Environmental Control of Respiratory Disease

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Introduction

Of all the organs, the respiratory tract presents the largest and most delicate surface for contact with potentially deleterious airborne material. Indeed, the alveolar and bronchiolar areas, which in the adult horse approximate 100 m² at end expiration and 300 m² at end inspiration, are exposed to approximately 30 million litres of air annually. Consequently, breathing exposes the lungs to the potentially adverse effects of a complex mixture of gaseous and particulate pollutants. The potential noxious consequences are diverse and the resulting effects on health may be immediate or delayed.

Respiratory tract defences are therefore of paramount importance in the maintenance of respiratory health. Consequently, several specific (primarily for biological agents) or non-specific (including defences against inorganic agents) mechanisms have evolved to remove inhaled agents and prevent their deleterious consequences. Unfortunately, the efficiency of these defences may be reduced, either because they are overwhelmed by extreme levels of exposure, or because they are impaired by airway diseases.

Several equine respiratory disorders are directly caused by, or exacerbated by inhalation of airborne dust. The most widely recognised disorder affecting adult horses is heaves (previously termed chronic obstructive pulmonary disease or recurrent airway obstruction). Heaves is caused by inhalation of agents in airborne organic dust including moulds and bacterial endotoxins. Inflammatory airway disease, another chronic pulmonary disease mainly encountered in young race horses subjected to strenuous exercise, may also be associated with inhalation of airborne pollutants including micro-organisms and organic dusts [1,2]. Additionally, pharyngeal lymphoid hyperplasia has been linked with inhalation of airborne dust in some studies [3], but not in others [2]. Acute respiratory diseases, including those caused by infectious agents, may be exacerbated and prolonged by concomitant dust inhalation. Furthermore, it has been proposed that heaves may develop after a respiratory viral infection, and that reduction of airborne dust is thus an important consideration in the management of horses with viral infection to prevent the potential subsequent development of heaves [4]. This chapter will review the mechanisms of deposition of inhaled particles, non-specific pulmonary clearance mechanisms, the potentially noxious airborne agents, and environmental management recommended for horses with airway diseases.

Particle Deposition in the Respiratory Tract

The respiratory tract has an important physiological role as a filter that prevents most of the inhaled dust from reaching the alveoli (Fig. 1). Most of the particles in the ambient air will be deposited on the respiratory epithelium during inhalation at a location that is determined by the particle size, nature (hydrophilic or hydrophobic) and shape (elongated particles may penetrate deeper in the lung than spherical particles of the same maximum diameter). The size of particles is most usefully defined by the aerodynamic particle diameter, which takes into account the size, shape and density of particles, all of which have significant effects on their deposition patterns.

Accurate measurement of particle size distribution requires complex equipment such as laser particle velocimeters and is rarely attempted on complex organic dust mixtures such as those encountered in horse stables. Three deposition mechanisms have been identified, namely gravitational sedimentation (the particle sediments with a speed proportional to its density and square of its diameter), inertial impaction (occurs where the airflow changes direction or velocity) and diffusion. Inertial impaction causes most large particles (≥ 5 µ) to deposit in the upper airway, mainly in the nasal turbinates, pharynx and...
The efficiency of the mucociliary system may be influenced by diverse factors that affect either the ciliary function, or the composition of the mucus which prevent it from being readily propelled by the cilia [5].

Each ciliated epithelial cell has approximately 200 cilia (density 6 - 8/µm²), which are approximately 6 µ in length in the large airways and 5 µ in length in the small airways. During the ciliary beat cycle (12 - 14 cycle/sec) there is an effective stroke and a recovery stroke. During the effective stroke, the mucus is swept proximally, while, during the recovery stroke, the cilia bend and flex near the cell surface and do not propel the mucus. Although it was previously thought that the mucus was uniformly thick (5 - 10 µ), present evidence suggest that it may be present in smaller amounts (droplets) in lower airways, while forming larger plaques in the large airways [6]. Mucus is continuously propelled to the pharynx at a speed approximating 1 mm/min in the peripheral airways and 2 cm/min in the trachea [7].

