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Occupational Exposure to Aflatoxin B$_1$ in Swine Production and Possible Contamination Sources

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Although the adverse health consequences of ingestion of food contaminated with aflatoxin B1 (AFB1) are known, relatively few studies are available on the adverse effects of exposure in occupational settings. Taking this into consideration, our study was developed aiming to elucidate the possible effects of occupational exposure to AFB1 in Portuguese swine production facilities using a specific biomarker to assess exposure to AFB1. In total, 28 workers participated in this study, providing blood samples, and a control group ($n = 30$) was composed of subjects without any type of agricultural activity. Fungal contamination was also studied by conventional methods through air, surfaces, and new and used floor coverage. Twenty-one workers (75%) showed detectable levels of AFB1 with values ranging from $< 1$ ng/ml to 8.94 ng/ml and with a mean value of 1.91 ± 1.68 ng/ml. In the control group, the AFB1 values were all below 1 ng/ml. Twelve different Aspergillus species were identified. Aspergillus versicolor presented the highest airborne spore counts (3210 CFU/m$^3$) and was also detected in higher values in surfaces (>300 CFU/cm$^2$). Data indicate that exposure to AFB1 occurs in swine barns, and this site serves as a contamination source in an occupational setting.

Swine confinement buildings are prone to contamination with fungi and their metabolites (Attwood et al., 1987). Studies normally performed in swine farms involve high dust aerosolization (Kim et al., 2008), and consequently result in wide spread of fungi and their metabolites, such as volatile organic compounds and mycotoxins (Milner, 2009; Tsapko et al., 2011; May et al., 2012).

Aflatoxin B1 (AFB1) is the most prevalent aflatoxin, usually found in cases of aflatoxicosis, and is responsible for acute toxicity, chronic toxicity, carcinogenicity, teratogenicity, genotoxicity, and immunotoxicity (Lizárraga-Paulín et al., 2011). This mycotoxin is activated by cytochromes P-450 to AFB1-$8,9$-exo-epoxide and AFB1-$8,9$-endo-epoxide, but it is the exo-epoxide that binds to DNA to form the predominant 8,9-dihydro-8-(N$^7$-guanyl)-9-hydroxy-AFB1 (AFB1-$N^7$-Gua) adduct (Iyer et al., 1994). AFB1-$N^7$-Gua is the metabolite that confers the mutagenic properties of the compound (Gopalakrishnan et al., 1990). Although investigations concerning food and feed contamination by AFB1 were reported, only a small number of studies examined...
mycotoxins exposure consequences in occupational settings (Halstensen, 2008). Under most conditions, the level of exposure to mycotoxins due to inhalation is lower compared to oral intake. However, it is important to consider that workers can be occasionally exposed to high airborne levels of mycotoxins during certain operations (Halstensen et al., 2004; Mayer et al., 2008; Degen, 2008; Cleave et al., 2010; Harting et al., 2012; May et al., 2012).

Inhalation of certain mycotoxins may be more harmful than oral exposure and combined exposure to multiple mycotoxins may occur, which may enhance adverse effects (Degen, 2011). Our knowledge in understanding the potential effects of inhaled mycotoxins is based essentially on animal studies and in vitro investigations, and extrapolation of such findings to humans is complex and needs to be viewed with caution (Mayer et al., 2008).

Epidemiological data noted that pulmonary exposure to AFB1 was associated with an increase in lung tumor incidence (Hayes et al., 1984). More recently, Yanga and colleagues (2012) demonstrated that AFB1 is activated in the lung by CYP2A13, subsequently to form AFB1–DNA adducts, and induced cytotoxicity and apoptosis via the mitochondrial signaling pathway. Considering the lack of studies on occupational exposure to mycotoxins in agricultural settings, the carcinogenic potential of AFB1, and recently confirmed occupational exposure to AFB1 in poultry farms being reported (Viegas et al., 2012), this study was developed aimed to assess AFB1 exposure in workers from swine production farms in Portugal.

MATERIALS AND METHODS

Detection of AFB1 in Serum of Swine Workers

In order to assess occupational exposure to AFB1, a biomarker of internal dose that measures AFB1 in serum was used in order to provide data regarding recent exposure to AFB1 and also its level of intensity. This approach may be useful for rapid screening of samples for acute exposures and also reflects chronic exposure in a way that is not available from other markers such as the aflatoxin–N7-guanine adduct in urine (Leong et al., 2012).

