

Detection of Horse Allergen around a Stable

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Key Words

Air sampling · Allergen dispersion · Horse allergen

Abstract

Background: Integrating horse stables with built-up areas may lead to conflicts. Dispersion of horse allergen may become a health risk for allergic people. The aim was to measure the dispersion of horse allergen around a stable, considering wind speed and direction and vegetation. The disturbance of staff at a workplace nearby a stable was investigated. **Methods:** Air sampling was performed around a stable (32 horses) at distances of 50–500 m in all directions. Sampling was done with a pump and an IOM sampler. Samples were collected at 50 points during all seasons. Horse allergen levels were determined using ELISA. Disturbance by horses was studied with a questionnaire handed to the employees in an office near the stable. **Results:** The median horse allergen level at the stable entrance was 316 U/m³, in the horse fields 40 U/m³ and in the whole source area 16 U/m³, which declined to <2 U/m³ at about 50 m from the source area. Downwind of the prevailing winds low levels of horse allergen (2–4 U/m³) could sometimes be detected at up to 500 m. The staff, including those allergic to horses, managed to tolerate horses close to the workplace. **Conclusions:** At low winds horse allergen spread in ambient air about 50 m from the stable and horse fields. At higher winds low allergen levels were sometimes found in open ar-

eas up to 500 m from the source area. These levels were similar to those found in the office after moving away from the stable area. The employees did not report more symptoms of allergy or asthma while working close to the stable compared to after the move.

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Introduction

There are about 280,000 horses in Sweden today. About half a million people (6%) ride and this number is steadily increasing. Therefore an increasing number of stables are integrated with built-up areas and this may lead to conflicts. The spread of horse allergen into the surroundings is of particular concern since this could become a potential health risk for people allergic to furred animals.

The second most common allergy in Sweden, after allergy to birch pollen, is to furred animals. The risk seems to vary between species and breeds [1]. Approximately 15–18% of Swedish schoolchildren are sensitized to cat, dog or horse allergen [2, 3] and in adults it is about 15% [4]. The prevalence of horse allergy in the general population is not well known. However, earlier studies indicated that 28–46% of children with asthma have horse allergy [5, 6].

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The most important source of horse allergen is horse dander [1] followed by hair and skin scrapings. Exposure to horse allergens can occur directly through horse riding or indirectly through the transport of allergens in the ambient air from stables [7] or by means of transfer on clothes or hair to public environments [8–11].

In Sweden the recommended safe distance between new schools, daycare centers or homes and stables is 500 m [12]. There were no data available on the spread of horse allergen when this distance was set and it has been questioned in township planning. A previous study conducted in a densely built-up area showed that airborne horse allergen spread no further than 20–40 m from a stable [7].

The main aim of this study was to estimate the dispersion of horse allergen by measuring allergens in the ambient air and dust settled around a stable, taking the influence of weather, such as wind speed and direction, and vegetation into account. A second aim was to investigate the disturbance by and infiltration of allergen from a stable into a workplace located nearby. Finally we validated a new standard for the horse allergen assay.

Material and Methods

Study Design

The stable with 32 horses was a riding school. The horses were outdoors between 8 a.m. and 5.30 p.m., most of the time in enclosed pasture areas 50 m south and 250 m southwest of the stable (fig. 1). A large university building was 50 m to the north and an office building was 50 m to the west. South of the offices there was open ground extending for several hundred meters, while in westerly and easterly directions there was forest close to the pastures.

Air samples were taken during April to June (13 days) and August to September (13 days) 2002 at 53 and 30 sampling points, respectively. The points were 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 (fig. 1). Samples were taken 3 times at each point. Sampling was done between 8 a.m. and 5.30 p.m., when activities around the stable were greatest. Samples of settled dust were collected from hard surfaces on five occasions: three during May to June and two during August to September.

Seasonal variations were investigated by measuring in 2003 on 3 days in February to March and 2 days in September and in 2004 on 4 days in March and 3 days in September to October. At these times a smaller number of measuring points were used. In total, 325 samples were collected over 3 years, of which 213 were from outdoor air.

