Concentrations of dust and endotoxin in equine stabling

A. G. Whittaker, K. J. Hughes, T. D. H. Parkin, S. Love

POOR stable management can directly cause or exacerbate a range of respiratory disorders in horses (Art and others 2002). Organic dust derived from bedding and forage is the most abundant and toxicologically significant inhalable agent present in equine housing (Davis and Foster 2002). The airborne concentration of dust is determined by its rate of release from the source material, the volume of the building and the rate of clearance of dust, which depends upon the amount of ventilation of the barn or stable (Webster and others 1987). In addition, stable management activities, such as mucking out and the sweeping of aisles, can lead to a large increase in the amount of airborne dust (Crichlow and others 1980, Clarke and others 1987, Webster and others 1987). However, obtaining a relevant sample to determine a horse’s exposure to inhalable dust requires direct sampling within the breathing zone of the horse. The concentrations of total and respirable dust are substantially greater in air in the breathing zone of a horse than in samples of air obtained from the general stable environment at fixed sampling points (Woods and others 1993). Respirable dust is defined as particles of sufficiently small aerodynamic size (<7 µm) to allow deposition within the lower respiratory tract (McGorum and others 1998). In well-ventilated stables, the primary determinant of exposure to dust is the dust content of forage and bedding and the rate of release of dust from these materials through disturbance by the muzzle/nose of horses during normal behaviour, and is unlikely to be influenced by the ventilation (Clarke 1993, Woods and others 1993, Davis and Foster 2002). This is supported by the finding that the composition of forage has a greater influence than the bedding on respirable dust concentrations in the breathing zone of horses (Clements and Pirie 2007a). However, in situations where stable ventilation is poor, the growth of mould on source materials (forage or bedding) in situ can lead to increased numbers of airborne particles and poor air hygiene (Clarke and others 1987). While the overall dust concentration is a valuable indicator of environmental risk, other airborne pollutants, such as bacterial endotoxin, can potentiate the proinflammatory effect of organic dust (Pirie and others 2003).

The aim of this study was to examine the total dust and endotoxin concentrations in the breathing zone of horses in stables. First, the impact of different management systems was investigated under experimental conditions (part A). Secondly, a field study was undertaken to obtain measurements of dust and endotoxin in equestrian properties within central Scotland (part B).

In part A, four stables were provided with different combinations of forage and bedding: hay/straw, hay/wood shavings, haylage/wood shaving and haylage/rubber matting. Each bedding material and forage used was obtained from a common source. The four stables were identical in design and size (70 m²) and were contained in the same block. The stables did not share a common airspace, as dividing walls extended to the ceiling of the block. Ventilation was provided by leaving the upper half of the stable door open. Four yearling Welsh Mountain ponies were housed, one in each stable, for four three-week periods between April and September 2008, and dust in the ponies’ breathing zone was sampled three times a week. The ponies were randomly assigned to the stables such that each pony was housed in the same stable but provided with each of the four forage/bedding combinations over the period of the study. Between each period of housing in the stables, the ponies were kept at pasture for three weeks.

For part B, stables were selected from those that responded positively to a posted invitation that was sent to 40 properties. Dust samples were collected from 75 stables at 15 properties over a five-month period (March to July 2008). The stables used a range of forages and bedding: soaked hay/wood shavings (17 per cent); dry hay/wood shavings (27 per cent); haylage/straw (25 per cent); dry hay/rubber matting (5 per cent); soaked hay/rubber matting (5 per cent); dry hay/straw (11 per cent); and haylage/wood shavings (10 per cent). The studies were approved by the Animal Ethics and Welfare Committee of the Faculty of Veterinary Medicine, University of Glasgow.

For both parts A and B, dust was collected for six hours on to pre-weighed polycarbonate filters within an IOM MultiDust Personal Sampler (SKC) positioned within the breathing zone of the horse and linked to a Sidekick sampling pump (2·0 1/minute) (SKC). The breathing zone was defined as the air in the immediate vicinity of the nares sampled by placing the sampler on the pony/horse’s head collar, approximately 5 to 10 cm from the nares. In part A, each sampling period included a single mucking out/feeding activity, performed at 09.00. It was not possible to standardise the timing of stable management procedures during part B, as the sampling was performed at a time convenient for the stable owners. Filters were re-weighed and stored at −20°C before

<table>
<thead>
<tr>
<th>Forage/bedding type</th>
<th>Dust (µg/m³)</th>
<th>Endotoxin (EU/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay/straw</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Hay/wood shavings</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Haylage/straw</td>
<td>b</td>
<td>c</td>
</tr>
<tr>
<td>Haylage/rubber matting</td>
<td>c</td>
<td></td>
</tr>
</tbody>
</table>

FIG 1: Concentrations of dust and endotoxin in the breathing zone of horses housed with four different combinations of forage and bedding. For each analyte, different letters denote a significant difference (P<0·05). EU Endotoxin units

