). As an employee of the environmental council, he was stationed in 1990 at the Veral bird and wildlife recovery center in Lugo (northwestern Spain). His work, which included transporting injured animals to the center, involved exposure to roe deer among other species. After an undetermined number of exposures, the patient began to experience rhinoconjunctivitis but was unable to recall contact urticaria (possibly because the patient wore gloves during his work as in the first case). On one occasion after he had been bottle-feeding a roe deer fawn, he suddenly developed dyspnea, wheezing, cough, rhinoconjunctivitis, and palpebral edema in both eyes. After that he avoided contact with this species and has not been exposed to roe deer for the last 9 years.

Extracts

Raw material (hair and dander) derived from roe deer epithelium was extracted at 2.5% in phosphate buffer during 90 minutes at 2°C to 8°C with magnetic shaking. After centrifugation the supernatant was dialyzed against phosphate buffer and filtered (pore size, 0.2 µm). For skin tests, the

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extract was 50% glycerinated. As horse and cow epithelium extracts, we used batches T0640 and S00001100, respectively (ALK-Abelló, SA, Madrid, Spain).

Skin Tests

Skin prick tests (ALK-Lancet, Hørsholm, Denmark) were performed with a battery of standard inhaled and epithelial allergens habitually used in our allergy unit: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, *Acarus siro*, *Alternaria alternata*, *Cladosporium herbarum*, *Betula verrucosa*, *Phleum pratense*, *Lolium perenne*, *Plantago lanceolata*, and dog, cat, horse, cow, and rabbit epithelium (ALK-Abelló SA). Histamine at 10 mg/mL was used as a positive control and saline solution as a negative control. The size of the wheal was measured after 15 minutes, and the test result was considered positive when the diameter of the allergen-induced wheal was at least as large as the diameter of the histamine-induced wheal. The roe deer extract was previously tested, with negative results in 5 atopic and 5 nonatopic patients.

Conjunctival Provocation Test

A drop of 1:100 extract solution prepared with the extract used in the skin tests was placed in the conjunctival sac. As a negative control, a drop of physiologic saline was placed in the conjunctival sac of the other eye. The test result was considered positive when sneezing or hyperemia, tearing, oculonasal itching, or nasal discharge appeared on the side of the extract solution according to the recommendations of the European Academy of Allergy and Clinical Immunology.⁴ One atopic and 2 nonatopic control subjects were also tested, with negative results in all 3 individuals.

Nonspecific Bronchial Hyperreactivity Test

Methacholine was used according to a modification of the Cockcroft method for the nonspecific bronchial hyperreactivity test.⁵ The test result was considered positive when forced expiratory volume in 1 second was reduced by 20% or more compared with the value recorded after inhalation of the control substance (physiologic saline).

IgE Immunodetection

Allergens in the battery habitually used in our allergy service were tested with the CAP System (Pharmacia, Uppsala, Sweden); for specific IgE determination to deer epidermal derivatives, red deer was used because no commercial roe deer solid phase was available. Values below 0.35 kU/L were considered negative.

Extracts and molecular weight markers were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (15% acrylamide) under nonreducing conditions. The polyacrylamide gels were transferred by electrophoresis to nitrocellulose membranes,⁶ and these were blocked with 1% bovine serum albumin in phosphate-buffered saline (PBS) for 1 hour at room temperature, then incubated for approximately 18 hours with serum from each patient

at a 1:5 dilution. As a negative control, nitrocellulose membranes, to which extract protein had been transferred, were incubated with 1% bovine serum albumin in PBS. The membranes were washed with 0.1% Tween-20 in PBS, then incubated for 2 hours at room temperature with human anti-IgE monoclonal antibody HE-2 ascites at a 1:3,000 dilution.⁷ The membranes were then washed and IgE-binding proteins were detected with a chemiluminescence method (ECL, Amersham Bioscience, Little Chalfont, Buckinghamshire, England) according to the manufacturer's instructions. For immunoblot inhibition assays, serum samples from the patients were preincubated for 2 hours at room temperature with 500 μ L (0.2 mg/mL of protein) of the inhibiting extract.

