




Article

Bacterial Burden in the Air of Indoor Riding Arenas

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Abstract: Airway diseases in horses are often multifactorial and have a strong environmental background because diseased horses react to inhaled agents. In this study, the air quality of closed riding arenas was analyzed monthly in four riding arenas over the course of one year with special emphasis on bacteriology. A standardized riding program with one horse was used to measure exposures to airborne bacteria. Air samples were taken from the heights of the riders' and the horses' breathing zone (2.5 m and 1.5 m, respectively) at four sampling points before and after the riding program. The bacterial loads in all four arenas significantly increased after the riding program. However, the results showed no differences between the breathing zones of the riders (2.5 m height) and those of the horses (1.5 m height). Gram-positive bacteria and especially *Staphylococcus* spp. occurred as the predominant aerobic mesophilic bacteria; 80% of the identified Staphylococci were *Staphylococcus xylosus*. The cultured samples from the ground of the arenas indicated that the ground was probably the main source of airborne *Staphylococcus* spp. during riding. The impact of an additional bacterial burden in riding halls on the health of riders and horses remains unknown; however, the air quality of riding arenas should be of special interest in future studies in terms of the high air consumption of horses during training periods.

Keywords: animal health; bacteria; bioaerosols; horse training; riding arena; respiratory diseases



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1. Introduction

Horses' health and well-being is influenced by environmental living and training conditions such as microclimatic conditions, temperature, air humidity, and hygiene [1]. Their requirements in terms of air quality are higher than those of other livestock animals [2]. Horses have large airways and lungs, providing a surface of about 1650 m² that allows them to be superb athletes [3,4]. During a 90 min training period, a horse consumes about 45,300 L of air, whereas about 81,000 L of air are inhaled over the rest of the day [5]. Thus, the air quality of riding arenas is of special interest [6].

Recurrent airway obstruction (RAO) or inflammatory airway disease (IAD) are major disease problems in horses and are correlated with bad air quality [7]. In northern countries with cool climates, the incidence of severe equine asthma (RAO) is estimated to be between 14 and 20% [8–10] and that of the mild to moderate form (IAD) reaches 68 to 80% [11–14]. In particular, IAD is considered to be underdiagnosed because it leads to performance intolerance, but is otherwise clinically subtle; cough only occurs in 38% of all cases [15–17]. In addition to genetic predisposition [11,18], exposure to dust, including a high number of pro-inflammatory components [19,20], plays a pivotal role in disease induction. Wood et al. showed that inhaled airborne particles resulted in an increased amount of tracheal mucus in thoroughbred horses [21]. Furthermore, the accumulation of mucus in the airways as

well as mild pulmonary inflammation, which causes impaired gas exchange, leads to poor performance in high-performing horses [22,23].

Fungi and bacteria and their cell wall structures (e.g., endotoxins and 1,3- β -glucans) are of major importance [16] and are under discussion as causative or promoting agents if inhaled [24–26]. They are inhaled etiological agents of airway obstructions, in addition to toxic gases and airborne antigens. The airborne transmission of infectious viruses may also play a role [27]. Bacteria in general are often associated with airway infections as secondary pathogens. The species that are isolated from the airways of diseased horses are *Streptococcus equi* subspecies *zooepidemicus*, *Actinobacillus/Pasteurella* species, and *Streptococcus pneumoniae* [21,28]. Non-hemolytic Streptococci and coagulase-negative Staphylococci are not associated with disease [29]. Bacteria in the air are often present as bioaerosols attached to dust and other particles [30,31] (Van Leuken et al. 2016, Clauss 2015). To be inhaled deeply by horses, the diameter of these particles has to be between 0.3 and 5.0 μm [32]. This was most common in indoor riding arenas with sandy ground [33].

