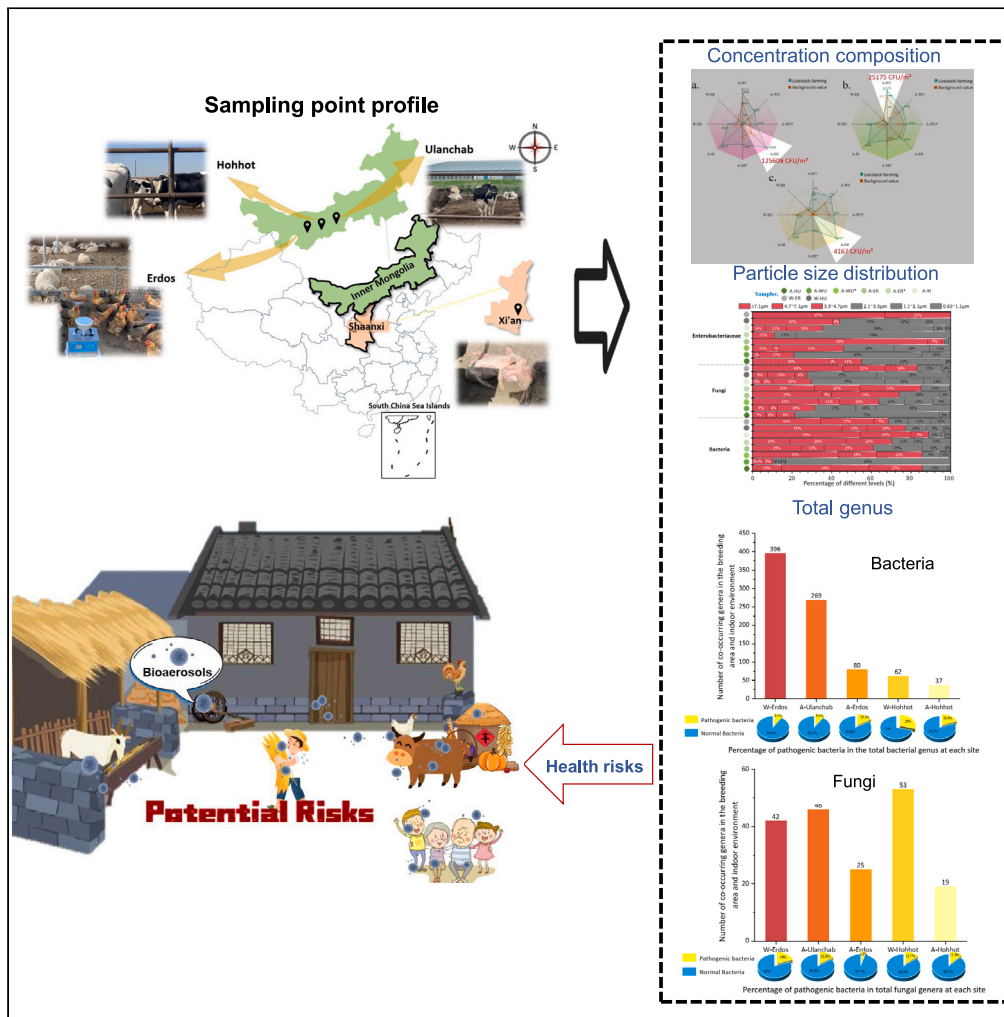


Article

# Distribution characteristics and potential risks of bioaerosols during scattered farming



Xuezheng Yu,  
Yunping Han,  
Jianguo Liu, ...,  
Ziyu Zhou, Ying  
Yan, Yuxiang  
Zhang

yphan@rcees.ac.cn (Y.H.)  
jgliu\_yc@163.com (J.L.)

**Highlights**  
Characteristics of  
bioaerosols in scattered  
livestock and poultry  
breeding were studied

71 bacterial genera and 16  
fungal genera of the  
potential pathogen were  
identified

Health risk of bioaerosols  
may be higher with human  
and animal co-habitation

Left-behind children and  
the elderly need more  
attention

Yu et al., iScience 26, 108378  
December 15, 2023 © 2023 The  
Authors.  
<https://doi.org/10.1016/j.isci.2023.108378>

## Article

## Distribution characteristics and potential risks of bioaerosols during scattered farming

Xuezheng Yu,<sup>1,2,3,4</sup> Yunping Han,<sup>1,2,4,5,\*</sup> Jianguo Liu,<sup>3,\*</sup> Yingnan Cao,<sup>3</sup> Ying Wang,<sup>1,2</sup> Zixuan Wang,<sup>1,2,3</sup> Jinxin Lyu,<sup>3</sup> Ziyu Zhou,<sup>1,2,3</sup> Ying Yan,<sup>1,2,3</sup> and Yuxiang Zhang<sup>3</sup>

## SUMMARY

**In most economically underdeveloped areas, scattered farming and human–livestock cohabitation are common. However, production of bioaerosols and their potential harm in these areas have not been previously researched. In this study, bioaerosol characteristics were analyzed in scattered farming areas in rural Northwest China. The highest bacteria, fungi, and Enterobacteria concentrations were  $125609 \pm 467$  CFU/m<sup>3</sup>,  $25175 \pm 10305$  CFU/m<sup>3</sup>, and  $4167 \pm 592$  CFU/m<sup>3</sup>, respectively. Most bioaerosols had particle sizes >3.3 μm. A total of 71 bacterial genera and 16 fungal genera of potential pathogens were identified, including zoonotic potential pathogenic genera. Moreover, our findings showed that the scattered farming pattern of human–animal cohabitation can affect the indoor air environment in the surrounding area, leading to chronic respiratory diseases in the occupants. Therefore, relevant government departments and farmers should enhance their awareness of bioaerosol risks and consider measures that may be taken to reduce them.**

## INTRODUCTION

During livestock farming, bioaerosols can be released during livestock activity, composting treatment of livestock manure, and by microorganisms attached to feed and bedding through the weathering and erosion process by combining with air, water, and dust particles.<sup>1–3</sup> Previous papers have found that livestock and poultry enclosures and manure composting areas produce elevated bioaerosol concentrations with potentially pathogenic bacteria, which can easily cause serious zoonotic diseases through respiratory irritation and pose a potential threat to human and animal health if not properly managed.<sup>4–6</sup> Studies have shown that even a small amount of bacterial aerosol production from the breeding environment can cause airborne infections, causing clinical signs or invisible infections in organisms<sup>6</sup> and posing a significant potential threat to both farmers and livestock.<sup>7</sup> In addition, bioaerosols can cause growth retardation, immune deficiency, and organ dysfunction.<sup>8,9</sup> Release of bacterial aerosols into the external environment can also result in environmental pollution.<sup>10</sup> Therefore, the distribution characteristics and health risks of bioaerosols in large-scale livestock and poultry breeding and fecal disposal areas have attracted considerable attention.

China has extensive livestock and poultry breeding, which is mostly concentrated in the rural areas of Northwest China. The livestock and poultry breeding industry is not only an important pillar industry in rural areas of Northwest China, but also an advantageous characteristic industry.<sup>11</sup> The number of rural farms in China is currently decreasing, with a gradual shift to a farming system based on large-scale farming.<sup>12</sup> However, a large number of scattered livestock and poultry farming patterns still exist in the rural areas of Northwest China, where China's economy is more backward. Under the influence of the traditional lifestyle of the older generation, the current scattered farming model in rural areas of Northwest China is mainly based on human–animal cohabitation and is characterized by small investment, small scale, small production, low demand for farming technology, and livestock enclosures being located in close proximity to the indoor living areas of villagers.<sup>13–15</sup> The number of livestock and poultry raised is generally not less than 2 and not more than 50, and the aim is not to obtain a large profit through breeding, but mainly to meet the needs of family members.<sup>16</sup> There are several unscientific livestock and poultry farming practices in rural areas of Northwest China that lead to high bioaerosol emissions, such as irregular manure cleaning, poor pen ventilation, rough pen cleaning, and irregular storage of livestock and poultry feed which leads to mold and deterioration.<sup>6,13,17</sup> In some instances, the human living environment and livestock enclosures are interspersed, and the nearest enclosures are located <5 m from the residential living area, which can impact the indoor environment in the case of irregular management.<sup>18,19</sup> In addition, animal feces contain numerous intestinal bacteria. Enterobacteria is often used as an important indicator of food quality and health safety, and inadvertent infection may cause diseases such as arthritis,

<sup>1</sup>State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, P.R. China

<sup>2</sup>University of Chinese Academy of Sciences, Beijing 100049, P.R. China

<sup>3</sup>Key Laboratory of Environmental Pollution Control and Remediation at Universities of Inner Mongolia Autonomous Region, College of Resources and Environmental engineering, Inner Mongolia University of Technology, Hohhot, Inner Mongolia 010051, PR China

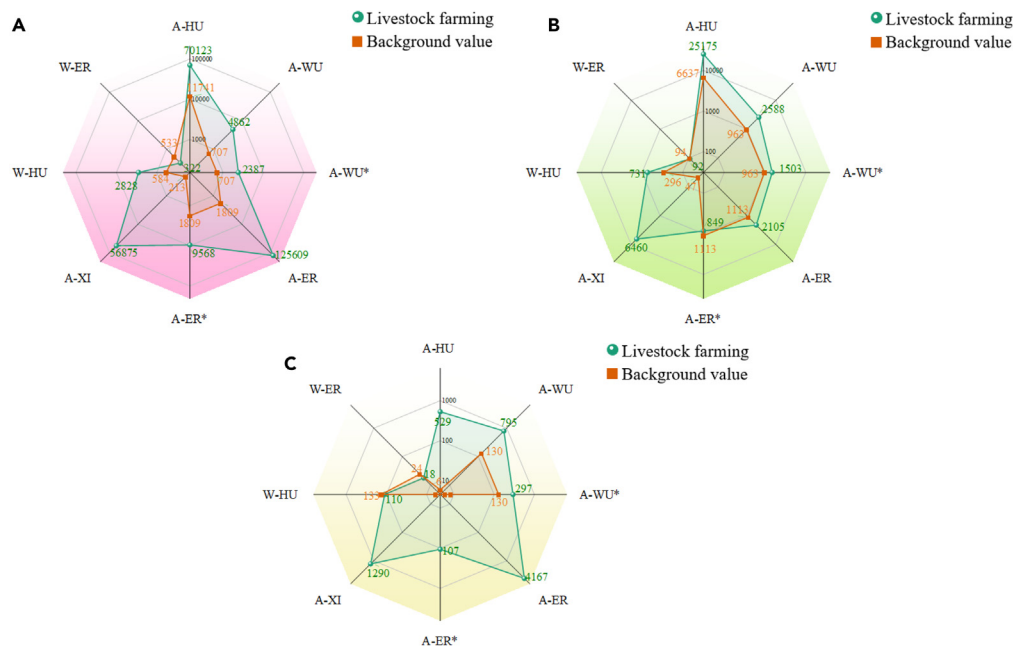
<sup>4</sup>These authors contributed equally

<sup>5</sup>Lead contact

\*Correspondence: [yphan@rcees.ac.cn](mailto:yphan@rcees.ac.cn) (Y.H.), [jgliu\\_ycx@163.com](mailto:jgliu_ycx@163.com) (J.L.)

