# Ventilation and air hygiene parameters in horse stables


## Summary

To assess ventilation, air hygiene and the influence of ventilation on air hygiene in horse stables, 11 barns on 4 separate premises were studied during four consecutive weeks in January and February. One of the premises used mechanical ventilation and woodshavings bedding, all others used natural ventilation and straw bedding. In all barns, inside and outside temperature and relative humidity were monitored for a week. Ammonia, stall wind speed, smoke clearance, airborne micro-organisms (fungi, gram-negative bacteria and total bacteria) and stall air and breathing zone air dust and endotoxin levels were measured. The aim was to find a correlation between the inside-outside temperature difference and air hygiene parameters.
In the naturally ventilated barns, inside temperature and humidity were 7.5(2.8-12.7)°C and 75(67-86)%, respectively. Ammonia ranged from <2 to 11 ppm, wind speed was <0.2 to 0.6m/s. Airborne micro-organism levels were 33561(2830-35043), 510(84-1111) and 2887(344-10809) colony forming units (CFU)/m3 for fungi, gram-negative and total bacteria, respectively in the naturally ventilated barns and 769(671-3960) and 4815(2121-6222) for fungi and total bacteria in the mechanically ventilated barns. Stall air and breathing zone air dust levels, in mg/m3, were, respectively 0.24(0.16-0.58) and 2.70(0.44-12.49) in the naturally ventilated barns, and 0.46(0.13-0.98) and 7.36(1.97-45.03) in the mechanically ventilated barns. Stall air and breathing zone air endotoxin levels, in EU/m3, were 268(86-800) and 1653(1273-48485) naturally ventilated barns, and 233(22-504) and 1974(453-15028) in the mechanically ventilated barns. Levels of dust and endotoxin were correlated (spearman correlation 0.884). Breathing zone levels of dust and endotoxin were significantly higher than and not correlated to stall air levels. CFU counts of fungi were significantly lower in the mechanically ventilated barns.
No correlation between barn outside-inside temperature difference and any of the air hygiene parameters was found, most likely due to a limited number of samples and variability in used source materials.


## Introduction

Airway disease is an important cause of loss of performance in horses[1,2,3]. As originally stated by Clarke[4] and reiterated by Vandenput[5], a horse's respiratory well-being is crucial for its short-term athletic ability and its long-term welfare. Studies have shown that organic dust, moulds and endotoxins play a role in the aetiology or recurrence of airway disease[6-10]. In human medicine, and more specifically occupational medicine, much research has been done regarding dust and endotoxin exposure and respiratory health effects[11,12]. Dust, airborne micro-organisms and endotoxin are present in horse stables, often in large quantities[13,14,15]. Inadequate ventilation contributes to those high levels of airborne pollutants[13,15]. However, in only one study was ventilation rate objectively measured, using a smoke cloud and particle counter to calculate air changes per hour[13]. Other studies often mention no more than having shut doors, windows and/or air vents to create a badly ventilated, dusty environment[6,15]. Likewise, in treatment protocols for equine heaves, veterinarians and owners are encouraged to ensure 'adequate' ventilation, without advice on what constitutes adequate ventilation and how ventilation adequacy should be assessed[16].
When investigating dust exposure one needs to include measurements of the animal's breathing zone, as total and respirable dust concentrations in breathing zone air are significantly higher than those measured in stall air in a (very) well ventilated stable[14]. For this reason, in the aforementioned study it was stated that in an adequately ventilated stable, no additional benefit to reduction in the horse's exposure can be expected by further improving ventilation. This has led others to assume that overall, ventilation is of no significant importance in efforts to decrease dust levels[5]. However,

it is by no means certain that all or even most horse stables are adequately ventilated[17]. Webster, in 1987 found that most boxes and barns had ventilation rates higher than what he believed to be an adequate rate of 4 air changes per hour (AC/h), but that these ventilation rates decreased greatly to usually less than 2 AC/h when box or barn doors were shut[18].

