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Air Quality in Horse Stables

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1. Introduction

A large number of people are highly engaged in the equine industry around the world, especially in industrialised countries. For example, in the USA about 4.6 million Americans are directly involved in the equine industry, and in Sweden the industry provides full-time employment to over 30 000 people. Horse riding is the second biggest sport in Sweden after soccer, engaging approximately 6% of the population, and is by far the biggest sport for girls. Consequently, many people spend a considerable portion of their day in stable environments, either as workers in the care and training of horses, or in their leisure time.

The type of buildings used for stabling horses can vary widely. Some are newly built and thus designed solely for housing horses, whereas others have been converted or "retrofitted" from barns used for other farming activities, such as housing cattle or for feed storage. However, regardless of design these buildings have many common requirements for indoor environment, including selection of type of feed and bedding, and the need for continual removal of faeces and urine. Moreover, the building ventilation must provide adequate exchange of fresh air to the horses' living space to regulate the stable temperature and remove moisture, stable gases and organic debris produced by the horses and their activities.

There is no need to remove excess heat in stables in the winter in northern climates. However, this puts more focus on ventilation, which must rid the stables of moisture, odour, and ammonia that have built up in the more closed environment of the stable. Moisture originates from horse's respiration, faeces and urine, and other stable activities such as showering the animals, and general stable cleaning. Accompanying this moisture build-up is increased risk of condensation, intense odour, and increased ammonia release from bedding that ventilation must remove. How much ventilation should be provided? Ventilation of one air change per hour (ACH) means that the total volume of air in the stable is replaced in one hour. Guidelines for horse stables suggest providing between 4 to 8 air changes per hour to reduce mould spore contamination, minimize condensation, and reduce moisture, odour, and ammonia accumulation (Horse Stable Ventilation, 2003). Thus, for the mean of 6 ACH a stable's ventilation system must be capable of a complete air change every 10 minutes.

With the inevitable seasonal appearance of subzero winter weather, horse owners occasionally work against these principles of ventilation in an unheated building by trying to maintain warm stable conditions for their personal comfort during animal care activities. In addition, horses in active competition throughout winter may also be kept in warmer housing, which is why many stables prevent a seasonal change to a thicker, winter hair coat. Thus, during winter conditions, the inflow of cold outside air is limited by the closure of windows, blockage of ventilation channels, or even closure of entire sections of the stable to provide a more comfortable (personal) working environment. This, however, can adversely impair the removal of excess moisture, stable gases and organic air particles that should be replaced by fresh outside air via ventilation.

The outside air is presumed to be the optimal in air quality for respiratory health, and ventilation of stables aims to approach outside-air quality for both the horse, and the horse owner. Curiously, there is recent evidence that horses spending time outdoors in cold climates can acquire airway inflammation (Robinson et al., 2006). During winter with subzero temperatures, the air holds very little water (≤2 g/m^3). Thus the horse's respiratory system must both warm and humidify the inhaled air before it reaches the alveoli. In particular, exercise in cold air has been associated with up-regulation of inflammatory cytokines in the airways (Davis et al., 2005; Riihimäki et al., 2008a) and increased inflammatory cells in the lung in actively training race-horses during winter (Riihimäki et al., 2008a). However, despite the theoretical disadvantages of the horse breathing cold air, the bulk of evidence suggests that many components of stable air, and its gaseous and particulate matter, such as endotoxin, are perhaps greater risk factors for induction of airway inflammation in both the stabled horses (Pirie et al., 2003; Berndt et al., 2010) and for those people working in the stable (Tutluoglu et al., 2002).

The air of farm buildings housing large animals contains a wide variety of gases and organic dusts from microbial, plant, and animal sources. The organic dust may contain bacteria that are pathogenic or non-pathogenic, living or dead, and fungi, high molecular weight allergens, bacterial endotoxins, 1-3-β-glucans, pollen, and plant fibres (Douwes et al., 2003). Two major pro-inflammatory components of organic dusts include bacterial endotoxin, a component of the cell wall of gram-negative bacteria, and mould 1-3-β-glucans (Douwes et al., 2003). Endotoxin has been shown to be present in the air of animal housings in amounts presumed to be a respiratory hazard to both humans and livestock animals (Pomorska et al., 2007). However, apart from proposed guidelines (Donham et al., 1989) there are no officially accepted limits for allowable endotoxin content in the air for either farm animals or humans. Even less is known regarding the critical threshold for 1-3-β-glucans before impacting on airway health. However, it is well known that exposure to the variety of organic dust particles, microorganisms and endotoxins present in different farm animal stable buildings can cause chronic airway diseases in both humans and horses. In particular, recurrent airway obstruction (RAO, heaves), which is an animal model of asthma, and the syndrome of inflammatory airway disease (IAD) in horses are major, non-infectious, largely environmental causes of reduced performance and persistent coughing in stabled horses. For the first-named disease, endotoxin appears to act in synergy with other airway challenges to induce excessive airway inflammation even with low dust challenge (Pirie et al., 2003).

Stable environments can also be influenced through selection of type of feed, bedding and sanitary procedures, any or all of which can have a complex influence on stable-air quality (McGorum et al., 1998). For example, when assessing the influence of bedding on concentration of ammonia in the stable air, wheat straw was preferred over other materials such as wood shavings or straw pellets (Fleming et al., 2008a, 2008b). In contrast, bedding material low in inhalable dust, such as shredded paper, appeared to be preferable to straw bedding as the former was associated with reduction of markers of airway inflammation in horses with RAO (Wyse et al., 2005). However, in practical terms use of paper as bedding in horse stalls is associated with cumbersome handling and manure disposal. Peat moss is another popular stable bedding product, touted as being low in organic dust. However, depending on its source this product has been shown to vary widely in dustiness and hygienic quality (Airaksinen et al., 2005). Even local differences of bedding between boxes within a stable can influence the overall respirable particle load in the common stable airspace (Clements & Pirie, 2007). Clearly the various forms of bedding in a stable and the differences in bedding between boxes within a stable can have a substantial, yet elusively defined effect on the stable air dust load, and consequently on the airway health of the horses and people present.

The type of feed and method of feeding can also influence the amount of dusts being inhaled via stable air. For example, providing a low-dust feed in the form of ensilage has been shown to reduce the markers of airway inflammation in stabled horses (Wyse et al., 2005). In addition, even short-term immersion of dusty feed (hay) can reduce the air particle load in the stabled horses' breathing zones (Clements & Pirie, 2007). It is not known if these reductions in dust from horse feed are also important to the respiratory health of people.

Even the methods used to routinely clean the stable, irrespective of bedding or feed type, can have a substantial influence on the stable air quality. It is advisable to carry out work when there are no horses in the stables because activities in the stable (feeding, mucking out, and sweeping) cause increased levels of ammonia and airborne particle concentrations (Fleming et al., 2008a, 2008b; Sadegh et al., 2009). However, while common sense would suggest that daily cleaning and replacement of bedding, or at least daily removal of manure, would minimize accumulation of undesirable stable organic and gaseous materials, this may not be the case. Fleming and colleagues (Fleming et al. 2008a) have recently found evidence to the contrary - that, for a 2-week period, regardless of bedding type, the least build-up of particles less than 10µm in diameter, and of ammonium, was achieved when there was no mucking out or even manure removal. Presumably a build-up of urine during the course of the trial increased the moisture of the bedding material, which led to increased binding of organic particles that were subsequently not released into the atmosphere. If ones' goal is solely to minimize stable air respirable dust then, based on these results, it would be advisable to dispense with a daily mucking-out regimen for week-long periods and simply add additional bedding regularly to absorb urine. However, the choice of mucking-out regimen should not be based solely on single, selected measurements of factors such as ammonia and particle concentrations since the growth of pathogenic germs and fungi would certainly be favoured if the same bedding were maintained for some weeks. Moreover, specific composition of the stable air particles, in particular endotoxin and glucans, are likely to be far more important to respiratory health than increased stable air particles or manure gases. To help clarify which factors in the stable air are important to respiratory health, ventilation capacity, organic dusts, microorganisms and/or fungi were measured and correlated to the respiratory health of the horses and people breathing the stable air daily.

