Occupational exposure to Aflatoxin B_1 in Portuguese swine farms

Susana Viegas[1]; Luisa Veiga[1]; Paula Figueredo[1]; Ana Almeida[1]; Elisabete Carolino[1]; Carla Viegas[1]
[1] ESTeSL-IPL, Portugal

1. INTRODUCTION

Aflatoxins were first isolated about 40 years ago after outbreaks of disease and death in turkeys (Williams et al., 2004) and cancer in rainbow trout (Rucker et al., 2002; Williams et al., 2004) fed with rations formulated from peanut and cottonseed meals. These toxins are secondary metabolites produced under certain conditions of temperature, pH and humidity predominantly by *Aspergillus flavus* and *Aspergillus parasiticus* fungi species (Bhatnagar et al., 2006).
Among 18 different types of aflatoxins identified, major members are aflatoxin B_1, B_2, G_1 and G_2. Aflatoxin B_1 (AFB_1) is normally predominant in cultures as well as in food products. AFB_1 was shown to be genotoxic and a potent hepatocarcinogen (IARC, 1993; Dash et al., 2007). This mycotoxin is metabolized by the mixed function oxidase system to a number of hydroxylated metabolites including the 8,9-epoxide. The latter is considered to be the ultimate carcinogen that reacts with cellular deoxyribonucleic acid (DNA) and proteins to form covalent adducts (Autrup et al., 1991; Richard, 1998; Brera et al., 2002; Dash et al 2007).
In 1987, the International Agency for Research on Cancer concluded that there was sufficient evidence for carcinogenicity of naturally occurring aflatoxins in humans (IARC, 1987). This conclusion was reaffirmed in two subsequent re-evaluations (IARC 1993, 2002), based upon results from several cohort studies in China and Taiwan that reported associations between biomarkers for aflatoxin exposure and primary liver-cell cancer.
Occupational exposure to this mycotoxin may occur by inhalation of dust generated during the handling and processing of contaminated crops and feeds. Therefore, farmers and other agricultural workers present a higher risk for occupational exposure due to airborne aflatoxin via inhalation of dust (Flannigan and Gillian, 1996; Ghosh et al., 1997; Brera et al., 2002).
To confirm exposure, mycotoxins and/or mycotoxin metabolites may be detected in biological samples using biomarkers (Hooper et al., 2008).
Swine production is known to be an occupational setting that involves high occupational exposure to particulate matter and fungi (Donham et al., 1989; Vogelzang et al., 2000; Duchaine et al., 2000; Kim et al., 2007; Kim et al., 2008). Thus it is conceivable that swine production workers are exposed via inhalation to aflatoxins. The aim of this study was to determine whether swine workers in Portugal were exposed to aflatoxin (AFB_1).

2. MATERIALS AND METHODS

This study was carried out in 7 swine farms located at the district of Lisbon, Portugal, between January and May 2011. The pig buildings investigated in this research were all classified as the manure removal system where manure can be removed from the pig building completely several times a day. In some swine's places, such as in the maternity and where the males were confine, the floor is cover with straw or journal paper.
The ventilation modes of the pig buildings were mechanical ventilation by wall exhaust fans and natural ventilation by operation of a winch-curtain.
Blood samples were collected from a total of 11 workers. In addition, a control group (n=25) was included that conducted administrative tasks in an educational institution without any type of agricultural activity. All subjects were provided with the protocol and signed a consent form.
For quantification of AFB_1 the RIDASCREEN®Aflatoxin B1 30/15 ELISA (R®Biopharm) was used. The assay is calibrated with aflatoxin standards ranging from 1 to 50 ng/ml. Values below 1 ng/ml are not detectable since these are below the detection limit. Absorbance was measured at 450 nm and results assessed with Ridasolf Win software version 1.73 (R®Biopharm).
Statistical analysis was performed with SPSS for Windows statistical package, version 17.0.

3. RESULTS AND DISCUSSION

Six workers (54.5%) had detectable levels of AFB_1 (values ranging between <1 ng/ml and 8.94 ng/ml, with a mean value of 1.61 ng/ml). In the control group, the AFB_1 values were all below 1 ng/ml. However, significant differences were not found between workers and the control group (Kruskall-Wallis test; p=0.723).
In the present study a biomarker of internal dose was used providing information regarding recent exposure to AFB_1 and its intensity. Therefore, the results obtained based on AFB_1 quantification are related to intensity of environmental contamination and absorption rates (Zhang et al., 2003). The findings corroborate the hypothesis that occupational exposure to AFB_1 by inhalation occurs in swine production.
Mycotoxins are not volatile but when found in respirable air are associated with mold spores or particulates (Robbins et al., 2004; Bush et al., 2006) and therefore, in occupational settings the preferential route of exposure to AFB_1 is through inhalation.
Among all aflatoxins, the AFB_1 is the most potent hepatocarcinogenic substance known, recently after a thorough risk evaluation, it has been proven to be also genotoxic (Van Egmond and Jonker, 2004; Zain, 2011; Ferrante et al.,2012).
For this type of carcinogen, it is normally consider that there is no threshold dose below which no tumour formation will occur. In other words, only a zero level of exposure will result in no risk (Ferrante et al., 2012).
Is also important to consider that there are sufficient experimental and epidemiological data to suggest that the lung is, in addition to the liver, a target for AFB_1 (Dvorackova and Pichova, 1986; Donnelly et al., 1996; Oyelami et al 1997; Massey et al., 2000).

Additionally, is necessary to take in account that, in this occupational setting, a potential exposure to more than one mycotoxin, since in addition to *A. flavus*, other fungal species recognize as mycotoxin producers were found (to be publish elsewhere). Therefore, the effects of possible interactions need to be considered in the risk assessment process (Sexton and Hattis, 2007; Ferrante et al., 2012).

4. CONCLUSIONS

Results obtained suggest that exposure to AFB_1 by inhalation occurs and represents an additional risk in this occupational setting that need to be recognized, assessed and, most important, prevented.

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