How to Do Air Quality Testing in the Equine Barn

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The way that we house and exercise our horses, especially in non-temperate climates such as New England, is well entrenched in the equine culture. It may conserve space to store hay overhead, it may be economically favorable and more comfortable to have the indoor arena attached to the main barn, and it may make us more comfortable to close up our barns in the winter, but this management style results in high organic dust and endotoxin exposure for our horses. This dust exposure results in exacerbation of signs in horses with recurrent airway obstruction (RAO) and is also strongly associated with inflammatory airway diseases (IADs). Organic dust exposure has been shown to result in long-term declines in respiratory function and increased prevalence of chronic lung diseases in humans—it is logical to expect that the same exposures will have similar effects on equine respiratory health. We believe that it is important to decrease this exposure to both horses and people in equine barns, and changing this exposure flies in the face of long-established equine culture. Hard numbers can make it easier for owners and trainers to accept hard facts, and it is possible for veterinarians to generate these hard numbers with air quality evaluation. Our ultimate aim is to improve the respiratory health and well being of all horses exposed to organic dust and, eventually, to develop strategies for decreasing organic dust exposure in equine management. Authors' address: Tufts University, Cummings School of Veterinary Medicine, 200 Westboro Road, North Grafton, MA 01536; e-mail: melissa.mazan@tufts.edu (Mazan). © 2006 AAEP.

1. Introduction

Horses not only provide us with a source of great pleasure, but horse sports and recreation contribute an estimated 140 billion dollars to the gross domestic product (GDP) of this country each year. Despite the intrinsic value of these animals to our economy, equine stables throughout the country expose the horse to a continuous barrage of airborne organic dust. In consequence, non-infectious inflammatory airway diseases (IADs) and recurrent airway obstruction (RAO) plague the stabled horse in this country, affecting an estimated 25–80%. Recurrent airway obstruction is clearly associated with exposure to organic dust, and the condition of most horses improves when they are removed from the dusty environment. Holcombe et al. have shown that stabling causes pulmonary neutrophilia in young horses, and a close inspection of studies in RAO horse shows that not only the affected horses, but also the controls develop pulmonary neutrophilia; highlighting the potential for widespread airway inflammation in the equine population. Barn dust is rich in dust from shavings, sawdust, manure, hay, animal hair and dander, and silica from dirt in indoor arenas. Indoor arenas may also be high in biologic material depending on the footing used. Respirable dust particles (<4 um), which predictably reach the lower airways, can range from 0.79 mg/m³ in “low dust” management to 8.8 mg/m³ in conventional stabling. Moreover, barn dust is also rich in endotoxin, which we know causes pulmonary neutrophilia in both RAO and...
control horses\textsuperscript{4,14} and which has been shown to be a strong risk factor for IAD in young horses.\textsuperscript{15} Respiratory exposure to organic dust has been shown to result in decreased growth in swine,\textsuperscript{16} and may consequently be very important for breeders raising young stock. Finally, exposure to horse barns has effects on human health as well—recent work from our laboratory shows that 55\% of people routinely exposed to horse barns report symptoms of lower airway disease compared with 15\% of age-matched controls (\(P<0.01\)).\textsuperscript{17}

How important is airway inflammation? In humans, airway inflammation is linked to lung dysfunction. Exposure to high levels of particulates has important functional consequences for people working in so-called dusty trades, such as the cotton industry or carpentry.\textsuperscript{18} People exposed to dust from swine barns develop airway inflammation and airway hyperreactivity, and long-term exposure results in poorer lung function than age-matched controls.\textsuperscript{19} This is with quite low dust exposures (0.16 mg/m\textsuperscript{3} of respirable dust).\textsuperscript{20} Most importantly, longitudinal, accelerated declines in lung function are documented in exposures to swine dust and other organic dust.\textsuperscript{18,21–24} This implies that early exposures to organic dusts can predict later dysfunction, regardless of initial presence of clinical disease.