The efficiency of the mucociliary system may be influenced by diverse factors that affect either the ciliary function, or the quality and quantity of mucus production. Factors affecting ciliary function include environmental factors (temperature and humidity), mechanical stimulation (e.g., dust deposition), pharmacological action (β-agonist, xanthine and cholinergic drugs increase activity while anaesthetics have the opposite effect [7], infectious diseases (bacterial infection and purulent secretion impair the ciliary function; viruses may destroy the ciliated cells), administration of mucolytics and expectorants (the efficiency of which have been proved in human medicine but not in equine medicine) and chronic inflammation (which causes goblet cell hyperplasia and metaplasia). In other species, exposure to high ammonia concentrations may reduce ciliary activity in the respiratory tract [8]. Furthermore, exposure of pigs to 50 ppm ammonia increased the severity of atrophic rhinitis [9], but did not reduce the resistance to experimental pulmonary bacterial infection [10]. Although the effect of inhaled ammonia on ciliary activity in horses is not known, some authors suggest that high levels may be...
detrimental to equine mucociliary clearance. In deep litter horse stables, the airborne ammonia levels 10 cm above the bedding may reach values as high as 50 ppm [11]. The quality of the mucus is critical for effective mucociliary clearance. The control of mucus secretion is still poorly understood. Mechanical stimulation (i.e., particle deposition) promotes gel layer production. Exposure to dusty hay and straw increased the mucus visco-elasticity and decreased both ciliary and cough clearability of mucus in heaves affected horses but not in controls [12]. Equine homologues of mucin genes have been recently identified. Expression of EqMUC5AC is higher in horses with heaves than in healthy subjects, and may persist for considerable periods of time after removal of the dust exposure [5]. Finally, head position has an important influence on clearance of mucus from the trachea of horses. Keeping the head elevated, such as occurs during transportation, leads to mucus accumulation in the large airways and trachea. This factor may contribute to the increased risk of bacterial pneumonia in transported horses. While the increased airflow occurring during exercise increases mucus clearance in man, it seems to have no influence on the efficiency of mucociliary clearance in horses [7].

Alveolar Macrophages - As there is no mucociliary clearance from the alveoli, particles deposited in this region are cleared by phagocytes including macrophages. Alveolar macrophages are a part of the mononuclear phagocyte system, originating from bone marrow, transported in the blood as monocytes and eventually becoming tissue macrophages. Following phagocytosis of dust particles, macrophages migrate to the small airways and are expelled via the mucociliary escalator. Alternatively, they exit the lung in the lymphatic vessels or via the blood (Fig. 3). Various factors such as toxic gases, alveolar hypoxia and glucocorticoids can impair normal macrophage activity.

Cough - Coughing has two major physiological functions, namely acting as the final pathway for mucociliary clearance, and expelling inhaled particles [13]. Since coughing is a desirable response triggered by physiological and pathological conditions, antitussive drugs should be avoided in horses with increased volumes of airway secretions. Coughing can rapidly expel inhaled particles, or excessive or abnormal respiratory secretions, down to the level of the fourth or fifth generation bronchi [14]. Coughing may be the most important clearance mechanism when the epithelium is deciliated, such as occurs during respiratory virus infections.

Pulmonary Clearance in Disease - Up to 50% of the cilia must be destroyed before there is a measurable decrease in mucociliary clearance rate [5]. However, despite this reserve capacity, mucociliary clearance may be reduced during viral infections. In man, the mucociliary clearance rate is severely reduced during the first week of a viral infection, and may remain reduced for up to 3 months [15]. In horses, herpes virus infection reduced mucus clearance by approximately 50%, while influenza infection reduced the clearance rate by 100% [16]. Furthermore, the mucociliary clearance returned to normal only after 4 weeks after the initial viral infection. This impairment is probably the result of viral cytotoxicity and epithelial shedding. In disease, the composition of the mucus may be altered, i.e., excessive production of viscoelastic mucus, which will consequently impair the efficiency of the mucociliary system. While respiratory diseases usually have a transient effect on mucus secretion, many chronic inflammatory airway diseases lead to epithelial metaplasia and mucus cell hyperplasia with resultant long term or permanent changes in mucus secretion (Fig. 4). Alveolar macrophages are very sensitive to alveolar hypoxia, and consequently, pulmonary diseases that cause partial obstruction of the small airways compromise macrophage function.