1.1 Aim

The aim of this chapter is to describe differences in indoor air in horse stables under winter and summer conditions. An intervention study is also described with measurements of stable air quality before and after installation of mechanical ventilation in a previously naturally ventilated riding school stable. In the same stable, detailed measurements of stable air quality were performed together with measurements of respiratory health of stable personnel and stabled horses. The hypothesis is that selected components of stable air will be correlated to indices of respiratory health in people and in stabled horses spending considerable time in the stable environment. Another aim is to determine if these indices are improved after changing the ventilation system of the stable.

2. Material and methods

2.1 Study designs

Seasonal changes in air quality were studied in a conventional racing stable with natural ventilation. The indoor stable environment, personnel and horses were investigated three times, first in the winter stabling period (February, year 1), second after the intervening summer (September, year 1), and the third time in the following winter (March, year 2). The second step was to perform an intervention study by introducing a mechanical ventilation system in a riding school stable.

2.2 Hygiene measurements in stable environment

Environmental monitoring was generally performed during the morning routines in the stables, when the personnel were cleaning the boxes or stalls by mucking-out the faeces and urine. Fresh straw was put in for bedding. Horses were typically fed three times a day with hay/haylage, pelleted fodder and oats: in the morning before going out to the fields, in the afternoon before training sessions, and in the evening. A second sampling period was performed in the afternoon, when horses were groomed before training sessions and made ready for riding, and when people and horses were going in and out sporadically.

Hygienic measurements included real-time monitoring (24h) of carbon dioxide (CO_2), temperature, relative humidity (RH%), ultra-fine particles and particulate matter (PM10, particle size 10 μm). Temperature, RH% and CO_2 were generally logged every minute with a Q-Trak™ (model 8550, TSI Inc., Minnesota, USA). Monitoring of ultrafine particles, which are defined as <0.1 μm particles, were performed with a P-Trak™ (model 8525, TSI Inc., Minnesota, USA). Airborne dust concentrations of PM10 were measured with a Dust-Trak™ aerosol monitor (model 8520, TSI Inc., Minnesota, USA) (Riihimäki et al., 2008a; Elfman et al., 2009).

Passive sampling is a convenient method for ammonia measurements. Passive samplers (IVL, The Swedish Environmental Research Institute, Gothenburg, Sweden) were put out at approximately 1.5m above the floor for 24 hours at three times during a week (Kirchner et al., 1999). Indoor samples were compared with outdoor sampling for 1 week at different distances from the stable (1-100m). Samples can be analysed by e.g. IVL, Sweden and values are expressed as $\mu g/m^3$.

Air sampling was performed with pumps for sampling of horse allergen, total and respirable dust, airborne microorganisms and products thereof such as endotoxin and 1-3-β-glucan. Generally, three samples for each factor were collected during a 4-7 hour sampling round. Pumps (SKC Inc., Eighty Four, PA, USA) were run with a flow of 2L/min, and placed at 1-1.5m above ground at representative places in the stable.

Airborne total and respirable dust were collected on a membrane filter in a cassette (∅ 25 mm, pore size 0.8 μm). For respirable dust, a metal cyclone (SKC Inc., USA) was used before the filter cassette. The pump was attached to the person's clothing and the filter head was placed at the breathing zone. For horses, the pump was put on a lunging-girth on the horse's back and the filter head was attached on the halter close to the horse's muzzle. Airborne dust samples were analysed by a gravimetric method and the organic portion were calculated after combustion of the filter and weighing of the remaining inorganic material. Results were expressed as mg/m^3.

Collection of microorganisms and endotoxins in the air were performed with cassettes with a sterile nucleopore filter (∅ 25 mm, pore size 0.4 µm). Surface sampling was performed with a Scotch-brite® or tape on the walls in stalls or boxes (Wålinder et al., 2011). The total concentration of airborne and surface bacteria and fungi was analysed by the CAMNEA method (Palmgren et al., 1986) based on acridine orange staining and epifluorescence microscopy. The detection limit for viable organisms was 30 colony forming units (CFU) per m^3 of air. Analysis of endotoxin was generally performed with the kinetic turbidimetric Limulus test. Results were expressed as ng/m^3 or EU/m^3. Analysis of 1-3-β-glucan was made with the Limulus test with glucan-specific lysate in the chromogenic kinetic version and results were expressed as ng/m^3 (Cape Cod Inc., MA, USA and Endosafe, Charles River Endosafe, Charleston, USA).

Horse allergen particles were collected with a pump fitted with an IOM-filter holder (SKC Inc., USA) with a fluoropore membrane filter (pore size 1.0 µm, Type FA, Millipore, USA). Airborne samples were analysed with an ELISA method using monoclonal antibodies for horse allergen (Mabtech, Nacka, Sweden) and results were expressed as Units/m^3 of air (Elfman et al., 2008).

2.3 Investigation of human respiratory health

2.3.1 Subjects

The first study was performed in a race-horse stable with 13 employees (Table 1). Initially, all personnel agreed to participate but, due to high turnover among the stable personnel, only one person fulfilled all three rounds of this study. The intervention study was performed in a riding school with 7 personnel and approximately 320 students aged 7 to 18 years attending the riding school (Table 1). All personnel agreed to participate, but one employee dropped out during the first sampling round and one left the workplace before the second sampling time point. Riding students older than 12 years, and who spent more than one day per week at the riding school, were asked to participate in the investigation. In the end, seven students gave their written consent to participate after permission was also obtained from their parents.

	Race-horse stable	Riding-school stable	
Number of	Personnel	Personnel	Students
Subjects	*n=13*	*n=7*	*n=7*
Males/Females	*6/7*	*1/6*	*1/6*
Mean age (range) years	*33 (22-66)*	*34 (21-51)*	*14 (13-17)*
Smokers	*3*	*0*	*1*
Atopics	*7*	*0*	*2*
Doctor-diagnosed asthma	*1*	*0*	*0*
Signs of bronchial obstruction*	*3*	*0*	*0*

*Signs of bronchial obstruction via increased PEF variability during daily measurements for two weeks

Table 1. Personal characteristics of stable personnel and riding school students.

2.3.2 Questionnaire studies

Personnel in the race-horse stable and the riding school stable as well as the riding-students were asked to answer a questionnaire regarding health issues. The questions were about current or earlier allergic diseases including asthma and medication, as well as current symptoms of eczema, eye irritation, nasal congestion and dyspnoea during the week prior to the investigation. Furthermore, they were asked to give their ratings of smell and dustiness in the stable.

2.3.3 Pulmonary function tests

Lung volumes and peak expiratory flow (Table 2) were measured by dynamic spirometry using a Spirobank G (MIR, Rome, Italy) (Figure 1) according to the American Thoracic Society standards (ATS, 1995).

Fig. 1. Portable spirometer (Spirobank G) for pulmonary function tests. Photo: Robert Wålinder

Parameter	What is measured?
FVC	Forced vital capacity (L)
FEV1	Forced vital capacity in one second (L)
PEF	Peak expiratory flow (L/min)
PEF-variability	Repeated PEF measurements, four times per day during two weeks (expressed as total variability between max. and min. in percent or coefficient of variation)
NO	Concentration of nitrogen oxide in exhaled air (ppb)

Table 2. Pulmonary function test parameters.

