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Sensitization to Horse Allergens in Italy: A Multicentre Study in Urban Atopic Subjects without Occupational Exposure

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Key Words

Respiratory allergy · Pets · Horse · Hypersensitivity · Sensitization · Rhinitis · Asthma

Abstract

Background: Horses play a significant role in people's leisure time in Italy and other countries, but few data are available on IgE-mediated sensitization to horse allergens in patients without occupational exposure. We assessed, in a multicentric survey, the prevalence of horse sensitization in atopic subjects and its clinical characteristics. ***Methods:*** Allergists from the whole Italian territory were required to collect the results of skin prick tests from at least 100 consecutive subjects. Those patients with a positive skin test to horse dander underwent a detailed interview concerning clinical history, pet ownership and possible exposure. ***Results:*** Data from 3,235 outpatients were collected and 2,097 had at least 1 skin positivity. Among them, 113 (5.38%) were sensitized to horse dander (9 monosensitized). Thirty patients reported direct horse contact (4 owners and 26 for riding or occasional contact), 23 patients were sometimes in contact with horse owners and 60 subjects denied any direct or indirect exposure. Among 9 horse monosensitized patients, 6 had intermittent and mild rhinitis and 3 persistent moderate/severe rhinitis plus asthma. Three of them were horse owners or riders and the remaining had no contact with the animal. ***Conclusions:*** Our data evidence that the rate of sensitization to horse dander is not negligible and probably underestimated. In susceptible, not occupationally exposed individu-

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als, horse contact, but also indirect or no apparent exposure, may induce sensitization. We recommend inclusion of horse allergen in the routine panel for the diagnosis of respiratory allergy.

Introduction

It has been observed that occupational exposure to horses increases the risk of allergic sensitization to horse hair itself and, according to different studies, the prevalence of this sensitization in occupational settings varies from 3.6 to 16.5% [1, 2]. Most studies on the sensitization to horse allergens in populations without professional exposure have been carried out in Northern Europe and in rural areas, where the rate of horse ownership is high and horse riding is popular [3–9]. As such, very few studies have been carried out in populations living in large urban areas (cities and suburbs) [10–12]. Thus, in these areas the prevalence of sensitization to horse, its clinical characteristics and modality of exposure are poorly known. This gap in the literature probably reflects the widespread view that prevalence of horse-related allergy is low in urban populations since they are not exposed to horses [13, 14]. On the contrary, we suggest that in urban areas, horse is a model useful to study potential modalities of exposure and sensitization to horse other than horse riding.

With cat and dog allergens, symptoms and allergic sensitization can occur also as a result of an indirect exposure [15], therefore it is reasonable that the same can happen with horse [16, 17]. Also in this case, no large study has so far been performed to evaluate the role of direct or indirect exposure to horse allergens in developing allergic sensitization to these antigens.

With the cooperation of the Allergy Study Group of the Italian Society of Respiratory Medicine, we aimed at investigating the prevalence and characteristics of allergic sensitization to horse in urban atopic population in Italy, and to asses the role of the modality of exposure.

Methods

Nineteen allergy units, uniformly distributed over the Italian territory, were involved in this cross-sectional study. Each center was required to collect the data of at least 100 consecutive outpatients referred for actual or suspected respiratory allergy (asthma and/or rhinitis) and living in the urban area. Data were collected from January 1 to June 30, 2008. All centers followed the same protocol and recorded the results in a previously agreed form.

Table 1. Classification of the exposure to horses

Direct:	horse owners, nonprofessional riders, occasional direct contact
Indirect:	patients who deny any direct contact, but who could be exposed through the contact with the subjects above
No contact:	patients who deny any apparent direct or indirect exposure

Subjects with occupational exposure to horses (farmers, stablemen, breeders or veterinary doctors) were not considered. Patients with chronic infectious diseases, malignancies or dysmetabolic diseases, severe cutaneous disorders, negative skin reaction to histamine, or treated with drugs interfering with the skin response were excluded as well [18, 19]. The standardized form reported demographic data, type and duration of respiratory symptoms, pets ownership, horse exposure, and results of the skin prick tests (SPTs). The forms had to be filled by the allergist, who also verified the consistency of clinical history and SPT results. Then, the same doctor confirmed the diagnosis of respiratory allergy according to the international guidelines [20, 21]. Since the absence of a pet at home does not exclude a direct exposure to pet outside [22] and considering the peculiarity of horse contact, we classified horse exposure into 4 categories (table 1).