The fact that the respiratory clearance system is compromised during acute respiratory diseases is too frequently ignored by owners and equine practitioners. Horses with infectious respiratory diseases which are kept in dusty environments may have increased coughing, mucus hypersecretion and bronchoconstriction, thereby prolonging the recovery period. Furthermore, it
is hypothesised that this may lead to sensitization to inhaled environmental allergens and lead to the development of heaves. Thus maintaining horses with infectious pulmonary diseases in dust free environments is an important managerial consideration.

**Airborne Pollutants**
The airborne dust present in equine stables is a variable and complex mixture including organic and inorganic contaminants. These include bacteria, viruses, moulds, mite debris and feces, plant material, bacterial endotoxins, β-glucans and inorganic dusts [17]. These agents are potentially able to induce airway inflammation, either by initiating infection, by inducing allergy, by direct toxicity, or indirectly by overwhelming the pulmonary defence mechanisms.

When assessing the risk associated with particle inhalation, three points must be considered. The quantity of inhaled particles is clearly important, and is assessed in term of mass of dust or number of particles per unit volume of air. However, the proportion of the non-respirable and respirable fractions must also be considered, since this will in part determine the location of particle deposition. The respirable fraction is the fraction that will enter the intrathoracic respiratory system during inhalation, and comprises particles with an aerodynamic diameter \( \leq 5 \mu m \). The respirable fraction is considered as a reliable index of the respiratory hazard associated with dust inhalation [18,19]. Also of paramount importance is the composition of the dust, and in particular the relative quantities of pro-inflammatory agents including microorganisms, endotoxins, aeroallergens and proteinases. The elimination of the airborne dust from the environment of horses must consequently focus not only on the quantity of residual dust but also on the quality of this residual dust. The primary sources of airborne dust in equine stables are the feed and bedding, and the highest respirable dust exposure results from mould-contaminated forage and/or bedding. Dust levels at mucking out may reach 10 - 15 mg/m\(^3\), comprising 20 - 60% respirable particles. This equates to 3000 respirable particles/ml or 12,000,000 inhaled particles/breath. It is thus clear that many stables contain concentrations of total dust which exceed the suggested provisional air hygiene standard for biologically active dust exposure in cotton mill workers of \(< 2.5 \text{ mg/m}^3 \) [20]. Thankfully, effective hygiene measures may reduce dust exposure by up to 90%.

**Quantification of Airborne Pollutants**
As previously mentioned, when considering air quality in horse stables, the challenge should be quantified in terms of (1) total dust levels, (2) respirable dust levels and, (3) dust composition. Measurements may be done on samples of dust collected from air in the horse’s environment [21,22], or on forage and bedding samples [23]. The first method gives a global appreciation of the quality of the air in a stall and of the challenge experienced by the horse. When sampling the stable air, the methodology, time and the place of sampling must be carefully standardised. The recommended methods for airborne dust quantification are published by HMSO [24]. Results are generally given in mg/m\(^3\). The second method allows an objective assessment of a given forage or bedding. Here too, the procedure must be carefully standardised to obtain reliable information [23] (Fig. 5). Results are usually given as the number of particles/litre of air.

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**Figure 5.** Material used by Vandenput et al., [23] to standardise the qualitative and quantitative assessments of dust in various forages and bedding. - To view this image in full size go to the IVIS website at www.ivis.org.

**Determination of Total and Respirable Dust Levels** - The simplest system to quantify total airborne dust levels are samplers that collect dust from set volumes of air onto paper filters that are then weighed [21,24]. Another more accurate but more expensive method employs samplers that count and size particles (e.g., optical Rion particle counter, Fig. 6). These samplers use light dispersion techniques or piezoelectric balances to collect data on masses of airborne dust. They can quantify total and respirable fractions but cannot discriminate between organic and inorganic dusts. They can be used to provide a real-time output of airborne dust levels.

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**Figure 6.** Rion optical particle counter. - To view this image in full size go to the IVIS website at www.ivis.org.