RESULTS

In patient 1, the skin prick test results were positive for roe deer (18 $\times$ 13 mm), cow (8 $\times$ 8 mm), and horse extract (12 $\times$ 8 mm) and negative for all other extracts. In patient 2, the skin prick test results were positive for *D pteronyssinus* (13 $\times$ 13 mm), *D farinae* (12 $\times$ 12 mm), *L destructor* (10 $\times$ 10 mm), *T putrescentiae* (6 $\times$ 6 mm), and roe deer extract (12 $\times$ 10 mm) and negative for all other extracts, including cow and horse.

In patient 1, specific IgE titers were 6.27 kU/L for cow, 1.51 kU/L for horse, 8.87 kU/L for red deer, and less than 0.35 kU/L for *D pteronyssinus*, *L destructor*, *P pratense*, cat, and dog. In patient 2, specific IgE titers were 14.30 kU/L for *D pteronyssinus*, 15.30 kU/L for *L destructor*, 0.65 kU/L for *T putrescentiae*, and below 0.35 kU/L for red deer, reindeer, fox, and cow.

Conjunctival provocation test results with a 1:100 dilution of roe deer extract were positive in both patients, with itching, palpebral swelling, hyperemia, and tearing. The results of the nonspecific bronchial provocation test with methacholine in patient 2, up to a concentration of 16 mg/mL, were negative. This patient was offered a specific bronchial provocation test but declined.

Figures 1 and 2 show the results of IgE immunodetection assays with serum from patient 1 against roe deer, horse, and cow extracts. The patient's IgE recognized roe deer epithelium proteins of approximately 16 to 20 kDa, 37 kDa, and 75 kDa. Proteins of similar molecular weights were detected when the patient's serum was assayed against cow and horse epithelium extracts, although immunodetection signals were weak for the latter. Duplicate assays with separate samples of serum IgE from patient 2 showed no reactivity to proteins in roe deer or cow epithelium.

Figure 3 shows the results of IgE immunoblot inhibition assays with serum from patient 1. This patient's IgE showed cross-reactivity for roe deer and cow extracts. Roe deer extract completely blocked IgE binding to cow extract (lane 3) to a degree similar to the inhibition obtained when serum was preincubated with cow extract (lane 4).

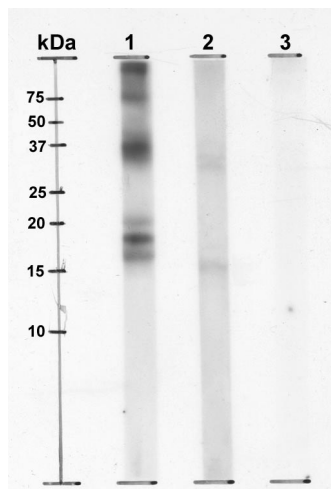


Figure 1. IgE immunoblotting with serum from patient 1. Lane 1, Roe deer extract; lane 2, horse extract; lane 3, negative control. Molecular weight markers (in kilodaltons) are shown.

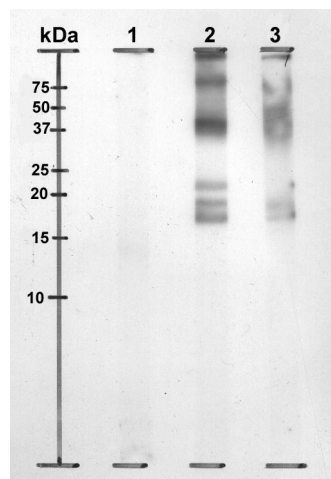


Figure 2. IgE immunoblotting with serum from patient 1. Lane 1, Negative control; lane 2, roe deer extract; lane 3, cow extract. Molecular weight markers (in kilodaltons) are shown.