So all in all, in spite of the importance of using 'clean' feed and bedding materials to reduce the release of particles[13,19], the importance of removing airborne particles, once released, via ventilation should not be overlooked[4,18,20,21]. An assessment of the state of ventilation in horse stables therefore seems warranted. Little is known regarding the ventilation requirements of horse stables[18,20,22], and standards cannot readily be extrapolated from the better researched field of farm animal housing because of vital differences in stocking density, animal longevity and intended use, and animal owners' personal desires regarding comfort and aesthetics. Furthermore, most of the aforementioned studies regarding airway disease compared differences in environmental risk factors such as dust, airborne micro-organisms and endotoxins within a combined variability of housing, feed, bedding, ventilation, and/or management. The aim of this study is to assess the current standards of ventilation and compare ventilation and air hygiene parameters within a common housing and management system: the widely used American barn system.

## Materials and Methods

The study was conducted during four consecutive weeks in the winter season (January - February 2007). In this period, barn stocking density is expected to be highest and many opportunistic ventilation openings such as doors and windows closed because of low outside temperatures. Ventilation then depends almost solely on the barn's intrinsic ventilation mechanisms, and is reduced compared to summer levels. This assumption is confirmed by a survey performed by Couetil et al, which showed that the incidence of equine Recurrent Airway Obstruction (RAO) was highest in winter and spring, with a peak in January[23].

*Included study locations*

The "American barn" also known as "stalled barn" or "covered yard" housing system consists of a variable number of individual boxpens in one large barn. Eleven barns on four separate premises were investigated in this study: two barns on one site, three on all other sites. Study premises were numbered 1-4, and barns on each premises were assigned labels A-C. Barns consisted of between 4 and 50 boxpens in a single barn. Barn height ranged from about 3m to about 20m. The boxes were mostly open-roofed with solid wall panels about 2.5 meters high with an open top front. Barn 3B had boxes with solid roofs, and in barn 4B boxes had open-framed wall panes. Only in barn 2A the top front had no frame, to allow horses to stand with their heads outside the boxes. Barns 2A and 4B had their boxes in rows facing a sand-filled riding arena. There was no activity in either arena during the time of sampling. Premises 1 used mechanical ventilation and wood shavings bedding, all others used natural ventilation and straw bedding.

*Ventilation*

For each barn, inside air temperature and humidity were continuously monitored for six consecutive days using thermohygrographs and measured values were compared to local outside air temperature and humidity data measured as reported by local weather stations of the KNMI Royal Dutch Meteorological Institute and obtained from the institutes's online database (www.knmi.nl). Barn ventilation was further assessed by general inspection and smoke clearance tests, by inside air velocity measurements with a Kestrel wind speed meter and measurements of ammonia concentration using Dräger tubes. Daily minimum, maximum, mean and maximal shift in temperature and humidity were recorded from the thermohygrograph readings, and mean values over the 6 monitored days were used in the analysis. In farm animal housing, the difference between outside and inside temperature is often used as an easily measurable, objective indication of ventilation efficiency. A temperature difference of up to 5ºC is considered acceptable[24].

*Dust and Endotoxin*

Ambient air and breathing zone inhalable dust and endotoxin concentrations were measured using PAS-6 sampler heads with Gilian Giliar5 personal samplers at a flow rate of 2.0 l min-1[12,25]. Ambient air samples were taken by placing a stationary sampler approximately 1.5 m above ground level pointing downward. Breathing zone samples were taken by attaching a sampler head to a horse's halter, near a nostril and pointing rostrally. Horses were allowed to move freely in their boxes during sampling. Sample duration was as long as stable personnel activity permitted.