Subjects were also instructed to make four daily measurements of peak expiratory flow (PEF) for two weeks, using an electronic disposable flow meter (Piko-1, Medeca Pharma, Uppsala, Sweden) (Table 2). In the intervention study performed at the riding school, concentrations of nitrogen oxide (NO) in exhaled air were measured with a NIOX MINO (Aerocrine AB, Solna, Sweden) (Figure 2).

Fig. 2. Measurement of nitrogen oxide in exhaled air (NIOX MINO). Photo: Robert Wålinder

2.3.4 Nasal lavage and analysis of biomarkers

Different reactions in the nasal mucosa to environmental exposures can be explored by lavage of the nose and subsequent analysis of proteins in the lavage fluid (Table 3). All the personnel at the two horse stables therefore underwent lavage of the nasal cavity. Five millilitres of isotonic saline solution were flushed back and forth five times via a nasal olive attached to a 20 ml syringe. The lavage fluid was kept on ice until centrifuged twice at 800xg and 1200xg, respectively (Wålinder et al., 1998). The remaining supernatant was frozen at -20 ^{o}C until biochemical analysis. Biomarkers analyzed included eosinophil cationic protein (ECP), which is a marker of eosinophil activity (Venge et al., 1987), myeloperoxidase (MPO) from neutrophils in the mucosa (Venge, 1994), lysozyme a marker of neutrophil activity and secretion from parasympathetically innervated mucosal glands (Raphael et al., 1989), and albumin, which is a marker of capillary leakage of plasma proteins (Raphael et al., 1991). Analyses of ECP, MPO and lysozyme were made by radio-immunoassays (RIA) and albumin by nephelometry at the University Hospital of Uppsala.

ECP	Eosinophil cationic protein: the concentration reflects the presence and activity of eosinophil granulocytes
MPO	Myeloperoxidase: the concentration reflects the presence and activity of neutrophil granulocytes
Lysozyme	Is mainly released by parasympathetic stimulation of glandular cells, and the concentration is a measure of the secretory activity of the nasal mucosa. Smaller amounts are also released by neutrophils, macrophages, and monocytes.
Albumin	The concentration reflects the vascular leakage and also glandular secretion.

Table 3. Biomarkers in nasal lavage reflect different reactions in the nose

1.4 Investigation of horses

There are several techniques used in research and practice to detect, quantify, and characterize the inflammatory process in the respiratory tract of horses. These include physiological measurements. There are also various analyses aimed to examine cellular and non-cellular immunologic components. Diagnosis of airway inflammation in horses is determined based on a combination of history, clinical examination, endoscopy and, in many instances, bronchoalveolar lavage (BAL) cytology (Derksen et al., 1985; Tremblay et al., 1993). Results from pulmonary function tests are used in research and for clinical purposes for disease monitoring and in grading of disease severity (Derksen et al., 1985). Additionally, exhaled breath condensate has been introduced to evaluate inflammation markers in the lung (Wyse et al., 2005). In the search for potential allergens involved in RAO, an intra-dermal skin test and serum IgE analysis have been evaluated, but these have not proven to be clinically reliable in horses (Lorch et al., 2001; Lebis et al., 2002). Bronchial brushings, BAL, tracheal lavage, exhaled breath condensate, and even lung tissue samples have been shown to be of value in the investigation of the inflammatory process in the equine lung (Bureau et al., 2000; Gerber et al., 2003; Wyse et al., 2005). Imaging techniques, such as radiographic examination of equine lung, however, have shown to be of little value for the diagnosis of lower grade inflammation (Mazan et al., 2005).

Tissue samples from the airways provide an opportunity to further study pathological changes related to structural and cellular morphology. The trans-thoracic lung biopsy, the classical way to obtain tissue material from the distal airways from RAO horses, (Schatzmann et al., 1974; Pusterla et al., 2007) has recently been complemented by thoracoscopically guided pulmonary wedge resection (Lugo et al., 2002; Lugo et al., 2006). However, both these techniques are invasive, and the former may be associated with fatal complications (Savage et al., 1998). Endobronchial biopsies, which are a safer alternative, have been used increasingly in human medicine as a complementary sample to BAL and induced sputum. This sampling method is used for studying the underlying pathogenesis of, for example, asthma and chronic obstructive pulmonary disease, and evaluation of effects of different treatments (Jeffery, 1998; Jeffery et al., 2003; Behndig et al., 2006). The utility of bronchial biopsy for study of RAO in horses has been reported recently (Ainsworth et al., 2006; Riihimaki et al., 2008a).

In the first study, we investigated respiratory health in race horses, stabled in a conventional stable environment, during different seasons (Riihimaki et al., 2008a; Elfman et al., 2009). Twelve standard-bred trotters (8 mares, 3 geldings, 1 stallion) aged 3-7 years were included in the study. Detailed clinical and respiratory examinations were performed including the following methods: pulmonary auscultation with and without re-breathing bag, cough provocation, routine venous and arterial blood sample analysis, upper and lower airway endoscopy, and BAL fluid collection under endoscopic guidance (Figure 3 and 4). The recovered BAL fluid was used for cytological examination and quantitative real-time multiplex PCR-analysis for cytokine mRNA expression of interleukins (IL) IL-6, IL-8 and IL-10. In the second study, the effects of stable air quality on equine airways were evaluated before and after installation of mechanical ventilation. From the riding school stable, twelve mixed-breed horses were included in the investigation. The respiratory health of horses was based on detailed clinical and respiratory examinations, including chest auscultation following re-breathing, scored from normal to markedly abnormal values. Routine blood sample analysis for white and red blood cell parameters and plasma fibrinogen was performed in order to detect any infections and/or general inflammatory processes. All horses were examined by an upper and lower airway endoscopy and BAL at each sampling occasion. The BAL samples were analysed for cytology and cytokine mRNA expression as stated above.

Fig. 3. Endotracheal bronchoscopy in a horse. Photo: Roland Thunholm

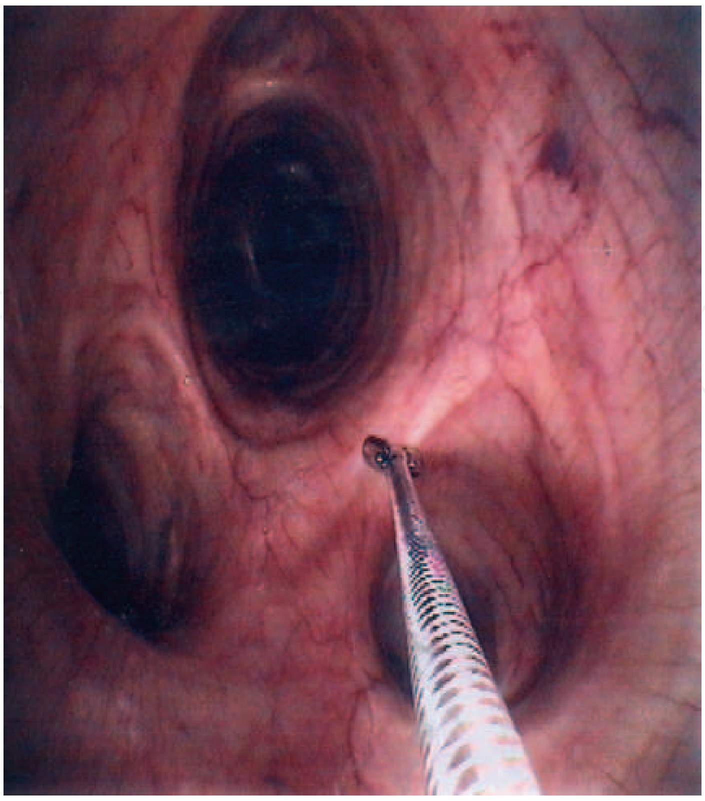

Fig. 4. Bronchoscopic view in a horse. Photo: John Pringle

3. Results

3.1 Hygienic measurements

3.1.1 Seasonal variations in air quality

Indoor climate in a stable can be monitored by using logging instruments. A typical example of logging temperature, relative humidity (RH%) and CO_2 over a week is shown in Figure 5. During winter, the outdoor temperature can be far below zero, but the aim is to keep indoor temperature above zero to prevent water freezing in the stable. The CO_2-levels in the stable generally fluctuate from about 250-300 ppm (the same as outdoor levels), when horses are not inside, to about 1500-3000 ppm during the night, depending on how many horses are kept in the stable and the type of ventilation. For horses, the upper acceptable limit value is 3000 ppm according to regulations on farm animal housing (Swedish Animal Welfare Agency, 2004), while for humans the recommended indoor value is 1000 ppm. In stables without insulation, as seen in Figure 5, the relative humidity should not exceed the outdoor RH% by more than 10% units. In stables with insulation, the relative humidity during winter should not exceed 80%, unless the temperature in the stable is less than 10 ^{o}C.