Local weather conditions were measured at about 1.5–2 m above ground level in the open pasture area. Temperature and relative and absolute humidity were measured with a TH-Calc instrument (TSI Instruments, USA). Wind speed was measured with a hand-held Silva Windwatch® and wind direction with a

compass (both from Silva, Sweden). Our local measurements were compared with data from the Swedish Meteorological and Hydrological Institute. Their collection site was 10 m above ground at Uppsala Airport situated 6 km north-northwest of the stable.

Annoyance by and infiltration of allergen were studied in an office building, originally located 30–150 m from the stable, before and after moving to a new location.

Methods for Sample Collection

Air samples were collected with stationary pumps placed 1–1.5 m above ground to represent the breathing zone. Allergen particles were collected with an IOM sampler with Fluoropore membrane filters (pore size 1.0 µm, Type FA, Millipore, Stockholm, Sweden) connected to a pump operating at 2.0 liters/min (SKC, Eighty Four, Pa., USA). Samples were taken for 7–8 h/day.

Samples of settled dust (29 samples) were collected on hard horizontal surfaces (e.g. window sills, roofs, post boxes) with an electrostatic cloth (4 · 4 cm, Swiffer™, Procter & Gamble, USA) [13]. New gloves were used for each sample to avoid contamination. Samples were stored at –20°C until extraction. The area of sampling was measured (0.01–0.25 m²) and allergen concentration was calculated per square meter.

Samples of indoor air (36 samples) and settled dust (5 samples each in February and September 2003) were collected in the office building and outdoors (10 samples), before and after moving the workplace. On the first occasion (February 2003), samples were also taken from the filters in the ventilation system. Indoor samples were taken at four different locations in the office used by many employees.

Analysis of Allergen Concentration

Sample Preparation

Air samples were extracted in 1 ml of phosphate-buffered saline containing 0.05% Tween 20 (PBS-T) and 1% bovine serum albumin (BSA, Sigma, USA) at 4–8°C overnight with agitation. The solutions were centrifuged at 10,000 rpm (7,000 g) for 10 min and supernatants were kept frozen at –20°C until analyzed. Samples of settled dust were extracted in 5 ml of PBS-T and then treated the same way as air samples.

Allergen Concentrations by ELISA

Horse allergen levels were determined using a two-site sandwich ELISA. The assay was basically performed according to the protocol published by Emenius et al. [7] (reagents were generously supplied by Mabtech, Stockholm, Sweden), except for the different horse allergen standard used in this study. The standard curve range was 2–64 U/ml. Air samples were analyzed undiluted, while settled dust samples were run at 1/5 to 1/125 dilution. Horse allergen levels were expressed as U/m³ air or U/m² for settled dust, where 1 unit is equal to 1 ng protein of the horse standard (Allergon, Valinge, Sweden and NIBSC, Potters Bar, Hertfordshire, UK). The detection limit for the ELISA assay was 2 U/ml meaning about 2 U/m³ or equal to 100 U/m².

Characterization of the Horse Allergen Standard

Horse allergen standard was prepared from horse dandruff (Allergon, Sweden, art. No. 0030116100, batch No. 204697001). Horse dander (100 mg) was extracted with 2 ml of PBS-T, containing thimerosal (0.1 g/l) as a preservative, for 2 h at room temper-