Flow rate was measured directly before and after sampling with a calibrated Brooks rotameter and if post-sampling flow rate differed from 2.0 l min-1 the mean of flow rates measured before and after sampling was used to calculate the total volume of air sampled. The PAS-6 personal sampler heads were fitted with pre-weighed 25 mm glass fibre filters. After sampling, filters were frozen at -20ºC and stored until further processing. Unexposed filters were brought to the study locations and used as blanks. Filters were analyzed gravimetrically in a conditioned weighing room. Filters with a negative weight difference were considered measurement errors and their weight was not included in the analysis. Endotoxin was analyzed by Limus Amoebocyte Lysate (LAL)[26] assay at the Institute for Risk Assessment Sciences (IRAS) in Utrecht. Samples were analyzed in duplicate and the mean value was calculated. Filters with an undetectable level of endotoxin were considered unreliable measurements and these were not included in the analysis. Dust and endotoxin were expressed as mg/m3 and endotoxin unit (EU)/m3, respectively.

*Micro-organisms*

Airborne viable micro-organism concentrations were determined using a N-6 single stage Andersen sampler[27] with Becker pumps at a flow rate of 28.3 l/min. Three different groups of micro-organisms were determined: fungi, bacteria ('total-bacteria') and gram-negative bacteria on Dychloran Glycerol Agar (DG18), Tryptone Soy Agar (TSA) and Crystal Violet agar plates respectively. Total bacteria and fungi sampling time was 30 seconds, gram-negative sampling time was 5 minutes. These sampling times were based on test runs performed to determine optimal sampling time. Two duplicate samples were taken for each group of micro-organism in each barn, at approximately 1.5 m above ground level in front of a box entrance, with the inlet pointed towards the box. On each study location, blank controls were taken. After sampling, fungi agar plates were incubated for 4 days at 24-25ºC and bacteria agar plates were incubated for about 24 hours at 37 ºC. Plate counts were corrected for blanks and for multiple hits[28]. Airborne micro-organism concentrations were expressed in colony forming units (CFU)/m3. For each barn the CFU/m3 for each group of micro-organisms was calculated as the mean CFU/m3 of the four samples.

Despite findings that the amount of respirable airborne particles increases dramatically during mucking out, and that different housing systems can have comparable levels of stall air readings during times of rest[13], all air samples were taken during the day at a time when there was no present or recent human activity in the barn. This was done in an attempt to look solely at the influence of ventilation on air hygiene and to minimize variability due to stable management e.g. differences in feed and bedding source materials or mucking out practices.

Sampling for micro-organisms and dust and endotoxins was always performed on the same day, except for premises 1, in which a different protocol was used. On premises 1, stationary samples of stall air were taken continuously for 4 days in 8-hour intervals to coincide with the shift changes of barn staff whose personal dust and endotoxin exposure was sampled for a separate study. On 1 day, air samples were taken over a period comparable to those on the other premises. In some barns, two horses were sampled rather than one. In these cases, the geometric mean of dust and endotoxin measurements of the two horses was used in the final analysis.

*Analysis*

All results were gathered in Excel 2003 and then exported and analyzed in SPSS 16. Temperature and humidity were read off the thermohygrograph sheets in 2h intervals. For each day, a mean, minimum and maximum temperature and humidity value were transcribed. Daily average temperature and humidity as reported by the KNMI were also transcribed. The mean of these values for each barn measuring period of six days was used in the final analysis.

Median, range and IQR of stable and breathing zone dust and endotoxin, and CFU/m3 of total bacteria, gram-negative bacteria and moulds were calculated. In the naturally ventilated stables the difference between outside and inside temperature was used as an index for the efficacy of ventilation. Correlations were made using Spearman's rho, comparisons for significant differences within premises 1 were done using the nonparametric Wilcoxon's signed rank test for related samples. Comparisons between different locations were done using the nonparametric Mann-Withney U test for independent samples. Statistical significance was assumed at $p<0.05$.