Results from logging particles in the air over a 24-hour period in a stable with three horses are shown in Figure 6. The activities that typically take place in the stable cause ultrafine particles to vary more and stay airborne longer than PM10 particles. Many factors affect the levels of particles, including horse and human activities, type of feed and bedding material. In this example the bedding material was a relatively new type of pelleted pinewood saw dust, which is watered to give a comfortable permanent bed. This type of bedding material is slightly less dusty than peat bedding, but there are no significant differences between the two types of bedding.

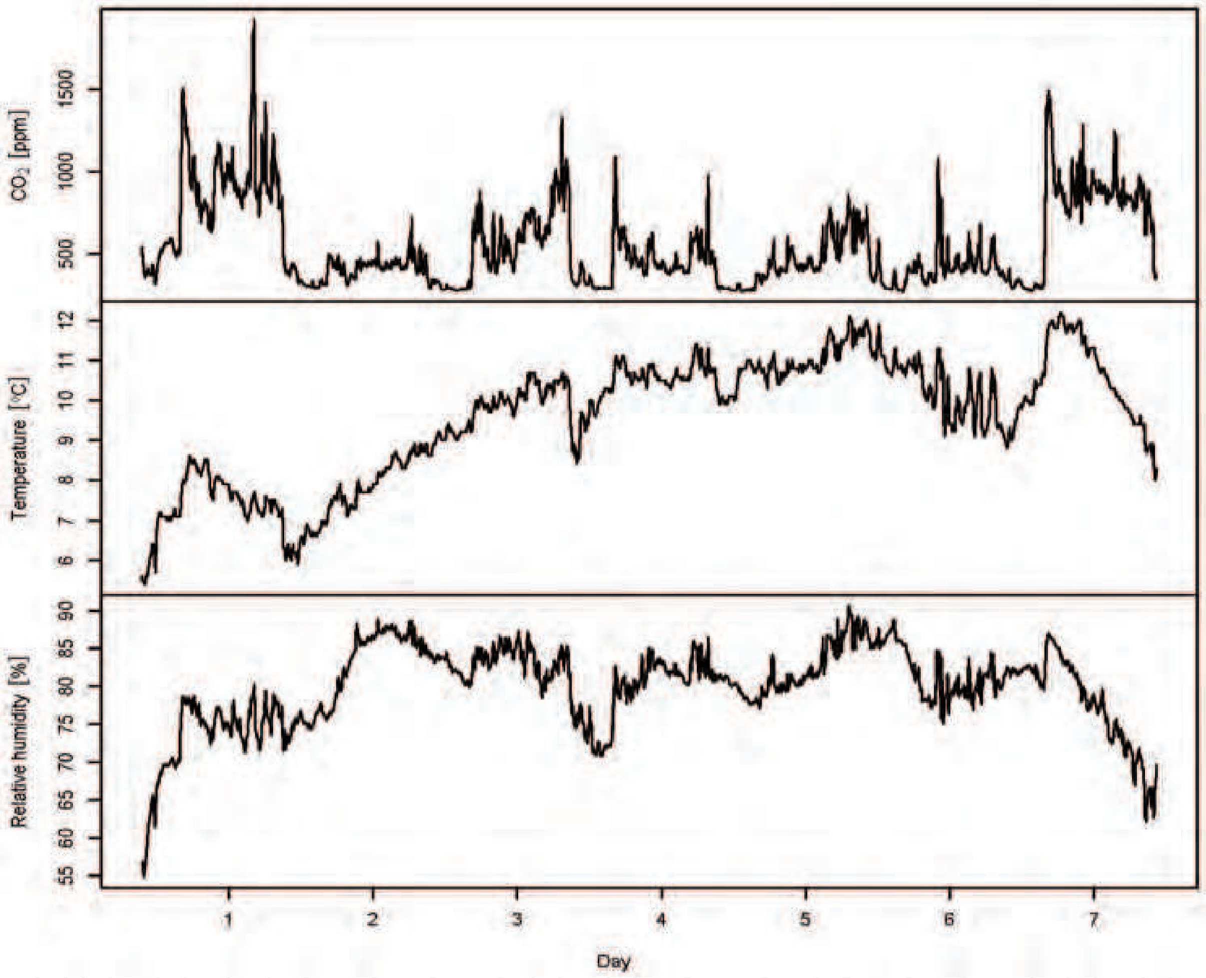

Fig. 5. Temperature, RH% and CO_2 in a stable logged over a week in winter with a Q-Trak™