<https://doi.org/10.1016/j.isci.2023.108378>





**Figure 1. Bioaerosol concentrations in rural scattered farming areas**

(A) Bacteria, (B) fungi, and (C) Enterobacteria concentrations (CFU/m<sup>3</sup>) in bioaerosols in rural scattered farming areas, and background concentrations in different villages. A: autumn; W: winter.

urethritis, and meningitis, among others, which can seriously affect the health of a patient.<sup>20–22</sup> Enterobacteria can accelerate cellular senescence, activate *in-vivo* inflammation, and damage mitochondria, thereby accelerating the aging process.<sup>23</sup> Furthermore, Enterobacteria lysis produces endotoxins, which are also a major source of serious health effects.<sup>24</sup>

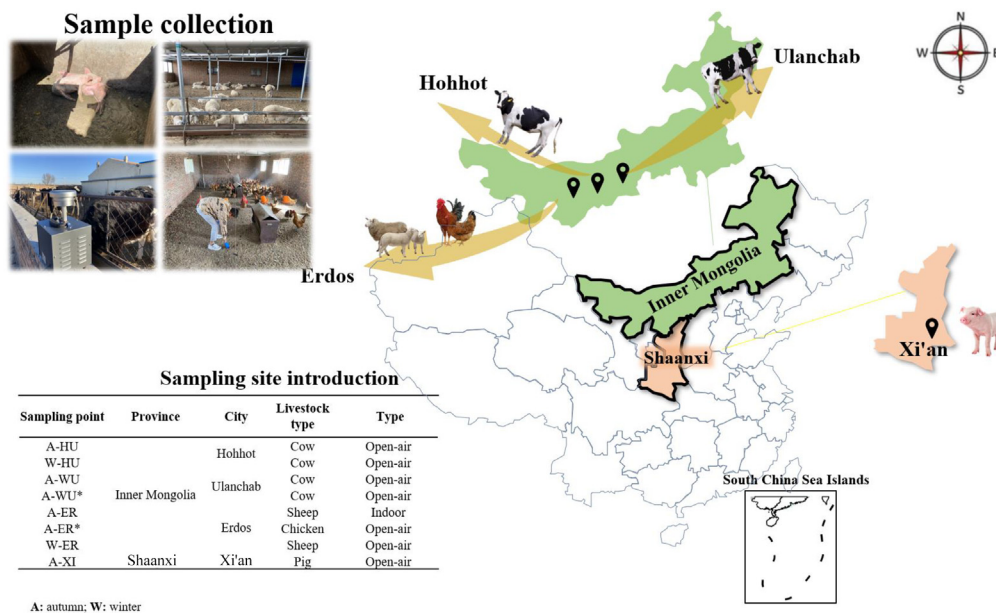
Currently, there is minimal concern regarding the potential hazards that may be caused by bioaerosols generated during scattered livestock and poultry farming in rural areas, mainly due to fixed traditional farming concepts coupled with a lack of relevant professional knowledge and management guidance.<sup>25,26</sup> Older generations in rural areas have been following this traditional scattered, human-animal cohabitation mode of farming, which has not been taken seriously due to the habitual belief that it does not pose potential risks to human health. This has led to a lack of awareness of the potential hazards of livestock farming among future generations and has created enormous problems, the most important of which are environmental pollution and health hazards, which are gradually showing an increasing trend.<sup>27,28</sup> Recently, pollution from farming has become the most important surface source of pollution in rural areas,<sup>29</sup> and local environmental protection departments receive numerous pollution complaints each year. It is imperative to raise awareness on the characteristics of pollution from farming to help change long-standing traditional practices, acquire conservation expertise, and develop evidence-based control measures to limit pollution.

Based on this, this study selected Ulanqab, Ordos, and Hohhot in Inner Mongolia and Xi'an, Shaanxi Province, China, as typical rural livestock farming areas, this study investigated bioaerosol concentration characteristics in different seasons, the particle size distribution, and the microbial composition under common rural livestock farming patterns. The aim was to elucidate the possible effects of rural scattered livestock farming on the living environment of villagers and the potential risks, and provide a scientific basis for point source information of potential pathogenic bacteria that may occur in rural areas and corresponding control measures.

## RESULTS

### Airborne culturable bacteria, fungi, and Enterobacteria concentrations during rural scattered farming

Figure 1 and Table S1 illustrate the concentration characteristics of bacteria, fungi, and Enterobacteria in bioaerosols in the rural scattered farming areas. The bioaerosol concentrations in the scattered farming areas were mostly greater than the background concentrations, with the exception of a few points. Bacterial concentrations in bioaerosols were higher than fungal concentrations at the same sampling locations. Among all sampling sites in autumn, the highest bacteria, fungi, and Enterobacteria concentrations were found in A-ER, A-HU, and A-ER at  $125609 \pm 467$  CFU/m<sup>3</sup>,  $25175 \pm 10305$  CFU/m<sup>3</sup>, and  $4167 \pm 592$  CFU/m<sup>3</sup>, respectively; the lowest concentrations were found in A-WU\*, A-ER\*, and A-ER\* at  $2387 \pm 1098$  CFU/m<sup>3</sup>,  $849 \pm 172$  CFU/m<sup>3</sup>, and  $107 \pm 12$  CFU/m<sup>3</sup>, respectively (see Figure 2 for sampling point details). At other sampling points, the bacterial concentrations were: A-HU:  $70123 \pm 25538$  CFU/m<sup>3</sup>; A-WU:  $4862 \pm 8$  CFU/m<sup>3</sup>; A-ER\*:  $9568 \pm 3824$  CFU/m<sup>3</sup>; and A-XI:  $56875 \pm 4047$  CFU/m<sup>3</sup>. The fungal concentrations were: A-WU:  $2588 \pm 548$  CFU/m<sup>3</sup>; A-WU\*:  $1503 \pm 1049$  CFU/m<sup>3</sup>; A-ER:  $2105 \pm 10$  CFU/m<sup>3</sup>; and A-XI:  $6460 \pm 2849$  CFU/m<sup>3</sup>. The Enterobacteria concentrations were: A-HU:  $529 \pm 171$  CFU/m<sup>3</sup>; A-WU:  $795 \pm 87$



**Figure 2. Study site locations and basic information on sampling sites**

CFU/m<sup>3</sup>; A-WU\*: 297 ± 33 CFU/m<sup>3</sup>; and A-XI: 1290 ± 244 CFU/m<sup>3</sup>. For all sampling sites in winter, the bacteria, fungi, and Enterobacteria concentrations were 2828 ± 425 CFU/m<sup>3</sup>, 731 ± 436 CFU/m<sup>3</sup>, and 110 ± 49 CFU/m<sup>3</sup>, respectively, at the W-HU site, and 322 ± 36 CFU/m<sup>3</sup>, 92 ± 26 CFU/m<sup>3</sup>, and 18 ± 6 CFU/m<sup>3</sup>, respectively, at the W-ER site.

Measurements of the bioaerosol concentrations in indoor environments where people reside showed that the bacteria, fungi, and Enterobacteria concentrations were higher in the case of scattered farming in the yard, i.e., 5476 ± 591 CFU/m<sup>3</sup>, 3384 ± 1789 CFU/m<sup>3</sup>, and 441 ± 59 CFU/m<sup>3</sup>, respectively, than in the absence of scattered farming in the yard, i.e., 2712 ± 514 CFU/m<sup>3</sup>, 819 ± 123 CFU/m<sup>3</sup>, and 24 ± 0 CFU/m<sup>3</sup>, respectively.

### Particle size distribution of airborne bacteria, fungi, and Enterobacteria in rural scattered farming areas

The impact of bioaerosol particles on human and livestock health varies depending on the particle size; the smaller the bioaerosol particle size, the deeper they enter the body of human and livestock. The particle size distribution of total bacteria, fungi, and Enterobacteria in bioaerosols from different sampling sites is shown in Figure 3. The particle size distribution of bioaerosols during rural scattered farming of different livestock types varied substantially.

The particle size distribution of bacteria in bioaerosols detected near pig, cattle, sheep, and chicken breeding sites was mainly >3.3 μm. Bacterial bioaerosol particle sizes of 0.65–2.1 μm were detected only at the cattle sites (A-WU), i.e., up to 85%.

For fungi, the particle size distributions of bioaerosols near sheep and chicken enclosures were similar, with both being dominated by particles >3.3 μm. The particle size distributions near pig and cattle enclosures were also similar, both mainly concentrated in the size range of 2.1–4.7 μm. The proportions of fungal bioaerosols with particle size distributions in the range of 2.1–4.7 μm were as high as 82%, particularly at the A-HU site.

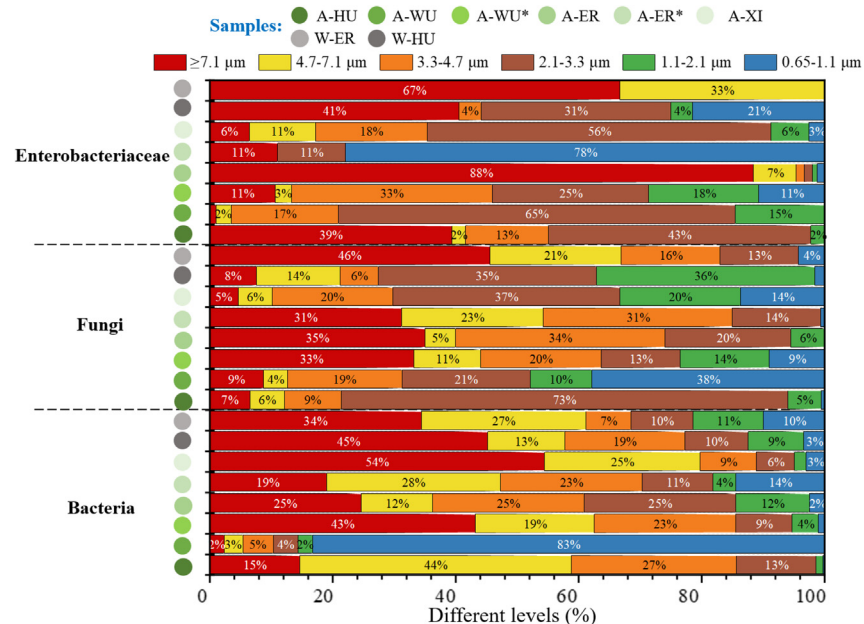
The particle size distribution of Enterobacteria was mainly ≥7.1 μm near sheep rearing enclosures and 2.1–4.7 μm near cattle and pig enclosures. The particle size distribution in chicken enclosures was relatively small, mainly concentrated in the range from 0.65 to 1.1 μm, accounting for approximately 78%; thus, close attention to risk prevention and control measures is needed to avert infection.