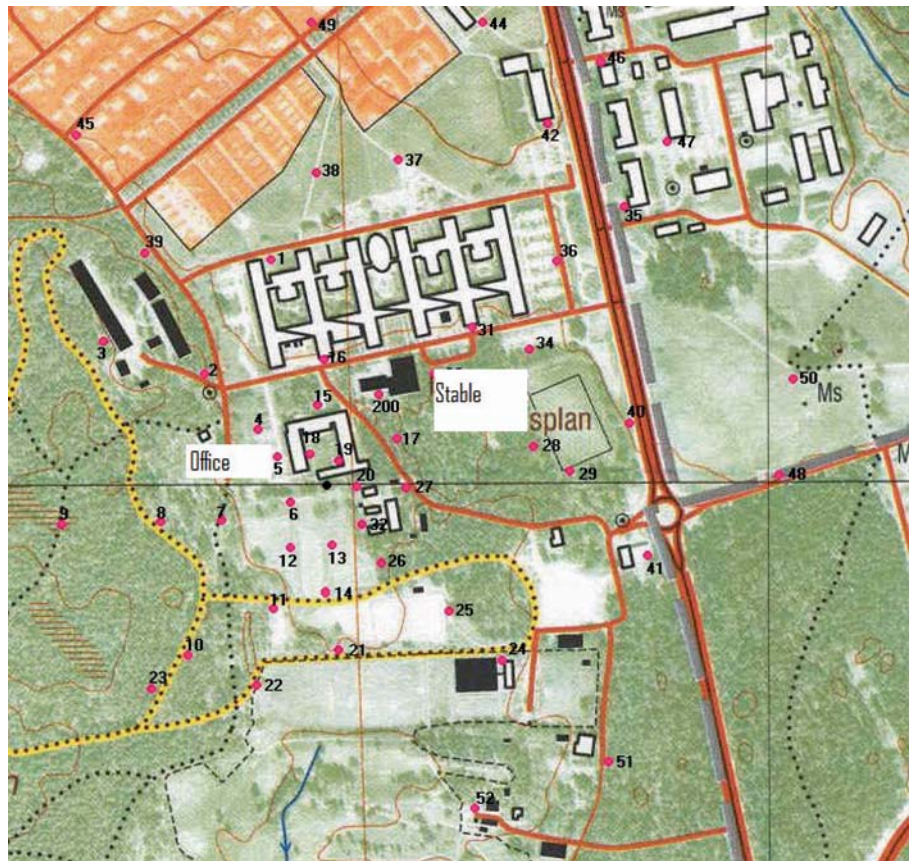


Fig. 1. Map of the stable area, horse fields, riding ground and sampling points (sample 43 was located 750 m north of the stable, i.e. outside this map).

ature with agitation. Insoluble material was removed by two centrifugation steps (at 3,500 g for 10 min and 7,000 g for 10 min). The extract was aliquoted and stored as a 1/5 dilution with thimerosal at 4–8°C. To compare levels of horse allergen (Allergen) with an earlier study [7] the standard was set to a horse standard from NIBSC (National Institute for Biological Standards and Control, UK). Protein in both standards were determined with the Micro BCA protein assay reagent kit (Pierce Chemical Company, USA) using BSA as a standard.

Analysis of Horse Dander Extract by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis and Immunoblotting

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of horse dander extracts (~300 ng protein/well) was performed on ExcelGel SDS (Multiphor II, GE Healthcare, Uppsala, Sweden). The extract was diluted with sample buffer with or without dithiothreitol (reduced and unreduced). LMW Calibration kit for SDS electrophoresis was used (GE Healthcare). The gel was silver stained following a slightly modified method [14] using Hoefer Automated Gel Stainer (GE Healthcare, Sweden). Immunoblotting analysis of horse dander proteins was run on Multiphor II as described by the manufacturer. Strips were incubated with monoclonal antibody 14G4 or 103 and control strips in only sample buffer. Bound monoclonal antibody was detected by incubating with sheep anti-mouse IgG conjugated with horse

radish peroxidase followed by chemiluminescence detection using ECL reagent and Hyperfilm ECL (GE Healthcare).

Questionnaire Study in an Office

Around 400 office employees were given questionnaires twice 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. The questionnaire was distributed and collected by the internal mail at the workplace, and was answered anonymously.

Apart from background factors such as age and gender, the questionnaire contained questions on annoyance to noise, dust and smell at the workplace. In addition, the participants were questioned on their health status, including symptoms of asthma and allergy and medication. The questions on annoyance were phrased as follows: 'To what degree have you, in the last week, been disturbed or annoyed by the following factors at your workplace?' The factors were noise, smell and dust. Annoyance by each factor was qualified by '...from activities in the surroundings', '...from the workplace', and '...from road traffic'. The respondents were asked to choose between the alternatives 'not at all, hardly at all, only a little, somewhat, quite, much, very much'. This paper only includes the answers concerning smell and dust from activities in the surroundings. Respondents who answered that they were disturbed at least 'somewhat' were considered to be disturbed.