The air quality in horse stables has been recently examined in several studies, with an emphasis on dust [34–37]. For instance, the effects of different bedding materials [38] or different feed types and feed-processing techniques [39] on the airborne particle concentration in the air of horse stables have been investigated [40,41]. Only a few studies on the air quality of closed riding arenas are available. Wheeler et al. collected data on the total and respirable dust in two indoor riding arenas in central Pennsylvania [42]. Overall, they observed higher levels of dust exposure during an exercise session where the horses were ridden faster in the examined indoor arenas. Vanable et al. analyzed the effect of recycled crumb rubber when applied on top of the existing riding arena sand. They observed reduced particulate matter in the air during an indoor riding class [43]. A study in an Irish equestrian center by Bulfin et al. characterized respirable dust and respirable crystalline silica exposures among equestrian workers [44]. Furthermore, air quality and dust particle concentrations in indoor riding arenas before and after use for riding were investigated in our previous research [33]. However, only a few studies analyzed dust samples from the horses' surroundings for bacteria: those results found that Gram-positive cocci (Micrococci, Staphylococci, and Streptococci) are the most common bacteria species groups in the horses' indoor environments [45,46]. However, these studies examined sedimented and brushed dust samples from stables and not bacteria directly sampled from the air of riding arenas.

For both the horses and their owners, ambient air is presumed to be critical for respiratory health. Thus, the ventilation of stables aims to approach ambient-air quality [37]. The air quality is worse in livestock housing than in human housing in terms of bacteria load [1]. The airborne microbial contamination depends on multiple factors, e.g., animal density or the presence of feces; thus, the bacterial burden on horses is obviously lower than on other livestock species [38]. However, in the air of stables as well as of indoor riding arenas, not only the horses, but also riders and riding instructors, are exposed to potential bacterial burden. Hitherto, work-related respiratory diseases related to air pollution in intensive livestock production facilities have been described in cattle [47], poultry [48], and especially pig farming [49].

There is a lack of knowledge of the bacterial burden in the air of riding arenas even though they might be of special importance for horses' and riders' health due to the intensive air consumption during training. Thus, this study aimed to identify the first results of additional bacteria exposure to horses as well as to horse riders.

2. Materials and Methods

2.1. Riding Arenas and Riding Programs

This study was conducted in four indoor riding arenas located in Saxony-Anhalt, Germany. These riding arenas were comparable in size, but they structurally differed in the footing material, the age of the ground, the direct proximity to the stable, and the position of doors and stands (Table 1). In Arena 2, surfaces were watered from April to October but not in months with lower temperatures (November to March).

Table 1. Characteristics of the four sampled riding arenas.

	Arena 1	Arena 2	Arena 3	Arena 4
Riding horses in stable	32	24	50	56
Size (m ²)	20 × 40	20 × 40	20 × 40	25 × 50
Direct proximity to stable ¹	Yes	No	No	Yes
Footing material	Sand	Sand/Wood shavings	Sand	Sand
Age of footing material	4 years	2 years	0.5 years	2 years
Underground	Natural ground	Natural ground	Natural ground	Natural ground

¹ Horse stable and arena are under one roof, divided by a half wall 4.0 m high, shared vent (approximately 0.5 m).

The riding program involved 1 horse and included a 12 min walk, a 5 min trot, and a 3 min gallop. It was created based on standardized riding figures to reach comparable usage in the four different arenas.

2.2. Sampling Methods

Samples of the air and footing material were taken monthly from September 2012 to August 2013. On sampling dates, the arenas were not used for riding for 10 h before the first sampling in the morning. The second sampling was performed directly after the described standardized riding program. To reduce air movement in the arena, all of the doors, windows, and gates were closed during monitoring.

Samples for bacteriological analyses ($n = 734$) were recorded at four measurement points in the middle of the short and the long sides of each arena before and after the riding program was performed. Airborne bacteria were collected with an impactor (airborne microorganisms sampler MAS–100 Eco MBV, Vevey, Switzerland) at a flow rate of 1 L min^{−1} at two different measuring heights. These characterized the expected height of the horses (1.5 m above ground) and that of the riders' noses (2.5 m above ground). One liter was sampled in total to avoid overgrown impaction media. For bacteriological analyses, airborne bacteria were impacted onto sheep blood agar plates (Oxoid, Wesel, Germany).

The agar plates were cooled to 4 °C during transport (<1 h). Aerobes were incubated for 24 h at 37 °C in the laboratory. The results for total culturable bacteria were expressed in colony forming units (CFU) per liter of air. The colonies grown were further identified via Gram staining, catalase and oxidase testing, and biochemical identification. For air samples showing CFU per liter values below a detection limit, the value was set to 0.5 CFU/L to keep these recordings and allow log10-transformation [50].