Twenty-eight workers from seven swine farms studied were enrolled in this study. As no apparent information was available on AFB1 background levels for the Portuguese population, a control group (n = 30) was included. This group was composed of subjects who conducted administrative tasks in an educational institution without any type of agricultural exposure. All participants signed a consent form and were provided with the study protocol.

Blood Sample Preparation

All blood samples were subjected to centrifugation to obtain serum stored at –20°C until analysis. Five hundred microliters of serum was incubated for 18 h at 37°C with pronase (Calbiochem, 50 U per 5 mg protein) before application to pre-wet C18 column (RIDA C18 column, R-Biopharm). The column was washed with 5 ml 5% methanol to remove small peptides and amino acids. The fraction containing aflatoxin was eluted with 80% methanol, which was posteriorly evaporated under a nitrogen stream and diluted to reach a 10% methanol solution. The eluate was then applied to an immunoaffinity aflatoxin column (Easi-Extract Aflatoxin; R-Biopharm) and the aflatoxin-containing fraction was eluted with 1 ml methanol in phosphate buffer 0.1 M, pH 7.4 (1:1), after rinsing the column with phosphate-buffered saline (PBS).

ELISA Assay

For AFB1 quantification, the RIDASCREEN Aflatoxin B1 30/15 enzyme-linked immunosorbent assay (ELISA; R Biopharm) was used, and was calibrated with aflatoxin standards from 1 to 50 ng/ml. Values below 1 ng/ml were considered nondetectable since these are below the detection limit. For testing, samples or standards were pipetted into the wells already coated with capture antibodies directed against
anti-aflatoxin. Prior to the addition of AFB1-antibody solution, AFB–enzyme conjugate was added. After 30 min of incubation, the wells were washed 3 times. Indicator color was obtained by adding a substrate/chromogenic solution to each well and the reaction was stopped after 15 min with a termination solution. Absorbance was measured at 450 nm and results were assessed with Ridasolf Win software version 1.73 (R Biopharm).

**Determination of Environmental Fungal Contamination in Swine Farms**

Fungal contamination was studied by conventional methods through air, surfaces, and new and used floor covering (Table 1). Air samples from 7 swine farms were collected at 140 L/min, at 1 m height, onto malt extract agar supplemented with chloramphenicol (MEA). Air collection was also performed outside premises, since this is the place regarded as the reference. Surfaces samples of the same indoor sites were collected by swabbing the surfaces using a 10 by 10 cm square stencil disinfected with 70% alcohol solution between samples according to the International Standard ISO 18593–2004. The obtained swabs were then plated onto MEA.

Samples from floor coverage were also collected from four of seven swine farms. After lab processing and incubation of the collected samples, quantitative (colony-forming units [CFU]: CFU/m³, CFU/cm², and CFU/g) and qualitative results were obtained, with identification of the isolated fungal species.

For species identification, microscopic observations were performed using tease mount or Scotch tape mount and lactophenol cotton blue mount procedures. Morphological identification was achieved through macro- and microscopic characteristics according to Hoog et al. (2002).

**Statistical Analysis**

The Mann–Whitney test was applied to compare the two study groups: swine workers and controls. SPSS (Statistical Package for Social Sciences) 21.0 was used to perform all the statistical analysis.

**RESULTS**

**Aflatoxin B₁ in Serum of Swine Workers**

Twenty-one workers (75%) showed detectable levels of AFB₁ with values ranging from <1 ng/ml to 8.94 ng/ml and with a mean value of 1.91 ± 1.68 ng/ml (Table 2). In the control group, the AFB₁ values were all below 1 ng/ml. Since the AFB₁ result is not a pure quantitative variable, it is considered an ordinal variable, which is the basis for using the nonparametric Mann–Whitney test to compare concentrations of AFB₁ between the two groups. When the concentration was less than 1 ng/ml, this was considered non-detectable. Significantly higher concentrations of mycotoxin were found in swine workers compared to controls.