### Pathogenic bacteria and fungi in bioaerosols in rural scattered farming areas

The dominant bacterial genera at the A-HU, A-WU, A-WU\*, A-ER, and A-ER\* sites were *Corynebacterium*, and those at the W-HU, W-ER, and A-XI sites were *Turicibacter*, *Christensenellaceae*, and *Lactobacillus*, respectively. For fungi, the dominant genera were *Cladosporium* at the A-HU, W-HU, A-WU, and A-WU\* sites, and *Alternaria* at the A-ER\* and W-ER sites. *Acaulium* and *Bjerkandera* were the dominant fungal genera at the A-ER and A-XI sites, respectively.

A total of 71 potentially pathogenic bacterial species were identified (Figure 4). The pathogenic bacterial strains with relatively high levels compared to other strains present at each sampling site included *Corynebacterium*, *Lactobacillus*, *Acinetobacter*, *Bifidobacterium*, *Staphylococcus*, *Sphingomonas*, and *Flavobacterium*. The relative abundance of pathogenic bacteria in the A-HU, W-HU, A-WU, A-WU\*, A-ER, A-ER\*, W-ER, and A-XI sites was 3.29%, 4.12%, 7.15%, 12.44%, 15.78%, 8.01%, 3.00%, and 9.01%, respectively.

Sixteen potentially pathogenic fungal genera were identified (Figure 5). The pathogenic fungal species with a relatively high abundance at each site included *Cladosporium*, *Alternaria*, *Aspergillus*, *Penicillium*, *Trichosporon*, *Verticillium*, and *Talaromyces*. The relative abundance of



**Figure 3. Particle size distribution of total bacteria, fungi, and Enterobacteriaceae in bioaerosols at different sampling locations**  
A: autumn; W: winter.

pathogenic fungi at the A-HU, W-HU, A-WU, A-WU\*, A-ER, A-ER\*, W-ER, and A-XI sites was 23.00%, 9.48%, 33.07%, 44.96%, 5.03%, 18.22%, 8.56%, and 7.58%, respectively.

This study found that environmental conditions (Table 1) had a substantial impact on the pathogenic bacterial genera in bioaerosols. Redundancy analysis (RDA) was used to investigate the effects of environmental parameters such as air temperature, relative humidity, particulate matter concentration, and wind speed on the concentration of potentially pathogenic bacteria (Figure 6). *Staphylococcus*, *Corynebacterium*, and *Penicillium* showed strong positive correlations with temperature, relative humidity, and particulate matter concentration, and significant negative correlations with wind speed and atmospheric pressure (Figures 6A and 6B).

The present study also confirmed the high similarity of species composition between indoor residential environments and rural dispersed breeding areas by NMDS analysis (Figure 7). After obtaining the species composition similarity, the number of shared genera was quantified and the proportion of potentially pathogenic bacteria in the shared genera for the human indoor environment and the rural scattered farming areas was calculated (Figure 8). The number of shared bacterial genera (Figure 8A) was significantly lower in the absence of scattered farming in the yard than in its presence, and that in winter was greater than that in autumn at the same points. The presence or absence of scattered farming in the yard had no significant effect on the number of shared fungal genera (Figure 8B). Moreover, this study found that the number of common fungal genera was less than that of bacteria at all points, and the number of potentially pathogenic fungal genera was also less than that of bacteria.

## DISCUSSION

Scattered livestock and poultry farming, as a traditional and basic industry in rural China, has always existed in the daily lives of villagers. It is only with the proposal of comprehensive rural environmental improvement and the occurrence of disease transmission incidents caused by airborne pathogenic microorganisms that people have gained a better understanding of the hazards that may arise in the process of scattered livestock and poultry farming in rural areas.

It has been shown that source characteristics of rural scattered farming are important factors influencing the concentration distribution characteristics and species composition of bioaerosols. In this study, the source tracing method (Figure 9) was used to analyze the sources of bioaerosols in rural scattered farming areas.<sup>30</sup> For bacterial bioaerosols, livestock manure and background air were important sources, accounting for 34.3% and 14.5%, respectively, similar to that reported in previous studies.<sup>31–33</sup> If livestock manure is not effectively managed, the numerous pathogenic microorganisms, parasitic eggs, and flies contained in it may increase the risk of diseases in humans and livestock.<sup>5,34</sup> Moreover, if manure is not treated adequately quickly, it leads to its accumulation and presence as a water, soil, and air pollutant.<sup>33,35</sup>

Some papers have argued that most of the fungal bioaerosols in rural scattered farming areas originate from the external environment, such as the soil outside enclosures.<sup>36</sup> This is similar to the results of the present study, which found that fungal bioaerosols originated from background concentrations through source tracing method, and that livestock manure may have accounted for 27.3% and 2.9%, respectively.





**Figure 4. Distribution of potentially pathogenic bacteria in bioaerosols in rural scattered farming areas, ranked at the level of the top 25 bacterial genera in terms of relative abundance**

A: autumn; W: winter.

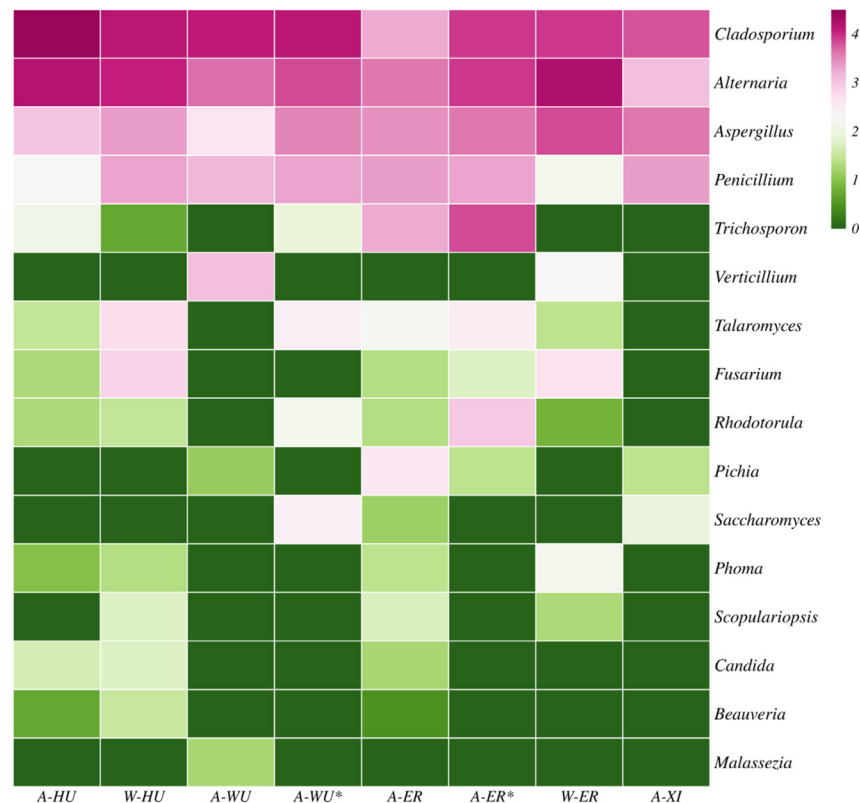
Another important factor affecting the bioaerosol concentration characteristics and airborne microbial species composition in rural scattered farming areas is the environmental conditions, such as meteorological conditions, livestock activity status, livestock housing environment, bedding material replacement cycle, and farming scale.<sup>4,13,37</sup>

This study found that temperature affected the bioaerosol concentration. In the same village, the bacteria, fungi, and Enterobacteria concentrations were lower in winter than in autumn, which may have been because temperature affected the metabolic activity of these organisms so that their growth and reproduction were somewhat inhibited.<sup>38,39</sup> The bacteria, fungi and, Enterobacteria concentrations in rural livestock breeding areas in winter in Hohhot (W-HU) decreased by 95.97%, 97.10%, and 79.17%, respectively, compared to those in autumn. The bacteria, fungi, and Enterobacteria concentrations in rural livestock breeding areas in winter in Erdos (W-ER) also decreased compared to those in autumn, i.e., by 99.74%, 95.62%, and 99.58%, respectively. The influence of other environmental factors on bioaerosol characteristics was also shown. Thus, attention should be given to the effect of weather conditions with high temperature, humidity, and particulate matter concentration on *Staphylococcus*.<sup>40–42</sup> *Staphylococcus* infection may cause acute or chronic infectious diseases in poultry, with common clinical manifestations of skin blisters and abscesses.<sup>43–45</sup>

Moreover, this study found that the livestock species is another important factor that causes large differences in bioaerosol concentrations. This study conducted field studies concurrently at the A-ER and A-ER\* sites in Erdos, where sheep and chickens were reared at relatively similar scales, and found considerable differences in bioaerosol concentrations, similar to previous results.<sup>46</sup> Not only does the livestock type have an effect, but some scholars have found a relationship between the livestock age and bioaerosol concentration.<sup>47,48</sup>

At site A-ER, unlike other sites, rural scattered farming is carried out indoors with a high number and density of farms and with ruminant sheep; ruminants can excrete numerous microorganisms in their stomachs, and animals can emit approximately  $10^5$  biological droplets per sneeze, all of which can form bioaerosols.<sup>49,50</sup> Combined with the fact that the site is in an indoor environment with poor air circulation, high bacteria and Enterobacteria concentrations were detected, i.e.,  $125609 \pm 467$  CFU/m<sup>3</sup> and  $4167 \pm 592$  CFU/m<sup>3</sup>, respectively.

In addition, the fungi concentration at the A-ER site was only  $2105 \pm 10$  CFU/m<sup>3</sup>, which may have been due to the timely change of bedding in this breeding area, reducing bioaerosol formation, as fungi are associated with organic material decomposition. This has also been demonstrated in studies where bedding materials can accumulate manure, are the best carrier for fungal attachment and growth, and are an important source of fungal bioaerosols in animal housing.<sup>51,52</sup>



**Figure 5. Distribution of all potentially pathogenic fungi in rural scattered farming areas aerosol in order of relative abundance**  
A: autumn; W: winter.

