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Endotoxin concentrations within the breathing zone of horses are higher in stables than on pasture

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ABSTRACT

Inflammatory airway disease is common in stabled horses, with a prevalence of 17.3% in Michigan pleasure horses. Stable dust is rich in endotoxin, which may induce neutrophilic airway inflammation. Climatological conditions (ambient temperature and relative humidity) may influence endotoxin concentrations in pastures. The aim of this project was to determine if endotoxin levels in the breathing zone of horses in stables were higher than of horses on pasture, and if the endotoxin on pasture was associated with climatological conditions. Endotoxin exposure of six horses that were stabled or on pasture was determined by a Limulus amebocyte lysate assay. Climatological data were obtained from the US National Climatic Data Center.

Endotoxin exposure was significantly higher (about 8-fold) in stables than on pasture. On pasture, endotoxin varied widely, despite constant climatological conditions. It was concluded that stabled horses are exposed to higher endotoxin concentrations than horses on pastures. Local endotoxin concentrations may be more important than ambient climatological conditions in determining endotoxin exposure of individual horses.

Introduction

In the Northern hemisphere, it is common to keep horses in stables, where they are fed hay. The economical advantage of this practice, however, can be diminished by the impairment of horse's welfare and exercise performance due to exposure to high concentrations of airborne pathogenic/inflammogenic materials. For example, stabled horses without clinical signs or a history of airway disease will experience influx of inflammatory cells into the airway lumen (Tremblay et al., 1993; Holcombe et al., 2001). Furthermore, feeding hay from round bales, from which horses are assumed to have a higher dust exposure than when fed from traditional square bales, increased the risk for >20% neutrophils in trachea by about 5-fold (Robinson et al., 2006). From these data, it can be inferred that hay dust exposure increases the risk of airway inflammation, a cardinal feature of equine inflammatory airway disease (IAD). The prevalence of IAD in Michigan pleasure horses has been reported to be 17.3% (Robinson et al., 2006).

Airway inflammation has functional consequences as increased mucus accumulation, which is associated with airway inflammation, is a risk factor for poor racing performance of stabled race horses (Holcombe et al., 2006). Dust exposure can have even more important consequences in horses with lung disease. For example, in horses with recurrent airway obstruction (RAO), organic substances that are frequently found in equine stable and hay dust can cause airway inflammation. Specifically, endotoxin and fungal products (from e.g. *Faenia rectivirgula, Aspergillus fumigatus*) within stable dust can trigger neutrophilic airway inflammation and obstruction in RAO-susceptible horses (Derksen et al., 1987; Pirie et al., 2003).

Woods et al. (1993) have previously reported that the respirable airborne dust concentrations in the breathing zone of horses on straw bedding and eating hay (conventional stables) are significantly higher than with shavings as bedding and a complete pelleted diet (Woods et al., 1993). McGorum et al. (1998) showed a strong correlation between dust concentrations and airborne endotoxin concentrations, and that the content of endotoxin is significantly higher in respirable dust from conventional stables than from pasture. However this study must be interpreted with caution because endotoxin concentrations were only measured in the breathing zone of a single pony. Endotoxin in horse stables contributes at least partially to the development of airway inflammation in both healthy and RAO-susceptible horses (Pirie et al., 2002). In addition to the amount of dust, endotoxin concentrations are also influenced by seasonal and climatological factors, such as temperature and relative humidity (Carty et al., 2003).

Endotoxin in horse stables arises from different point sources (e.g. hay, manure, etc.) (Pickrell, 1991). Due to differences in the individual behavior of horses or in stable hygiene, area samplers

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may not be adequate to reflect the actual endotoxin exposure of stabled horses. Indeed, Woods et al. (1993) reported that by using personal samplers, total and respirable dust concentrations in the breathing zone of horses in conventional stables were higher than concentrations measured in stables with area samplers (Woods et al., 1993).

In the present study we therefore determined the endotoxin concentration in the breathing zone of horses by use of a personal sampler device. We hypothesised that the endotoxin concentrations in the breathing zone of stabled horses are higher than of horses on pasture. We also hypothesised that ambient temperature and relative humidity are associated with endotoxin concentrations in the breathing zone of horses on pasture.

