

Direct and Indirect Exposure to Horse: Risk for Sensitization and Asthma

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Abstract Most studies on the sensitization to horse allergens in populations without professional exposure have been carried out in geographical areas where the rate of horse ownership is high and horse riding is popular. Very few studies have been carried out in populations living in large urban areas. This gap in the literature probably reflects the widespread view that prevalence of horse-related allergy is low in urban populations because the latter are not regularly exposed to horses. On the contrary, we suggest that urban areas constitute a model useful to study potential modalities of exposure and sensitization to horse allergen by other routes of exposure than horse-riding. In this article, we describe the risks related to various modalities of exposure to horse allergen, clinical aspects of airway sensitization to horse allergens in patients living in urban areas, and non-occupational exposure to horse allergen. In addition, we

illuminate some aspects related to dispersion of horse allergens from sources such as stables to indoor environments.

Keywords Allergic rhinitis · Allergic sensitization · Animal allergy · Asthma · Bronchial asthma · Furred animal sensitization · Indirect exposure to horse · Horse · Horse allergens · Horse allergen dispersion · Risk factor for sensitization · Risk factor for asthma

Introduction

It has been shown that occupational exposure to horse increases the risk of allergic sensitization to horse dander and, according to different studies, the prevalence of this sensitization in occupational settings varies from 6.4 % to

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12.8 % [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]. However, in Sweden horse keeping is expanding rapidly, particularly in the vicinity of cities and other densely built-up areas. Sweden is a horse-dense country with 362,700 horses—on average 39 horses per 1000 inhabitants for the whole country. Very few studies have been carried out in populations living in large urban areas (cities and suburbs) [10–12]. This lack of studies in the literature probably reflects the widespread view that prevalence of horse-related allergy is low in urban populations because the latter are not regularly exposed to horses [13, 14]. On the contrary, we suggest that urban areas constitute a model useful to study potential modalities of exposure and sensitization to horse other than horse-riding.

In this article we describe the risks related to various modalities of exposure to horse allergen, clinical aspects of airway sensitization to horse allergen in non-occupationally exposed patients living in urban areas as well as some aspects related to dispersion of horse allergens from sources such as stables, and areas with horses, to indoor environments.

Aspects Related to Dispersion of Horse Allergens

Horse keeping is expanding rapidly in Sweden, particularly in the vicinity of cities and other densely built-up areas, which means that after Iceland, Sweden is the most horse-dense country in Europe, calculated as the number of horses per capita. Approximately 75 % of all horses and two-thirds of all establishments with horses are, however, located in city areas and adjoining rural areas, as horses nowadays play an important role in people's leisure time, and are not primarily used for agricultural purposes. Consequently, this situation and, hence, a growing concern about potential health implications has had an impact on the Swedish authority guidelines concerning animal farming.

Initially, in 1990, a report from the Swedish Ministry of Health and Social Affairs recommended a distance of at least 500 m between homes, schools, and day care centers and places where horses are kept or are present. It was followed by a report by Planning's General Council, "*A better place for work*", which provided guidelines for the recommended safe distance as one of several measures to prevent conflicts between residential areas, schools, day care centers, etc and husbandry, despite a total lack of knowledge about how to analyze horse allergen and, as follows, the dispersion of horse allergen in the environment. However, it had previously been shown that very low concentrations of other inhalant allergens could activate markers of inflammation in sensitive individuals, indicating

that passive exposure to allergens could hypothetically maintain or worsen allergic reactions [15–17].

Horse Allergens and Lipocalins

There are several horse allergens: Equ c 1, Equ c 2, Equ c 3, Equ c 4, Equ c 5. The major horse allergen, Equ c 1, is found in horse dander and the sublingual salivary glands but is also expressed in the liver and the submaxillary salivary glands. It is also present in urine, but to a much smaller extent. The molecular weight of Equ c 1 is 22 kD, whereas the other horse allergens range between 16 and 18.7 kD [18].