Bioaerosol particles sized from 2.1 to 4.7  $\mu\text{m}$  can enter the human trachea and bronchus, and those with a particle size ranging from 0.65 to 2.1  $\mu\text{m}$  can freely enter human alveoli and cause human respiratory infections, posing a threat to human health.<sup>53–56</sup>

Analysis of the particle size distribution characteristics showed that most of the bacterial and fungal bioaerosol particle size distributions were dominated by those sized  $>3.3 \mu\text{m}$ , which is similar to that reported in previous studies.<sup>6,15,57</sup> This parameter can be influenced by various factors, such as the mode of aerosolization and hygroscopicity of the particles, animal activity, ventilation, and bedding materials.<sup>50,58,59</sup> Larger cell particles may form clumps of cells that can avoid detection by the immune system of the host, and large cell clumps are also favorable for evading phagocytosis.<sup>60</sup>

One study found that results from stables were similar to those detected in cattle yards (A-WU) in this study. Stables had the highest proportion of 0.65–2.1  $\mu\text{m}$  size bacterial aerosols.<sup>13</sup> At the A-WU site, the particle size distribution of 0.65–1.1  $\mu\text{m}$  accounted for up to 83%, which may have been mainly related to the weather conditions on the sampling day, whereby minor rainfall at the sampling time can cause rapid deposition of large-size bioaerosols, resulting in a high percentage of particles sized from 0.65 to 1.1  $\mu\text{m}$ .<sup>61</sup> Unlike the present study, however, some researchers have found that airborne fungi comprise a low percentage of particulate matter ( $<4.7 \mu\text{m}$ ) in pig barns, similar to the environment in which humans live and work.<sup>44</sup>

In addition, the percentage of respirable particle size was higher in autumn than in winter, which may have been mainly due to the effects of humidity and wind speed on particle size. Based on the measured meteorological data (Table 1), it was found that in autumn, with high humidity and low wind speeds, large particles with high moisture content were less susceptible to being blown up by wind speeds. Whereas, in winter, with low humidity and high wind speed, large-size particles with low water content are easily blown up by the carrying effect of the wind, and thus a relatively high percentage of large-size particles are detected.<sup>62</sup> Finer particles are more likely to enter deeper into the human respiratory tract and pose a health risk,<sup>54,63</sup> which may cause some degree of pulmonary effects on farmers.<sup>7</sup>

In addition to the concentration and particle size distribution, microbial community composition is also an important feature of bioaerosols in rural scattered farming areas.<sup>17</sup> This study found zoonotic pathogens including *Bacillus*, *Campylobacter*, *Alternaria*, *Aspergillus*, and *Penicillium*.<sup>64–66</sup> Among these, *Bacillus* causes cutaneous, abdominal, or gastrointestinal anthrax in humans and livestock.<sup>64</sup> *Campylobacter* causes reproductive and intestinal infections in livestock and poultry, and may also cause abortion and infertile fetuses in cattle and sheep; it can also infect humans, causing miscarriage, premature births, septicemia, and similar symptoms.<sup>67,68</sup> It has been demonstrated that domestic animals serve as the largest potential reservoir of zoonotic viral diseases.<sup>69</sup> The relative abundance of zoonotic pathogens at the A-HU, W-HU, A-WU, A-WU\*, A-ER, A-ER\*, W-ER, and A-XI sites was 4.85%, 3.58%, 5.96%, 10.07%, 2.72%, 5.23%, 4.87%, and 2.44%, respectively.

**Table 1. Meteorological parameters measured during sampling**

Sampling point	Sample ID	T (°C)	RH (%)	BP (kPa)	WS (m/s)	TSP ( $\mu\text{g}/\text{m}^3$ )
Hohhot	A-HU	22.88	49.15	89.22	1.65	97.09
	W-HU	4.34	33.80	89.88	2.00	1044.62
Ulanqab	A-WU	24.44	67.80	86.19	2.03	106.50
	A-WU*	24.56	67.03	83.46	0.93	94.60
Ordos	A-ER	23.18	58.50	86.09	0.01	1816.36
	A-ER*	21.32	60.35	86.08	4.30	103.26
	W-ER	-7.57	41.50	87.16	2.35	241.49
Xi'an	A-XI	30.76	44.70	91.91	0.50	143.08

A: autumn; W: winter.

In addition to zoonotic pathogens, other disease-causing bacteria that have been detected at the top of the relative abundance list can also cause serious health effects. *Corynebacterium* can parasitize the human nasal cavity, throat, conjunctiva, and skin, and is generally non-pathogenic and mostly conditionally pathogenic.<sup>70</sup> *Cladosporium* is a mycobacterium capable of producing conidia, which can cause widespread allergic reactions in patients with asthma or similar respiratory diseases, and long-term exposure may lead to immune system degeneration.<sup>71,72</sup> Regarding epidemiological results, it has been shown that children living or attending school near farms are at increased risk of developing asthma.<sup>4</sup> Infection by *Aspergillus* usually occurs in immunocompromised hosts, but sometimes affects immunocompetent populations, especially patients with acute illnesses who are treated in intensive care units (ICUs), and less commonly in patients with chronic illnesses.<sup>73</sup>

At the same sites (Figure 8), this phenomenon of higher numbers of shared genera in winter than in autumn may be mainly due to insufficient indoor ventilation in winter.<sup>74,75</sup> This suggested that scattered farming in the yard can affect the indoor environment of residents. However, the lack of significant effect on the number of shared fungal genera is due to the fact that fungi are usually associated with the decomposition of organic matter and the atmosphere contains a large number of naturally occurring fungi.<sup>76</sup> Nevertheless, it is recommended that livestock farming areas should be located at least 200 m from residential areas.<sup>77</sup>

Agricultural activities or livestock rearing may be associated with higher indoor bioaerosol concentrations in rural areas.<sup>78</sup> Rural indoor bioaerosol concentrations were similar to those found in dormitory environments and urban courtyards.<sup>79,80</sup> This study was conducted in rural areas of Northwest China, where the majority of residents were elderly with poor health and preschool children with imperfect immune systems. These individuals have limited abilities to prevent and control potentially pathogenic bacteria. This was combined with the unreasonable planning of breeding areas, most of which were chosen to be very close to residential areas (<100 m), which may have resulted in elevated levels of potentially pathogenic microorganisms in the residential indoor environments of residents as they adhere to the clothes of workers, the soles of their shoes, the tools they use, or are carried indoors by air currents. This could lead to elevated health hazards for the elderly and children living indoors.<sup>5,13,34</sup> These genera pose a much greater risk of allergies and respiratory diseases in the elderly and children than in adults.<sup>81–84</sup>

This study shows that scattered livestock farming in rural areas does pose a potential health risk to the population, and that this potential risk can be further exacerbated if reasonable and effective controls are not put in place. Notably, operators in England are required to carry out targeted preventative measures if the farming area is within 100 m of a sensitive human receptor (such as a home or workplace).<sup>4</sup>

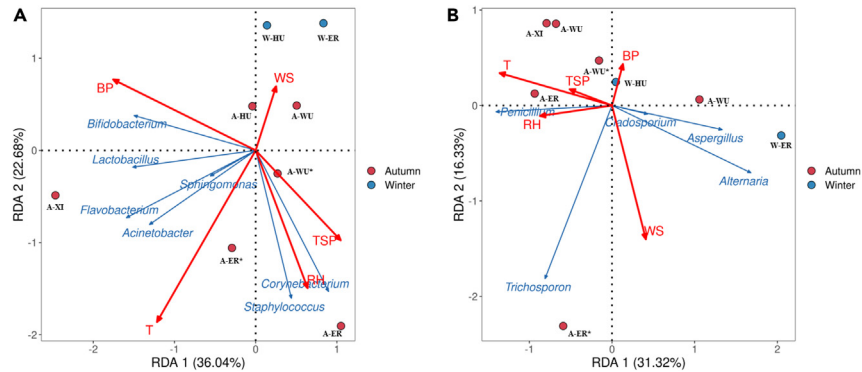
Currently, the quantitative microbial risk assessment method is used for bioaerosol risk assessment.<sup>54</sup> Respiratory exposure is only one route, and the risk of dermal exposure is also possible; the risk of these exposures is dose-dependent and not limited to the respiration rate.<sup>85</sup> Some elderly people or children may be exposed via dermal contact for longer time periods, and the exposure risk may be indirectly increased by the increased frequency of child interactions, resulting in increased concentrations of pathogenic microorganisms deposited on the upper and lower extremities of the accompanying elderly person, as well as on the inside of the arm. This, combined with differences in physical fitness, may lead to a more complex risk assessment that requires more in-depth studies.

## Conclusions and prospects

Based on our study of bioaerosol characteristics in scattered farming areas in rural Northwest China, the following conclusions were drawn.

1. The indoor living environment under human–livestock cohabitation was affected by air transmission or by pathogens carried by people. Bacteria, fungi, and Enterobacteria concentrations in the indoor living environment with scattered farming in the yard were  $5476 \pm 591$  CFU/m<sup>3</sup>,  $3384 \pm 1789$  CFU/m<sup>3</sup>, and  $441 \pm 59$  CFU/m<sup>3</sup>, respectively; those in the indoor living environment without scattered farming in the yard were  $2712 \pm 514$  CFU/m<sup>3</sup>,  $819 \pm 123$  CFU/m<sup>3</sup>, and  $24 \pm 0$  CFU/m<sup>3</sup>, respectively.
2. Bioaerosols with a small particle size (<3.3  $\mu\text{m}$ ) and carrying a variety of pathogenic microorganisms (87 species) from rural scattered farming areas posed a potential health risk to residents in economically disadvantaged and poorly housed rural areas in Northwest China, particularly the elderly and children.





**Figure 6. Influences of potentially pathogenic bacterial genera in bioaerosols from rural scattered farming areas**

Influencing factors of potentially pathogenic (A) bacterial and (B) fungal genera in bioaerosols from rural scattered farming areas, and the relatively top-ranked genera selected for RDA analysis. A: autumn; W: winter.

Having obtained the above conclusions from the analysis, we provide the following outlook for future research.

- At present, the lack of data on pathogenic microorganisms in rural scattered farming environments hinders comprehensive risk assessment, and risk assessment methods applicable to bioaerosols in rural scattered farming areas should be established as a matter of urgency in future research.
- In the future, it is important to increase awareness among farmers regarding the potential risks associated with bioaerosols released during the process of rural scattered farming. Relevant governmental departments should strengthen their educational efforts regarding the management of rural decentralized farming, so that farmers can communicate more with each other, exchange ideas, and share good experiences. These efforts will help to mitigate the potential impacts of bioaerosols on human and livestock health and reduce environmental pollution in rural areas.