Materials and methods

Animals and study design

Horses were selected from a herd maintained by the Pulmonary Laboratory at Michigan State University. In all horses, physical examination revealed no abnormalities. Six horses (three mares, three geldings, aged (±SD) 21 ± 6.7 years and consisting of two Quarter horses, one Quarter Horse/Arabian mix, one Grade horse, one Thoroughbred horse and one Standardbred horse) were studied.

Endotoxin exposure was measured when horses were stabled in conventional stables and subsequently on pasture. For determination of endotoxin concentrations in the breathing zone of stabled horses, animals were brought into the stable and were allowed to adjust to the environment for 1 h before the personal sampler for endotoxin collection was attached (see 'Determination of endotoxin concentrations in the breathing zone'). The stable used for the study was an enclosed 10-stall building. The straw-bedded stalls were cleaned and horses were fed hay between 0700 and 0800 h, and fed again in the afternoon between 1600 and 1700 h. For the first measurement, the horses were brought from pasture into the barn between 0800 and 1000 h. After the 1 h adjustment period and the 4 h stable measurement, horses were returned to the pasture. The second 4 h measurement (pasture) was performed with all horses on the same grass pasture. Horses had water available ad libitum and there were no trees or shrubs.

Horses were observed throughout the 4 h measurement period, but were allowed to move around freely with the personal sampler attached to the halter and surcingle. Stable and pasture measurements were separated by at least 1 day. The study was performed between the 15th and 26th of September, 2005. The protocol was approved by the All-University Committee for Animal Use and Care of Michigan State University.

Determination of endotoxin concentrations in the breathing zone

For the collection of endotoxin in the breathing zone around the horse nostril, personal samplers were attached to each horse (Fig. 1). In every horse, the end of the sampling tube was within 10 cm of one of the nostrils and breathing zone endotoxin concentrations are defined as endotoxin concentrations measured through this sampling tube. The personal sampler consisted of a filter cassette that was attached to the halter and an AirCheck 52 Personal Sample Pump (both provided by EMSL Analytical) that was attached to a surcingle. A rubber hose connected the filter cassette and the air pump. Endotoxin was sampled as described previously (Walters et al., 1994). Briefly, the breathing zone air was sampled at a flow rate of 1.5 L/min for an average of 4 h. Endotoxin that was collected on the air filter was quantified by the IH Laboratory at EMSL Analytical using the *Limulus* amebocyte lysate (LAL) kinetic chromogenic assay (Lindsay et al., 1989) and reported in EU/m^3.

Climatological data

Climatological data for Lansing, MI, were obtained from the US National Climatic Data Center (http://www7.ncdc.noaa.gov/IPS/). We report four dew point temperature and relative humidity measurements at 3 h intervals (0700, 1000, 1300 and 1600 h) on the days when endotoxin measurements were taken.

Statistical analysis

Raw data of endotoxin concentrations were not normally distributed (Kolmogorov–Smirnov $P \leqslant 0.05$). Therefore, data were $\log_{10}$-transformed. Means of endotoxin concentrations in stables and on pasture were compared using Student's *t* test. Data are reported as geometric means (anti-log-transformed) with the 5% and 95% values. Mean values were calculated for the climatological data. Data were analyzed by use of SAS version 8, SAS/STAT Software (SAS Institute, Cary, North Carolina, USA). Differences were considered significant for *P* values $\leqslant$0.05.

Results

Geometric mean (and their 5% and 95% values) of endotoxin concentrations in the breathing zone of stabled horses (EC = 7.08 (2.82; 36.3) $\times 10^3$ EU/m^3) were significantly higher than of horses on pasture (EC = 0.85 (0.45; 2.57) $\times 10^3$ EU/m^3) (Fig. 2). The mean difference was more than 8-fold. Endotoxin concentrations for each horse were always less on pasture than in the stable.

Data for temperature and percent relative humidity are shown in Table 1. The average ambient (out door) temperature during stabling (10.4 ± 1.3 °C) was not significantly different from the average ambient temperature on pasture (11.4 ± 1.8 °C) ($P = 0.35$; Table 1). In addition, there was no significant difference between the ambient relative humidity on days when horses were stabled (60.75 ± 6.66%) as compared to days when horses were kept on pasture (68 ± 0.24%; $P = 0.3$). Endotoxin concentrations did not correlate with the ambient temperature or the relative humidity when horses were stabled or kept on pasture

Discussion

In this study, we showed that the mean endotoxin concentration measured in the breathing zone of stabled horses is more than 8-fold higher than that of horses kept on pasture (Fig. 2). Endotoxin in the horse environment probably arises from point sources, concentrated sources of endotoxin, such as manure (Pickrell, 1991). Factors such as individual behavior determine the proximity of the horse's breathing zone to these point sources. It is therefore important to measure endotoxin in the breathing zone rather than in the general area. Endotoxin exposure on pasture was less than in the stables, presumably because of fewer point sources on pasture.