The commercial allergen extracts used for screening SPTs were provided by ALK Abello Group (Milan, Italy). All centers used the same batch and standard panel of allergens including: *Dermatophagoides pteronyssinus* and *D. farinae, Alternaria alternata, Cladosporium herbarum,* cat, dog and horse dander, *Parietaria,* grass mix, *Artemisia vulgaris, Olea europaea, Betula pendula, Cupressus sempervirens* and *Corylus avellana*. These allergens cover the majority of causative agents of respiratory allergy in Italy. Positive (10 mg/ml histamine HCl) and negative (saline solution in glycerine-phenol solution) controls were used as well. SPTs were carried out and interpreted according to current guidelines [23]. The result was read after 15 min and expressed as the mean of the major wheal diameter plus its orthogonal. A skin reaction of 3 mm or greater was considered positive. SPTs were always performed by the same operator at each center. The profiles of the wheals were outlined using a fine-point marking pen and transferred by adhesive tape onto patient's form.

SDS-PAGE and immunoblotting procedures were performed routinely in the allergenic extracts of horse, cat and dog to avoid the possibility of mite allergen contamination.

Results

The participating centers were homogeneously distributed over the Italian territory, as shown in figure 1. A total of 3,235 patients were included in the database. Out of them, 2,097 (64.82%) had a SPT positivity for at least one allergen and were diagnosed as having respiratory al-