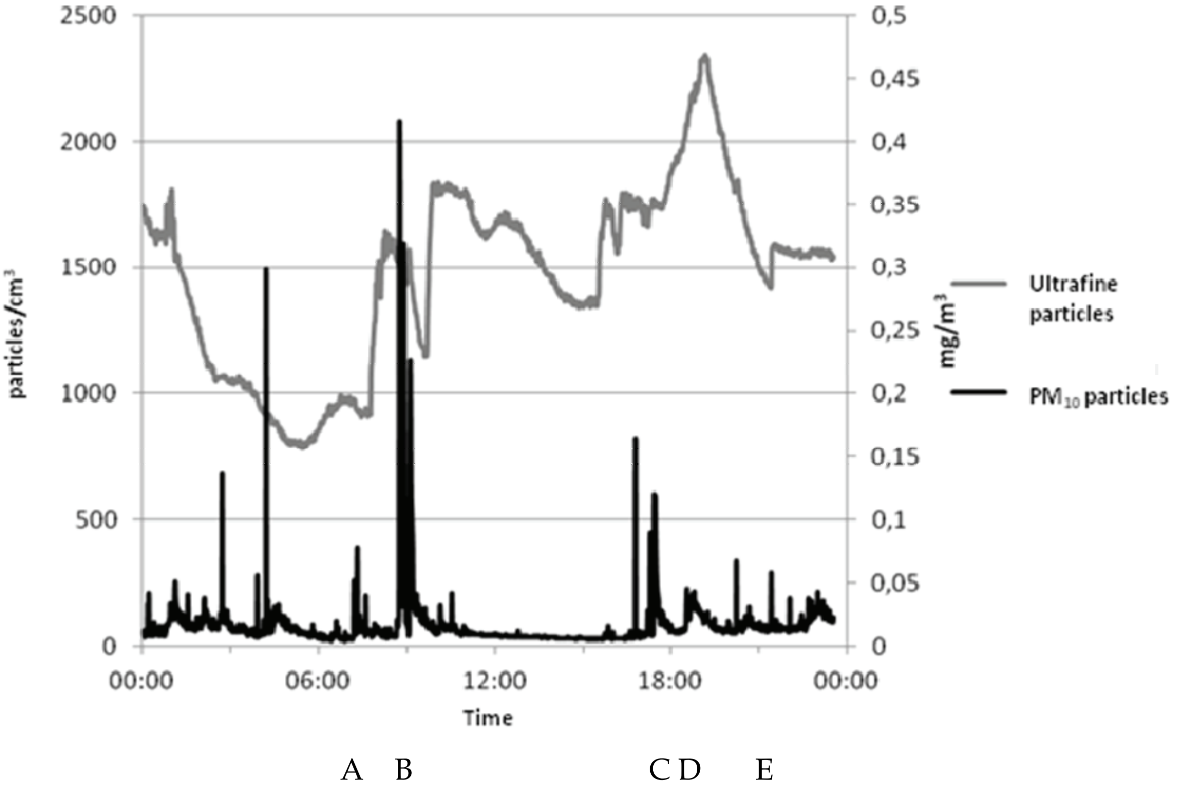

Fig. 6. Ultrafine particles (P-Trak) and PM10 particles (Dust-Trak) logged during 24 hours A=horses let out; B=measurements in stable; C=cleaning the stables from urine and faeces D= horses let into stable; E= evening feed

Results from total and respirable dust measurements are shown in Figure 7, and show variation between seasons: higher in winter when doors and windows are closed and lower in summer when they are kept open. These results are from a stable housing 18 horses and without mechanical ventilation. The bedding was straw. Horses were fed three times a day with haylage and pelleted fodder. The organic dust level was approximately 70% of total dust (range 0.4 – 0.8 mg/m^3), which is well below the Swedish hygienic limit value for humans (5 mg/m^3) (Swedish Work Environment Authority, 2005) or the Dutch proposed occupational exposure limit of nuisance dust which is the same as for horses (10 mg/m^3). According to Sadegh and co-workers (Sadegh et al., 2009) dust, endotoxin and 1-3-β-glucan are considerable in horse stables, while bacterial and fungal exposures are moderate. They reported that sweeping the floor is the predominant task that explains high levels of dust, endotoxin, and 1-3-β-glucan, and feeding the horses is a specifically important contributor for high 1-3-β-glucan levels.

Levels of airborne bacteria were slightly increased in February and September of year 1, but were normal in March of year 2, while fungi were slightly increased at all three sampling times compared to reference environments without microbial damage (Figure 8a). Samples from the indoor walls showed slightly increased levels of bacteria in February, year 1, but were normal in September, year 1 and March, year 2, compared to unaffected building materials. Levels of fungi were slightly increased on the inner wall surfaces at all three sampling times (Figure 8b). Endotoxin levels were lower in winter (February year 1, median 5 ng/m^3) and increased somewhat in the summer (September year 1, median 14 ng/m^3). Levels of 1-3-β-glucan, on the other hand, were higher in the winter sampling time

(February year 1, 1.85 ng/m³), and lower in summer (September year 1, 1.21 ng/m³) (Riihimäki et al., 2008).

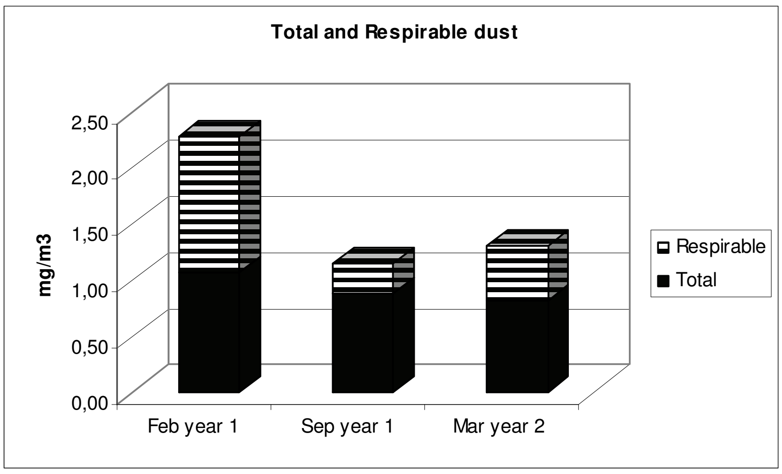

Fig. 7. Levels of total and respirable dust at three sampling time points.

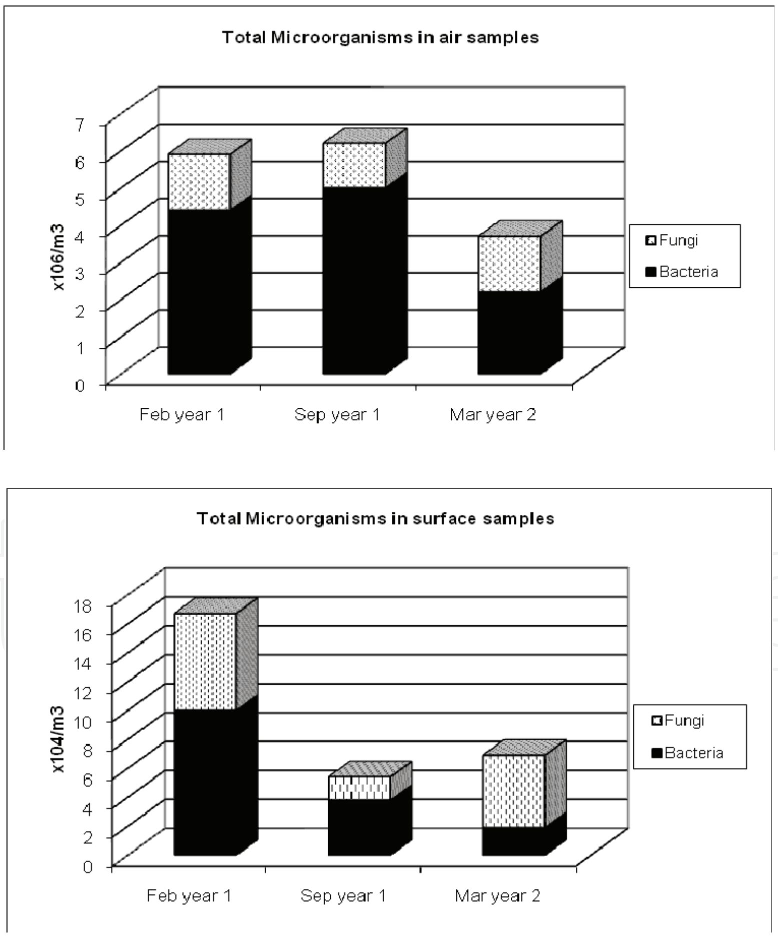

Fig. 8. a) Levels of total microorganisms in air samples; b) levels of total microorganisms in surface samples, at three sampling points: Feb, year 1; Sep, year 1; and Mar, year 2.

3.1.2 Bedding material and influence on air quality

The choice of bedding material affects the air quality in stables. Some materials are dustier than others and the capability to reduce ammonia varies a lot (Fleming et al., 2008a, 2008b; Airaksinen et al., 2005). We have gained some experience by evaluating two bedding materials: peat and pelleted pine sawdust. The latter material is also used for heating houses. The pellets are distributed over the floor in the stall and then watered until they fall apart and form a soft bed. This material can retain a lot of water or urine, without feeling wet, and is a very suitable material for permanent bedding. The following hygienic measurements were investigated: particles, temperature, air humidity, carbon dioxide, volatile organic compounds, horse allergen and ammonia. Samples from the two bedding materials were also analyzed for microorganisms.