Equ c1 was first identified as a member of the lipocalin protein family by Gregoire et al [19]. In animals, lipocalin proteins are produced in the liver or secretory glands and act as carriers of molecules. They are extracellular proteins that can bind small hydrophobic molecules and, apart from acting as transport proteins, are also involved in enzyme activity and other biological functions [20]. Besides horse allergens (Equ c 1 and Equ c 2), almost all of the mammalian respiratory allergens are lipocalins, including dog (Can f 1, Can f 2, Can f 6), mouse (Mus m 1), rat (Rat n 1), and cow (Bos d 2). Also other allergens, for instance cockroach (Bla g 4) and lactoglobulin from cow's milk (Bos d 5) are lipocalins. Equ c 2 is structurally similar to the cow allergen Bos d 2. Common features for all the animal allergens mentioned above are that they stick easily to surfaces and clothes, become airborne, are efficiently dispersed in the environment via different modes of transport, and are therefore often found indoors in public environments [21].

Sampling Methods Used For Exposure Assessments of Horse Allergens

We have made an attempt to summarize the methods used to collect environmental samples for analysis of horse allergens (Table 1). In 1998, Egmar et al collected dust from mattresses on display in a furniture store [22]. Dust was collected by vacuuming the whole mattress for 2 min, using a vacuum cleaner attached to a collector with filter. Kim et al and Zhao et al collected dust in a similar way from desks, chairs, and floors in classrooms [23, 24]. Reservoir dust is the most common method for collecting horse allergens and other animal allergens. The dust is weighed and extracted and horse allergen is analyzed and expressed as µg allergen/g dust or µg allergen/sample.

In 1992, a new sampling method for collection of airborne settling dust using Petri dishes was introduced by Tovey and colleagues [25]. This method has since been frequently used for sampling of horse allergens [24, 26, 27, 28, 29]. However, the gold standard for collecting airborne dust is using pumps with the so called Institute of Occupational Medicine (IOM) inhalable dust sampler and

Table 1 Cited studies related to analysis and dispersion of horse allergen

Author	Environments	Sampling method
Merritt et al 2011	Schools, Sweden	Petri dishes, Reservoir dust (vacuum cleaning)
Norbäck et al 2011	Schools, Sweden	Petri dishes, electrostatic sampler
Emenius et al 2009	Nearby stables and horse tracks outdoors, dwellings, Sweden	Petri dishes, Aspen leaves
Elfman et al 2008	Inside and outside a stable and an office, Sweden	Air samples/air pumps Reservoir dust (vacuum cleaning) Ventilation filters
Kim et al 2007	Schools, Korea and Sweden	Reservoir dust (vacuum cleaning), Petri dishes
Zhao et al 2006	Schools; Sweden and China	Reservoir dust (vacuum cleaning), Petri dishes
Kim et al 2005	Schools, Sweden	Reservoir dust (vacuum cleaning)
Emenius et al 2001	Inside and outside a stable, Sweden	Air samples (air pumps) Cloths
Egmar et al 1998	Bed mattresses in furniture stores, Sweden	Reservoir dust (vacuum cleaning)

filter, and this method has been used by some for measuring horse allergens in and nearby horse stables [30–32]. Other sampling methods include sampling of settled dust with electrostatic cloths or equivalent [30] and airborne particles with electrostatic samplers or ionizers [28, 33]. In Sweden, a more unconventional sampling method using aspen leaves has also been assessed for studying deposition of horse allergens at different distances from horse tracks. Aspen leaves are suitable since they are often in a horizontal position [26].

Analyzes Methods for Detection of Horse Allergen in Environmental Samples

Before monoclonal antibodies (mAbs) to horse allergens were developed, horse allergens were analyzed with counter-current immunoelectrophoresis (CCIE), using polyclonal rabbit antibodies to horse allergen. The results were “expressed as titers (ie, as the reciprocal value of the most diluted extract that yielded a precipitate)” [22].

In 2001, a sandwich enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies was established, with the purpose of detecting horse allergen in environmental analyzes of ambient air and settled dust [30]. The 2 monoclonal antibodies, 103 and 14G4, recognized different epitopes of the same molecule and were hence used as capture and detection antibodies, respectively. The detection limit of the ELISA assay was 5 units/mL where 1 unit equals 1 ng protein of the NIBCS (National Institute for Biological Standards and Control, Hertfordshire, UK) standard extract of horsehair and dander. In the study, the horse allergen recognized by the mAbs, was demonstrated to be IgE-binding, but was not further characterized.