Ground samples from the footing material ($n = 376$) were taken in sterile tubes on each measuring point before the first and after the last impactor measurements of bacteriological analyses. Samples were transported to the laboratory within 1 h, and 1 g of ground was dissolved in 100 mL of 20 °C warm water. A total of 50 µL of this solution was streaked out on the sheep blood agar plates, incubated for 24 h at 37 °C, and the CFU were counted. Selected colonies were further identified via Gram staining, catalase and oxidase testing, and by biochemical identification as described in the next section.

2.3. Identification of Bacteria

After the first incubation, we picked and further differentiated 900 colonies (312 from Arena 1, 170 from Arena 2, 240 from Arena 3, and 178 from Arena 4) from the agar plates of air samples and 218 colonies (93 from Arena 1, 48 from Arena 2, 46 from Arena 3, and 31 from Arena 4) from the agar plates of ground samples. Colonies were selected by different morphology (color, shape, and size) from plates that were not contaminated by fungi. Between two and seven colonies were selected from uncontaminated plates for further differentiation. Almost all isolated colonies (93.3%) were Gram-positive, catalase-positive, and oxidase-negative. Subsequently, species were, if applicable, identified by biochemical differentiation via an API system (API strip ID 32 STAPH, bioMérieux, Nürtingen, Germany).

2.4. Statistical Analyses

Data were processed with Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and statistically analyzed with SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). First, to evaluate the colony forming units (CFU/L), a logarithmic transformation was performed with conversion to \log_{10} -values (CFU/m³). Analyses on a descriptive basis were carried out using the PROC MEANS procedure. The time point of measurement and the different heights of the Wilcoxon rank sum test (PROC NPAR1WAY) were used to compare the bacterial loads between the arenas. All results of the statistical tests were considered significant at $p < 0.05$.

3. Results

3.1. Total Number of Culturable Aerobic Bacteria in Air Samples

There were 1335 air samples that could be evaluated. Others could not be evaluated due to technical problems at two measurement sessions in Arena 4 (September and October), mismeasurements, as well as values that were overgrown after incubation and hence were not countable colonies (Arena 1: $n = 109$; Arena 2: 0; Arena 3: $n = 6$; Arena 4: $n = 33$). For these samples, the number of aerobic growing bacteria was determined based on 1 L of impacted air (CFU/L) multiplied by 1000 L to obtain the more common unit (m³) for air constituents. Figure 1 shows the logarithmic colony forming units per liter of sampled air given in \log (CFU/m³) of the four arenas at two different measurement heights. There were no statistically significant differences in the CFU values between the two measurement heights in the four indoor riding arenas.

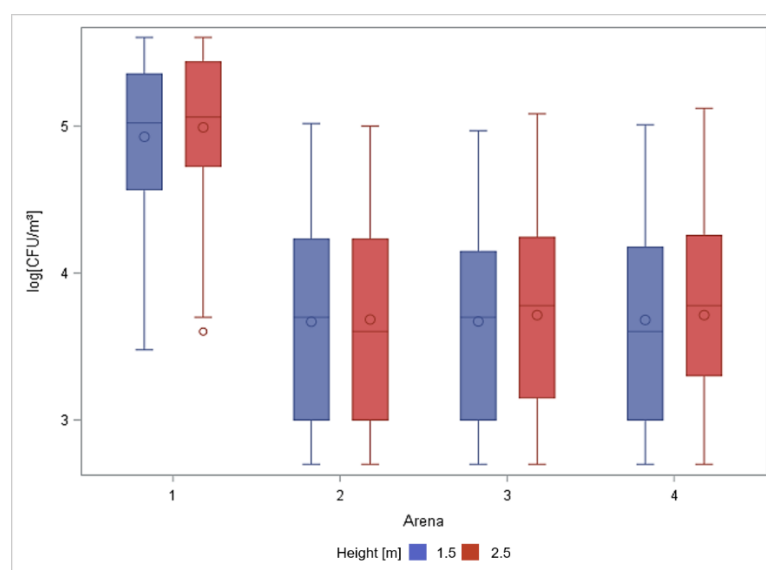


Figure 1. Logarithmic colony forming units per liter of sampled air given in \log (CFU/m³) of the four arenas at two different measurement heights (1.5 m and 2.5 m).