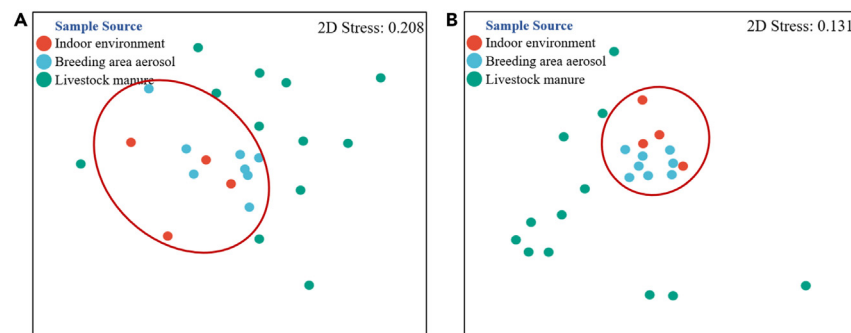
### Limitation of the study

Although the above findings were obtained from a field study in a scattered farming area in rural China, the study still has some shortcomings. They are mainly in the assessment methods, including the number of sample sites selected, the location covered by the sampling sites, and the time of sampling site selection.

### STAR★METHODS

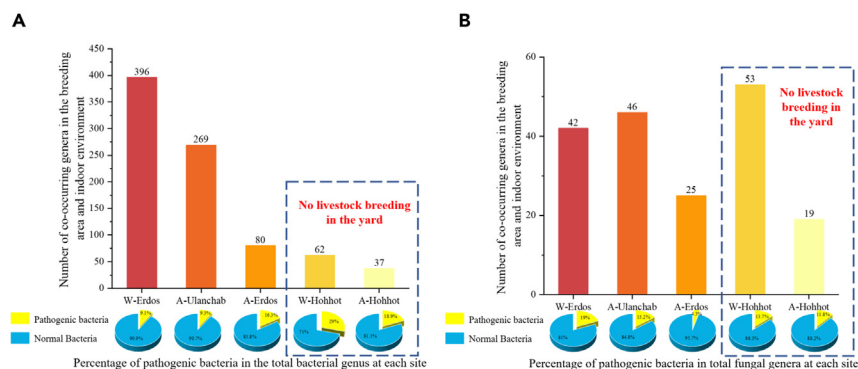
Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability



**Figure 7. NMDS-based analysis of bioaerosol variability in rural scattered farming areas**

Variability of (A) bacteria and (B) fungi in bioaerosols in rural scattered farming areas based on NMDS analysis.



**Figure 8. Population distribution of common bacterial genera in scattered farming and residential areas in rural areas**

Distribution of the number of shared genera of (A) bacteria and (B) fungi in rural scattered farming areas and residential environments. Pie charts show the proportion of potentially pathogenic genera among the shared genera in different areas. A: autumn; W: winter.

- Data and code availability
- **METHOD DETAILS**
  - Study sites
  - Bioaerosol collection
  - Microbial analysis
  - Statistical analysis
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

### SUPPLEMENTAL INFORMATION

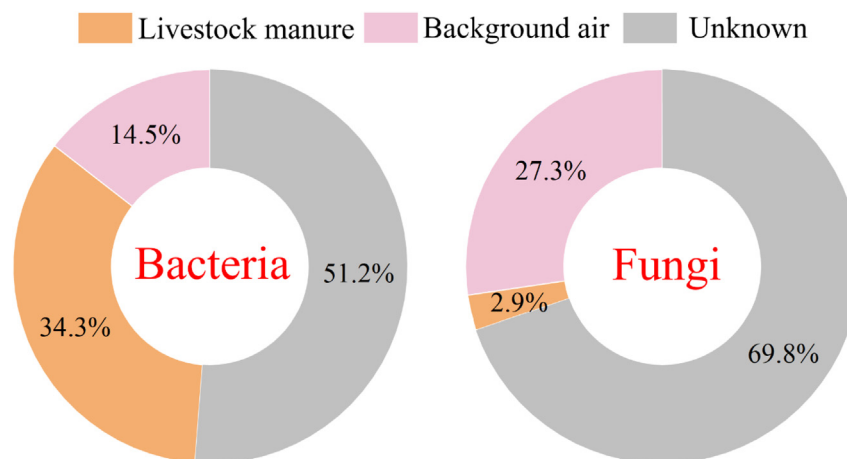
Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2023.108378>.

### ACKNOWLEDGMENTS

This work were supported by the National Natural Science Foundation of China (No. 41961134033), the National Key R&D Program of China (grant number: 2020YFD1100500), and Basic Research Operating Expenses Program for Colleges and Universities directly under the Autonomous Region (No. ZTY2023039), Innovation and Practice Training Program for Undergraduates of the Chinese Academy of Sciences (No. 20234001075). The authors express their sincere gratitude to Editage for their professional editing support of the manuscript.

### AUTHOR CONTRIBUTIONS

Conceptualization, X.Z.Y. and Y.P.H.; Methodology, J.G.L. and Y.N.C.; Investigation, Y.W., Z.X.W., and J.X.L.; Writing – Original Draft, X.Z.Y., Z.Y.Z., Y.X.Z., and Y.Y.; Writing – Review and Editing, Y.P.H.; Supervision, J.G.L.



**Figure 9. Sources of bacteria and fungi in bioaerosols in rural scattered farming areas**

## DECLARATION OF INTERESTS

The authors declare no competing interests.

## INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

Received: June 27, 2023

Revised: October 6, 2023

Accepted: October 27, 2023

Published: November 1, 2023

## REFERENCES

- Martinez, J., Dabert, P., Barrington, S., and Burton, C. (2009). Livestock waste treatment systems for environmental quality, food safety, and sustainability. *Bioresour. Technol.* 100, 5527–5536. <https://doi.org/10.1016/j.biortech.2009.02.038>.
- Robles-Jimenez, L.E., Aranda-Aguirre, E., Castelan-Ortega, O.A., Shettino-Bermudez, B.S., Ortiz-Salinas, R., Miranda, M., Li, X., Angeles-Hernandez, J.C., Vargas-Bello-Pérez, E., and Gonzalez-Ronquillo, M. (2021). Worldwide Traceability of Antibiotic Residues from Livestock in Wastewater and Soil: A Systematic Review. *Animals*. 12, 60. <https://doi.org/10.3390/ani12010060>.
- Zhou, L., Li, S., and Li, F. (2022). Damage and elimination of soil and water antibiotic and heavy metal pollution caused by livestock husbandry. *Environ. Res.* 215, 114188. <https://doi.org/10.1016/j.envres.2022.114188>.
- Douglas, P., Robertson, S., Gay, R., Hansell, A.L., and Gant, T.W. (2018). A systematic review of the public health risks of bioaerosols from intensive farming. *Int. J. Hyg Environ. Health* 221, 134–173. <https://doi.org/10.1016/j.ijheh.2017.10.019>.
- Lee, S.J., and Kim, K.Y. (2021). On-Site Investigation of Airborne Bacteria and Fungi According to Type of Poultry Houses in South Korea. *Processes* 9, 1534–1537. <https://doi.org/10.3390/pr9091534>.
- Nguyen, X.D., Zhao, Y., Evans, J.D., Lin, J., and Purswell, J.L. (2022). Survival of *Escherichia coli* in Airborne and Settled Poultry Litter Particles. *Animals*. 12, 284. <https://doi.org/10.3390/ani12030284>.
- Youssef, D.M., Wieland, B., Knight, G.M., Lines, J., and Naylor, N.R. (2021). The effectiveness of biosecurity interventions in reducing the transmission of bacteria from livestock to humans at the farm level: A systematic literature review. *Zoonoses Public Health* 68, 549–562. <https://doi.org/10.1111/zph.12807>.
- Delabouglise, A., Guerin, J.L., Lury, A., Binot, A., Paul, M., Peyre, M., Roger, F., and Bonbon, E. (2022). Intensification of livestock systems and pandemic risks. *Cah. Agric.* 31, 1–7. <https://doi.org/10.1051/cagri/2022012>.
- Yitagesu, E., Fentie, T., Kebede, N., Jackson, W., and Smith, W. (2022). The magnitude of calf morbidity and mortality and risk factors in smallholder farms across livestock production systems in central Ethiopia. *Vet. Med. Sci.* 8, 2157–2166. <https://doi.org/10.1002/vms3.877>.
- Zhen, Y., Ge, L., Chen, Q., Xu, J., Duan, Z., Loo, J.J., and Wang, M. (2022). Latent Benefits and Toxicity Risks Transmission Chain of High Dietary Copper along the Livestock-Environment-Plant-Human Health Axis and Microbial Homeostasis: A Review. *J. Agric. Food Chem.* 70, 6943–6962. <https://doi.org/10.1021/acs.jafc.2c01367>.
- Jiang, Y., Zhang, Q., Niu, J., and Wu, J. (2019). Pastoral Population Growth and Land Use Policy Has Significantly Impacted Livestock Structure in Inner Mongolia-A Case Study in the Xilinhot Region. *Sustainability* 11, 7208–7217. <https://doi.org/10.3390/su11247208>.
- Hu, Z., Yang, Q., Tao, Y., Shi, L., Tu, J., and Wang, Y. (2023). A review of ventilation and cooling systems for large-scale pig farms. *Sustain. Cities Soc.* 89, 104372. <https://doi.org/10.1016/j.scs.2022.104372>.
- Grzyb, J., Podstawski, Z., and Bulski, K. (2022). Bacterial aerosol, particulate matter, and microclimatic parameters in the horse stables in Poland. *Environ. Sci. Pollut. Res. Int.* 29, 26992–27006. <https://doi.org/10.1007/s11356-021-18142-6>.
- Loyon, L., Burton, C.H., Misselbrook, T., Webb, J., Philippe, F.X., Aguilar, M., Doreau, M., Hassouna, M., Veldkamp, T., Dourmad, J.Y., et al. (2016). Best available technology for European livestock farms: Availability, effectiveness and uptake. *J. Environ. Manage.* 166, 1–11. <https://doi.org/10.1016/j.jenvman.2015.09.046>.
- Sowiak, M., Bródka, K., Buczyńska, A., Cyprowski, M., Kozajda, A., So Ba La, W., and Szadkowska-Stańczyk, I. (2012). An assessment of potential exposure to bioaerosols among swine farm workers with particular reference to airborne microorganisms in the respirable fraction under various breeding conditions. *Aerobiologia* 28, 121–133. <https://doi.org/10.1007/s10453-011-9216-0>.
- Mathinya, V.N., Franke, A.C., Van De Ven, G.W.J., and Giller, K.E. (2022). Productivity and constraints of small-scale crop farming in the summer rainfall region of South Africa. *Outlook Agric.* 51, 139–154. <https://doi.org/10.1177/00307270221091839>.
- Awad, A.H.A., Elmorsy, T.H., Tarwater, P.M., Green, C.F., and Gibbs, S.G. (2010). Air biocontamination in a variety of agricultural industry environments in Egypt: a pilot study. *Aerobiologia* 26, 223–232. <https://doi.org/10.1007/s10453-010-9158-y>.
- Pillai, S.D., and Ricke, S.C. (2002). Bioaerosols from municipal and animal wastes: background and contemporary issues. *Can. J. Microbiol.* 48, 681–696. <https://doi.org/10.1139/W02-070>.
- Van Leuken, J.P.G., Swart, A.N., Havelaar, A.H., Van Pul, A., Van der Hoek, W., and Heederik, D. (2016). Atmospheric dispersion modelling of bioaerosols that are pathogenic to humans and livestock - A review to inform risk assessment studies. *Microb. Risk Anal.* 1, 19–39. <https://doi.org/10.1016/j.mran.2015.07.002>.
- Amare, A., Eshetie, S., Kasew, D., and Moges, F. (2022). High prevalence of fecal carriage of Extended-spectrum beta-lactamase and carbapenemase-producing Enterobacteriaceae among food handlers at the University of Gondar, Northwest Ethiopia. *PLoS One* 17, e0264818–e0264821. <https://doi.org/10.1371/journal.pone.0264818>.
- Delahoy, M.J., Wodnik, B., McAliley, L., Penakalapati, G., Swarthout, J., Freeman, M.C., and Levy, K. (2018). Pathogens transmitted in animal feces in low- and middle-income countries. *Int J Hyg Environ Health* 221, 661–676. <https://doi.org/10.1016/j.ijheh.2018.03.005>.
- Markovic, K.G., Grujovic, M.Z., Koracevic, M.G., Nikodijevic, D.D., Milutinovic, M.G., Semedo-Lemsaddek, T., and Djilas, M.D. (2022). Colicins and Microcins Produced by Enterobacteriaceae: Characterization, Mode of Action, and Putative Applications. *Int. J. Environ. Res. Public Health* 19, 1–18. <https://doi.org/10.3390/ijerph191811825>.
- Boopathi, S., Kumar, R.M.S., Priya, P.S., Haridevamuthu, B., Nayak, S.P.R.R., Chulenbayeva, L., Almagul, K., and Arockiaraj, J. (2023). Gut Enterobacteriaceae and uraemic toxins - Perpetrators for ageing. *Exp. Gerontol.* 173, 112088–112089. <https://doi.org/10.1016/j.exger.2023.112088>.
- Wei, D.J., Liu, W.T., Chin, H.T., Lin, C.H., Chen, I.C., and Chang, Y.T. (2021). An Investigation of Airborne Bioaerosols and Endotoxins Present in Indoor Traditional Wet Markets before and after Operation in Taiwan: A Case Study. *Int. J. Environ. Res. Public Health* 18, 2945. <https://doi.org/10.3390/ijerph18062945>.
- Maye, D., and Chan, K.W.R. (2020). On-farm biosecurity in livestock production: farmer behaviour, cultural identities and practices of care. *Emerg. Top. Life Sci.* 4, 521–530. <https://doi.org/10.1042/ETLS20200063>.
- Nyoni, N.M.B., Grab, S., and Archer, E.R.M. (2019). Heat stress and chickens: climate risk effects on rural poultry farming in low-income countries. *Clim. Dev.* 11, 83–90. <https://doi.org/10.1080/17565529.2018.1442792>.
- Hocquette, J.F., Ellies-Oury, M.P., Lherm, M., Pineau, C., Deblitz, C., and Farmer, L. (2018). Current situation and future prospects for beef production in Europe - A review. *Asian-Australas. J. Anim. Sci.* 31, 1017–1035. <https://doi.org/10.5713/ajas.18.0196>.
- Tullo, E., Finzi, A., and Guarino, M. (2019). Review: Environmental impact of livestock