**Environmental Fungal Contamination**

In the seven swine farms studied, _Aspergillus versicolor_ presented the highest indoor spore counts (>2000 CFU/m³) and highest overall prevalence (40.5%), followed by _Scopulariopsis brevicaulis_ (17%) and _Penicillium_ sp. (14.1%) (Table 3). _Aspergillus versicolor_ was also among the most frequent species in surfaces and new floor coverage. Within the _Aspergillus_ genus, _A. versicolor_ presented the highest airborne spore counts.

<table>
<thead>
<tr>
<th>Swine farms</th>
<th>Number of air samples</th>
<th>Number of surfaces samples</th>
<th>Number of floor covering samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12</td>
<td>10 (6 from floor and 4 from walls)</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>11</td>
<td>10 (from walls)</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>7 (from walls)</td>
<td>—</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>7 (from walls)</td>
<td>1</td>
</tr>
<tr>
<td>E</td>
<td>6</td>
<td>5 (from walls)</td>
<td>—</td>
</tr>
<tr>
<td>F</td>
<td>7</td>
<td>6 (from walls)</td>
<td>—</td>
</tr>
<tr>
<td>G</td>
<td>4</td>
<td>3 (from walls)</td>
<td>2</td>
</tr>
</tbody>
</table>
TABLE 2. Results of Mann–Whitney Test to Compare AFB1 Between Swine Workers and Control Group

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Range values and median</th>
<th>Mean rank</th>
<th>Mann–Whitney U</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB1 (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swine workers</td>
<td>28</td>
<td>&lt;1–8.9 1.91</td>
<td>18.50</td>
<td>90</td>
<td>−5.89</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>&lt;1</td>
<td>41.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 3. Fungi Most Frequently Identified at Swine Farms

<table>
<thead>
<tr>
<th>Air</th>
<th>Frequency (%)</th>
<th>Surfaces</th>
<th>Frequency (%)</th>
<th>New floor covering</th>
<th>Frequency (%)</th>
<th>Used floor covering</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. versicolor</td>
<td>40.5</td>
<td>A. versicolor</td>
<td>26.6</td>
<td>Chrysosporium sp.</td>
<td>38.5</td>
<td>Mucor sp.</td>
<td>25.1</td>
</tr>
<tr>
<td>S. brevicaulis</td>
<td>17.0</td>
<td>Cladosporium sp.</td>
<td>22.4</td>
<td>Trichoderma sp.</td>
<td>28.0</td>
<td>Geotrichum sp.</td>
<td>20.6</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>14.1</td>
<td>S. brevicaulis</td>
<td>17.5</td>
<td>A. versicolor</td>
<td>14.0</td>
<td>Trichoderma sp.</td>
<td>18.3</td>
</tr>
<tr>
<td>Others</td>
<td>28.4</td>
<td>Others</td>
<td>33.5</td>
<td>Acremonium sp.</td>
<td>14.0</td>
<td>Others</td>
<td>36.0</td>
</tr>
</tbody>
</table>

Note. Adapted from Viegas et al. (2013).

FIGURE 1. A. versicolor distribution in the studied swine farms.

(>2000 CFU/m³) and highest overall prevalence (41.9%), followed by A. flavus and A. fumigatus (8.1%). This species presented as a differing distribution (CFU/m³ and CFU/m²) at the seven swine farms analyzed (Figure 1).

DISCUSSION

According to L’etourneau and colleagues (2010), the presence of species like A. versicolor is sufficient to raise the risk of acquiring mycotoxicosis. Considering data in this study on fungal contamination and the presence of elevated concentrations of A. versicolor detected in swine farms through environmental sampling, this findings raises concerns that the workers of this occupational setting present are at increased risk for mycotoxicosis (L’etourneau et al., 2010). Data obtained showed 75% of the blood samples from swine farm workers displayed elevated AFB1 levels and all individuals from the control group showed no detectable AFB1 levels. Since no information on population background levels of AFB1 is available in Portugal, this control group was included, enabling us to presume exclusion of exposure by diet of the swine workers.
There is currently little requirement for manual work in the systems of pig production because most of the procedures needed in confinement pig houses are done automatically. In Portugal, however, there are some activities that still need farmers’ intervention, such as cutting piglets’ tails and vaccination. Because of that, there is an increase in time spent in those places and, consequently, intensification of exposure to particulate matter (PM) (Viegas et al., 2013) and to AFB1. These may contribute to the higher levels of AFB1 obtained in swine workers compared to poultry workers, who normally spend less time near the animals and within the pavilions (Viegas et al., 2012). In addition, this occupational setting may be related to high PM aerosolization, enhancing exposure to this mycotoxin (Kim et al., 2008). The characteristic of dust acting as a carrier of AFB1 to the breathing zone and mouth has been well documented in other studies (Astrup et al., 1999; Brera et al., 2002). This was affirmed by the fact of PM was characterized in a range (5–10 µm) that may not be respirable, thus promoting exposure probably via the oral route rather than directly by inhalation. The PM characteristics in the swine farms studied (Viegas et al., 2013) and data published in other articles (McDonnell et al., 2008; Kim et al., 2008) support the same conclusion that exposure is by both the inhalation and oral routes.