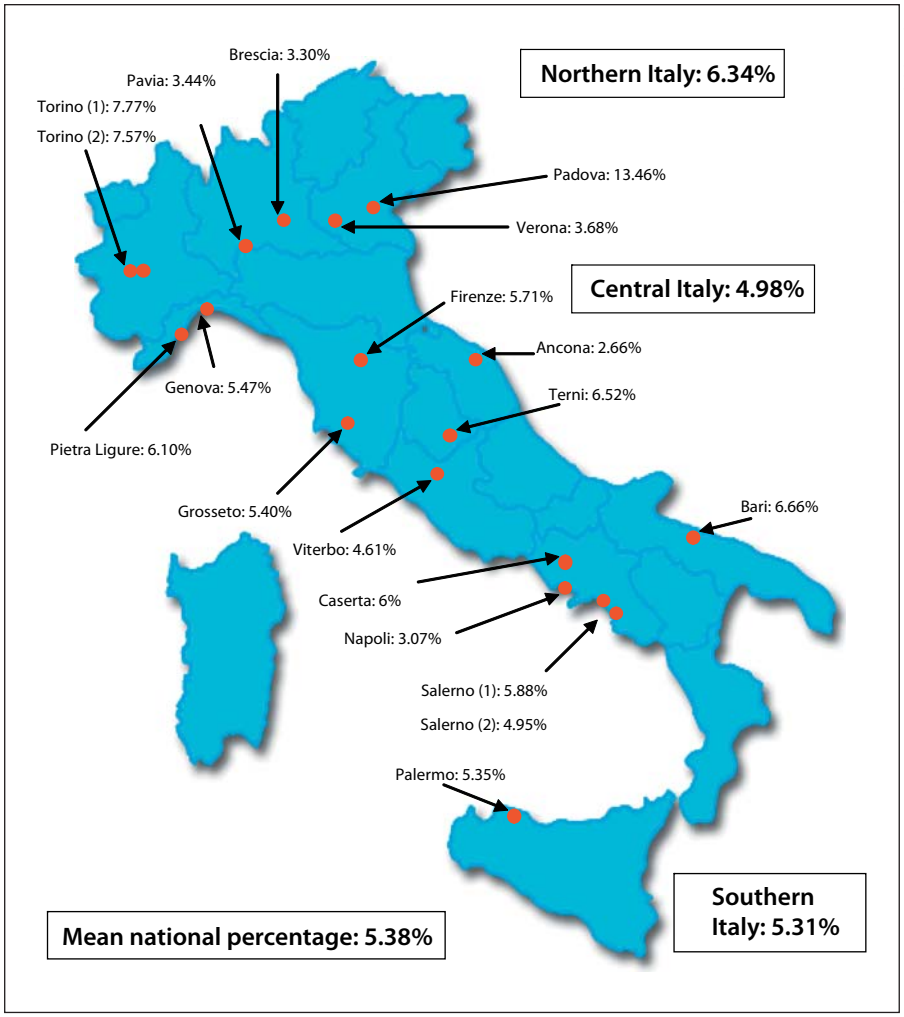

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Fig. 1. Geographic distribution of the centers, with the percentages of subjects having positive skin reactions to horse dander.

lergy. The 2,097 SPT-positive subjects had a mean age of 35.5 years (range 5–76) and 1,149 (54.79%) of them were female. One hundred thirteen were sensitized to horse dander, thus, the overall sensitization prevalence in subjects with respiratory allergy was 5.38%, ranging between 2.66 and 13.46% among centers (fig. 1). The main characteristics of the patients sensitized to horse are summarized in table 2. Of note, only 9 of the 113 patients were monosensitized. Thirty patients reported a direct horse contact (4 for ownership and 26 for riding or occasional contact), 23 patients excluded any direct exposure to the animal but were sometimes in contact with horse owners, and 60 subjects denied any apparent direct or indirect exposure to horse or horse allergens. Among the 9 monosensitized patients, 6 had intermittent and mild rhinitis and 3 persistent moderate/severe rhinitis plus asthma. Three were horse owners or riders and the remaining 6 individuals had no horse contact at all. Since 104 horse-sensitized patients showed multiple cutaneous positivity to other common allergens (mites, pollens and pets), we could not directly quantify the role of horse sensitization in eliciting symptoms. A comparison between the horse-positive and horse-negative subjects is reported in table 2. The most common sensitizing allergens associated in horse-allergic individuals were dust mites (64 patients, 10 monosensitized) and pollens (50, 17)/cat and dog epithelia (66, 0).

An interesting observation is the higher percentage of current smokers in horse-sensitized in comparison to atopic non-horse-sensitized individuals (24.8 vs. 9.5%). Moreover, in both groups, higher percentages of current smokers have been found in cat/dog-sensitized individuals in comparison to those sensitized to other allergens such as pollens and mites (in the first group 21.5 vs. 17.6 and 16.7%, respectively; in the second group 14.3 vs. 10.1 and 11.2%, respectively).

Because monoclonal antibody-based methods to measure the amount of horse allergen in the dust of indoor environments are not available in Italy with respect to other indoor allergens (that is, *Der p* 1, *Fel d* 1 and *Bla g* 1) [24, 25], we have no information about the levels of indoor exposure to this allergen.

Discussion

Allergic sensitization to horse allergen is considered unusual in patients living in urban areas because of the lack of exposure to the animal and its allergens [13, 14]. The results of our report show that the prevalence of allergic sensitization to horse dander in Italian urban atopic people is not negligible and can occur even without any apparent direct or indirect contact with horses. It is not easy to explain these findings. Emenius et al. [26] recently showed that horse allergens are abundant in the immediate proximity of stables or other places where horses are present, but the allergens dispersed from horses decline rapidly outdoor and are barely found indoors. As a consequence, it is likely that horse allergen could be transferred to 'horse-naive' individuals from clothing of individuals who are in contact with horses or horse-related environments. This passive transmission could account for the presence of horse allergen in domestic dust samples from urban environments [16, 17], and has been previously demonstrated for cat [15] or rabbit allergens [27]. It has been shown that the main horse allergen Equ c 1 (molecular weight 22 kDa) is a lipocalin protein [28]. Other allergens include Equ c 2, Equ c 3, Equ c 4, Equ c 5 and albumin [29].

Our findings may also indicate a cross-reaction between horse allergen [30] and the major allergens of several mammalian species, like cows [31], dogs [32], guinea pigs [33], rabbits [34], rodents [35] and, recently, cats [36]. In fact, all the allergens of these animals are lipocalins, which are typically small proteins whose major feature is the ability to bind small hydrophobic molecules such as steroids [37].

