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Research Article

Occupational Exposure to Mycotoxin-Producing Fungi in Respirable and Surface Dust of Farms and Feed Factories Located in the State of São Paulo, Brazil: A Pilot Study

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Abstract

In the present study, a preliminary investigation was conducted on the occurrence of mycotoxin-producing fungi in respirable air and surface dust from farms (N = 4) and feed factories (N = 3) located in the state of São Paulo, Brazil. The main genera detected were *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., and *Cladosporium* sp. *Aspergillus* sp. was identified in more than 50% of samples analyzed. The values of colony forming units (CFU) were higher in feed factories than in farms, with a maximum of 7.10 Log CFU/m^2. In samples of respirable dust, average values were 3.12 Log CFU/m^3 in animal feed factories and 2.95 Log CFU/m^3 in farms. The study demonstrated that the levels of mycotoxin-producing fungi found in the work environments studied may be of concern to the health of the workers.

Keywords: Fungi; Occurrence; Workplace; Breathable Dust; Settled Dust

Abbreviations

CFU: Colony-Forming Unit; NSF: Non-Sporulating Fungus

Introduction

Fungi contamination of workplaces has attracted considerable attention in the last decades, considering the health concerns regarding de human exposure to pathogeinic and toxigenic species. Previous studies have shown the occurrence of occupational exposure to mycotoxigenic fungi in rural workers from several countries, mainly among individuals involved in animal production, especially pig farmers and poultry breeders, evidencing high rates of asthma and inflammatory manifestations in the respiratory system [1-4]. In recent years, animal management techniques start to involve closed facilities, densely packed with animals, resulting in indoor air with high levels of dust, gases, microbes, and microbial metabolites, which increase potential health risks [5,6]. Cote., *et al.* [7] presented experimental evidence that, in humans, inhalation of fungal spores can cause asthma and rhinitis, while dust contaminated with mycotoxins had immunosuppressive effects. In addition, epidemiological studies have revealed high frequency of liver, lung, and biliary tumors in workers handling food contaminated with mycotoxins [8]. Thus, inhaling dust contaminated with certain mycotoxins can be very harmful to the health of workers and can potentiate adverse effects by means of combined exposure to multiple mycotoxins [9].

The main genera of mycotoxin-producing fungi are *Aspergillus* sp., *Fusarium* sp., and *Penicillium* sp. [10]. Aflatoxins are mainly produced by *A. flavus*, *A. parasiticus*, and *A. nomius* [8]. Fumonisins are produced by *F. verticillioides*, *F. proliferatum*, and *A. niger*, usually [11]. Trichothecenes and zearalenone are produced by *F. graminearum* and *F. culmorum* [12], and ochratoxin may be produced by *A. ochraceus*, *A. niger*, *A. carbonarius*, and *P. verrucosum* [13]. Recently, studies have demonstrated possible occupational exposure to mycotoxins resulting from the inhalation of contaminated dust in work environments in several countries [14-17]. However, to our knowledge, this issue has never been addressed in rural working environments in Brazil.

Aim of the Study

The present study aimed to conduct a preliminary investigation on the presence of mycotoxin-forming fungi genera in respirable air and surface dust from Brazilian farms and feed factories.

Material and Methods

Four sampling procedures were conducted in 7 locations in the state of São Paulo - Brazil, totaling 4 farms (2 poultry-producing farms, 1 pig-producing farm, and 1 dairy farm), and 3 feed factories. Sample collections started in October 2020 and ended in May of the following year. A total of 78 samples were collected, 26 of respirable dust and 52 surface swabs. Collection of breathable dust samples was performed with particles of size from 0.5 to 10 µm (PM0.5 a PM10), as described by Viegas., *et al*. (2013a, 2013b), with modifications. This is in accordance with the sampling procedures as recommended by Vincent and Mark [18], who indicated that respirable dust is a fraction of the dust transported by air that reaches gas exchange regions in the lungs, with aerodynamic diameter of less than 7 µm.

For collection of breathable dust, an Accura-2 Criffer air sampling pump was used, accompanied by a support for a membrane cassette with a flow rate of 2.50 L/min placed at a height close to the nasal region of the workers. Collection was carried out continuously for 8 hours. Fungal contamination of samples was analyzed on Dicloran Rose Bengal Chloramphenicol (DRBC) agar plates at dilutions of 10^{-1} and 10^{-2}. Surface dust samples were collected by rubbing the surfaces using a sterile dehydrated cellulose sponge with buffer solution, in accordance with the International Standard ISO 18593-2004 (ISO, 2004). Smears were placed in DRBC agar plates; 100 mL of peptone water were added to the sponge and stirred for a minute in a homogenizer. Dilutions of 10^{-2}, 10^{-3} and 10^{-4} were made for plate analysis.

All DRBC agar plates of collected breathable and surface dust samples were incubated at 27°C for 10 days. After incubation, results were determined, both quantitatively (in colony forming units, CFU/m^3, CFU/m^2) and qualitatively, with identification of the fungi genera of fungus. All colonies were subcultured on potato dextrose agar (Oxoid, Basingstoke, UK) at 25°C for 7 days. Fungus identification was performed by the classical method using macroscopic and microscopic characteristics [19]. Hyaline fungi were colored by lactophenol cotton blue and observed in a light microscope (400x).