Further descriptive statistics for CFU per meter cubed in the four arenas are shown in Supplementary Material Table S1.

The four analyzed riding arenas differed in the amount of \log (CFU/m³), with Arenas 2, 3, and 4 significantly varying from Arena 1 ($p < 0.001$). Arena 2 had the lowest average amounts of \log (CFU/m³), and Arena 1 had the highest amounts of \log (CFU/m³).

Differences within and between the arenas are represented in Figure 2. Over all four arenas, the amount of \log (CFU/m³) was significantly higher ($p < 0.0001$) after the riding program than before. Arena 2 showed the lowest number of CFU before the riding program. The levels of CFU after the riding program in Arenas 2, 3, and 4 were lower than the levels in Arena 1 before the program. Further results on the comparisons between the individual riding arenas at different points in time are listed in Supplementary Material Table S2.

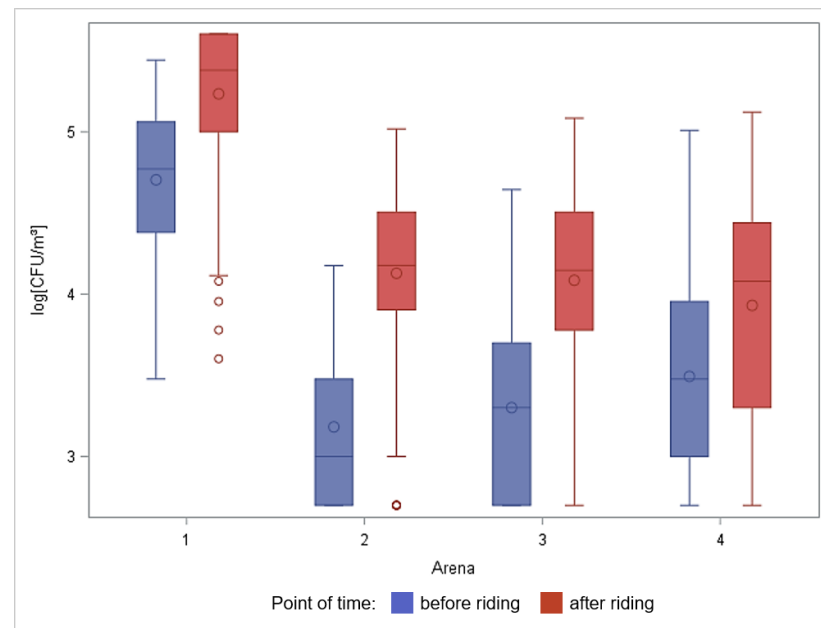


Figure 2. Logarithmic colony forming units per liter of sampled air log (CFU/m³) of the four arenas at two different points in time (before the riding program and after the riding program).

Figure 3 presents the curves of the air sample measurements (log (CFU/m³)) of the four arenas at two different heights before and after the riding program on a monthly basis. No data could be assessed in Arena 4 in September and October. Over all months, no significant differences were detected between the measurement heights in all four arenas.



Figure 3. The results of air samples measurements (log (CFU/m³)) before (b) and after (a) riding at different heights (1.5 m vs. 2.5 m) per arena over the months studied. In Arena 2, the watering time is also highlighted in blue.

3.2. Total Number of Culturable Aerobic Bacteria in Ground Samples

There were 210 ground samples evaluated. In these samples, the number of aerobic growing bacteria was determined based on 1 g of ground (CFU/g). Descriptive statistics for log (CFU/g) in the four arenas are shown in Table 2. Comparing the mean values of colony forming units per g sampled ground using the Wilcoxon rank sum test, there was a statistical difference between Arena 1 and Arenas 2–4 ($p < 0.0001$).

Table 2. Logarithmic colony forming units per gram of sampled ground (CFU/g) of the four arenas with mean; standard deviation (Std); minimum (Min); and maximum (Max).