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endotoxin analysis (McGorum and others 1998). Endotoxin was determined using the pyrochrome Limulus amoebocyte lysate test (Associates of Cape Cod). The filters were submerged in 5 ml endotoxin-free water containing 0·05 per cent Tween-20 and rotated for two hours. The samples were centrifuged at 1000 g for 20 minutes, diluted and stored at –80°C. For analysis, lyophilised pyrochrome was reconstituted in Glucashield glucan inhibiting buffer (Associates of Cape Cod). A dilution series of control standard endotoxin (potency 9·0 endotoxin units [EU]/ng), together with the diluted samples in 50 µl triplicate, was added to a 96-well microplate; and 50 µl of the reconstituted pyrochrome was added. The plate was agitated for 30 seconds and then incubated at 37°C for 20 minutes. The reaction was stopped with 10 per cent acetic acid and the optical density was determined at 405 nm (Mullrison Ascent; Thermo Scientific). For statistical analysis, the non-parametric Mann-Whitney test was used to compare datasets. Significance was set at P<0·05. Spearman’s rank correlation coefficients were calculated to identify relationships between the two continuous non-parametric variables, dust concentration and endotoxin concentration.

For both parts A and B, respirable dust concentrations were below the detection limit of the equipment used and only total dust concentrations were recorded. In part A of the study, the dust concentrations in the breathing zone of the ponies appeared to be influenced more by the type of forage than the type of bedding. Stables containing hay forage had significantly higher dust concentrations than those containing haylage (P<0·05). There was no significant difference in the dust concentrations in stables containing hay/straw and hay/wood shavings, or between stables containing haylage/wood shavings and haylage/rubber matting (Fig 1). When the data were pooled, the endotoxin concentration was significantly correlated with the dust concentration (Spearman’s rank correlation 0·754). The endotoxin concentrations were influenced predominantly by the type of forage, although there was also a significantly higher endotoxin concentration in stables with straw bedding compared with wood shavings (Fig 1). The dust concentrations varied over the study period (Fig 2) in a way that was suggestive of a slight seasonal trend; however, considerable day-to-day variation was also present.

In part B, the use of hay as opposed to haylage did not influence dust or endotoxin concentrations in the breathing zone of horses. When the data were pooled, there was a correlation between the dust and endotoxin concentrations (Spearman’s rank correlation 0·917). There was a trend towards lower dust and endotoxin concentrations when the hay was soaked before feeding (Table 1), similar to the findings of Clements and Pirie (2007b). However, due to the high degree of variation, this finding was not statistically significant (P=0·20).

Overall, the results of the present study support the findings of previous experimental studies that forage has a greater effect on the total and respirable dust and endotoxin concentrations in the breathing zone of horses than the type of bedding (Webster and others 1987, Woods and others 1993, Clements and Pirie 2007a). These findings have important implications for stable environment management for maximising the respiratory health of horses, and horse owners should be advised that the choice and preparation of forage has a greater influence on the dust and endotoxin concentrations in the stable than the bedding type. In part A of this study, changing from hay forage/straw bedding to haylage/wood shavings was associated with an approximate halving of airborne dust in the breathing zone, and an approximate fivefold decrease in endotoxin concentrations (Fig 1). It has been established that the clinical signs of recurrent airway obstruction can be induced by the exposure of susceptible horses to dusty stable environments (Jackson and others 2000) or inhalational challenge with hay dust constituents (Pirie and others 2003), but the threshold concentrations of dust or dust constituents for the development of respiratory disease are unknown. However, considering the likely duration of exposure of horses to dust during long periods of stabling, it is possible that the concentrations of airborne dust and endotoxin in stables using hay/straw could result in a cumulative exposure sufficient for the development of pulmonary disease. Long-term exposure to dust or endotoxin has been shown to be important for the development of occupational pulmonary diseases in human beings (Khan and Nanchal 2007), and a similar situation may exist in both horses that are susceptible to respiratory disorders and horses that are otherwise healthy.

Assessing the quality of air in stables is technically challenging because the majority of dust is derived from forage rather than bedding. The only practical means by which exposure of the horse’s airways to dust and endotoxin can be estimated is by making measurements within the breathing zone, as has been reported previously (Woods and others 1993, McGorum and others 1998, Clements and Pirie 2007a). In the present study, attempts were made to measure the respirable dust concentrations in the breathing zone; however, the amounts obtained were below the lower detection limit of the equipment used (data not shown), and so the total dust concentrations are reported. It is likely that respirable dust would have been detected if a collection period longer than six hours had been used. There are no available data in relation to direct measurement or the contribution of respirable dust to total dust concentrations, but previous studies indicate that respirable dust may account for 30 to 60 per cent of total dust in both the stable and the breathing zone of horses (Woods and others 1993, McGorum and others 1998, Riihimaki and others 2008). As such, total dust concentrations in the breathing zone may be cautiously interpreted to determine the horse’s possible exposure to respirable dust and its influence on respiratory health. Similar to the results of part A, previous studies have determined that stable management influences exposure to total (and respirable) dust in an