It can be difficult to persuade owners that the everyday experience of a horse in even the best-kept of “traditional” barns can be injurious to the horse’s lungs. It can be even more difficult and unwelcome to a horse owner, trainer, or groom to realize that exposure to a barn can be dangerous for human respiratory health. We therefore extended our environmental testing from the research arena to clinical testing. We found that hard numbers can be convincing when mere words fail. The purpose of this paper is to explain the steps that can be followed to provide an environmental assessment for horse owners and others in the equine environment.

2. A Short Course on Air Sampling
Air sampling is, literally, taking a sample of air to test. This can be done gravimetrically or with a laser photometer. When we sample air gravimetrically, we draw air through a pre-weighed filter at a known rate, for a known period of time.

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\text{Flow rate} \times \text{time} = \text{volume of air (expressed as cubic meters)}
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\[
\text{Filter post - filter pre} = \text{mg of particulate}
\]

\[
\text{Mg of particulate/volume of air} = \text{mg/m}^3
\]

After that period of time, we weigh the filter and determine how many milligrams of particulate was in a certain amount of air; this is expressed as milligrams of sample per cubic meter of air. There are many devices with which the filters can be used, including button samplers, cyclones, and cascade impactors. Which type of sampling device you use is dependent on the size of the particulate you are interested in. Volatiles in the air (such as ammonia) are sampled either with rather expensive dedicated optical or electrochemical instruments or, more simply, by sorbent sampling. Air is drawn through sorbents that are contained within a glass tube. Sorbents are materials that react with specific volatiles, often by colorimetric changes—they are simple to use, although they are not as precise as other gas measuring devices.

Air in the typical horse barn includes particulates, endotoxin, and ammonia; thus, these are the specifics for which you are testing. Particulates are, literally, particles that are found in the air. Particles outside the barn generally come from the earth crust—think of a rundown paddock on a windy day—which is primarily silica, and from combustion—especially diesel engines and factories. Both local and remote production of particulates are very important with such air pollution, because the winds can bring particulates thousands of miles. Particulates within the barn arise from hay and straw molds and fungi, sawdust and shavings, dust from indoor arenas, and manure and animal dander and hair. Ammonia levels can be high in enclosed barns.

Particulates can be split into those that are inhalable, those that are thoracic, and those that are respirable. They are designated as such based on their size, because the size of particles determines whether they can reach and interact with the airways and parenchyma of the lung. As the name implies, inhalable particles can be inhaled and encompass all particles whose aerodynamic diameter is \(\leq 100 \mu \text{m}\) (abbreviated as PM100). Many of these particles are large and will be caught in the nose and upper airways. These particles have been largely ignored but have gained attention in the past several years, because they are responsible for inflammation of the nose and throat in humans. When PM100 is measured, it is often referred to as total dust. Thoracic dust has a smaller aerodynamic diameter and, as its name implies, is dust small enough to reach the thorax. The aerodynamic diameter of thoracic dust is \(\leq 30 \mu \text{m}\). Respirable particles are variably considered to have an aerodynamic diameter of \(\leq 4 \mu \text{m}\) and are small enough to reach the smallest airways. Particles \(<2.5 \mu \text{m}\) are able to reach the smallest alveoli. Respirable dust is thus thought to be the most important for lung damage. Ultrafine particles, \(<1 \mu \text{m}\) in aerodynamic diameter, are less commonly measured, but are thought to be responsible for much of the morbidity and increased mortality associated with air pollution. These particles can be carried throughout the body and have recently been shown to be associated with cardiovascular disease in humans and rodents.