The endotoxin concentrations measured in this study are higher than recognized occupational thresholds for adverse health problems in people (Castellan et al., 1987; Donham et al., 1989; Zock et al., 1998). In those studies the authors showed that endotoxin concentrations in the range from 40 to 1000 EU/m^3 can cause pulmonary function changes and airway inflammation. We recently demonstrated that horses with RAO have up-regulation of the endotoxin receptor toll-like receptor 4 (TLR4) in airway epithelial cells during stabling (Berndt et al., 2007). Toll-like receptor 4 expression correlated with interleukin 8, a potent neutrophilic chemoattractant, and with numbers of neutrophils in the airways. Thus, endotoxin in barn dust may be in part responsible for the characteristic airway neutrophilia during acute exacerbations of RAO-affected horses during stabling.

When measured with area samplers, outdoor endotoxin concentrations have been reported to be positively correlated with ambient temperature and negatively with percent relative humidity (Carty et al., 2003). Others have shown that environmental endotoxin is positively associated with both ambient temperature and percent relative humidity (Wickens et al., 2002). Here we report that the endotoxin concentration in the breathing zone of horses on pastures, which were measured with personal samplers, varies greatly despite constant ambient temperature or the percent of relative humidity. This suggests that endotoxin concentrations in the breathing zone of horses are more dependent on the immediate environment in which the horse keeps its nose than on environmental conditions, such as temperature or relative humidity. This supports the contention that differences in the concentration of endotoxin in the breathing zone, caused by factors such as head positioning, sniffing and frequency of moving, is the main factor determining individual endotoxin exposures.

The literature on measuring the natural endotoxin exposure of horses by using personal samplers is limited. Our results are in general agreement with a previous study, in which the authors