**Determination of Dust Composition** - The endotoxin [25], β-glucan, and allergen [22] content of total or respirable dusts may be determined, although this usually requires expensive laboratory kits. The number of viable bacteria and moulds in dust samples may be determined by culture of dust collected using impingers, cyclones or impactors (e.g., Andersen
sampler, Fig. 7). Alternatively, dust samples may be collected directly on a microscope slide, or into liquids, and then plated out onto culture media. While dust may be collected directly onto culture media, an open Petri dish exposed to stable air will lead to a disproportionate collection of large particles. It should be noted that such cultures will quantify only those bacterial and moulds which are viable. It will not detect microbes which are dead, but which retain their antigenicity and thus their pro-inflammatory properties.

Figure 7. Cascade impactor (Andersen sampler) used to determine the biological content of dust. - To view this image in full size go to the IVIS website at www.ivis.org.

The microflora of hay which is baled with a high moisture content is dominated by thermotolerant actinomyces and fungi, including *Aspergillus fumigatus*, *Faenia rectivulgula* (previously termed *Micropolyspora faeni*) and *Thermoactinomyces vulgaris*. These moulds are considered to be largely responsible for induction of pulmonary inflammation in heaves affected horses [19,26-29]. These mould spores pose special problems for identification because most of the spores of fungi and actinomyces cannot be identified by microscopic examination alone [30]. Therefore, identification may require culture on Sabouraud’s media at 46 to 56°C [23].

Inhaled airborne endotoxin contributes to the severity of the pulmonary inflammatory response in heaves [31]. Indeed there are striking similarities between heaves and endotoxin mediated lung diseases in other species. Both are characterised by reversible airway obstruction, airway neutrophilia, bronchial hyper-responsiveness and increased mucus production, have similar time courses, and result in minimal permanent structural lung changes such as emphysema and fibrosis [32,33]. Horse stables have total and respirable airborne dust endotoxin levels that may exceed the threshold for induction of inflammation and hyperresponsiveness in healthy human subjects [25]. Further, lipopolysaccharide (LPS) inhalation induced a dose dependent neutrophilic airway inflammatory response in asymptomatic heaves affected horses and in control horses [31]. Inhalation of high doses of LPS also induced detectable lung dysfunction in the heaves group. The no-response thresholds for induction of airway inflammation and dysfunction were lower for the heaves group than for the control group. Furthermore, the pulmonary neutrophilia which was induced by inhalation of an aqueous extract of mouldy hay dust was attenuated by depletion of its endotoxin content, and re-established when the endotoxin was added back [34]. This data indicates that endotoxin contributes to the pulmonary neutrophil recruitment in heaves. In other species, experimental endotoxin inhalation can induce pulmonary inflammation and dysfunction and can potentiate the pulmonary inflammatory response to inhaled allergens [35-37].

**Standardisation of Time and Place of Sampling** - The time of airborne dust sampling is critical to identify the level of challenge experienced by horses. For example, the highest airborne dust levels occur during mucking out [2]. Because horses spend a considerable period of time with their muzzles in close contact with the feed and bedding, dust levels in the breathing zone (i.e., close to the nostrils) more accurately represent the respiratory challenge encountered by the horse than levels measured at a fixed point in the stable. Indeed, dust level in the breathing zone may be 20 fold higher than in the stall in some situations [22] (Fig. 8). Sampling of dust in the breathing zone is achieved by mounting personal dust samplers on the head collar adjacent to the nostrils, as described by Woods et al., [22].

**Environmental Prevention**

The most logical way to prevent and treat pulmonary diseases which are caused or exacerbated by inhaled organic dusts, including heaves, is to reduce the level of exposure to airborne dusts [38]. Many studies report clinical and/or functional improvements solely as a result of environmental control [39,40]. For example, clinical remission of symptomatic heaves occurred 4 to 24 days after horses were moved to a controlled environment, with the time being dependent on the age, duration of illness and initial severity of the clinical signs [39]. Pulmonary function of symptomatic heaves affected horses was significantly improved within 3 days following a reduction in airborne dust levels [40]. Thus when treating a horse suffering from pulmonary disease, owners should be educated that environmental management is at least as important as
Reducing Dust Levels by Use of Low Dust Food and Bedding

**Pasture** - Keeping horses permanently outside, with a shelter against inclement weather and no supplementary hay feeding, is the ideal dust-free regime (unless the horse is suffering from summer pasture-associated obstructive pulmonary disease (vide infra)). Alleviation of clinical signs and lung dysfunction in most heaves-affected horses occurred after 3 to 7 days on pasture [41]. Unfortunately, for numerous practical reasons, sport horses are often stabled indoors. Therefore, a strict control of stable dust levels by use of dust free food and bedding is essential if heaves horses are to remain in clinical remission [42].