## Results

*Ventilation parameters*

A summary of the data collected using thermohygrographs is shown in Table 1:

| | | median | range |
|---|---|---|---|
| mean inside temperature in ºC | mechanically ventilated | 14.7 | 13.8 – 20.8 |
| | naturally ventilated | 7.5 | 2.8 – 12.7 |
| daily shift in inside temperature in ºC | mechanically ventilated | 2.8 | 1.2 – 3.0 |
| | naturally ventilated | 2.5 | 0.7 – 5.8 |
| outside temperature in ºC | mechanically ventilated | 2.1 | -5.3 – 10.8 |
| | naturally ventilated | 6.6 | -4.0 – 11.8 |
| difference between outside and inside temperature in ºC | mechanically ventilated | 12.6 | 11.8 – 18.7 |
| | naturally ventilated | 2.3 | 0.3 – 6.1 |
| mean inside air humidity | mechanically ventilated | 49% | 38% - 51% |
| | naturally ventilated | 75% | 67% - 86% |

Air velocity in barn corridors was below a level detectable by the equipment used (<0.2 m/s) in 8 (64%) of the 11 studied locations. In the remaining barns corridor air velocity ranged from 0.2 to 1.2 m/s. Stall air velocity was below 0,2 m/s in all barns except one, in which stall air velocity ranged from 0.3 to 0.6 m/s. Stall smoke clearance time ranged from 5 seconds to 5 minutes, median clearance time was 45s. Smoke clearance time in the mechanically ventilated barns was significantly higher than in the naturally ventilated barns. Ammonia was undetectable in 9 (82%) of the study locations. The remaining barns, all on the same premises which used mechanical ventilation, had ammonia concentrations ranging 2-11 ppm. Average inside temperature was significantly higher and inside air humidity was lower in the mechanically ventilated barns compared to the naturally ventilated barns. Only in two of the naturally ventilated barns, both on premises 2, did the difference between outside and inside temperature exceed the recommended 5ºC.

*Air hygiene parameters*

Air hygiene parameter measurements are summarized in Table 2:

| | | | median | range (min - max) | percentiles ($25^{th}$ & $75^{th}$) |
|---|---|---|---|---|---|
| dust mg/m3 | mechanically ventilated | stall air | 0.46 | 0.13 - 0.98 | 0.29 & 0.59 |
| | | breathing zone air | 7.36 | 1.97 - 45.03 | 3.17 & 44.31 |
| | naturally ventilated | stall air | 0.24 | 0.16 - 0.58 | 0.17 & 0.51 |
| | | breathing zone air | 2.70 | 0.44 - 12.49 | 1.32 & 5.20 |
| endotoxin EU/m3 | mechanically ventilated | stall air | 233 | 22-504 | 76 & 325 |
| | | breathing zone air | 1974 | 453 - 15028 | 1567 & 9262 |
| | naturally ventilated | stall air | 263 | 86 - 800 | 94 & 540 |
| | | breathing zone air | 1653 | 1273 - 48485 | 1375 & 8410 |
| EU/mg | mechanically ventilated | stall air | 491 | 160 - 1287 | 278 & 832 |
| | | breathing zone air | 295 | 120 - 846 | 230 - 592 |
| | naturally ventilated | stall air | 800 | 539 - 1916 | 585 & 1656 |
| | | breathing zone air | 861 | 519 - 3756 | 585 - 2527 |
| total bacteria CFU/m3 | mechanically ventilated | stall air | 4815 | 2121 - 6222 | 2121 & 6222 |
| | naturally ventilated | stall air | 2887 | 344 - 10809 | 1323 & 5612 |
| gram-neg bacteria CFU/m3 | mechanically ventilated | stall air | not included | not included | not included |
| | naturally ventilated | stall air | 510 | 84 - 1111 | 130 & 743 |
| fungi CFU/m3 | mechanically ventilated | stall air | 769 | 671- 3960 | 671 & 3960 |
| | naturally ventilated | stall air | 33561 | 2830 - 35043 | 4126 & 34612 |

On premises 1, gram-negative sampling time was 30s rather than 5 min as Crystal Violet had not been available previously for the test run and its optimal sampling time had to be guessed. As a result, all Crystal Violet agar plates used on premises 1 were empty, and excluded from analysis. There were no fungi measurements on premises 4 due to equipment fault.