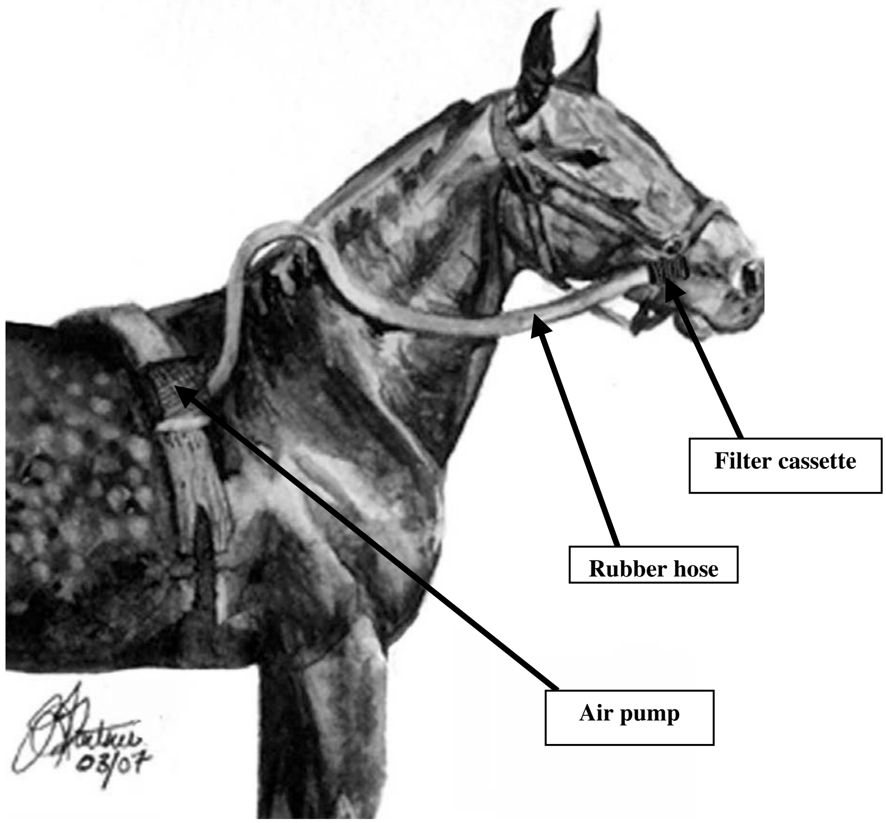

Fig. 1. Personal sampler devise for endotoxin measurements in the breathing zone around the horse nostril. The personal sampler consisted of a filter cassette that was attached to the halter, and an AirCheck 52 Personal Sample Pump (both provided by EMSL Analytical) that was attached to a surcingle. The filter cassette and the air pump were connected through a rubber hose. Endotoxin was sampled at a flow rate of 1.5 L/min for an average of 4 h.

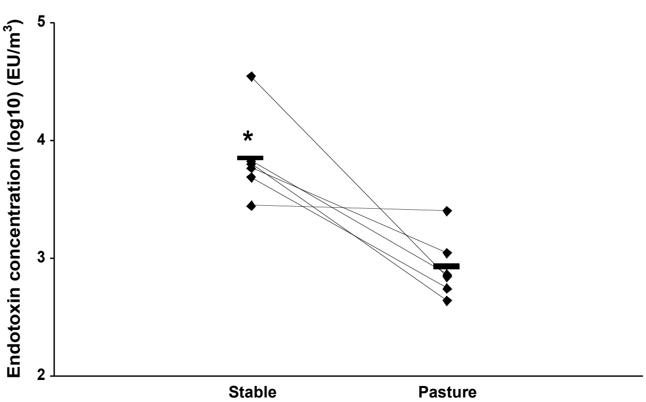

Fig. 2. Endotoxin concentrations ($\log_{10}$ $10^3 \times$ EU/m^3) in the breathing zone of stabled horses and horses on pasture. Individual diamonds represent individual measurements. Horizontal bars represent the geometric means. (*) indicates significant different ($P \leqslant 0.05$) from endotoxin concentration measured on pasture.

Table 1
Average ambient temperature (°C) and average relative humidity (%) of four measurements in 3 h intervals (0700, 1000, 1300, and 1600 h) on days of endotoxin measurements

	E	Date	Average temperature (°C)	Average relative humidity (%)
Horse 1	S	15 September 05	9.4	56.5
Horse 2	S	15 September 05	9.4	56.5
Horse 3	S	16 September 05	12.0	80.75
Horse 4	S	16 September 05	12.0	80.75
Horse 5	S	21 September 05	9.7	45.0
Horse 6	S	21 September 05	9.7	45.0
Horse 1	P	23 September 05	9.2	67.5
Horse 2	P	23 September 05	9.2	67.5
Horse 3	P	17 September 05	13.0	68.75
Horse 4	P	17 September 05	13.0	68.75
Horse 5	P	24 September 05	12.0	67.75
Horse 6	P	24 September 05	12.0	67.75

Data were obtained from the US National Climatic Data Center. E, Environment, in which horses were maintained; S, Stable; P, Pasture.

showed an about 15-fold higher airborne endotoxin concentration in conventional stables as compared to concentrations on pasture (McGorum et al., 1998). However, in that study average endotoxin concentrations in stables and on pasture were higher than in our study (1.67 mg/m^3 (139.2 $\times$ 10^3 EU/m^3) and 0.11 mg/m^3 (9.2 $\times$ 10^3 EU/m^3), respectively). It is important to note that measured endotoxin concentrations are highly sensitive to the sampling technique, experimental design, and analytical method (Thorne et al.,1997, 2003; Liebers et al., 2007). While in the present study, we used a pump flow rate of 1.5 L/min over 4 h to collect endotoxin concentrations in the horse's breathing zone, McGorum et al. (1998) collected dust at a flow rate of 2 L/min over periods of 4–10 h (McGorum et al., 1998). Furthermore, whereas McGorum et al. (1998) obtained measurements by using one pony, we attached personal samplers to six individual horses.

Conclusions

Stabled horses are exposed to 8-fold higher concentrations of endotoxin than horses maintained on pastures. Endotoxin concentrations on pasture as well as in the barn are sufficiently high to cause airway inflammation in other species. Therefore, it is likely that endotoxin exposure plays a role in airway inflammation of stabled horses as well, particularly those affected with RAO. Local endotoxin concentrations are more important than ambient climatological conditions in determining endotoxin exposure levels in individual horses.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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