It isn’t necessary to measure everything—a reasonable approach is to measure respirable dust—the particulate thought to be most responsible for airway and parenchymal disease. However, it makes
sense to base your testing on the problems that you detect in a particular barn. If the animals have a problem with upper airway inflammation, you probably want to measure total inhalable dust. If they have disease primarily affecting the small airways, you will want to measure respirable dust. This is where it is important to understand the distinctions between the various measuring devices. For total dust, a sampler that is essentially a holder for a filter is needed—you are really not trying to discriminate among sizes. For respirable dust, you are usually going to be using some type of cyclone or cascade impactor. A cyclone is a small device that literally causes air going in it to swirl in a pattern such that heavier (larger) particles drop into a grit pot and are discarded, whereas lighter (smaller) particles are deposited on the filter. These are relatively inexpensive and easy to use but are (as with any filter device that discriminates size) exquisitely sensitive to flow rate. It is absolutely essential that the pumps used with these be calibrated before each test. Each of these devices uses a filter that is held in a polystyrene cassette. The cassette alone with a filter can be used if all you want to know is what the total dust load is.

Finally, laser photometers are light scatter devices that can tell you in real time what type of particulate load you are experiencing. These come in more and less sophisticated versions, many of which can give readouts for PM10, PM5, and PM2.5 that can be downloaded onto a computer. We use the TSI Sidepak, a small device that can clip onto the belt. The only down side of this type of air sampling is that you cannot determine what is in the particulate—what amount of endotoxin, silica, or heavy metals.

Equine barns are high in endotoxins—it comes from manure, of course, but is also high in grain dust and hay. Grain dust is so high in endotoxins that it is associated with occupational asthma in humans. Stagnant water or manure-contaminated pools of water are also good sources of endotoxins. Finally, indoor arenas, depending on their footing, can be an important source of particulates and endotoxin particulates. In humans, long-term exposures to relatively low levels of endotoxin (10–28 EU, or endotoxin units/m³) have been linked to chronic decreases in lung function.

Fungal molds and yeasts are yet another type of specific particle, and there is considerable evidence that fungal spores are implicated in RAO (heaves), although we don’t know if this is an allergen-mediated phenomenon or if fungi are simply acting as particulates.

3. Materials and Methods

Measuring Total Dust Concentrations to Which Horses Are Exposed

Determine Which Dust Fraction to Measure

If horses are primarily experiencing symptoms of lower respiratory inflammation, you will want to measure the respirable fraction. This is easily done using a cyclone, which, using a 2.75 l/min flow...
rate, will capture particles in the range of 4 µm and lower. If horses have upper and lower respiratory inflammation, you will wish to measure inhalable dust using a button type assembly.

**Calibrate Your Pump**

This can be done with a flowmeter, or, if you choose to rent equipment, will be done by the company you work with.

**Affix the Equipment to the Horse**

In addition to the sampling equipment you will rent or buy, you will need a good surcingle—the leather kind with D rings are best. The pump is affixed to the surcingle using duct tape, and a long piece of Tygon tubing (ask the particular company/laboratory which diameter you will need) will extend from the pump to the air sampler. We generally place braids in the horse's mane and run the tubing through the mane to keep it from becoming dislodged. The tubing attaches to the sampler, which is affixed to the horse's halter—again, attach it firmly with duct tape. It may help to pad the monitor with cotton. It is also important to make sure that someone will be in the barn throughout the day to make sure that the equipment stays attached. It would be ideal, but difficult, to monitor for a 24-h period. We try to monitor for 6–8 h; if you monitor for a shorter time, you may not have accumulated enough dust to have a measurable result. If you have access to a laser photometer, it is very useful to take “spot” readings at different times throughout the day—during stall cleaning, feeding, aisle sweeping, etc.—to find out what is causing the most exposure. Remember to record the time that you start and the time that you finish and to make sure that the flow stays constant, so that you will be able to determine a mass concentration for exposure.

**Measure the Weight of the Filters**

To do this accurately, you must be able to weigh the filters with great accuracy and precision; thus, the filters are almost always sent out to a qualified laboratory for analysis. Even the analytical scales used in a molecular biology laboratory are not sufficient for this measurement. Moreover, the laboratory must have a dry room, where the filters are pre-weighed and where filters equilibrate after sampling in to make sure that you are not just measuring water weight. An alternative to preweighing each filter is to include at least one blank for every five sampled filters.