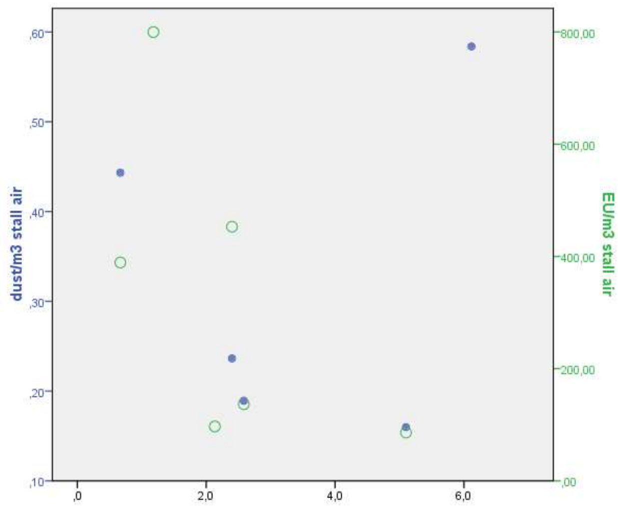


Figure 1: Difference between inside and outside temperature (C) and stall air dust and endotoxin levels
Dots represent dust samples, circles represent endotoxin samples

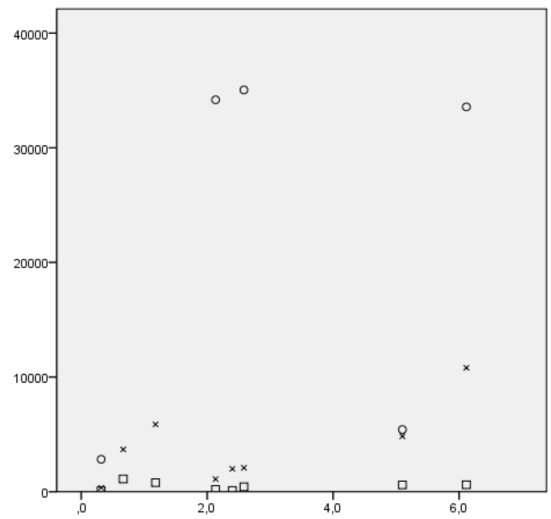


Figure 2: Difference between inside and outside temperature ( C) and total bacteria (crosses), gram-negative bacteria (squares) and fungi (circles)

There was no significant correlation between stall air levels and breathing zone air levels of either dust or endotoxin on any of the study locations. Overall, breathing zone air levels of both dust and endotoxin were significantly higher than stall air levels. There was a significant correlation between air dust and endotoxin content in both breathing zone and stall air with a spearman correlation of 0.884 ($p < 0.01$). On premises 1, where stall air dust and endotoxin concentrations were measured continuously for several days, evening and night endotoxin levels were significantly lower than daytime levels. Median evening and night dust levels were lower than daytime levels but this difference was non-significant. Endotoxin levels were nearly statistically significantly ($p = 0.056$) lower in the mechanically ventilated barns compared to the naturally ventilated barns. In the naturally ventilated barns air endotoxin levels were correlated to dust endotoxin content (EU/mg) with a correlation value of 0.771 and levels of airborne gram-negative bacteria were correlated to levels of airborne total bacteria (spearman correlation = 0.810).

Because the inside-outside temperature difference failed to deliver satisfactory correlations to any of the air hygiene parameters, other ventilation parameters were assessed for possible correlations. Of these, box smoke clearance time looked most promising, as can be seen in figure 3.