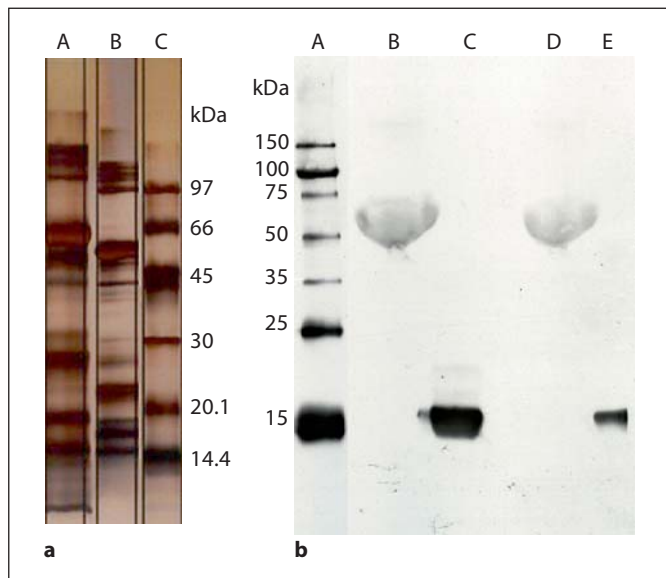


Fig. 2. **a** SDS-PAGE: lane A = reduced horse dander extract; lane B = unreduced horse dander extract; lane C = molecular weight standards. **b** Immunoblotting: lane A = molecular weight standards; lane B = mAb14G4 (reduced SDS-PAGE); lane C = mAb14G4 (unreduced SDS-PAGE); lane D = mAb103 (reduced SDS-PAGE); lane E = mAb103 (unreduced SDS-PAGE).

Questions on health status included: 'Have you been diagnosed by a physician as having asthma?', 'Do you presently take any medicine for asthma?', 'Do you have allergy to...' with separate questions about cat, dog, horse, birch, and grass. Respondents answering yes to at least one of these questions were regarded as having respiratory allergy. These questions were followed by: 'Have you had any symptoms of allergy during the last week?'

Statistical Methods

Concentrations of horse allergen are presented as median values for each sampling point. Allergen levels below the detection limit were assigned a value of 1.0 U/m^3 (half the detection limit) for statistical calculations. The degree of disturbance between seasons and building location was analyzed for employees with and without asthma or allergy using the χ^2 test. Correlations between local wind measurements and official wind data were calculated using Spearman's rank correlation coefficient. A two-tailed significance level of 5% was used.

Results

Horse Allergen Assay

Validation tests, using both stressed and real-time studies, showed that the horse allergen standard was stable for at least 2 years at $4\text{--}8^\circ\text{C}$ instead of only 1 week as for the NIBSC standard. The standard used in this study was twice as high as the NIBSC standard, which means

that values should be divided by 2 when comparing it with the earlier study [7].

Figure 2a shows the result of SDS-PAGE of the horse allergen standard. Lane A and B show the extract under reducing and nonreducing conditions. Lane C shows the molecular mass protein markers. In figure 2a the following horse allergens could be identified: Equ c 1 (22 and 31 kDa, nonreduced and reduced form), Equ c 2 (16 and 18 kDa), Equ c 3 (65 kDa), Equ c 4 (17 and 4.5 kDa), and Equ c 5 (17 and 20.5 kDa). Immunoblotting (fig. 2b) showed that both monoclonal antibodies 103 and 14G4 bound to one of the horse allergens Equ c 2, 4 or 5. The horse allergen proteins could not be distinguished since they are very close in molecular mass. Neither of the two antibodies bound to the reduced form of the allergen, indicating that both antibodies bind to conformational sites on the allergen.

Detection of Horse Allergen in Ambient Air

The median level of horse allergen inside the stable was $4,300 \text{ U/m}^3$ ($n = 12$, range $1,926\text{--}6,272 \text{ U/m}^3$). The level at the ventilation outlet on the roof of the stable was $14,500 \text{ U/m}^3$. At the outside of the stable entrance the median level in summer was 316 U/m^3 and in winter 123 U/m^3 . In the source area the level of horse allergen was 16 U/m^3 in summer and 8.3 U/m^3 in winter. The horse allergen levels at 50–100 m around the stable were less than 5% of the level obtained at the stable entrance (table 1).

We regarded the whole area where the horses moved about during the day as the source area of horse allergen. The median level of horse allergen in the pastures was 40 U/m^3 and around 10 U/m^3 at the riding grounds, used only in the afternoons. The horse allergen levels outside this source area rapidly declined to $<2 \text{ U/m}^3$ (median; range $<2\text{--}41 \text{ U/m}^3$), where the highest level of 41 U/m^3 was measured at point 11, which was about 25 m south of the pasture and at the riding track. Levels of allergen in samples from all other points in the forest were below the detection limit.