Arena	No.	Mean	Std	Min	Max
1	49	5.44 ^a	0.25	4.70	5.90
2	56	5.19 ^b	0.32	4.62	5.90
3	56	4.99 ^b	0.33	4.08	5.58
4	49	4.91 ^b	0.35	4.15	5.72

^{a,b} different superscripts indicate a significant difference.

3.3. Bacteria Species in Air and Ground Samples

Bacteria species were identified from both the air samples and the ground samples. Thirty different species were identified and are listed in Table 3. *Staphylococcus xylosus* was the predominant species in both air and ground samples, followed by *Staphylococcus capitis* and *Staphylococcus equorum*.

Table 3. Occurrence of identified bacteria species in air (n total colonies = 900) and ground (n total colonies = 218) samples from four indoor riding arenas along with numbers and percentages (in parentheses).

Bacteria Species	Air Samples	Ground Samples
<i>Staphylococcus xylosus</i>	528 (59%)	154 (71%)
<i>Staphylococcus capitis</i>	65 (7%)	8 (4%)
<i>Staphylococcus equorum</i>	52 (6%)	12 (5%)
Others ¹	255 (28%)	44 (20%)

Others¹: *Dermococcus nishinomiyaensis*, *Kocuria kristinae*, *Kocuria rosae*, *Kocuria varians*, *Kocuria rosea*, *Micrococcus luteus*, *Micrococcus lylae*, *Staphylococcus arlettae*, *Staphylococcus aureus*, *Staphylococcus cohnii* subspecies *choinii*, *Staphylococcus chromogenes*, *Staphylococcus epidermidis*, *Staphylococcus gallinarum*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus hyicus*, *Staphylococcus intermedius*, *Staphylococcus kloosii*, *Staphylococcus lentus*, *Staphylococcus sciuri*, *Staphylococcus saprophyticus*, *Staphylococcus schleiferi*, *Staphylococcus simulans*, *Staphylococcus warneri*, and *Viridans streptococci*.

4. Discussion

This study gives the first insights into the bacterial content of the air in riding arenas as well as the impact of riding on its bacterial burden. Air samples were taken before as well as after a standardized riding program with one horse. Measurements were taken at two different heights: the nose of the horse and that of the rider. In Germany, the four investigated riding arenas were representative concerning their size, footing material (sand and wood chip surfaces), watering, and other maintenance. Nevertheless, when discussing the results, we must consider that the bacterial burden would certainly be higher if more horses had been ridden indoors and if the training sessions had been longer.

4.1. Aerobe Bacteria in Air Samples

When we analyzed the aerobe bacteria in the air samples of riding arenas, Arena 1 had the highest CFU (4.96 log (CFU/m³)). Arena 1 had a direct connection to the adjacent stables and had a shared vent. In contrast, Arenas 2, 3, and 4 had lower CFU values (3.68 to 3.79 log (CFU/m³)). A previous study investigating the ventilation and air hygiene in horse stables found similar values from 2.8 to 4.8 log (CFU/m³) [36]. The measured CFU amounts from the stables differ from those in the riding arenas as the constant walking, trotting,

and cantering of heavy horses on the riding surface causes resuspensions of particulate matter and exposure to airborne pollutants [51]; thus, there are higher values. However, the average amounts of bacteria before the riding program were comparable to those showed by Elfman et al. (2011): 3.2 log (CFU/m³) (Arena 2) and 4.7 log (CFU/m³) (Arena 1). They did not consider the possible influence of bedding, footing material, or the number of animals. Furthermore, several factors that can affect the tenacity and concentrations of airborne microorganisms, such as dilution, sedimentation, temperature, humidity, etc., may have had impacts on the results [52].

Arena 4 was also in the same building as the horse stables (but separated by a door and with no shared vent). Here, higher values before riding were recorded (3.5 log (CFU/m³)). These differences of the amount of CFU between Arenas 1 and 4 and the other two arenas could be explained by the results of Rapp et al. [5], who showed that the amount of air-carried pollutants is higher in openly connected arenas and stables than in disconnected ones. Furthermore, it is conceivable that the age of the footing material in Arena 1 impacted the increased values of air measurements because it was at least twice as old as the others and therefore probably also had more particles that were small enough to be lofted.