4. **Measuring Mold**

If you suspect that horses are being subjected to a mold load, you will want to test specifically for molds and fungi. We usually choose to test for total fungal spores using a spore trap. The spore trap, as the name implies, draws air through a cassette and allows spores and other particulate material to be trapped. As with other air sampling devices, to have a quantitative analysis of the exposure to fungal spores, it is critical to calibrate the pump and to have the ability to regulate the flow precisely. In this way, the number of spores and other particulates per cubic meter of air can be determined. The trapped material can be placed directly onto the microscope for analysis and enumeration. Spore traps such as the Air-O-Celle have in-built reticules so that it is easy to count the particles. It is important to remember that spore traps tend to need very high air flows (15 l/min) as opposed to the low airflows needed for personal air monitors (usually in the 1.8- to 2.7-l/min range), so it will be necessary to rent more than one pump. We perform fungal spore analysis inside several representative stalls: for instance, the stall closest to the doors, a stall in the middle of the barn, a stall near the indoor arena, a stall near the hay drop, etc. We also do at least one outdoor measurement, and, if horses have signs associated with outdoor exposures, we perform analyses in multiple different areas of the farm. Fungal spore testing is much less time consuming, because most spore traps require only a 5-min exposure. It is important to send at least one field blank (a cassette that has traveled with you but has not been exposed to the air) with every five samples that are taken.

5. **Measuring Endotoxin**

It is hard to imagine a stable that is not high in endotoxin; however, it is still important to establish baseline levels so that the results of environmental interventions can be assessed. To begin with, filters should be endotoxin-free before sampling, and PVC and polypropylene (two common filter materials) should not be used because they bind endotoxin, resulting in falsely lowered levels. 'Teflon can be used, but the filter of choice is a 0.2-µm polycarbonate, with glass-fiber backing pads (these pads are necessary, because the filters are very flimsy). You must be careful not to touch the filters or even to breathe on them, because you don’t want to contaminate your sample. For endotoxin sampling, a device such as a cyclone or an impactor is not necessary—you may simply attach the tubing from the pump to the cassette containing the filter, because you will usually wish to capture all airborne endotoxin. Once the sample has been collected, it should be handled and packed using gloves and sent to the laboratory.

6. **Measuring Volatiles**

We use the Drager sorbent tubes for ammonia; each test takes <1 min, thus multiple samples can easily be taken. As with fungi, we like to test multiple representative stalls as well as the aisles of the barn. You will be sent thin glass tubes that have a material that adsorbs the gas that is drawn through them, generally with a small manual bellows-type pump. If ammonia is present, the material inside the tube will change color, and the approximate
ammonia level can be read from lines marked on the outside of the glass.

7. How to Obtain Equipment
It makes much more sense to rent this equipment than to buy it, because it is difficult to justify outright purchase economically. There are multiple companies that will rent equipment that is pre-calibrated and will analyze the samples for you—several of these companies are listed below. These companies tend to be an excellent resource for questions as well. What they aren’t as familiar with is how to use personal air monitors on horses.

Environmental Testing Laboratories
TestAmerica Analytical
Phone: 800-344-5759
info@testamericainc.com

EMSL Analytical, Inc
Suite 107, 7 Constitution Way
Woburn, MA 01801
Phone: 781-933-8411
http://www.emsl.com

Emlab
1150 Bayhill Drive, Suite 100
San Bruno CA 94066
Phone: 650-829-5800
Fax: 650-829-5852

Columbia Analytical Services, Air Quality Laboratory
Suite D
2665 Park Center Drive
Simi Valley, CA 93065-8200
Phone: 805-526-7161
Fax: 805-526-7270
Contact: Kate Aguiler
Email: kaguilerasimi.caslab.com
Website: www.CASAirLab.com