Ever since, this ELISA assay has been used, with minor alterations [23, 27•, 28, 29]. The horse allergen detected by

the mAbs has yet not been fully characterized and is often referred to as Equ c x. However, Elfman et al demonstrated that the mAbs 103 and 14G4 bind to one of the horse allergens, Equ c 2, 4, or 5, which are all similar in molecular mass [31] but it is believed that the antibodies are directed to the Equ c 4 allergen [26]. In addition, Elfman and colleagues used another standard, produced from horse dandruff (Allergon), and twice as high as the NIBSC standard, yielding a lower detection limit of 2 units/mL [31].

In the most recently published study, the ELISA assay has been amplified, mainly by using K-Blue peroxidase substrate, instead of alkaline phosphatase substrate (ALP). The detection limit of the assay is 0.2 units/mL, which is approximately 10-fold more sensitive than the “standard” ELISA assay [27•]. This facilitated the analysis of horse allergens in school environments where allergen levels are low.

The Dispersion of Allergens

In a study by Egmar et al [22], the main purpose was to examine the presence of cat and dog allergens in public environments, but horse allergen was also measured, although the method to analyze horse allergen was different compared with cat and dog allergen (CCIE vs ELISA, see above). A test room where customers could test displayed beds and mattresses in a furniture store was chosen as a model, and levels of pet allergens in 15 factory-new mattresses were compared with 15 mattresses tested by customers. Samples were collected by vacuuming mattresses, which had been available for testing during a period of 2–78 weeks. Low concentrations of cat allergen, Fel d 1, and dog allergen, Can f 1, were found in most of the brand new mattresses. Horse allergen could, however, not be detected in any of the factory new mattresses, although 6 of them

were stuffed with horsehair, but was found in 11 of the 15 customer-tested mattresses, in a titer ranging from 32 to 64 (median 4). The difference between new and tested mattresses was significant ($P=0.006$), and there was a positive correlation between testing (exposure) time (weeks) and the concentrations/titers of horse dander, $r=0.64$, $P=0.008$.

Emenius et al [30] evaluated the newly developed horse-allergen-specific mAb assay, described above. Seventeen air samples were collected (using portable air pumps and pieces of fabric for samples of settled dust, respectively) inside 1 stable and outside, in the vicinity (<40 m) of the stable. Airborne levels of horse allergen were over 500-fold higher inside the stable (~ 100 horses) compared with just outside the stable, and over 3000-fold higher than outside a residential building located only 12 m from the stable. Horse allergen could not be detected in air samples collected at 40 m, nor in the reference area over 500 m from the stable, indicating a very rapid dilution of horse allergen levels in the ambient air, ranging from a mean of 438,980 units/m³ indoors in the stable, to 1140 units/m³ outside the stable entrance, to 150 units/m³ (mean) 12 m from the stable.

This first study by Emenius et al was followed by a second study [26], also investigating the transfer of allergens into homes located nearby 3 stables. The same horse-dense area (>200 horses) adjacent to downtown Stockholm was chosen for the study. In total, samples were collected at 49 different sites and resulted in 45 indoor and 26 outdoor samples (Petri dishes; sites outdoors sheltered from rain). Twenty-seven of the sampling sites were located in areas where horses were often present and 22 sites in areas where horses were uncommon. This paper also includes data from horse allergen samples collected near a training track for trotting horses. All outdoor samples, from housing areas as well as from the horse-training track, confirmed previous results; outdoor levels of horse allergen seem to drop quickly when the distance from stables increases. Only 6 apartments had measurable concentrations of horse allergen indoors. Three of those had horse allergen indoors, although none of the inhabitants reported any contact with horses. All 3 homes were located less than 20 m from the nearest stable, and facing the stable. Measured indoor levels were about 1 %–2 % of the outdoor levels (measured on 2 balconies, <10 m from the ventilation outlet of the stable). For the other homes, detectable indoor horse allergen levels could be explained by self-reported horse contact among the inhabitants.