- farming and Precision Livestock Farming as a mitigation strategy. *Sci. Total Environ.* 650, 2751–2760. <https://doi.org/10.1016/j.scitotenv.2018.10.018>.
29. Nsenga Kumwimba, M., Zhu, B., Stefanakis, A.I., Ajibade, F.O., Dzakpasu, M., Soana, E., Wang, T., Arif, M., Kavida Muyembe, D., and Agboola, T.D. (2023). Advances in ecotechnological methods for diffuse nutrient pollution control: wicked issues in agricultural and urban watersheds. *Front. Environ. Sci.* 11, 1–37. <https://doi.org/10.3389/fenvs.2023.1199923>.
30. Li, P., Li, L., Yang, K., Zheng, T., Liu, J., and Wang, Y. (2021). Characteristics of microbial aerosol particles dispersed downwind from rural sanitation facilities: Size distribution, source tracking and exposure risk. *Environ. Res.* 195, 110798. <https://doi.org/10.1016/j.envres.2021.110798>.
31. Ezugworie, F.N., Igboke, V.C., and Onwosi, C.O. (2021). Proliferation of antibiotic-resistant microorganisms and associated genes during composting: An overview of the potential impacts on public health, management and future. *Sci. Total Environ.* 784, 147191. <https://doi.org/10.1016/j.scitotenv.2021.147191>.
32. Kyakuwaire, M., Olupot, G., Amoding, A., Nkedi-Kizza, P., and Basamba, T.A. (2019). How Safe is Chicken Litter for Land Application as an Organic Fertilizer? A Review. *Int. J. Environ. Res. Public Health* 16, 3521–3523. <https://doi.org/10.3390/ijerph16193521>.
33. Venglovsky, J., Sasakova, N., and Placha, I. (2009). Pathogens and antibiotic residues in animal manures and hygienic and ecological risks related to subsequent land application. *Bioresour. Technol.* 100, 5386–5391. <https://doi.org/10.1016/j.biortech.2009.03.068>.
34. Vyskocil, J.M., Turgeon, N., Turgeon, J.G., and Duchaine, C. (2020). Ozone treatment in a wind tunnel for the reduction of airborne viruses in swine buildings. *Aerosol Sci. Technol.* 54, 1471–1478. <https://doi.org/10.1080/02786826.2020.1790495>.
35. Zubair, M., Wang, S., Zhang, P., Ye, J., Liang, J., Nabi, M., Zhou, Z., Tao, X., Chen, N., Sun, K., et al. (2020). Biological nutrient removal and recovery from solid and liquid livestock manure: Recent advance and perspective. *Bioresour. Technol.* 301, 122823. <https://doi.org/10.1016/j.biortech.2020>.
36. Kasprzyk, I. (2008). Aeromycology-main research fields of interest during the last 25 years. *Ann. Agric. Environ. Med.* 15, 1–7. <https://doi.org/10.2105/AJPH.2006.100131>.
37. Guo, L., Zhao, B., Jia, Y., He, F., and Chen, W. (2022). Mitigation Strategies of Air Pollutants for Mechanical Ventilated Livestock and Poultry Housing-A Review. *Atmosphere* 13, 452. <https://doi.org/10.3390/atmos13030452>.
38. Kalwasińska, A., Burkowska, A., Swiontek Brzezinska, M., Brzezinska, and Swiontek, M. (2014). Exposure of Workers of Municipal Landfill Site to Bacterial and Fungal Aerosol. *Clean* 42, 1337–1343. <https://doi.org/10.1002/clean.201300385>.
39. Madhwal, S., Prabhu, V., Sundriyal, S., and Shridhar, V. (2020). Distribution, characterization and health risk assessment of size fractionated bioaerosols at an open landfill site in Dehradun, India - ScienceDirect. *Atmos. Pollut. Res.* 11, 156–169. <https://doi.org/10.1016/j.apr.2019.10.002>.
40. Kallawicha, K., Wongsasuluk, P., Chompuchan, C., Suksatan, W., Chao, H.J., Siritwong, W., and Nilsalai, W. (2021). Influence of meteorological condition during rainstorm periods on the ambient concentrations of fungi in Bangkok, Thailand. *Hum. Ecol. Risk Assess.* 27, 2224–2234. <https://doi.org/10.1080/10807039.2021.1967109>.
41. Yin, Y., Qi, J., Gong, J., and Gao, D. (2021). Distribution of bacterial concentration and viability in atmospheric aerosols under various weather conditions in the coastal region of China. *Sci. Total Environ.* 795, 148713. <https://doi.org/10.1016/j.scitotenv.2021.148713>.
42. Zoran, M.A., Savastru, R.S., Savastru, D.M., and Tautan, M.N. (2020). Assessing the relationship between surface levels of PM<sub>2.5</sub> and PM<sub>10</sub> particulate matter impact on COVID-19 in Milan. Italy. *Sci. Total Environ.* 738, 1–12. <https://doi.org/10.1016/j.scitotenv.2020.139825>.
43. Brar, B., Marwaha, S., Poonia, A.K., Koul, B., Kajla, S., and Rajput, V.D. (2023). Nanotechnology: a contemporary therapeutic approach in combating infections from multidrug-resistant bacteria. *Arch. Microbiol.* 205, 62. <https://doi.org/10.1007/s00203-023-03404-3>.
44. Liu, T., Li, G., Liu, Z., Xi, L., Ma, W., and Gao, X. (2023). Characteristics of aerosols from swine farms: A review of the past two-decade progress. *Environ. Int.* 178, 108074. <https://doi.org/10.1016/j.envint.2023.108074>.
45. Silva, V., Araujo, S., Monteiro, A., Eira, J., Pereira, J.E., Maltez, L., Igrejas, G., Lemsaddek, T.S., and Poeta, P. (2023). *Staphylococcus aureus* and MRSA in Livestock: Antimicrobial Resistance and Genetic Lineages. *Microorganisms* 11, 1–15. <https://doi.org/10.3390/microorganisms11010124>.
46. Hong, P.Y., Li, X., Yang, X., Shinkai, T., Zhang, Y., Wang, X., and Mackie, R.I. (2012). Monitoring airborne biotic contaminants in the indoor environment of pig and poultry confinement buildings. *Environ. Microbiol.* 14, 1420–1431. <https://doi.org/10.1111/j.1462-2920.2012.02726.x>.
47. Brooks, J.P., McLaughlin, M.R., Scheffler, B., and Miles, D.M. (2010). Microbial and antibiotic resistant constituents associated with biological aerosols and poultry litter within a commercial poultry house. *Sci. Total Environ.* 408, 4770–4777. <https://doi.org/10.1016/j.scitotenv.2010.06.038>.
48. Just, N., Blais Lecours, P., Marcoux-Voiselle, M., Kirychuk, S., Veillette, M., Singh, B., and Duchaine, C. (2013). Bacterial diversity characterization of bioaerosols from cage-housed and floor-housed poultry operations. *Environ. Res.* 59, 46–50. <https://doi.org/10.1016/j.envres.2011.01.009>.
49. Castro, V.S., Carvalho, R.C.T., Conte-Junior, C.A., and Figueiredo, E.E.S. (2017). Shiga-toxin Producing *Escherichia coli*: Pathogenicity, Superspreading, Diagnostic Methods, Occurrence, and Foodborne Outbreaks. *Compr. Rev. Food Sci. Food Saf.* 16, 1269–1280. <https://doi.org/10.1111/1541-4337.12302>.
50. Stärk, K.D. (1999). The Role of Infectious Aerosols in Disease Transmission in Pigs. *Vet. J.* 158, 164–181. <https://doi.org/10.1053/tvj.1998.0346>.
51. Kaliste, E., Linnainmaa, M., Meklin, T., Torvinen, E., and Nevalainen, A. (2004). The bedding of laboratory animals as a source of airborne contaminants. *Lab. Anim.* 38, 25–37. <https://doi.org/10.1258/00236770460734362>.
52. Li, H., Yang, L., Shan, C., Qiu, D., Guo, J., and Gao, Y. (2017). The microbial growth and decline of fermented cow dung bedding for dairy cow. *J. Agri. Univer. Hebei* 40, 88–92. <https://doi.org/10.13320/j.cnki.jauh.2017.0040>.
53. Falcon-Rodriguez, C.I., Osornio-Vargas, A.R., Sada-Ovalle, I., and Segura-Medina, P. (2016). Aeroparticles, Composition, and Lung Diseases. *Front. Immunol.* 7, 1–9. <https://doi.org/10.3389/fimmu.2016.00003>.
54. Han, Y., Li, L., Wang, Y., Ma, J., Li, P., Han, C., and Liu, J. (2020). Composition, dispersion, and health risks of bioaerosols in wastewater treatment plants: A review. *Front. Environ. Sci. Eng.* 15, 38. <https://doi.org/10.1007/s11783-020-1330-1>.
55. Humbal, C., Gautam, S., and Trivedi, U. (2018). A review on recent progress in observations, and health effects of bioaerosols. *Environ. Int.* 118, 189–193. <https://doi.org/10.1016/j.envint.2018.05.053>.
56. Liu, J.F., and Ma, W.L. (2010). Characteristics of Microbial Aerosol Pollution in Pig Houses. *Anim. Husb. Feed Sci.* 2, 41–44. <https://doi.org/10.1016/j.plaphy.2006.09.008>.
57. Chinivasagam, H.N., and Blackall, P.J. (2005). Investigation and application of methods for enumerating heterotrophs and *Escherichia coli* in the air within piggery sheds. *J. Appl. Microbiol.* 98, 1137–1145. <https://doi.org/10.1111/j.1365-2672.2005.02560.x>.
58. Preller, L., Heederik, D., Kromhout, H., Boleij, J.S., and Tielen, M.J. (1995). Determinants of dust and endotoxin exposure of pig farmers: development of a control strategy using empirical modelling. *Ann. Occup. Hyg.* 39, 545–557. <https://doi.org/10.1093/annhyg/39.5.545>.
59. Verreault, D., Moineau, S., and Duchaine, C. (2008). Methods for Sampling of Airborne Viruses. *Microbiol. Mol. Biol. Rev.* 72, 413–444. <https://doi.org/10.1128/mmb.00002-08>.
60. Crosby, H.A., Kwiecinski, J., and Horswill, A.R. (2016). *Staphylococcus aureus* Aggregation and Coagulation Mechanisms, and Their Function in Host-Pathogen Interactions. *Adv. Appl. Microbiol.* 96, 1–41. <https://doi.org/10.1016/bs.aambs.2016.07.018>.
61. Rajput, P., Anjum, M.H., and Gupta, T. (2017). One year record of bioaerosols and particles concentration in Indo-Gangetic Plain: Implications of biomass burning emissions to high-level of endotoxin exposure. *Environ. Pollut.* 224, 98–106. <https://doi.org/10.1016/j.envpol.2017.01.045>.
62. Wang, Y., Fu, Y., Wang, C., and Wen, N. (2018). Dissimilar Emission Characteristics between Bioaerosol and Suspended Particles from Gaseous Biofilters and Bioaerosol Health Risk Evaluation. *Aerosol Air Qual. Res.* 18, 1874–1885. <https://doi.org/10.4209/aaqr.2017.11.0485>.
63. Han, Y., Yang, K., Yang, T., Zhang, M., and Li, L. (2019). Bioaerosols emission and exposure risk of a wastewater treatment plant with A<sup>2</sup>O treatment process. *Ecotoxicol. Environ. Saf.* 169, 161–168. <https://doi.org/10.1016/j.ecoenv.2018.11.018>.
64. Chmielowiec-Korzeniowska, A., Trawińska, B., Tymczyna, L., Bis-Wencel, H., and Matuszewski, Ł. (2021). Microbial contamination of the air in livestock buildings as a threat to human and animal health – a review. *Ann. Anim. Sci.* 21, 417–431. <https://doi.org/10.2478/aoas-2020-0080>.