The results showed that there were no differences in levels of PM10-particles (<10 µm) or ultrafine (<1µm) particles (Figure 6), when data had been adjusted for temperature and air humidity. The concentration of carbon dioxide did not differ between the bedding materials. The level of volatile organic compounds in the air was higher when pelleted wood was used than with peat, and δ-limonen was only detected with pelleted wood. After four weeks, high amounts of microorganisms were present in both bedding materials, with levels in peat 10-100 times higher than in pelleted wood. The conclusion is that there are no significant differences between these two bedding materials regarding amount of dust in the stable environment. Peat tends to give a better stable environment due to higher absorption of ammonia and lower levels of volatile organic compounds, while wood pellets have other advantages: they are easier to handle, use half the storage space, do not freeze in winter, and are the most cost-effective product.

3.1.3 Hygienic measurements before and after installation of mechanical ventilation

The most striking effect of installing mechanical ventilation in a stable was the reduction of carbon dioxide. The CO_2-level in a riding school stable with only natural ventilation was 950 ppm (median), with a range of 990 – 1280 ppm. At the summer sampling time in August, when indoor stable conditions were the best possible, with open windows and doors, the median CO_2-level was only slightly reduced (800 ppm, range 670 – 900 ppm). After installation of a balanced supply and exhaust mechanical ventilation system, the CO_2-levels had improved to 510 ppm (range 350 - 750 ppm).

Other parameters that improved with mechanical ventilation included ammonia, horse allergen and ultrafine particles. The median level of ammonia dropped from 3200 µg/ m^3 before intervention to 1330 µg/ m^3 after intervention. The median horse allergen level was 5 170 U/m^3 in the winter with natural ventilation, which dropped to 790 U/ m^3 with mechanical ventilation, which was almost as good as in the intervening summer sampling time point of 750 U/ m^3. The median level of ultrafine particles was 8000 particles (pt)/cm^3 in the winter before intervention and 5 400 pt/ cm^3 after intervention (Figure 9), while the median summer value was 1 500 pt/cm^3.

The factors that did not significantly change after intervention were total and respirable dust. The median total dust was 210 µg/m^3 in February before intervention, and 220 µg/m^3 in the winter after intervention, while the median respirable dust level was 100 µg/m^3 the winter before and 130 µg/m^3 the winter after intervention. Microorganisms in air samples showed almost the same level for bacteria (approx 20 x 10^5/m^3), while fungi increased from 3.5 x 10^5/m^3 before to 6.6 x 10^5/m^3 after intervention. Median endotoxin levels in air samples increased after intervention from 96 to 275 EU/m^3. We have no real explanation for this increase, but it could depend on increased levels of endotoxin due to handling of slightly mouldy hay or straw. Glucan levels were not investigated in this study (Wålinder et al., 2011).

We believe that it is best to compare before and after intervention (for example, installation of mechanical ventilation) during the winter stabling period, since this is the season with the most challenging indoor climate. Despite the need for air exchange, doors and windows have to be kept closed most of the time to avoid freezing of drinking water and drainage pipes. In the study presented here we chose to install an automatic system regulated by the indoor temperature in the stable. That means that the airflow runs at maximum (2 200 L/s) at high temperature and at a minimum of 400-500 L/s when the temperature is very low, otherwise the indoor temperature would go below zero. Ventilation systems have to be run this way in cold temperate climates since it is too expensive to warm up the incoming air. In many stables, ventilation systems are even shut down in winter to reduce the inlet of cold air and the result is often bad air quality with a lot of dampness kept indoors. This means that seasonal variations, such as differences in hygienic measurements between winter and summer, are often bigger than differences seen before and after installation of mechanical ventilation. In summer, stable doors and windows are kept open and horses spend most of their time outdoors in pastures, which is why the indoor stable environment is usually at its best. Levels of microorganisms can be elevated reflecting the generally higher outdoor levels in late summer, i.e. end of August to beginning of September, in temperate climates in the northern hemisphere.

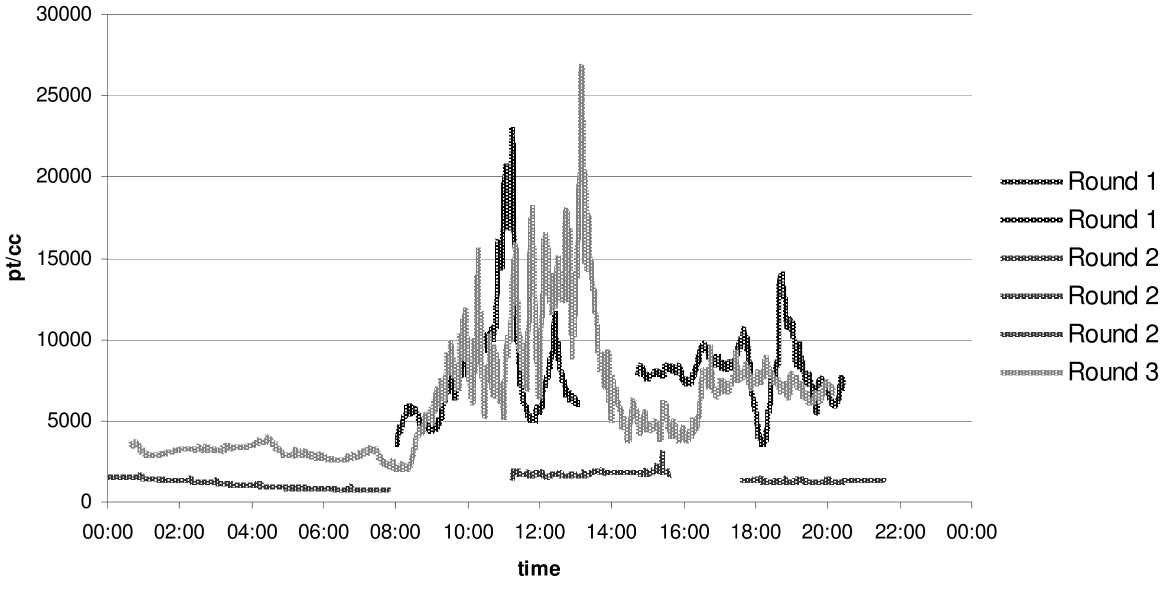

Fig. 9. Levels of ultrafine particles (P-Trak) logged over 24 hours. Round 1=in winter before intervention with only natural ventilation, Round 2= in summer before intervention, Round 3=in winter after installation of mechanical ventilation.

3.2 Air quality in stables and effects on human airways

3.2.1 Respiratory health in personnel at a conventionally managed race-horse stable

In this study one of the subjects reported a history of asthma. However, 3 out of 13 subjects had increased PEF-variability (CV>20%), indicating bronchial obstruction. These individuals also had increased levels of ECP in nasal lavage, indicating allergic inflammation equivalent to allergic asthma. Only two of the personnel reported work-related airway symptoms. Compared with teachers (Wålinder et al., 2000) the stable personnel in the present study (9 out of 13) also had increased levels of both lysozyme and myeloperoxidase (MPO) in the nasal mucosa (Table 4).

Biomarker	Race-horse stable personnel	Riding school students	School teachers	
No of subjects	n=13	n=7	Males n=59	Females n=352
ECP (µg/L)	<2	<2	<2	<2
MPO (µg/L)	77	26	20	10
Lysozyme (mg/L)	5.5	3.7	2.0	1.7
Albumin (mg/L)	6	<5	<5	<5

Table 4. Comparison of biomarker concentrations (median) in nasal lavage in personnel (adults) at a race-horse stable, students at a riding school stable, and among teachers in schools.

3.2.2 Respiratory health in personnel and riding-students before and after installation of mechanical ventilation in a stable

All personnel and riding-students, enrolled in the study at the riding school stable, had normal pulmonary function. None reported a history of asthma, but two of the students had hay-fever (Table 5). This means that all people included in this study had better respiratory health than the personnel at the race-horse stable. A growth-related increase of pulmonary function among the riding-students (forced vital capacity, FVC, increased as a mean 0.3 L in the students during the study period of 13 months) was observed. There was a slight, but not significant, decrease in PEF-variability in both personnel and riding-students (Table 5).