Results and Discussion

Table 1 presents the main fungi identified in the four samplings from farms and feed factories located in the state of São Paulo, Brazil. High concentrations of fungi were detected on surface and breathable dust from both sampling sites. Average count found on the surface dust of feed factories (5.99 Log CFU/m^2) was higher than in farms (5.26 Log CFU/m^2). However, maximum values detected were very similar, with 7.10 Log CFU/m^2 and 7.04 Log CFU/m^2 in feed factories and farms, respectively. In samples of respirable dust, mean values of CFU observed in factories (3.12 Log CFU/m^3) were slightly higher than in farms (2.95 Log CFU/m^3). However, maximum counts presented in Farms were greater, with 4.73 Log CFU/m^3. The main fungi found throughout the samples were from the genera *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Cladosporium* sp., as well as yeasts.

The high frequency of *Cladosporium* sp. found in the present study was also observed by Despot and Klarić [20] in air samples from grain mills in Croatia. In a study on occupational exposure in Irish bakeries and a pizza restaurant, the most common genera identified were similar to those found in the present study: personal air samples showed *Penicillium* sp. (91.1%), *Aspergillus* sp. (4.8%), and *Cladosporium* sp. (4.1%), and settled dust samples showed *Penicillium* sp. (88.3%), *Aspergillus* sp. (7.8%), and *Chrysosporium* sp. (2.9%) [17].

The highest frequency of the genus *Aspergillus* in farms and feed factories observed in our study is agreement with the findings by Viegas., *et al.* [21], who also most frequently identified the genus *Aspergillus* in pig farms (40.5%), at high concentrations (> 2,000 CFU/m^3). A study of respiratory filters of protection devices among

Type of fungus	Percentage of positive samples			
	Feed factories (N = 3)		Farms (N = 4)	
	Respirable dust samples (N = 12)	Surface dust samples (N = 24)	Respirable dust samples (N = 14)	Surface dust samples (N = 28)
Aspergillus sp.	67	67	64	79
Fusarium sp.	50	54	36	79
Cladosporium sp.	58	75	64	82
Penicillium sp.	16	29	29	39
Rhizopus sp.	16	38	7	21
Trichothecium sp.	0	4	0	7
Trichoderma sp.	0	0	0	7
Mucor sp.	0	0	0	4
NSF	67	38	28	21
Yeasts	16	71	21	64
Average count (range), Log_{10} CFU/m^3	3.12 (2.00 - 3.98)	5.99 (4.04 - 7.10)[1]	2.95 (2.30 - 4.73)	5.26 (3.30 - 7.04)[1]

Table 1: Main fungi detected in respirable dust and surface dust samples in four samplings conducted in feed factories and farms in the state of São Paulo, Brazil.

[1]Log10 CFU/m^2.

N: Number of Total Samples Collected; CFU: Colony-Forming Units; NSF: Non-Sporulating Fungus.

workers from a waste sorting industry detected *Aspergillus* sp. most frequently, with maximum counts of 208,500 CFU/m^2 (5.32 LogCFU/m^2) [22]. Air samples from poultry farms in Portugal showed 40.5% *Scopulariopsis brevicaulis*, 30% *Rhizopus* sp., 10.1% *Penicillium* sp., and 9.7% *Aspergillus* sp. and samples from surfaces in the same places showed 47.9% *Aspergillus* sp., 15.3% *Fusarium* sp., and 8.8% *Penicillium* sp. [23].

High frequency of *Aspergillus* sp. is a health concern for the workers, since this genus is the most common producer of aflatoxins [10], which is among the most toxic mycotoxins for humans due to its genotoxic and carcinogenic potential [24]. In addition, species from the genus *Aspergillus* can produce ochratoxin A and fumonisins, both classified as possible carcinogens to humans [24]. Furthermore, *Fusarium* sp. and *Penicillium* sp. are also important producers of mycotoxins, such as fumonisins, ochratoxin A, zearalenone and deoxynivalenol, among other mycotoxins [8,11-13].

Fungi counts reported in the present work were well below those reported in environmental samples collected in places that do not handle grains or animal feed, including Irish bakeries [17], a veterinary practice in Krakow [25], a waste-sorting plant in Poland [26], a flour mill in Egypt [27], and apartments and basements in Croatia [20]. However, in air samples from grain mills in Croatia, fungi counts were close to those found in the present study, ranging from 1,700 to 40,000 CFU/m^3 (3.23 to 4.6 Log CFU/m^3) [20]. Counts above 3.30 Log CFU/m^3 or m^2 were also identified in pig farms and waste sorting industries [21,22]. These findings, taken together, confirm the presence of large amounts of fungi in environments where grains and animal feed are handled in Brazil, raising concerns about the risk posed to the health of workers.

Conclusion

The present study shows a high presence of fungi producers of mycotoxins on farms and feed factories in São Paulo, Brazil. However, the presence of fungi does not necessarily mean the presence of mycotoxins in the environment, thus this is exactly the reason further studies are necessary. Further studies should evaluate the levels of mycotoxins produced by the commonly occurring species from the fungi genera detected in the environments of the studied workplaces. Furthermore, in these workplaces, the use of protective equipment should be mandatory to avoid possible contamination and inflammatory reactions in the respiratory system.

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Competing interests

The authors declare that there is no conflict of interest.

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