65. Dungan, R.S. (2010). BOARD-INVITED REVIEW: Fate and transport of bioaerosols associated with livestock operations and manures. *J. Anim. Sci.* 88, 3693–3706. <https://doi.org/10.2527/jas.2010-3094>.
66. Sajid, M.S., Kausar, A., Iqbal, A., Abbas, H., Iqbal, Z., and Jones, M.K. (2018). An insight into the ecobiology, vector significance and control of *Hyalomma ticks* (Acari: Ixodidae): A review. *Acta Trop.* 187, 229–239. <https://doi.org/10.1016/j.actatropica.2018.08.016>.
67. Bunduruş, I.A., Balta, I., Ştef, L., Ahmadi, M., Peş, I., McCleery, D., and Corcionivoschi, N. (2023). Overview of Virulence and Antibiotic Resistance in *Campylobacter* spp. *Livestock Isolates. Antibiotics* 12, 1–19. <https://doi.org/10.3390/antibiotics12020402>.
68. Robyn, J., Rasschaert, G., Pasmans, F., and Heyndrickx, M. (2015). Thermotolerant *Campylobacter* during Broiler Rearing: Risk Factors and Intervention. *Compr. Rev. Food Sci. Food Saf.* 14, 81–105. <https://doi.org/10.1111/1541-4337.12124>.
69. Tomori, O., and Oluwayelu, D.O. (2023). Domestic Animals as Potential Reservoirs of Zoonotic Viral Diseases. *Annu. Rev. Anim. Biosci.* 11, 33–55. <https://doi.org/10.1146/annurev-animal-062922-060125>.
70. Ruzmir, A.V., Paunescu, I.A., Martis, S., Latcu, S., Novacescu, D., Bardan, C.R., Bob, F., Licker, M., Botoca, M., Cumpănas, A., and Bardan, R. (2022). Encrusted Uretero-Pyelitis Caused by *Corynebacterium urealyticum*: Case Report and Literature Review. *Diagnostics* 12, 2239. <https://doi.org/10.3390/diagnostics12092239>.
71. Forkel, S., Beutner, C., Schröder, S.S., Bader, O., Gupta, S., Fuchs, T., Schön, M.P., Geier, J., and Buhl, T. (2021). Sensitization against Fungi in Patients with Airway Allergies over 20 Years in Germany. *Int. Arch. Allergy Immunol.* 182, 515–523. <https://doi.org/10.1159/000512230>.
72. Sharpe, R.A., Bearman, N., Thornton, C.R., Husk, K., and Osborne, N.J. (2015). Indoor fungal diversity and asthma: A meta-analysis and systematic review of risk factors. *J. Allergy Clin. Immunol.* 135, 110–122. <https://doi.org/10.1016/j.jaci.2014.07.002>.
73. Muldoon, E.G., Strek, M.E., and Patterson, K.C. (2017). Allergic and noninvasive infectious pulmonary aspergillosis syndromes. *Clin. Chest Med.* 38, 521–534. <https://doi.org/10.1177/2324709623117544>.
74. Cao, L., Yang, L., Swanson, C.S., Li, S., and He, Q. (2021). Comparative analysis of impact of human occupancy on indoor microbiomes. *Front. Environ. Sci. Eng.* 15, 89. <https://doi.org/10.1007/s11783-020-1383-1>.
75. Rai, S., Singh, D.K., and Kumar, A. (2021). Microbial, environmental and anthropogenic factors influencing the indoor microbiome of the built environment. *J. Basic Microbiol.* 61, 267–292. <https://doi.org/10.1002/jobm.202000575>.
76. Amara, A.A., and El-Baky, N.A. (2023). Fungi as a Source of Edible Proteins and Animal Feed. *J. Fungi* 9, 73. <https://doi.org/10.3390/jof9010073>.
77. Green, C.F., Gibbs, S.G., Tarwater, P.M., Mota, L.C., and Scarpino, P.V. (2006). Bacterial Plume Emanating from the Air Surrounding Swine Confinement Operations. *J. Occup. Environ. Hyg.* 3, 9–15. <https://doi.org/10.1080/15459620500430615>.
78. Nasir, Z.A., Colbeck, I., Sultan, S., and Ahmed, S. (2012). Bioaerosols in residential micro-environments in low income countries: A case study from Pakistan. *Environ. Pollut.* 168, 15–22. <https://doi.org/10.1016/j.envpol.2012.03.047>.
79. Li, Y., Wang, X., Cao, G., WANG, Y., Miao, Q., and He, J. (2022). An Assessment of Airborne Bacteria and Fungi in the Female Dormitory Environment: Level, Impact Factors and Dose Rate. *Int. J. Environ. Res. Public Health* 19, 6642. <https://doi.org/10.3390/ijerph19116642>.
80. Wu, D., Zhang, Y., Qin, W., Tian, Y., Li, A., Hou, L., Hou, Y., Han, B., Wang, T., Xiong, J., and Gao, R. (2021). Assessment of seasonal variations in concentration, particle-size distribution, and taxonomic composition of airborne fungi in a courtyard space. *Atmos. Pollut. Res.* 12, 113–121. <https://doi.org/10.1016/j.apr.2020.10.014>.
81. Mia, M.M., Hasan, M., and Pory, F.S. (2022). Occupational exposure to livestock and risk of tuberculosis and brucellosis: A systematic review and meta-analysis. *One Health* 15, 100432. <https://doi.org/10.1016/j.onehlt.2022.100432>.
82. Rivera-Torres, J., Giron, N., and San Jose, E. (2023). COVID-19: A Comprehensive Review on Cardiovascular Alterations, Immunity, and Therapeutics in Older Adults. *J. Clin. Med.* 12, 1–20. <https://doi.org/10.3390/jcm12020488>.
83. Sigurdarson, S.T., and Kline, J.N. (2006). School Proximity to Concentrated Animal Feeding Operations and Prevalence of Asthma in Students. *Chest* 129, 1486–1491. <https://doi.org/10.1378/chest.129.6.1486>.
84. Zinatizadeh, M.R., Zarandi, P.K., Ghiasi, M., Kooshki, H., Mohammadi, M., Amani, J., and Rezaei, N. (2023). Immunosenescence and inflamm-ageing in COVID-19. *Ageing Res. Rev.* 84, 101818. <https://doi.org/10.1016/j.arr.2022.101818>.
85. Yan, C., Gui, Z.C., and Wu, J.T. (2021). Quantitative microbial risk assessment of bioaerosols in a wastewater treatment plant by using two aeration modes. *Environ. Sci. Pollut. Res. Int.* 28, 8140–8150. <https://doi.org/10.1007/s11356-020-11180-6>.
86. Han, Y., Yang, T., Yan, X., Li, L., and Liu, J. (2020). Effect of aeration mode on aerosol characteristics from the same wastewater treatment plant. *Water Res.* 170, 115324. <https://doi.org/10.1016/j.watres.2019.115324>.
87. Uhrbrand, K., Schultz, A.C., Koivisto, A.J., Nielsen, U., and Madsen, A.M. (2017). Assessment of airborne bacteria and noroviruses in air emission from a new highly-advanced hospital wastewater treatment plant. *Water Res.* 112, 110–119. <https://doi.org/10.1016/j.watres.2017.01.046>.
88. Mentese, S., Otkun, M.T., and Palaz, E. (2017). Comparison of dichloran rose bengal chloramphenicol and Sabouraud dextrose agar with cycloheximide and chloramphenicol for airborne mold sampling. *Aerobiologia* 33, 211–219. <https://doi.org/10.1007/s10453-016-9462-2>.
89. Wang, Y., Li, L., Xue, S., Han, Y., and Yang, K. (2019). Characteristics and formation mechanism of intestinal bacteria particles emitted from aerated wastewater treatment tanks. *Water Res.* 163, 114862. <https://doi.org/10.1016/j.watres.2019.114862>.
90. Andersen, A.A. (1958). New sampler for the collection, sizing, and enumeration of viable airborne particles. *J. Bacteriol.* 76, 471–484. <https://doi.org/10.1128/JB.76.5.471-484.1958>.
91. Yang, Y., Zhang, R., and Lou, Z. (2022). Bioaerosol emissions variations in large-scale landfill region and their health risk impacts. *Front. Environ. Sci. Eng.* 16, 158–159. <https://doi.org/10.1007/s11783-022-1593-9>.
92. Lu, R., Frederiksen, M.W., Uhrbrand, K., Li, Y., Østergaard, C., and Madsen, A.M. (2020). Wastewater treatment plant workers' exposure and methods for risk evaluation of their exposure. *Ecotoxicol. Environ. Saf.* 205, 111365. <https://doi.org/10.1016/j.ecoenv.2020.111365>.
93. Yang, T., Jiang, L., Cheng, L., Zheng, X., Bi, X., Wang, X., and Zhou, X. (2021). Characteristics of size-segregated aerosols emitted from an aerobic moving bed biofilm reactor at a full-scale wastewater treatment plant. *J. Hazard Mater.* 416, 125833. <https://doi.org/10.1016/j.jhazmat.2021.125833>.
94. Cao, C., Jiang, W., Wang, B., Fang, J., Lang, J., Tian, G., Jiang, J., and Zhu, T.F. (2014). Inhalable microorganisms in Beijing's PM<sub>2.5</sub> and PM<sub>10</sub> pollutants during a severe smog event. *Environ. Sci. Technol.* 48, 1499–1507. <https://doi.org/10.1021/es4048472>.
95. Cuervo-Sierra, J., Gómez-Almaguer, D., Jaime-Pérez, J.C., Martínez-Hernández, R.A., García Sepúlveda, R.D., Sánchez-Cárdenas, M., Ortiz-López, R., Ignacio-Ibarra, G., Muciño-Hernández, G., Arana-Trejo, R.M., et al. (2013). Prevalence of FLT3 Mutations in Acute Myeloid Leukemia: A Multicenter Latin America Study. *Blood* 122, 4979. <https://doi.org/10.1182/blood.V122.21.4979.4979>.
96. Dennis, K.L., Wang, Y., Blatner, N.R., Wang, S., Saadalla, A., Trudeau, E., Roers, A., Weaver, C.T., Lee, J.J., Gilbert, J.A., et al. (2013). Adenomatous polyps are driven by microbe-instigated focal inflammation and are controlled by IL-10-producing T cells. *Cancer Res.* 73, 5905–5913. <https://doi.org/10.1158/0008-5472.CAN-13-1511>.
97. Han, Y., Yang, T., Xu, G., Li, L., and Liu, J. (2020). Characteristics and interactions of bioaerosol microorganisms from wastewater treatment plants. *J. Hazard Mater.* 391, 122256. <https://doi.org/10.1016/j.jhazmat.2020.122256>.
98. Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., and Fierer, N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J.* 4, 1340–1351. <https://doi.org/10.1038/ismej.2010.58>.