The dispersion of horse allergen in ambient air was also investigated by Elfman et al in 2008 [31], using the same methods as above. In this study, air samples were collected with stationary pumps placed 1–1.5 m above ground level at 2 different seasons (spring and autumn) and at various sampling sites (spring 53 sites,

and autumn 30 sites) distributed in a radial pattern from the middle of the area and at distances of 50, 100, 200, 300, 400, and 500 m in all directions. A further 29 samples of settled dust were collected outdoors and indoors, on hard horizontal surfaces with an electrostatic cloth. Some samples were also taken from filters in the ventilation system. Around 400 office employees working in an office located close to the stable area also answered questionnaires on 2 occasions in 2003 (February and September) and a third time in September 2004, after the office had been moved to new premises 500 m east of the stable. Similarly, measured levels of horse allergen declined rapidly with the distance from the stable, even though low levels, less than 5 % of the level obtained at the stable entrance, were also detected at 50–100 m distance from the stables (levels below the detection limit in the studies by Emenius et al). The level of horse allergen in most airborne samples from the office building was below the detection limit, and could only be detected in 1 room very close to the stable area. However, various levels of horse allergen could be measured in ventilation inlet filters in this building, equipped with a mechanical ventilation system. Among the staff, only a few experienced any annoyance, and there were few significant differences between the 3 time points.

In a newly published study [27•], the 2 research groups (Stockholm and Uppsala) together investigated the presence of horse allergen in schools, and correlation between allergen levels in schools and number of children with regular horse contact in their leisure time. Air and dust samples from 3 different school exposure studies were analyzed for the content of horse allergen Equ c x. In total, >400 samples from classrooms located in 235 schools were collected. Petri dishes and/or vacuum cleaners were used to collect airborne and settled dust, respectively. Furthermore, in 20 classrooms (4 schools), airborne settled dust was collected with Petri dishes in classrooms every week for 10 weeks. In classrooms, where the percentage of children with horse contact was above the median rate of reported horse contact, the levels of horse allergen sampled with Petri dishes were significantly higher compared with those in classrooms where children reported less or no horse contact.

A positive relationship between horse allergen levels and rate of horse contact was also shown for vacuumed dust samples. Most schools were situated far away [>500 m] from stables, which shows that close vicinity to horses is not a requirement for obtaining detectable levels of horse allergen indoors. Hence, as has been shown previously for cat allergen, it was suggested that transfer of horse allergens occurs via contaminated

clothing [34, 35]. The authors conclude that measures should be taken to minimize possible transfer and deposition of allergens in pet-free environments, such as schools.

Kim et al [29] measured levels of horse allergen in 8 primary schools. Settled dust was collected from desks, chairs, and floors, by using a vacuum cleaner. The results showed frequent contamination of both cat, dog, and horse allergens in classrooms. All had cat and most had dog and horse allergens; median level of horse allergen was 945 U/g (<200–31 000 U/g). The authors had no information about the prevalence of horse contact in the families, but due to geographical and socioeconomic factors, horse contact was expected to be common.

From the same research group in Uppsala, Sweden, there are also 2 articles comparing differences between levels of furred pet allergens in schools in China (Taiyuan city), Korea (Namyangju and Chuncheon city), and Sweden (Uppsala), which include both measurements of horse allergen in settled dust from desks, chairs, and floors and airborne dust in Petri dishes.

Zhao et al [24] compared measured allergen levels in settled and airborne dust from Taiyuan schools (China) with levels in dust from Uppsala schools (Sweden). None of the Chinese airborne dust samples contained horse allergen (Equ c x). In contrast, most of the Swedish airborne dust samples contained both cat, dog and horse allergens, often in high levels. Median level of airborne horse allergen in dust was 1250 U/g dust. Data on contact with furred pets seems unavailable in the present study. Kim et al [27••] presented a project with a similar aim as above. Data obtained from 65 classes in Korea, was compared to schools in the region of Uppsala, Sweden. In Korea, 19.9 % of the pupils had at least one pet at home, whereas in Sweden it was 58.8 %. The most common pet in Korea was dog (14.0 %), whereas in Sweden it was cat (25.9 %). Only 1 pupil (0.04 %) had contact with a horse at home in Korea and 2 (0.45 %) in Sweden. However, as above, all Swedish classrooms had cat allergen and most had dog and horse allergens above 1000 ng/g. In Korea, cat allergen was found in only 3 of 68 dust samples (4 %), dog allergen was found in most samples (88 %), and only 1 sample (1 %) contained horse allergen.

All the above studies show the same pattern; horse allergen mainly seems to be distributed by humans to various, normally pet-free, indoor environment via contaminated clothing and hair, as has been elegantly demonstrated by others for cat allergen [34–37]. The outdoor dispersion of horse allergen seems to be of subordinate importance, with the exception for [humans and] buildings in the very close vicinity to animal handling.