DISCUSSION

Allergy to deer epidermal derivatives has usually been described as the result of occupational exposure and incidental contacts with animals. Symptoms are diverse and can consist of asthma,^{3,8-10} rhinitis,^{1,3,8-10} urticaria,^{1,3,10} angioedema¹¹ conjunctivitis,^{3,10,11} laryngeal edema,¹¹ or anaphylaxis.¹²

Only 3 cases of allergy to roe deer have been described previously. In 1963, Munro-Ashman and Frankland³ described a case of allergic rhinoconjunctivitis and asthma caused by roe deer scurf. Later, Geyer and colleagues² described a case of occupational contact urticaria in a hunter, who did not have respiratory symptoms. More recently, Spiwak and Dutkiewicz¹ reported a case of contact urticaria and rhinitis in a hunter whose condition was diagnosed on the

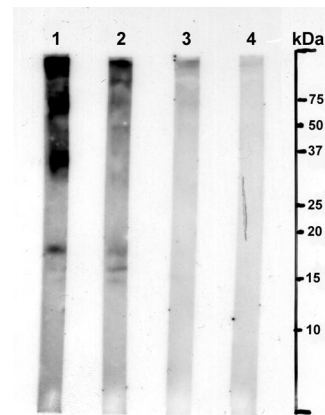


Figure 3. IgE immunoblotting inhibition assay with serum from patient 1. Lane 1, Roe deer extract; lane 2, cow extract; lane 3, cow extract overlaid with patient's serum preincubated with roe deer extract; lane 4, cow extract overlaid with patient's serum preincubated with cow extract. Molecular weight markers (in kilodaltons) are shown.

basis of clinical symptoms and a rubbing test with roe deer skin. To our knowledge, no roe deer-specific allergen has been identified.

We describe 2 patients with allergy to roe deer epithelium. The first patient had clinical symptoms of rhinoconjunctivitis, and the second had rhinoconjunctivitis and probable bronchial asthma. The latter patient had had a single episode of respiratory distress with dyspnea, wheezing, and cough after exposure to a roe deer but had no further clinical manifestations of asthma. Because of the medicolegal implications of occupational illness, the patient was offered a specific bronchial provocation test with roe deer extract, which he declined.

The causal relationship between exposure to roe deer and allergy in our patients was confirmed with skin tests and conjunctival provocation tests with roe deer hair and dander extract. One patient was also shown to have IgE to roe deer proteins of approximately 16 to 20 kDa, 37 kDa, and 75 kDa. The 16- to 20-kDa proteins were probably lipocalins (ie, proteins present in epidermal derivatives, such as hair, danders, and secretions, of a number of mammals). The other proteins may be lipocalin aggregates. Recent work has shown that most major allergens in mammals that are able to cause respiratory sensitization (with the notable exception of cat allergens) belong to this family of proteins.^{13,14} Our patient also had IgE to proteins of similar molecular weight from horse and cow, and the corresponding major allergens from these sources (Equ c 1 and Bos d 2, respectively) are lipocalins.^{15,16} Furthermore, immunoblot inhibition assays demonstrated cross-reactivity between roe deer and cow allergens (Fig 3). This finding is consistent with earlier reports of possible cross-reactivity between deer allergens and other ungulate allergens, notably, cow protein allergens with a molecular weight of 22 and 25 kDa.^{8,10-12}

In our second patient, we were unable to demonstrate specific IgE to roe deer epithelium. Because of the approximately 9-year period between the patient's last exposure to roe deer and the date of the in vitro assays, serum IgE levels may have diminished because of the lack of further allergic stimulation. This would account for the discrepancy between specific circulating IgE and the results of the skin prick and conjunctival provocation tests. In conclusion, our results show that roe deer epithelium is a source of allergens able to cause sensitization and lead to allergy in exposed individuals.

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