## STAR★METHODS

## KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Bacteria	Rural environmental conditions	N/A
Fungi	Rural environmental conditions	N/A
Chemicals, peptides, and recombinant proteins		
MO-BIO Power Soil DNA Isolation Kit	Shenzhen Anbisheng Technology Co.	N/A
Deposited data		
original sequence	NCBI Sequence Read Archive database	SUB13041698
Software and algorithms		
PRIMER 7.0	Han, Y.P. (2020)	N/A
R	The R Project for Statistical Computing	<a href="https://github.com/danknights/sourcetracker/">https://github.com/danknights/sourcetracker/</a>

## RESOURCE AVAILABILITY

## Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Yunping Han ([yphan@rcees.ac.cn](mailto:yphan@rcees.ac.cn)).

## Materials availability

This study did not generate new unique reagents.

## Data and code availability

- Raw reads were deposited into the NCBI Sequence Read Archive database (Accession Number: SUB13041698) and are publicly available as of the date of publication.
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.
- This paper does not report original code.

## METHOD DETAILS

## Study sites

This study was conducted in rural areas of four cities in two provinces in Northwest China: Erdos, Hohhot, and Ulanqab in Inner Mongolia, and Xi'an in Shaanxi Province. [Figure 2](#) shows the specific location and type of livestock farming in the surveyed areas, and the abbreviations used for sampling points. Field surveys were conducted in autumn (September) and winter (December), 2022, to reflect the seasonal effects on the bioaerosol characteristics generated by livestock and poultry farming patterns in rural Northwest China. This study also measured microbial profiles in dwellings in agricultural areas during different seasons to assess the potential impact of rural livestock production processes on indoor environments. Finally, a site less influenced by external environmental factors was selected to measure the background concentrations in the village.

Meteorological parameters were determined during sample collection using portable instruments. Temperature, relative humidity and barometric pressure were monitored using a temperature and humidity monitor (LTP-202, Longtuo Instruments, China), wind speed was monitored using a portable anemometer (PH-1, Longtuo Instruments, China), and particulate matter concentration was monitored using a particulate matter sampler (JCH-120F, JuChuang, Qingdao, China). The detailed monitoring results are shown in [Table 1](#).

## Bioaerosol collection

Total culturable bacteria, fungi, and Enterobacteria were collected using an aerodynamic cut size of 7.0, 4.7, 3.3, 2.1, 1.1, and 0.65- $\mu\text{m}$  diameter Anderson six-stage impingement sampler (228–9530 K, SKC Gulf Coast Inc. USA) at a flow rate of 28.3 L/min for 3 min to allow airborne microorganisms for culture.<sup>86,87</sup> Total bacteria and fungi were collected using nutrient and Sabouraud mediums, respectively, and incubated at  $36 \pm 1^\circ\text{C}$  for 24–48 h and then at  $20^\circ\text{C}$  for 48–72 h.<sup>63,88</sup> In addition to total bacteria and fungi, the study focused on Enterobacteria, which



increase with human age and are consistently observed in patients with metabolic diseases.<sup>23</sup> Enterobacteria were collected using McConkey medium and incubated at  $36 \pm 1^\circ\text{C}$  for 24–48 h.<sup>86,89</sup> Three replicate samplings were performed at each livestock breeding site and microorganisms were collected simultaneously at all sites. Finally, the results of the six-stage Andersen impactor were processed using the positive pore correction method.<sup>90</sup> A total of 864 culturable samples were obtained.

A particulate sampler (JCH-120F, JuChuang, Qingdao, China) was used to collect total suspended particulate matter (TSPs) at a flow rate of 100 L/min. The sampling time was set to 8 h; TSPs were collected on the surface of a quartz membrane (90-mm diameter).<sup>91</sup> Cross-contamination was avoided by strict control of the environment and handling procedures during preparation, collection and preservation.<sup>92</sup> A total of 13 TSP samples were collected, including 8 samples from livestock breeding areas and 5 samples from living rooms. The collected quartz membranes were weighed and calculated to obtain the TSP concentration at the sampling sites.<sup>93</sup> Subsequently, the quartz membranes were cut and cleaned with sterile deionized water. A mixture of small enriched membranes with each sample was centrifuged at  $200 \times g$  for 3 h at  $4^\circ\text{C}$  and re-enriched using 0.22- $\mu\text{m}$  super polyethersulfone (PES) membranes for microbiological analysis.<sup>94</sup>

### Microbial analysis

DNA extraction was performed on the microbial enrichments that had undergone PES enrichment using an MO-BIO Power Soil DNA Isolation Kit. The DNA purity and mass concentration were measured by microspectrophotometry.<sup>95</sup> Bacterial and fungal amplifications were performed in three replicates using primers 338F/806R and 0817F/1196R, respectively; the bacterial amplification regions were the V3 and V4 regions of 16S rRNA, and the fungal amplification region was the ITS1 region of 18S rRNA.<sup>96–98</sup> High-throughput sequencing was performed using the Illumina Miseq PE300 platform (Illumina, San Diego, USA).

### Statistical analysis

Heatmaps show the species classification of bacteria and fungi among the dominant potentially pathogenic bacteria in bioaerosol samples from livestock and poultry farming areas. Non-parametric multidimensional statistical analysis (NMDS) retains the rank order of differences between sample pairs based on the rank of the sample two-by-two distance, with a greater distance between sample pairs indicating greater variability.

Source tracking of aerosols in livestock farming areas was performed using the source tracker in R (<https://github.com/danknights/sourcetracker/>), a statistical method based on Bayes' theorem for analyzing potential aerosol sources.<sup>30</sup>

## QUANTIFICATION AND STATISTICAL ANALYSIS

Appropriate statistical analyses were chosen depending on the experimental setting, number of replicates and type of data. In this study, heatmaps were constructed and NMDS analyses were performed using PRIMER 7.0 software.<sup>86</sup> Source tracking of aerosols in livestock farming areas was performed using the source tracker in R.<sup>30</sup> For the NMDS two-dimensional analyses, it is usually considered to have some explanatory significance when  $\text{stairstress} < 0.2$ ; when  $\text{stairstress} < 0.1$ , it can be considered to be a good ordering; and when  $\text{stairstress} < 0.05$ , it is well represented.