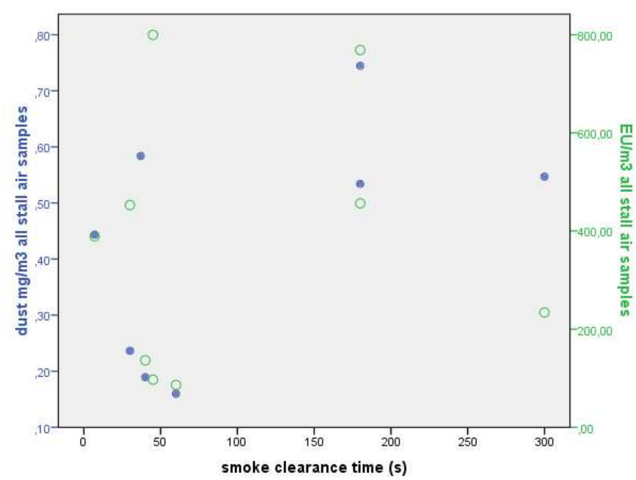


Figure 3: Smoke clearance time in seconds and its relation to stall air dust and endotoxin content. There is a slight, non-significant correlation between clearance time and dust levels (corr value = 0.549, p=0.79)
Dots represent dust samples, circles represent endotoxin samples

## Discussion

### *Ventilation*

With only 2 of the 8 studied naturally ventilated barns having a -slightly- higher than recommended difference between outside and inside temperature, it would appear that barns are generally adequately ventilated. It has been stated before that horses do not need a warm environment and that maintaining adequate ventilation is more important than maintaining a high temperature, as horses can comfortably sustain temperatures up to -10ºC if given reasonable time to adapt[21,29]. Perhaps the results of this study show that horse's caretakers have acknowledged this.

Only one barn had stall air velocities that would be considered a draft by either Clarke's or Wathes' arbitrary standards of 0.4 m/s and 0.3 m/s respectively[20,22], although it should be noted that these velocities were measured during windy outside conditions. All in all it seems horses in barns are rarely exposed to drafty conditions.

As for air humidity, no recommended value for horse stables is known. Clarke[21] doesn't think air humidity levels are likely to stress a horse, unless they are extremely high or low. However, the acceptable range of 30% to 90% posed by Wathes[22] is unlikely to be a realistic one. Recommended air humidity levels for porcine and avian housing range from 50-80%, and values below 50% have been shown to increase the incidence of respiratory problems[24].

Ammonia is unlikely to be as much an important factor in horse stables as it is in production animal housing. Horse stables typically have a much lower stocking density and much more air- and floorspace per animal. Horse stables may also be cleared of excrements more fervently, leading to less production of ammonia. A review of relevant literature showed no definitive maximum threshold level for ammonia. In human occupational health standards, ammonia has as a time weighted average (TWA) for an 8 hour working day of 25 ppm[30]. In 1983, a 'safe' limit of 20 ppm ammonia in horse stables was proposed[22]. The most recent notion is that ammonia concentrations in horse stables should not exceed 10ppm, partly because of welfare reasons[31]. Ammonia levels were highest on premises 1. It is unclear whether these higher levels are caused by the significantly slower air clearance, or whether they reflect the use of a different bedding material or different management practices. Concerning the bedding material, popular opinion states that use of woodshavings leads to higher ammonia levels, but a study in bedding materials for cattle actually showed that shavings emitted significantly less ammonia than straw[32]. Still, ammonia levels in none of the studied barns appear to be high enough to cause respiratory damage to their inhabitants.

### *Air hygiene*

The levels of dust and endotoxin measured in this study show some resemblance to those reported elsewhere, although levels in this study overall seem to be somewhat higher than those measured in other studies[3,6,8,14,15,31].