	Personnel n=7		Riding-students n=7	
	Before	After	Before	After
PEF (L/min) (SD)	426 (65)	407 (25)	387 (50)	416 (40)
PEF-variability (CV)	9.7%	3.5%	10.3%	7.2%
NO (ppb)	12	11	8	13

Table 5. Pulmonary function values before and after intervention.

Exhaled NO was low before, and was mainly unaltered after intervention. Levels of biomarkers of inflammation in nasal lavage from the personnel were somewhat lower in comparison with results from the personnel at the race-horse stable (Table 4) but did not change after intervention. Although air exchange increased after intervention, the mean ratings of smell and dustiness were slightly higher (not significantly) among personnel and students. The number of subjects having eye irritation, nasal obstruction, dyspnoea, or eczema was either slightly lower or unchanged after intervention (Table 6).

Symptoms	No. of subjects with symptoms before n=14	No. of subjects with symptoms after n=12
Eye irritation	*5*	*4*
Nasal obstruction	*13*	*7*
Dyspnoea	*2*	*1*
Eczema	*3*	*3*

Table 6. The prevalence of subjects having symptoms of eye irritation, nasal obstruction, dyspnoea, or eczema before and after installation of mechanical ventilation in a horse stable.

Apart from a growth-related increase of pulmonary function among the riding-students, intervention did not show any significant health effects on the airways. Although the hygienic measurements did show increased air exchange rate, reduced dust, ammonia and horse allergen levels, the absence of measurable health effects after intervention could be due to either a low statistical power, because of a small study-population, or a healthy-worker effect in this stable, or both. It could also depend on the shortness of the interval between installation of the mechanical ventilation and the follow-up.

3.3 Air quality in stables and effects on horse airways

The aim was to examine possible effects of seasonal changes, that is summer versus winter conditions, on horse airways. Despite the complexity of the study we were able to detect a significant up-regulation of IL-6 mRNA expression during both winter periods compared to summer, which coincided with a trend of increased neutrophils in BAL fluid. It is, however, unclear whether the increased IL-6 mRNA expression in BAL during winter was an effect of the stable environment and/or exercise in cold weather conditions. We also found significant alterations in IL-10 mRNA expression between summer and one winter. The change was, however, detected in samples from only one of the two winters. Furthermore, there was no correlation between cytokine mRNA expression and BAL cell cytology. There were no significant seasonal differences in any other clinical parameters, including blood parameters, PaO_2 and subjectively-graded appearance of mucous in trachea (Riihimaki el al., 2008a).

Unfortunately, it was discovered later that three out of twelve horses showed transient pulmonary eosinophilia when the horses were sampled in late summer (Riihimaki et al., 2008b). However, this was probably related to factors other than stable environment. Our initial research plan and hypotheses were based on the twelve horses, but since three horses had obvious pulmonary eosinophilia they were not included in the analyses. It was not possible to perform measurements in the following summer due to a change in ownership of the stable. It would also have been valuable to have data on race-horses not being stabled, but kept in pastures over the summer.

3.3.2 Respiratory health in horses before and after installation of mechanical ventilation in a stable

In horses, a significant decrease in accumulation of tracheal mucous was the strongest indication of reduced airway inflammation after intervention (Wålinder et al., 2011). Cytological results from BAL were normal except for in two horses that showed a slightly increased percentage of neutrophils after intervention, and one of the horses also had increased respiratory rate and respiratory sounds. Of the cytokines investigated, the expression of IL-6 mRNA in BAL cells was significantly lower in the winter after intervention compared to the winter before. The cytokine IL-6 is a pre-inflammatory marker and indicator for exposure to stable environment per se. It has also been shown to be associated with increased mucous secretion (Chen et al., 2003; Gerber et al., 2003). Therefore, a decreased amount of mucous in trachea together with a lower expression of IL-6 mRNA in BAL cells may indicate improvement of horse airway health after intervention. However, after intervention, one of nine horses had increased respiratory rate and respiratory sounds, and this horse, together with another animal, had a mild elevation in BAL neutrophils after intervention. Therefore, the effect of intervention in the stable on clinical outcome in horses is not clear cut.

4. General discussion

The battery of methods that we have presented here for monitoring indoor air quality in stables seems to be adequate for the task. Many of the methods have been used for extended times and have been well validated. However, one should be aware of the fact that there are many pitfalls in pursuing studies like this. It is well known that there is a large day-to-day variability and also a substantial measurement error in many hygienic measurements (O'Meara & Tovey, 2000). There is a high turnover of both personnel and horses, especially in race-horse stables, which makes follow-up studies very cumbersome and difficult to perform.

In the future, we will hopefully progress to better and quicker techniques to identify, for example, moulds using DNA-based technology. Dust from large animal farming is a complex mixture, and factors other than endotoxin and glucan, such as muramic acid and 3-hydroxy fatty acid, may contribute to respiratory inflammation and these components should therefore be added to the list of markers (Poole et al., 2010).

Regarding the horses being examined, for most management systems in our northern climates stabled horses spend far more time indoors than their keepers on a daily basis. Thus, they are likely to have a larger cumulative exposure to the variety of potentially irritating components of stable air and dusts. Much of the research on air quality in relation to respiratory problems in the horse has had its focus on the asthma like disease recurrent airway obstruction (RAO), mentioned earlier, in which as yet poorly defined components of stable air trigger airway inflammation solely in selected susceptible horses, while stablemates breathing the same stable air remain clinically normal. While desirable, defining specific allergens or irritants in this stable air that triggers airway inflammation in these RAO horses has yet to be identified. Moreover, initiation of airway inflammation (and obstruction) appears to occur in concert with the presence of endotoxin in the stable air, which interacts synergistically with other ill defined components of stable and hay dusts to greatly enhance the inflammatory effects of stable air on RAO susceptible horses (Pirie et al., 2003). For these highly reactive horses, there are as yet no clear specific markers of stable air quality that can be used to distinguish stables with “poor “ air quality from those in which the horse with RAO will not overreact. Additionally, even clinically normal horses during stabling have more recently been shown to develop lower airway inflammation (Holcombe et al., 2001), in which endotoxin has been incriminated as one key inflammatory component (Berndt et al., 2010). Unfortunately, specific guidelines for air quality in stables for the resident horses, even if clinically normal are not available.

Turning to the actual measurement of airway problems in relation to air quality, the sensitivity and precision of methods used for the horse lag considerably behind those available to physicians for their human subjects. For example, the use of forced expiratory volume measurements to detect small changes in pulmonary function over time, as done on the human subjects in our studies, is not feasible in the horse, as it requires conscious cooperation and patient compliance in performing respiratory maneuvers on command. Alternative pulmonary function measurements in the horse that do not require patient compliance, such as simple measurement of changes in intrapleural pressure, or the noninvasive but more sophisticated measurements using forced oscillation techniques (Young & Tesarowski, 1994) that we have in our laboratory are in sufficiently sensitive to small but clinically relevant changes in pulmonary function in relation to changes in stable air quality.

On the other hand, measurement of airway inflammation in the horse is becoming all the more sensitive (Riihimäki, 2008; Berndt et al., 2010) and in the future will likely provide better objective data on the true state of airway health or inflammation in the stabled horse. Moreover, in horses we are able to obtain samples (bronchoalveolar lavage cytology, bronchial biopsy (Riihimäki, 2008)) that can directly identify alterations in level of pulmonary inflammation, but for ethical reasons are not feasible in human subjects.