Of all the arenas, the CFU values were significantly higher after the standardized riding program than before. This result showed that the amount of air pollutants increased due to the movement of the horses and the resuspension of surface material. Grzyb et al. investigated the bacterial bioaerosol concentrations in air samples before and after adding straw bedding to horse stables. The results showed the highest values at about 181,132 CFU/m³ after bedding, and thus, after activity in the stable [1]. Wheeler et al. recorded the greatest dust concentrations in the examined arenas during riding activity [42]. Accordingly, the exposure of horses and riders to dust increased in our previous study with increased particle concentrations in indoor riding arenas [33]. Bacteria in the air are often present as bioaerosols attached to dust and other particles [53]; thus, the increase in bacterial burden associated with the increase in dust was expected. This burden is obviously high compared with the bacterial concentrations (<10³ CFU/m³) measured outside of stables or in paddocks [54]. Therefore, exposure to air pollution is worse when horses are hard at work. While resting, the ventilation of an average horse is in the region of 80 L per minute [4]. This may increase more than 20-fold to a rate of 1800 L per minute during exercise. Horses also breathe more deeply, and thus there is a greater intake of potential atmospheric contaminants. Here, a standardized riding program of just 20 min was completed. Therefore, we assume that performing a longer training would increase the bacterial exposure for the horse, rider, and instructor even more.

Air samples were collected at two different heights corresponding to the approximate breathing zone of the horse (1.5 m) and the rider (2.5 m). In previous studies, a height of 1.5 m was used to represent the height of the horse's nose as well as the breathing zone of a standing person, e.g., an instructor or a human lungeing a horse [1]. Bulfin et al. investigated the potential risk for equestrian workers and exercising horses in terms of respirable crystalline silica and respirable dust exposure [44]. They showed the highest concentrations at a height of 1.5 m on one day. Some people spent over 80% of their time working indoors and, inter alia, graded the surface of the arena—this led to an excessive amount of dust [44]. Millerick-May et al. did not estimate the approximate heights to measure horse or human exposure, but used a personal monitor, which was affixed to the horse and recorded the concentration of particles in the breathing zone [55]. To the best of our knowledge, and apart from our first study [33], this is the first investigation to examine the bacterial burden in the air at horse and rider altitudes. While no significant differences were found, future studies may confirm this by measuring particle fractions of bioaerosols [31].

4.2. Aerobe Bacteria in Ground Samples

The footing material and thus the ground samples in all arenas consisted of sand, except in Arena 2, where the sand was mixed with wood shavings. Sand is a common

material as a surface or footing material in indoor riding arenas. Because horses are exposed to the airborne pollution arising from the footing material used in the riding arena surface, the ground samples impact the bacterial burden of such indoor arenas.

The concentration of aerobic bacteria in the analyzed ground samples ranged from an averaged log (CFU/g) of 4.92 to 5.44. Accordingly, Arena 1 showed the highest value in the ground samples as well as the highest bacterial load in the air. In contrast, Arena 3 had no connection to the horse stable and had the freshest footing material; it had the lowest maximum value.

The bacterial burden in the air of the riding arenas was higher after the riding program than before; thus, aerobic bacteria in the analyzed ground samples impacted the air load, and the footing material was probably a considerable source of airborne *Staphylococcus* spp. during riding. Enteropathogenic strains of *Escherichia coli* and other fecal bacteria can dwell in the litter and footing material or settle on the dust particles. This can pose a health threat in the form of bioaerosols [54]. Therefore, keeping the ground of the riding arena clean can reduce the risk of inhaling harmful microorganisms for both horses and riders.