For comparison, an overview of dust and endotoxin levels found in previous reports is shown in Table 3:

| Article, type of environment | Endotoxin ng/m3 | Dust mg/m3 | Endotoxin content of dust (ng/mg) | Measuring area |
|---|---|---|---|---|
| [8] hay/straw challenge | 160 (87-581) | 2.83 (0.83-6.83) | 56 (31-164) | BZ |
| [6] hay/straw challenge* | 147 (36-427) | 1.3 (0.5-1.9) | 75 (30-150) | BZ |
| [15] conventional housing system | 19.76 (7.52-60.53) | 2.74 (2.19-5.48) | 7.57 (3.43-11.04) | BZ |
| [15] low dust housing system | 3.91 (2.12-17.41) | 0.80 (0.29-2.78) | 6.9 (3.07-12.40) | BZ |
| [3] straw bedding | 43.53 **EU**/m3** | | | stall |
| [3] sawdust bedding | 19.08 **EU**/m3** | | | stall |
| [14] conventional housing system*** | | 17 (10-35) | | BZ |
| | | 2.5 (2.5-2.6) | | stall |
| [14] low dust housing system*** | | <1 | | BZ & stall |
| [31] sawdust bedding | 24.9(0.1-153) | 0.47 (0.17-1.56) | | stall |

* Two challenges, geometric mean of the two medians used

** 10 EU equals 1 ng of endotoxin[33]

***Estimated off graphics. Mean ± sd, all others median and range

As can be seen in Table 3, measured dust and endotoxin levels can vary greatly, up to 10-fold, both within and between studies. Human occupational health studies in agricultural environments have shown endotoxin exposure levels of up to 6000 EU/m3 in animal environments. Dutch human occupational health standards for inhalable dust exposure are 10 mg/m3 dust, as an 8 hour TWA (MAC/OES, www.ser.nl). The median levels of dust measured in the present study are lower than this standard. However, horses typically spend up to 24 hours a day in their dusty environment instead of just 8. As for endotoxin, human occupational health standards allow for a maximum endotoxin concentration of 50 EU/m3, again as a TWA for an 8 hour working day. Human studies have shown that respiratory effects can be caused by chronic exposure to these relatively low levels[12]. The levels reported in the present study greatly exceed this human standard. However, it has been stated before that horses may not respond to the same levels of airborne endotoxins as do human subjects[15]. On the other hand, levels reported by Pirie[6,8] of 147-160 ng/m3 of breathing zone endotoxin exposure during hay/straw challenges were sufficient to induce symptoms in previously asymptomatic heaves horses. It is interesting to note that these levels reported by Pirie, in deliberately dusty stables, equal about 1470-1600 EU/m3[33], which is lower than the median levels of breathing zone endotoxin found in the present study. Unfortunately, endotoxin analysis is not well standardized yet, or in any case wasn't at the time when most of the aforementioned studies were performed, so the extent to which results from different studies can be compared to each other remains questionable.

The correlation between airborne dust levels and airborne endotoxin levels measured in the present study is greater than another reported correlation coefficient of 0.66 for measurements in an “Animal Production” environment. This may reflect the lesser number of study locations in the present study, as dust endotoxin content varies greatly between different measuring locations[12].

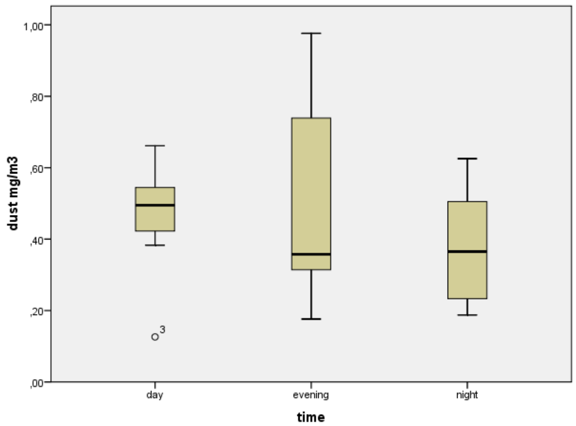


Figure 4: Stall air dust levels over 24 hours. Markers represent median, IQR and range.