Concerning fungal contamination data, some of the fungal isolates more frequently identified in air belong to A. versicolor, followed by Scopulariopsis brevicaulis and Penicillium sp. Similar results were reported by Jo and Kang (2005), where Aspergillus sp. and Penicillium sp. were also the most frequently found in swine barn air, and by Duchaine and colleagues (2000) with Scopulariopsis, Aspergillus, and Penicillium the most frequently recovered in summer and winter. It is noteworthy that sampling was taken only on one day in each farm, and variations in fungal contamination are expected. Nevertheless, the prevalent genera noted are common in several studies (Jo and Kang, 2005; Duchaine et al., 2000). All seven swine farms showed high counts of A. versicolor in air and on surfaces, with surfaces less susceptible to variation to fungal concentration than air, since it is harder to deactivate spores on surfaces than in the air (Burton et al., 2008). This may account for less variation regarding fungal contamination in this setting.

Sterigmatocystin, a mycotoxin produced by A. versicolor, is not cytotoxic by itself, but, similar to AFB1, becomes carcinogenic after activation in the liver by the cytochrome P-450 monooxygenase (McConnell and Garner, 1994; Nielsen, 2003). In addition to these toxic properties, sterigmatocystin also acts as a potent inhibitor of tracheal ciliary movement (Nielsen, 2003). Consequently, exposure to multiple mycotoxins needs to be considered and interactions may occur, modifying and potentiating adverse health effects.

There are specific sources of AFB1 in this setting that need to be considered. High contamination of swine feed has been well documented (Kabak et al., 2007; Simas et al., 2007; Pereyra et al., 2011; Gerbaldo et al., 2011; Gowda et al., 2013; Asurmendi et al., 2013). This may result in direct exposure of workers when they handle the feed that is given to the animals. Further, the production of this mycotoxin is released during feed harvesting, storage, and/or transport, due to the high temperature and humidity (Abramson et al., 1999; Cotty and Jaime-Garcia, 2007; Gerbaldo et al., 2011; Pereyra et al., 2011). A recent study of 53 Danish swine farms demonstrated that handling of feeding materials was the task that resulted in higher exposure to PM and increased the exposure by 17% (Basinas et al., 2013). Further, mycotoxins may be present in the environment or feed long after death and disintegration of the producer (Halstensen 2008; Alborch et al. 2011). This might explain how swine workers demonstrated high levels of AFB1 exposure while the fungal load produced by A. flavus was low in the swine farms considered.

Taking into consideration all aspects regarding exposure—high exposure occurs during some specific tasks, is related to different factors, and AFB1 is a genotoxic carcinogen—it would seem appropriate to apply the ALARA
principle, which implies keeping exposure to carcinogenic substances at the lowest achievable level (ALARA = as low as reasonably achieved). This may be accomplished through some specific measures: (1) Perform regular survey of toxinogenic strains and aflatoxins to identify and assess contamination sources (feed, air, litter, etc.); (2) monitor potential indoor sources of fungal contamination and spread; and finally, (3) define health surveillance programs oriented for swine workers to detect health changes that may be related with this specific risk factor.

CONCLUSIONS

Data indicate that exposure to AFB1 occurs in swine barns and may be related to different causes and contamination sources. Preventive and protective measures need to be developed to avoid exposure to this carcinogetic agent.

REFERENCES