4.3. Bacteria Species

Overall, 900 colonies from airborne bacteria samples and 218 colonies from ground bacteria samples were identified. The predominant species were *Staphylococcus xylosus*, *Staphylococcus capitis*, and *Staphylococcus equorum* in the air and footing material. These species are coagulase-negative Staphylococci (CNS). Generally, *Staphylococcus xylosus* resides as a commensal on the skin of humans and animals as well as in the environment. The dominant skin flora of animals (including pigs, cows, chickens, and horses) include CNS; *Staphylococcus xylosus* is more common in animals than in humans [56,57]. For instance, investigating the microbiological causes of dermatosis in racehorses, Shimozawa et al. found *Staphylococcus xylosus*, *Staphylococcus hyicus*, and *Staphylococcus aureus* in lesions on horses' skin [58]. *Staphylococcus xylosus* and *Staphylococcus equorum* were also found in the nostrils of horses, and *Staphylococcus xylosus* and *Staphylococcus capitis* were identified in fecal samples from horses in previous studies [59,60]. These commensals probably accumulate in the footing material and may be released by the animals themselves during riding. Although the impact of airborne CNS on the health of riders and horses in the present study remains unknown, the identification of these bacteria showed that the animals themselves and the contaminated footing material are sources of high bacterial burden in the air of riding arenas.

Newton et al. found that U.K. thoroughbred racehorses had a strong inverse association between clinical respiratory disease and the presence of low numbers of some species of non-pathogenic bacteria including *Staphylococcus* spp. [61]. Equine pleuropneumonia cases are most commonly associated with aerobic and facultative anaerobic bacteria including β -hemolytic *Streptococcus* spp., members of the Pasteurellaceae family, Enterobacteriaceae, *Pseudomonas* spp., and *Staphylococcus* spp. [62].

Horses, riders, and trainers spend much of their time in indoor riding arenas and consume ambient air with forced breathing; thus, air quality is critical to their health status. In horses with repeated inhalation of respirable dust, microorganisms and their cell wall components as well as toxins can cause inflammation (IAD or RAO) of the lower respiratory tract [16].

4.4. Limitations and Future Research

We assume that other bacteria such as Enterococci or Streptococci occur in the air of riding arenas. Here, the focus was air samples and their levels of aerobic culturable microbiota. Therefore, we suggest expanding the analysis spectrum in future studies. For instance, further microbiological spectra can be prospected by using other cultivation methods.

One core issue of this study is the low sampling volume, which might be a disadvantage of the direct impaction method. Methods sampling bioaerosols in fluids or on filters offer options to sample higher volumes and higher bioaerosol concentrations. For

instance, Beck et al. investigated bioaerosols in beef slaughter facilities by wetted wall cyclone bioaerosol samplers and collected air at 100 L/min for 15 min [63].

Another study concerning the microclimate conditions in horse stables, analyzed 1 m³ of air, i.e., 1000 L [1]. Therefore, the possibility of finding different microorganisms in the samples increases with the volume of sampled air. To assess the microbiological quality of air in different horse stables, Wolny-Koladka sampled 100 L of air collected over 1 min at a height of 1.5 m [54]. This study showed the presence of airborne *E. coli*, *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp., and fungi in the analyzed samples. No virus analysis was performed here.

Thus, further studies with higher sampling volumes and extended analysis (e.g., additional cultivation methods) are recommended for more detailed information on airborne microbial communities, including pathogens, in the air of riding arenas.

5. Conclusions

This study examined the air of four indoor riding arenas and provides the first results of bacteria exposure to horses as well as to horse riders. The investigation showed no difference in the bacterial burden between the breathing zone of riders (2.5 m height) and horses (1.5 m height). However, the bacterial loads in all four arenas significantly increased after the riding program. Direct connection between the riding arena and the stable as well as the footing material seem to impact air quality in the riding arenas. Further investigations that examine a higher air volume with an expanded microbiological spectrum and a higher number of riding horses could verify these first results and could give more information on the actual bacterial burden.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture12122111/s1>, Table S1: descriptive statistics for CFU/m³ in the four arenas, Table S2: comparisons between the individual riding arenas at the different point of times.

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Institutional Review Board Statement: Horses used in this study were undertaking their usual exercise. In accordance with the animal welfare unit of the Martin-Luther-University Halle-Wittenberg, the protocol of this study was not subject to ethical committee approval. Owners gave informed consent for the inclusion of their horses.

Data Availability Statement: The data presented in this study are available on request from the corresponding author on reasonable request.

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Conflicts of Interest: The authors declare no conflict of interest.

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