Clinical Aspects of Allergic Sensitization to Horse Allergens According to Modality of Exposure to Horse in Patients Living in Urban Areas

Respiratory Symptoms

In 2005, Liccardi et al described 2 unusual cases of severe bronchospasm following horse exposure in a stable in 2 individuals living in Naples without previous relevant direct/indirect contact with this animal. Skin prick tests [SPTs] and evaluation of serum specific IgE antibodies revealed intensive, exclusive allergic sensitization to horse allergens [38]. After this preliminary observation, the same group (Liccardi et al) aimed to seek possible relationships between allergic sensitization to horse allergens and different modalities of exposure to horse by studying an urban atopic population living in the Naples area and not occupationally exposed to horse or horse-derived materials [39]. A total of 1822 patients were examined; of the 1201 individuals with a positive SPT for at least one allergen, 35 adults (3.43 %) were sensitized to horse dander. Among the horse allergic patients, 6 subjects reported direct horse contact and 10 denied direct exposure to the animal but had only occasional contact with horse owners (indirect exposure). Lastly, 19 patients denied apparent direct or indirect exposure to horses or horse allergens. Twenty of the 35 horse-sensitized patients reported both nasal and bronchial symptoms, 14 reported rhinitis without asthma, and 1 reported asthma without rhinitis. The results of SPT and specific IgE levels against allergen extract from horse dander (e3) did not differ significantly between patients with direct contact with horses vs those with indirect exposure; between patients with direct contact vs those with no apparent contact, or between patients with indirect exposure vs those with absence of apparent contact with horse. On the contrary, specific IgE antibodies against Re205 (horse serum proteins) were significantly higher in patients with direct contact vs those with indirect exposure, and vs those with no apparent contact. Apart from 1 patient, all individuals exposed directly to horse had specific IgE against horse serum proteins, whereas no patient with indirect exposure or no apparent contact had serological responses to horse serum proteins. Other observations emerging from the present study are the high prevalence of female sex (25/35), a family history of allergy (25/35), and a concomitant allergic sensitization to dog and cat (23 and 25, respectively/35).

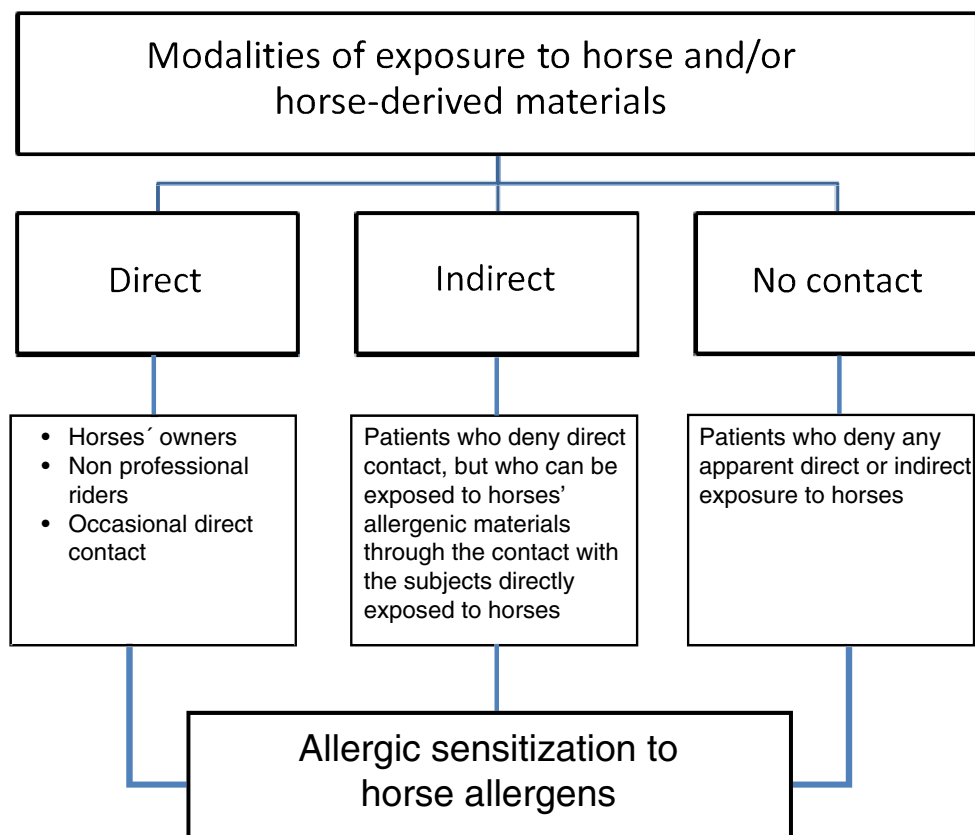
Moreover, a multi-center study was carried out involving 19 allergy units belonging to the Allergy Study Group of the Italian Society of Respiratory Medicine [SIMeR], uniformly distributed over the Italian territory to confirm our previous results at a national level [40••]. A total of 3235 patients were included in the database. Out of these, 2097 (64.82 %) had a SPT positivity for