**Low-dust Forage** - Some degree of fungal contamination is present in all batches of hay, regardless of their quality. Hay that has heated and is very dusty (Fig. 8) may contain very high levels of many different pro-inflammatory agents including mould spores, bacteria, endotoxins, proteinases and forage mites [22,43,44]. Mould spores are aerodynamically small enough to reach the bronchioles when inhaled [30] and are consequently particularly dangerous for heaves-affected horses. The most critical factor determining the microbial content of hay is the moisture content at baling. When hay is baled at less than 15 - 20% moisture content, it is virtually dust free. However, when baled at 35 - 50% moisture content, hay may heat up to 50 - 60 ºC and consequently become heavily contaminated with thermotolerant microbes. A horse consuming heated hay may inhale $10^{10}$ dust particles per breath [43]. Unfortunately, as the quality of hay cannot be assessed accurately by visual and olfactory examinations, objective assessment is required.

Artificial drying of hay, using a drying kiln with air blowers, or rapid high-temperature drying immediately after cutting, may significantly decrease the microbial growth. Soaking hay reduces the respirable dust challenge, although it does not always reduce the dust challenge to a level that results in complete resolution of symptoms of heaves. Soaking is efficient only when it is done for several hours and when the hay is completely soaked (with the bale’s strings cut). However, prolonged soaking of hay reduces its nutritive content due to leaching of soluble nutrients. The water used to soak the hay should be changed daily to prevent build up of endotoxins and microbes. The dust content of hay may alternatively be reduced by mechanical agitation in conjunction with vacuum removal of liberated airborne particles. Alternatively, the dust content of hay may be reduced by thorough steaming before feeding.

Complete pelleted diets are a suitable alternative to forages since they contain all the required nutrients, have a very low dust content, and contain few mould spores [23,45] (Fig. 9 and Fig. 10). Unfortunately some horses dislike pellets, and since they are consumed very quickly, they may promote the development of stable vices.

Silage is currently the best alternative to hay (Fig. 9 and Fig. 10) [23,42]. Increasing numbers of horses are being fed on silage to minimise airborne dust, regardless of whether they suffer from heaves or not. To minimise the possibility of a horse developing botulism from silage feeding, bags must be airtight and the silage must be consumed within 5 days after the bag is opened. Alternatively horses may be vaccinated against botulism. Botulism is mainly associated with the use of big bale silage [46,47]. Grass harvested for silage is inevitably contaminated with soil, which is the main source of Clostridium botulinum. Silage with a high dry matter (DM) content may have a reduced risk of causing botulism, since when DM exceeds 25% the fermentation of water soluble carbohydrates reduces the pH to values (4.2 - 4.6) which inhibit clostridial growth. Reduction of the silage pH may be enhanced by addition of molasses or formic acid. When silage DM is too low, secondary fermentation occurs and lactate is metabolised to acetic and butyric acids. Deamination and decarboxylation of amino acids produce amines, ammonia and carbon dioxide, resulting in an unpleasant odour. This secondary fermentation increases the pH (up to 8.5 in some cases) and allows Clostridia to multiply. Clostridial growth may also be favoured when
the plastic bag is broken. Thus silage should not be fed to horses if it has a smell of ammonia, if it comes from a damaged bag, or if the bale was opened more than 5 days previously. The risk of botulism may be minimised by feeding commercial silage (e.g., Horshage®), which is carefully harvested, often treated with preservatives, and ensiled at high DM.

Low-dust Concentrates - While concentrates are rarely a significant source of airborne organic dust, the respirable dust challenge from concentrates may be reduced by feeding molassed concentrates and grains. A less efficient option is to humidify the concentrates prior to feeding.