Influence of Weather Conditions

The median temperature was 14°C (range -4 to 26°C), median relative humidity was 49% (23–84%) and median absolute humidity was 5.8 g/m^3 ($1.5\text{--}10.5 \text{ g/m}^3$). Allergen levels were not correlated to temperature, or relative or absolute humidity.

The horse allergen level measured at the midpoint of the pastures varied with the season with a median level of 9 U/m^3 in winter, 43 U/m^3 in spring to summer and 20 U/m^3 in autumn. It was possible to assess the influence of

Table 1. Spread of horse allergen around a stable

	Summer			Winter		
	n/N	median U/m ³	range U/m ³	n/N	median U/m ³	range U/m ³
Stable entrance	10/10	316	169–706	2/2	123	67–179
Source area	42/49	16	<2–203	11/13	8.3	<2–165
50 m	12/27	1	<2–41	4/10	1	<2–21
100 m	6/15	1	<2–19	2/8	1	<2–5.0
200 m	4/21	1	<2–24	0/2	1	<2
300 m	1/16	1	<2–3.5			
400 m	2/19	1	<2–2.9			
500 m	3/21	1	<2–4.1			
Total	80/178			19/35		

n = Number of samples >2 U/m³; N = total number of samples.

wind on the dispersion of horse allergen by correlating allergen levels at certain points with the variation in wind speed and direction. The median wind speed was 2.2 m/s (range 0–6 m/s). The prevailing wind was from the south, occurring about 3 times more often than winds from the north, east, west or calm conditions. From table 2 it can be seen that the difference in horse allergen levels could be 2–10 times higher with a favorable wind compared to a contrary wind.

The correlations between our measurements of local wind and the official weather data was 0.672 for speed ($p = 0.000$) and 0.681 ($p = 0.000$) for direction. On average, the local wind speed at ground level was 60% of the official wind speed data for Uppsala. In 72% of the measurements, the main wind direction was the same as in the official data.

Horse Allergen in Settled Dust

The median level of horse allergen in the surface samples taken within the source area was 27 kU/m² (range 3–97 kU/m²). Outside the source area (25–50 m from stable and pastures) the median level was 30 kU/m² (range 5–195 kU/m²) and the highest level was detected in samples from the roof of the goods office, located at the side of the office building 30 m west of the stable and the manure heap. At distances of more than 100 m from the source area only 1 in 16 of samples was over the detection limit. It was 3 kU/m².

Allergen Levels in the Office Building

The level of horse allergen in most airborne samples from the office building was below the detection limit.

Table 2. Influence of wind direction and speed on dispersion of horse allergen

Point No.	Direction from source	Distance m	Wind direction	Wind m/s	Horse allergen U/m ³
0	N	0	ENE SW	2 0.5	4 40
5	N	25	variable SSE	2 2	<2 16
21	S	50	SE ENE S	3 2 2	<2 19 17
31	NE	100	calm SSW	0 5	<2 4
10	SW	150	SE ENE	3 2	<2 2
1	N	200	calm SSE	0 2	<2 8

Detectable levels were measured in only one room which was closest to the stable (2.2 U/m³). Allergen levels in the ventilation filters were 2,600, 30,200 and 24,500 U/m² in supply air and 85,700, 6,900 and 27,800 U/m² in exhaust air filters. There was no relationship between the location of air intake and the amount of allergen in the supply air filters. After the move, only one sample from the canteen (3.7 U/m³) was above the detection limit.

Questionnaire Study

Results are presented in table 3. The response rates were 69% (February 2003), 72% (September 2003) and 65% (September 2004). On average, staff had been working at this office for 7.9 years, 73% of the respondents were women and the mean age was between 40 and 49 years. Horse allergy was reported by 7.8 (February 2003), 6.4 (September 2003) and 6.2% (February 2004), while any respiratory allergy was reported by 24, 25 and 27% of respondents, respectively. Furthermore, asthma was reported by 6.8, 6.5 and 7.2%.