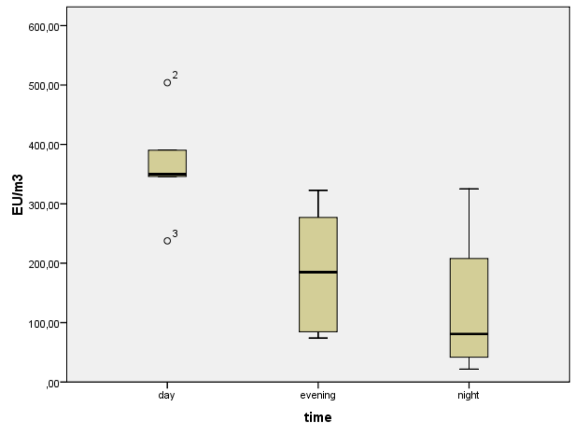


Figure 5: Stall air endotoxin levels over 24 hours. Markers represent median, IQR and range.

Stall air dust and endotoxin levels were significantly lower during the evening compared to daytime levels on premises 1 (figures 4 and 5), which is in agreement with similar findings by Woods[14]. Woods also measured the respirable fraction (particles of a size small enough to reach the lower airways) of total dust and found that the respirable dust fraction was not significantly lower during nighttime, most likely because these particles, because of their small size, are slower to gravitate out[21]. However, respirable dust fractions were not measured in the present study. Dust endotoxin content (EU/mg) did not differ significantly between day, evening or night in the present study.

Levels of airborne micro-organisms measured in the present study seem lower than those reported by others, albeit to different extends. For example, Seedorf[31] reports a median CFU/m3 of 19,992 (range 829-165,220) for total bacteria and 15,878 (range 2750-114,531) for fungi in stables with wood shavings. On the other hand, Tanner[3] reports values of CFU/m3 more on par with those found in the present study (1831±1054 for gram-negative bacteria and 5845±5113 for fungi in straw-bedded stables). Both these reports do show however, that measured levels of micro-organisms can vary widely, as they do in the present study. No data is available regarding levels of airborne micro-organism sufficient to induce respiratory symptoms. There were significantly lower levels of fungi in the mechanically ventilated barns, and although median dust levels in both breathing zone and stall air were higher, dust endotoxin content and airborne endotoxin levels were lower. Whether this is due to the significantly lower air humidity (providing less favorable conditions for fungal growth) or to the use of woodshavings instead of straw in the mechanically ventilated barns remains uncertain. It has been shown before that woodshavings can contain as much fungal spores and more dust than (good quality) straw[3,5].

No significant correlation between any of the ventilation parameters and any of the air hygiene parameters was found in this study. This is will be partially due to the relatively low number of samples and as well as the considerable variability in source materials. The latter caused a variability in contaminant release which may have been larger than anticipated. Ideally, source materials should be identical on all study locations so release of airborne contaminants is roughly the same anywhere. Unfortunately, the current study setting didn't allow for such a setup. On the other hand, as previously stated, most of the studied naturally ventilated barns were sufficiently ventilated by the standards in the present study design, so there may simply be not have been further influence of ventilation on airborne pollutant levels.

Overall, this study was limited by a low number of samples, a significant proportion of which was lost during storage or lab processing. This made it hard to find statistically significant correlations or differences. Another problem were plentiful apparent outliers. However, since this was a descriptive, rather than inferential study and the number of samples was already limited, no samples were excluded solely based on apparent extreme values.

Future studies with greater numbers of study sites and samples and more standardized measurement protocols may be more successful in finding significant results. Also, future studies should move from the descriptive stage and focus on more narrow aims such as the quantification of the relationship between smoke clearance time and dust and endotoxin levels. The use of real-time continuous particle monitors[34] may prove useful in the assessment of the influence of ventilation on peak levels of dust exposure. Ultimately, a comparison between different housing systems would be useful in showing show which housing system is most favorable towards equine respiratory health.

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