Low-dust Bedding - Bedding is an important source of airborne fungal spores, endotoxins and other pro-inflammatory agents. When straw is baled with high moisture content, it may develop a microbial flora similar to that of heated hay. Even when grossly "clean", straw may become a source of respirable mould spores and endotoxins when used under deep litter management, or in hot and humid and poorly ventilated stables. Wood shavings, paper bedding, peat moss and sawdust are suitable alternatives to straw [23,48,49]. However, as with straw, they can become a source of moulds and endotoxins when used in deep litter systems, or in warm and poor ventilated stables [50]. Moreover, poor quality wood shavings may result in higher airborne dust and fungal spore levels than good quality straw (Fig. 11) [23]. Flax straw has a very low dust content, but unfortunately it may cause intestinal obstruction when eaten. Cardboard bedding (e.g., Ecobed®) and large wood shavings produced especially for horse bedding (e.g., Cleanbox®) are almost free of contaminants and are thus useful low dust bedding materials (Fig. 12) [51] (van Erck, personal communication). Non-biological beddings are very clean, thermally efficient and, unlike the aforementioned organic beddings, do not provide a medium for fungal growth. They are however expensive and some of them, such as plastic bedding, need to be cleaned, thus requiring additional labour [52].

Reducing Airborne Dust Originating from the Surrounding Environment - Ideally, the stalls adjacent to the heaves affected horse should be managed similarly. Additionally, owners must be informed that the beneficial effect of a careful management in one stable may be totally lost by the proximity of hay or straw storage, which is a potential source of inhaled particles. The storage of hay and straw in a loft above the stable is particularly detrimental. Manure and other organic waste is another source of organic dust that should not be positioned close to stables. Lastly, it is essential to warn owners that a very brief exposure to organic dust is sufficient to induce pulmonary dysfunction and inflammation in heaves horses [29,53], and that the resultant inflammatory response may take days or weeks to resolve, even following removal of the challenge [41].

Reducing Airborne Dust Levels by Improving Stable Ventilation - In a stable with clean forage and bedding, there is no need for a ventilation rate exceeding 8 changes per hour [18]. In the majority of the stables, this ventilation rate is obtained without mechanical intervention, provided the top half of the stable door is kept open at all times [44]. Additional ventilation will be required when the doors and windows are closed. Properly placed and adequately sized wall and roof vents are required to permit natural environmental factors to provide adequate ventilation. Ideally, stables should have permanent openings of 0.3 m²/horse in the walls and 0.15 m² in the roof [54]. Since healthy adult horses tolerate a wide range of temperatures, owners should be informed that it is highly beneficial for their animal’s health to keep the window open, even during cold weather. When a classic management system is applied (i.e., hay and straw), airborne dust level is 6 to 7 times greater in the horse’s breathing zone than in the stall itself [22]. Since ventilation does not fully remove the dust challenge in the breathing zone when the horse is eating and sniffing, changing the forage and bedding material is essential to significantly decrease the respirable dust level (Fig. 13 and Fig. 14).
Infectious Airway Diseases and Controlled Environment - The occurrence of an infectious pulmonary disease depends on the level of airborne bacteria, viruses or mycoplasma, on the pathogenicity of a given agent, and on the resistance of the host. Prevention of infectious respiratory diseases is difficult, given that these diseases are highly contagious (especially equine influenza), the fact that many are endemic, their ability to be transmitted by routes other than aerosol, and the fact that asymptomatic carriers or horses during the incubation phase may transmit infections. Stable design and management is unlikely to reduce the incidence of highly infectious respiratory diseases such as influenza, however, it may decrease the transmission of less contagious infections. Regular disinfection of stables is recommended to prevent the persistence of infectious agents that can be transmitted via fomites, including *Streptococcus equi*.

Summer Pasture-associated Obstructive Pulmonary Disease - In some geographical regions, a syndrome that resembles heaves is recognised in horses at pasture during summer months [55]. The cause of the disease is unclear, but it may be a pulmonary hypersensitivity to inhaled allergens such as pollens or outdoor moulds, in conjunction with non-specific triggers such as dry air and irritant dusts. Currently, the treatment of this condition is symptomatic and one option is to stable the horse during the summer period. Silage, haylage or pelleted feed should be provided and wood shavings used for the bedding since (1) a dusty environment exacerbates summer pasture-associated obstructive pulmonary disease (SPAOPD), and (2) some horses may suffer from both SPAOPD and heaves [55]. In addition, medical treatment may be required.

References


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