Unfortunately, field studies aiming to determine the influence of stable air quality on the horse respiratory health are plagued with confounding external factors, such as changes in bedding or feeding regimens for horses in adjacent stalls in the same stable, the potential for occurrence of subclinical respiratory viral infections or transient parasitic infections in competition horses (Riihimäki et al., 2008b). Even the influence of cold air and exercise can influence gene regulation of inflammation in the horses' airways (Davis et al., 2005; Riihimäki et al., 2008a). Such uncontrolled external facets in these field studies of the horse are difficult to control, and cloud the scientific interpretation of results. As such, future studies on the influence of the quantitative and qualitative indices of stable air on the respiratory health of the horse need to ensure more rigorous control and standardization of the actual stable environment, both in terms of bedding and feed throughout the stable, as well as of the timing and intensity of exercise regimen.

For those working and living on the horse farm, whether the farm environment is a risk factor for developing allergic disease and asthma is under debate. There are conflicting epidemiological results that state, on one hand, that children growing up in farm environments have less allergic disease, especially if they are growing up on a farm with animals (Bråbäck et al., 2004; Ege et al., 2011). On the other hand, there are studies showing that farmers are among the occupations having the highest prevalence of asthma (Lembke et al., 2004). Furthermore, exposure to organic dust may cause rhinitis, asthma, bronchitis, or intrinsic lung disorders such as farmer's lung/allergic alveolitis. There are several studies now showing that working in a stable can have adverse health effects on the respiratory function similar to those experienced by farmers (Kristiansen & Lahoz, 1991; Mackiewicz et al., 1996; McGorum et al., 1998; Tutluoglu et al., 2002; Elfman et al., 2009).

A majority of the personnel in the race horse stable had higher levels of lysozyme and MPO in nasal lavage than white collar workers. Since lysozyme is a marker of nasal mucosal secretion this could be a reaction to the relatively high levels of airborne dust present in the stables. MPO, a marker of neutrophil activity, may indicate a response to high exposure to bacteria in straw bedding and horse dung. Today, transmission of parasites, worms and infections from horses to humans is rare. Instead the exposures to organic dust, gases, microbes, mould and storage mites in the stable environment might have a greater health impact. Since 3 out of 13 personnel had objective signs of asthma without actually having reported asthma, this may indicate neglect of respiratory symptoms and disease among this category of workers in the race-horse stable. Asthma is the most common chronic disease among children and young adults, affecting about 5-10% of school children and 6-7% of adults, of which 30-50% is attributed to allergy (The National Board of Health and Welfare, 2009). Therefore, screening of asthma and allergy among stable-workers may be a way of detecting sensitive individuals who can then be informed of preventive measures to reduce worsening of their disease.

Primary prevention in the stable should involve reduced exposure to organic dust, irritating gases, and microbial agents. Feeding and cleaning activities can be done in a way that creates less dust in stable air. Also ventilation is important, where the installation of mechanical ventilation could increase the air exchange rate and reduce particles and gaseous substances in stable air (Wålinder et al., 2011).

Secondary preventive measures of stable-related airway disease could involve pre-employment testing of stable workers followed by medical advice for those with a risk of developing airway-related diseases, such as hay fever and asthma. The examination could include a medical history, especially of allergy and airway disease, physical examination of the airways, and skin disorders such as eczema. This could be followed by a pulmonary function test and an allergen test. This regime may also be applicable for riding-school students who often spend a lot of time in the stables. The purpose of these investigations would be to protect persons with atopy and airway disease from impairment caused by the stable environment. This screening could be of importance since a possible disregard among stable personnel having respiratory problems has been described (Elfman et al., 2009).

In order to protect sensitive inhabitants living in the neighbourhood of horses from the spread of horse allergens and smell, there is a recommended distance between horse establishments and nearby dwellings. In Sweden, the official recommendation since 1989 is 500m (National board of housing, building and planning 1998), but not much was known about dispersion of horse allergen in ambient air at that time and no assays were available for detection of horse allergen. Later studies have shown that horse allergens do not disperse more than about 50-100m from source, which is from stables and fields where horses are kept (Emenius et al., 2001; Elfman et al., 2008). On days with increased wind speed and with prevailing winds, horse allergen levels can sometimes be detected up to 200-450m from the source. Other importance factors for spread of horse allergens are typography, vegetation and buildings (Elfman et al., 2008). In fact, dense vegetation can also be used to reduce dispersion of horse allergen to nearby houses. The best choice of vegetation for shielding is conifers, which can reduce particle levels by 15-25%, compared with 3-17% by deciduous trees (Freer-Smith et al., 2004).

5. Conclusions

Many people spend a considerable time in stable environments, either as workers in the care and training of horses, or in their leisure time. Therefore, the indoor stable environment must provide adequate exchange of fresh air to the horses' living space to regulate the temperature of the stable and remove moisture, stable gases and organic debris produced by the horses and stable activities.

The most striking effect of installing mechanical ventilation in a stable was a reduction of the carbon dioxide level by half. Other parameters that improved with mechanical ventilation included levels of ammonia, horse allergen and ultrafine particles. Many factors affect the levels of particles, including horse and human activities, type of feed and bedding material.

In a conventionally-managed race-horse stable, 3 out of 13 workers had increased PEF-variability (CV>20%), indicating bronchial obstruction. These individuals also had increased levels of ECP in nasal lavage, indicating allergic inflammation equivalent to allergic asthma. Only two of the personnel reported work-related airway symptoms. After, installation of mechanical ventilation in a stable, there was a slight, but not significant, decrease in PEF-variability in both personnel and riding-students. The absence of measurable health effects after intervention may be due to a low statistical power because of a small study-population, a healthy-worker effect in this stable, or both.

In horses, we could see a significant up-regulation of IL-6 mRNA expression during both winter periods compared to summer, which coincided with a trend of increased neutrophils in BAL fluid. It is, however, unclear whether the increased IL-6 mRNA expression in BAL during winter was an effect of the stable environment and/or exercise in cold weather conditions. After intervention, a significant decrease in accumulation of tracheal mucous was the strongest indication of reduced airway inflammation. Of the cytokines investigated, the expression of IL-6 mRNA in BAL cells was significantly lower in the winter after intervention compared to the winter before. Therefore, a decreased amount of mucous in trachea together with a lower expression of IL-6 mRNA in BAL cells may indicate improvement of horse airway health after intervention.

This paper contributes to the identification of suitable biomarkers to monitor the indoor horse stable environment and respiratory health in humans and horses. An improved management of horse stable climate will be beneficial for the airways of workers, riding-students and horses spending considerable time in confined stabling systems with variable air quality.

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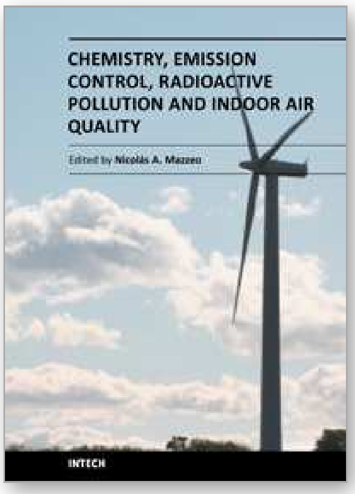

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The atmosphere may be our most precious resource. Accordingly, the balance between its use and protection is a high priority for our civilization. While many of us would consider air pollution to be an issue that the modern world has resolved to a greater extent, it still appears to have considerable influence on the global environment. In many countries with ambitious economic growth targets the acceptable levels of air pollution have been transgressed. Serious respiratory disease related problems have been identified with both indoor and outdoor pollution throughout the world. The 25 chapters of this book deal with several air pollution issues grouped into the following sections: a) air pollution chemistry; b) air pollutant emission control; c) radioactive pollution and d) indoor air quality.

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