Among the staff few experienced any disturbance or annoyance and there were few significant differences between the three investigations. Among those with horse allergy, 2 persons reported annoyance due to smells from the surroundings in both the 2003 questionnaires, and none in 2004 after the move. Among those with asthma there was a nonsignificant increase in annoyance due to smells after the move. Among asthmatics, the prevalence of symptoms was higher in February 2003, and this coincided with a lower use of asthma medicine.

Discussion

In this study we found that horse allergen generally spread up to about 50 m outside the source area, i.e. the stable and outdoor areas where horses were kept. Depending on wind speed and direction low levels of horse allergen could sometimes (6 samples 2–4 U/m³) be detected between 300 and 500 m away. In total, we analyzed 58 samples from these distances.

The result from the SDS-PAGE of the horse allergen standard was comparable with earlier studies [15], in which the same source material was used but had a different batch number (204695001, Allergon). Immunoblotting studies showed that two monoclonal antibodies raised against horse allergen bound to a molecular mass of around 16 kDa. The horse allergen could not be identified as Equ c 2, c 4 or c 5. Further investigations are needed to determine which specific allergen the antibodies are directed against.

In this study, we chose to use a horse allergen standard different from the one used by Emenius et al. [7]. The reason was that our preparation was stable for 2 years compared to 1 week for the NIBSC standard. There is a factor of 2 between the two preparations, meaning that our levels should be divided by 2 to be comparable with this earlier study.

Table 3. Annoyance to smell and dust from the outdoor environment and allergic symptoms among employees working in an office located close to the stable (2003) and after moving to a new building (2004)

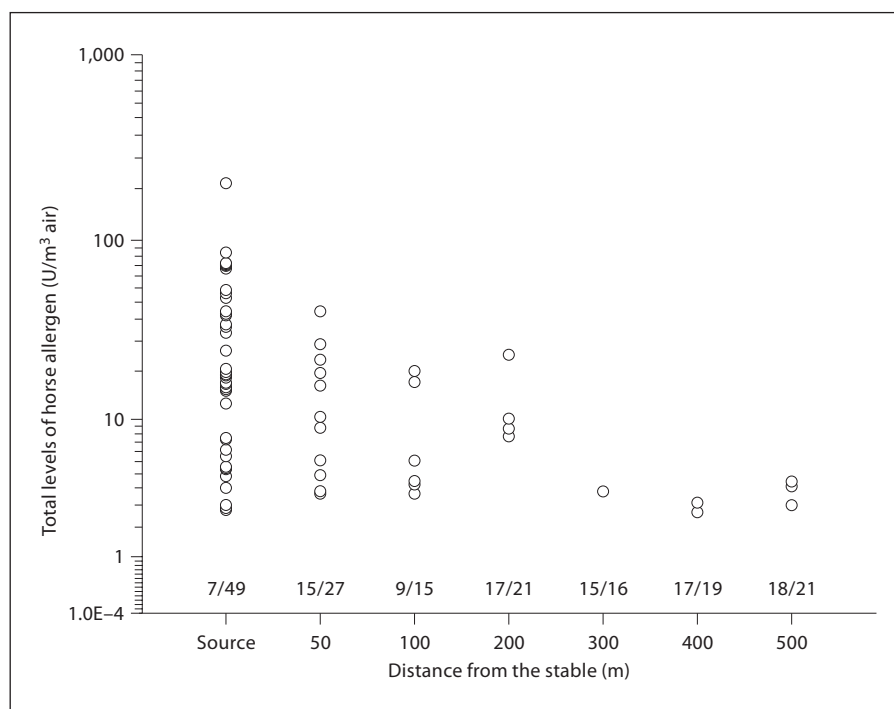
	Feb 2003	Sep 2003	Sep 2004
All respondents, n	262	284	272
Annoyance to smell, %	2.7	2.2	2.2
Annoyance to dust, %	1.5	2.2	1.9
Present symptoms of allergy, %	7.8	4.3	6.4
Present asthma medication, %	0.8	3.8	4.2
Respondents with respiratory allergy, n	57	64	70
Annoyance to smell, %	5.3	3.1	5.9
Annoyance to dust, %	3.5	4.7	4.4
Present symptoms of allergy, %	22	15	17
Respondents with horse allergy, n	18	16	16
Annoyance to smell, %	11	13	0.0
Annoyance to dust, %	5.6	0.0	0.0
Present symptoms of allergy, %	24	13	25
Respondents with asthma, n	16	17	19
Annoyance to smell, %	6.3	5.9	11
Annoyance to dust, %	6.3	5.9	5.3
Present symptoms of allergy, %	33	12	16
Present asthma medication, %	6.3	47	42

The detection limit for the ELISA assay was 2 U/ml protein meaning 2 U/m³ or 3 U/ml equal to 100 U/m². Emenius et al. [7] used a detection limit of 5 U/m³, while our detection limit, divided by 2, would be 1 U/m³. Our lower detection limit is mainly obtained by a longer time of sampling, 7 h instead of 3.