at least 1 allergen and were diagnosed as having respiratory allergy. 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. Only 9 of the 113 patients were mono-sensitized. Thirty patients reported a direct horse contact (4 for ownership and 26 for riding or occasional contact), 23 patients denied 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 mono-sensitized 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. 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) [40•]. In both these studies we have classified possible modalities to exposure to horses as suggested in the Fig. 1.

A recent Italian pediatric study by Novembre et al [41] reported that the prevalence of horse sensitization in children was 2.7 %. Moreover, the authors outlined the importance of indirect exposure to horse allergens in inducing allergic sensitization and recommended horse inclusion in the routine panel of allergens used for the diagnosis of respiratory allergy. On the basis of all these reported studies in children and adults [39, 40•, 41], Italian Adaptation of 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 [42]. Liccardi et al [43•] have also shown that high percentages of patients sensitized to several furry animals [including horses] exhibit only indirect exposure/no contact to animals.

It is not easy to explain allergic sensitization to horse allergens in individuals with no apparent direct or indirect exposure to horse. It is likely a mechanism of cross-reaction between horse allergen and the major allergens of other mammalian species, namely dogs, cats, cows, guinea-pigs, rabbits, etc [44, 45]. All these allergens are lipocalins [20], and a recent study by Nordlund et al [46] has shown that IgE antibodies to animal derived lipocalin, kallikrein, and secretoglobulin are markers of bronchial inflammation in severe

Fig. 1 Suggested modalities of exposure to horse or horse-derived materials



childhood asthma. Moreover, Nilsson et al [47] have demonstrated Can f 6 as a new lipocalin dog allergen that cross-reacts with lipocalins from horse and cat. Serum albumin (a 68 kDa pan-allergen involved in milk, meat, and epithelial allergy) may constitute another cross-reacting allergen [48•]. Moreover, a recent study demonstrated that first contact with serum albumin was through cow's milk and that patients might have developed sensitization to epithelia serum albumin even without direct contact with animals [49].

Systemic Symptoms

Anaphylactic reactions have been described after a simple exposure to horse [50] and after a horse bite [51] in highly sensitized individuals.

Conclusions

Allergic sensitization to horse allergen is often considered unusual in patients living in urban areas because of the lack of exposure to the animal and its allergens. However, the results of the studies in the present review shows that horse allergen are transferred into various indoor environments by humans in contact with horses, and potential sensitization following indirect exposure to horse allergen transferred into normally horse-free environments cannot be totally avoided. The results of the studies have further shown that the prevalence of allergic sensitization to horse dander in the urban atopic population may be consistent and may occur even without any apparent direct or indirect contact with horses due to a cross-reacting mechanism between horse allergens and allergens produced by common pets or other furry animals. In some geographical areas, such as Northern Europe, where the number of horses is very high and animals are located near city areas, the presence of horse allergens is common in public places and the indirect modality of exposure to horse or horse-related materials inducing allergic sensitization may be consistent. As a consequence, many allergic individuals are unaware of the exposure and that they are at risk of exacerbation of respiratory symptoms following this indirect exposure to horse since it is likely that animal-sensitized patients constitute an “allergic phenotype” [52•], individuals suspected of being highly atopic or those already sensitized to pet allergens should undergo SPTs and evaluation of serum specific IgE antibodies to horse before beginning an activity involving regular direct horse contact and before entering environments containing horses such as stables, riding schools, and race courses. Hence, we suggest that horse allergens

should be included in the standard panel for the diagnosis of respiratory allergy.

In addition, measures should be taken to minimize the transferal and deposition of allergens in pet-free environments to protect susceptible individuals from involuntary exposure to horse allergen. For instance, stables and riding schools should be provided with facilities such as dressing rooms and showers.

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Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

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