### TABLE 1: Median (range) concentrations of dust and endotoxin per m³ air in the breathing zone of horses from 73 stables using different types of forage and bedding

<table>
<thead>
<tr>
<th>Bedding type and forage type</th>
<th>Number of</th>
<th>Organic dust (µg/m³)</th>
<th>Endotoxin (EU/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubber matting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soaked hay</td>
<td>4</td>
<td>448 (298-751)</td>
<td>317 (177-1984)</td>
</tr>
<tr>
<td>Hay</td>
<td>4</td>
<td>890 (236-1074)</td>
<td>1298 (165-1889)</td>
</tr>
<tr>
<td>Straw bedding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hay</td>
<td>8</td>
<td>181 (94-735)</td>
<td>81 (18-869)</td>
</tr>
<tr>
<td>Haylage</td>
<td>19</td>
<td>548 (183-1884)</td>
<td>510 (36-3184)</td>
</tr>
<tr>
<td>Wood shavings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soaked hay</td>
<td>13</td>
<td>332 (143-719)</td>
<td>157 (49-761)</td>
</tr>
<tr>
<td>Hay</td>
<td>20</td>
<td>555 (118-1878)</td>
<td>548 (62-6946)</td>
</tr>
<tr>
<td>Haylage</td>
<td>7</td>
<td>573 (80-1297)</td>
<td>863 (123-1738)</td>
</tr>
</tbody>
</table>

EU Endotoxin units
experimental setting (Woods and others 1993, McGorum and others 1998, Clements and Pirie 2007a, 2007b). However, part B of the present study is the first to quantify the exposure to both dust and endotoxin in the breathing zone of horses in the UK not under controlled experimental conditions. In part B, large variations in the dust and endotoxin concentrations were measured; it is likely that these variations were a result of the forage and bedding coming from a variety of sources with differing dust content. Additionally, the design of the stables used in part B varied between properties, which may have influenced the ventilation; the ventilation rates were not determined in this study. These variables, which could not be avoided, may explain the differences in the dust and endotoxin concentrations measured in parts A and B for similar management systems, and highlights the likelihood that bedding/forage from different sources will differ in their dust content. For example, good-quality hay may contain less dust than a poor-quality haylage. Furthermore, there is a need for repeated sampling and consistency in stable activities such as mucking out during the collections of dust samples.

Earlier studies performed in the UK and Canada found that the concentrations of total and respirable dust in the ambient air of stables were influenced by stable activity, for example, mucking out of stables and cleaning of aisles; however, the concentrations in the breathing zone of the horses were not determined in either study (Crichlow and others 1980, Clarke and others 1987). While documentation of the influence of stable management on the concentrations of ambient dust and its constituents is useful for assessment of the overall stable environment, the concentrations in the breathing zone are more likely to indicate the potential impact on the respiratory health of horses (Woods and others 1993, McGorum and others 1998). Airborne dust concentrations measured in stables may underestimate the concentration in the breathing zone of horses considerably, particularly when conventional materials such as hay forage and straw bedding are used (Woods and others 1993). Riihimaki and others (2008) reported total and respirable dust concentrations in the breathing zone of Swedish standardbred horses fed haylage and bedded on straw in conventional racing stables that were similar to those of a ‘low dust’ management system (silage forage and shavings) reported by McGorum and others (1998). Similarly, the concentrations of dust and endotoxin found in part B of this study were consistent with those of the ‘low dust’ environment reported by McGorum and others (1998). Taking into account the slightly differing potencies of the control standard endotoxin used in this study and the study of McGorum and others (1998), and the expression of endotoxin concentration in terms of EU rather than ng, the concentrations of endotoxin in the present study were higher than those reported by McGorum and others (1998). However, the detergent Tween-20 that was used in the extraction procedure in the present study is thought to disrupt hydrophobic interactions between the endotoxin and the filter, disaggregate endotoxin micelles and dissociate cell-bound endotoxin, and increase the extraction efficiency approximately sevenfold (Douwes and others 1995). Taking these methodological differences into consideration, the endotoxin concentrations measured in the breathing zone of horses in the present study were considered comparable to those in a ‘low dust’ environment described by McGorum and others (1998). However, there are limitations when comparing endotoxin values measured in studies that use different units, due to the likely heterogeneity in the biological activity of endotoxins in the stable environment.

Given the potential detrimental effects of aerosolised irritants on the welfare and exercise capabilities of stabled horses, the present findings suggest that the conditions in the stables studied were conducive to maintaining respiratory health in the horses. Further studies are required to determine whether these findings are similar to the conditions for horses stabled in other locations; however, the results of the present study may reflect good awareness of the owners of the importance of optimising air hygiene in stables.

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