The allergen levels in ambient air declined rapidly with the distance from the stable and the riding grounds. Sampling points in the forest showed undetectable levels of horse allergen, indicating that foliage effectively stopped the spreading of horse allergens. Earlier studies have shown that trees can trap particles and their efficiency depends on wind speed, and type and density of vegetation, where conifers are most successful and reduce levels by 15–25% and deciduous trees by 3–17% [16].

At higher winds, allergens were found in open areas further away from the stable and riding grounds. At distances around 250 m northwest to northeast of the stable behind the university building very low levels (2–4 U/m³) could sometimes be detected, but only during prevailing winds (southwest to southeast). These levels were of the same order as those found in the office building, both before and after the move to the new location.

Fig. 3. Airborne levels of horse allergen at the stable entrance, source area and the spread at different distances. The figures above the x-axis indicate the number of samples below detection limit/total number of samples collected.



High levels of horse allergen were found in the exhaust air from the ventilation system of the stable. By improving the capture of allergen in the ventilation system this source may be reduced.

Allergen levels were influenced by the season: levels in autumn were half those in summer and winter levels were a quarter compared to summer. This may be explained by more rain during autumn, which washes away horse allergen, and frozen ground in winter that reduces airborne levels of allergen. Furthermore, horses mostly have covers in winter, which should reduce the spread of allergen.

Calculations of dispersion of pollutants are mostly performed using official meteorological data. In this study, local and official wind speeds and directions were fairly well correlated, even though local wind speeds were generally lower. Thus, local conditions ought to be taken into account when developing dispersion models.

Ventilation filters from the office building confirmed the presence of allergen both in intake and exhaust air, but showed no relationship to the location of the air intake. Differences in the amount of allergen may be more related to how long the filter had been used. Inside the building, levels of airborne horse allergen were of the same magnitude as after the move. This indicates that transfer of allergen on the clothes of employees with horse contact was

more predominant compared to direct infiltration from the stable. Several studies have shown that cat and dog allergens carried on the clothes and hair of pet owners contribute to the presence of allergen also in pet-free environments [8, 10]. We also reported the presence of horse allergens in schools [11], suggesting that a similar transport is valid for horse allergen. The office building was equipped with mechanical ventilation with the air intake high above the ground. It is possible that allergen levels would be higher in buildings with natural ventilation only, i.e. with windows that are open more often.

The presence of horses close to the workplace was generally well tolerated by the staff, including those with horse allergy. However, the number of persons allergic to horses was low (16–18 persons) and it cannot be ruled out that sensitive persons have avoided working in these premises. Persons with allergy or asthma were more annoyed by smells from the surroundings and had more allergic symptoms after the move to the new building, but the study does not indicate the cause of this. An increase in respiratory symptoms, attributed to exposure to emissions from newly painted indoor surfaces, has previously been found among office workers [17]. In February 2003 symptoms were also high, but our data imply that this might be due to the lower use of medication when it is 'out of pollen season'.

When establishing a safe distance around a horse stable, it should be important to take into account not only general data on allergen dispersion in ambient air, but also on odor dispersion and local conditions such as topography and vegetation, presence of buildings, and handling of manure. As regards exposure indoors, the construction of the building, including ventilation, must also be considered. Also more knowledge about the dose-response relationship between sensitization and symptoms and exposure to horse allergen is needed.

In conclusion, our study has shown that on calm days horse allergen spreads in ambient air as far as about 50 m outside the stable and outdoor area where horses are kept. With higher winds low levels of allergen were occasionally found in open areas up to about 500 m from the sta-

ble. These levels were similar to those found indoors in the office after the move from the stable area. The office employees did not report more symptoms of allergy or asthma while working close to the stable compared to after the move.

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