Serum albumin, a thermolabile protein of approximately 68 kDa, is an important panallergen involved in milk, meat and epithelia allergy [38, 39]. A recent study showed that patients might develop sensitization to epithelia serum albumin even without direct contact with animals [40] because of sensitization to serum albumin through cow's milk. Given these findings, it is conceivable that our horse-sensitized and non-occupationally exposed patients may have a specific susceptibility to allergens of this animal [41]. We previously found that direct contact with a horse triggered severe respiratory symptoms in horse-sensitized (but unaware) patients [42]. We can hypothesize that in the 3 horse monosensitized patients with reported direct and regular horse ex-

Table 2. Patients' characteristics

	Horse+ (n = 113)		Horse– (n = 1,984)	
	n	%	n	%
Sex, F/M	58/55	51/49	1,091/893	55/45
Mean age, years	35.5		30.3	
Age range				
0–20	22	19.5	496	25
21–41	58	51.3	804	40.5
41–60	27	23.9	595	30
>60	6	5.3	89	4.5
Family history of allergy				
Yes	66	58.4	982	49.5
No	47	41.6	1,002	50.5
Pet at home				
Cat	9	8.0	145	7.3
Dog	16	14.2	258	13
Cat + dog	3	2.6	24	1.2
Other animals	10	8.8	159	8
None	75	66.4	1,398	70.5
Modality of exposure to horse				
Direct (ownership, riding, etc.)	27	23.9	44	2.2
Indirect (with horse owners)	23	20.4	179	9
Occasional	3	2.6	30	1.5
No direct or indirect contact	60	53.1	1,731	87.3
Smoking				
Yes	28	24.8	188	9.5
No	76	67.3	1,617	81.5
Ex-smokers	9	7.9	179	9
Clinical symptoms				
Rhinitis only	18	15.9	486	24.5
Asthma only	9	8.0	109	5.5
Rhinitis + asthma	24	21.2	89	4.5
Rhinitis + conjunctivitis	21	18.6	893	45
Rhinitis + conjunctivitis + asthma	22	19.5	318	16
Other	19	16.8	89	4.5
Seasonality of symptoms				
Intermittent	49	43.4	1,081	54.5
Persistent	64	56.6	903	45.5
Asthma severity				
Mild	51	45.13	1,301	65.6
Moderate/severe	62	54.87	683	34.4
Sensitization				
Monosensitized (to horse)	9	8	–	–
Monosensitized (other allergens)	–	–	203	10.2
Sensitized to 2 allergens	9	8	153	7.7
Sensitized >2 allergens	95	84	1,628	82.1

posure a primary sensitization may be involved, in the remaining 6 individuals it is likely an indirect exposure as a mechanism. In the remaining 104 patients sensitized also to other allergens, it is likely that a prevalent cross-reacting mechanism might be involved in 66 individuals with animal allergy (cat, dog and rabbit), in the last 38 pollen-sensitized patients it is likely a prevalent indirect mechanism of exposure and consequent sensitization.

It is difficult to give a plausible explanation of the higher percentage of smokers in individuals sensitized to animals (horse, dog, cat) and further studies should be carried out on this topic. We can hypothesize that associated inhalation of cigarette smoke and animal allergens might induce a synergic effect on airway allergic sensitization.

The results of our multicentre study prompt two other considerations. First, allergic sensitization to horse allergens is more frequent than expected in subjects without direct exposure to horses. In fact, the overall prevalence of this condition in the whole country (5.38%) is higher than that found by us in Naples (3.45%) and in Florence (2.7%) in adults and in children [43, 44]. Second, individuals suspected of being highly atopic or those already sensitized to pet dander should undergo SPTs and evaluation of serum-specific IgE before beginning an activity involving regular direct horse contact and before entering environments containing horses such as stables, riding schools and race courses.

On the basis of the last reported studies in children and adults [43, 44], the Italian adaptation of the international GINA guidelines has recognized the relevance of horse as an underestimated risk for allergic sensitization and suggested to include it in the standard panel for the diagnosis of respiratory allergy [45, 46].

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