Travelers Property Casualty Industrial Hygiene Laboratory
90 Lamberton Rd.
Windsor, CT 06095
Phone: 800-842-0355
Fax: 860-687-7430
Contact: Marcel Baril
Email: www.travelerslab.com

8. Results From Our Laboratory
Particulate Exposure—Gravimetric Measurements
We measured exposure to respirable dust (<0.4 um) using gravimetric analysis in five lifelong stabled and three lifelong pastured horses for a 4-h period during quiet stall or paddock rest. Stabled horses were housed on sawdust and fed good-quality hay; windows and main doors were open. Pastured horses lived in a dirt paddock with access to a three-sided shelter and were fed good-quality hay. Stabled horses had considerably higher dust exposure [0.921 ± 0.148 versus 0.017 ± 0.011 (SD) mg/m³].

Particulate Exposure—Fungal Spores
We performed volumetric analysis of fungal spores within the barn and outdoor environments five times in the summer of 2005 using Air-O-Cell® cassette technology. Stabled horses were exposed to a markedly higher fungal spore load than pastured horses (27,900 ± 12,366 versus 939 ± 601) and >90% of these fungal spores are in the respirable range (1–5 µm).

9. Measurements During Exercise
Horses are exposed to greater volumes of particulates during exercise because of the vastly greater ventilation rate (60 l/min during rest, 1500 l/min during breezing or racing). Therefore the quantity and nature of particulate exposure during exercise might be relevant to disease outcome, such as inflammatory airway disease. We hypothesized that the training position of horses on a racetrack would effect particulate exposure, i.e., the lead horse in a pack would have the least, and the followers progressively greater particulate exposure. Thoroughbred racehorses (n = 6) were fitted with personal air samplers (flow rate, 2.9 l/min), and particulates (i.e., total dust) collected on filters (Environmental Profiles, Baltimore, MD) were enumerated using standard light microscopy followed by detailed qualitative differentiation of particle types using polarized light microscopy, transmission electron microscopy, and energy dispersive X-ray microanalysis (EMSL Analytic, Beltsville, MD). Four horses at a time were set about jogging in a line (positions 1–4) on a dirt warm-up track (1 mi) followed by a breeze on a grass track (6 furlongs), which is standard for the farm (Tapeto Farms, Northeast, MD). Sampling was started when the horses reached the warm-up track. In addition, two horses were run alone on the tracks (position 0) to enhance our collection of “undisturbed” exposure data. Samples were also taken on several locations along the length of the tracks immediately before horses working on the track. The following particulates were quantified in parts per meter cubed (ppcm): total mold, total pollen, cellulose, quartz, clay (aluminum silicate), silica, hair, and skin fragments. Individual and combined particle exposure for individual horses, as well as area data, was plotted as a function of training position: 0 for lead, first, second, third, fourth (i.e., last).

There was a significant (p < 0.05) effect of training position on exposures to mold/fungi (Spearman’s two-tailed correlation coefficient r = 0.733), quartz (0.681), clay (0.661), silica (0.686), skin (0.618), and total particulates (0.748), but no correlation with pollen, fiberglass, natural cellulose, or hair.
10. Costs

Depending on the extent of testing done, costs may run from $500 to $5000.

References and Footnotes


aSKC cyclone, SKC Inc., Eighty Four, PA 15330
bTwo-piece 37-mm polystyrene cassette, SKC Inc., Eighty Four, PA 15330.
cSidepak, TSI Inc., Shoreview, MN 55126.
dDC-Lite, SKC Inc., Eighty Four, PA 15330.
eAir-O-Cell, SKC Inc., Eighty Four, PA 15330.
fPolycarbonate filter, SKC Inc., Eighty Four, PA 15330.
gDrager accuro measurement system, SKC Inc., Eighty Four, PA 15330.