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Oral exposure to culture material extract containing fumonisins predisposes swine to the development of pneumonitis caused by *Pasteurella multocida*

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Abstract

Fumonisin B_1 (FB_1) is a mycotoxin produced by *Fusarium verticillioides* and *F. proliferatum* that commonly occurs in maize. In swine, consumption of contaminated feed induces liver damage and pulmonary edema. *Pasteurella multocida* is a secondary pathogen, which can generate a respiratory disorder in predisposed pigs. In this study, we examined the effect of oral exposure to fumonisin-containing culture material on lung inflammation caused by *P. multocida*. Piglets received by gavage a crude extract of fumonisin, 0.5 mg FB_1/kg body weight/day, for 7 days. One day later, the animals were instilled intratracheally with a non toxin producing type A strain of *P. multocida* and followed up for 13 additional days. Pig weight and cough frequency were measured throughout the experiment. Lung lesions, bronchoalveolar lavage fluid (BALF) cell composition and the expression of inflammatory cytokines were evaluated at the autopsy. Ingestion of fumonisin culture material or infection with *P. multocida* did not affect weight gain, induced no clinical sign or lung lesion, and only had minimal effect on BALF cell composition. Ingestion of mycotoxin extract increased the expression of IL-8, IL-18 and IFN-γ mRNA compared with *P. multocida* infection that increased the expression of TNF-α. The combined treatment with fumonisin culture material and *P. multocida* delayed growth, induced cough, and increased BALF total cells, macrophages and lymphocytes. Lung lesions were significantly enhanced in these animals and consisted of subacute interstitial pneumonia. TNF-α, IFN-γ and IL-18 mRNA expression was also increased. Taken together, our data showed that fumonisin culture material is a predisposing factor to lung inflammation. These results may have implications for humans and animals consuming FB_1 contaminated food or feed.

Keywords: Mycotoxin; Swine; Fumonisin; Susceptibility; Infection; Immune response

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1. Introduction

Mycotoxins are secondary metabolites of fungi that may contaminate animal and human feeds at all stages of the food chain. Since they contaminate approximately 25% of the World cereal crop production, they are representing a major risk factor affecting human and animal health (Finks-Gremmels, 1999; Oswald et al., 2005).

Fumonisin B_1 (FB_1) belongs to the fumonisin family of toxins which are produced by *Fusarium verticillioides* and *Fusarium proliferatum*, fungi that commonly contaminate maize (Bezuidenhout et al., 1988). Recent surveys on fumonisins in food and feed throughout the World, including the United States and most European countries, have raised concerns about the extent of FB_1 contamination of maize and its implications for food safety (Shephard et al., 1996; Scudamore et al., 1998). FB_1 was found in 50% of maize samples collected between 1988 and 1991 from the midwestern United States (Murphy et al., 1993). Up to 10% of these samples had toxin concentrations between 10 and 50 ppm. Similarly, another survey of fumonisins in maize gluten and other maize products in the United Kingdom found these mycotoxins in almost every sample at concentrations of up to 32 ppm (Scudamore et al., 1998).

Fumonisin B_1 causes a variety of species-specific toxicological effects in domestic and laboratory animals. It induces leukoencephalomalacia in horses, pulmonary edema in pigs, nephrotoxicity in rats, rabbits and lambs as well as hepatotoxicity in all species examined (reviewed in Bolger et al., 2001). This toxin has also been reported to be a carcinogen in rodents and a contributing factor in human esophageal cancers (Howard et al., 2001; Rheeder et al., 1992). The inhibition of ceramide synthase was shown to be the primary biochemical effect of fumonisin. As a result of this inhibition, sphingoid bases and sphingoid base metabolites accumulate leading to the depletion of more complex sphingolipids (Merrill et al., 1996).

In pigs, *Pasteurella multocida* type A is the most frequent secondary pathogen, which can generate a respiratory disorder, called swine pneumonic pasteurellosis (Chung et al., 1994). Unless some predisposing damage has occurred, *P. multocida* is considered incapable of invading the lung (Ciprian et al., 1994). Primary infections with bacteria such as *Mycoplasma hyopneumoniae*, *Bordetella bronchiseptica*, or viruses (pseudorabies virus, porcine reproductive and respiratory syndrome virus) have been shown to predispose pigs to *P. multocida* pneumonia (Amass et al., 1994; Carvalho et al., 1997; Brockmeier et al., 2001). Cytotoxin from *Actinobacillus pleuropneumoniae*, lipopolysaccharide from gram negative bacteria or ammonia are also known to promote *P. multocida*-induced pneumonia (Chung et al., 1994; Hamilton et al., 1999; Halloy et al., 2004a).

Ingestion of high doses of FB_1 induces pulmonary edema in pigs (reviewed in Haschek et al., 2001). However, only few data are available on the effect of ingestion of low doses of this toxin on the development of pulmonary inflammatory processes (Smith et al., 1996; Zomborszky-Kovacs et al., 2002). The objective of the present paper was to determine if ingestion of fumonisin culture material could predispose conventional piglets to the development of pneumonia induced by *P. multocida* type A.

2. Materials and methods

2.1. Animals

Twenty conventional piglets (9.6 ± 2.1 kg) were used in this study. They were acquired locally from a herd not infected by mycoplasma and vaccinated against PRRS virus. Animals acclimatized for seven days in a room of the animal facility of the University Liege with minimal air pollution. They were weighted at days 0, 7, 13 and 20, the last day of the experiment. Animals were fed with pellets and had access to water ad libitum. The experimental protocol was approved by the Ethics Committee of the University of Liege.

2.2. Toxin and bacterial isolate

Fumonisin was administrated as a soluble crude extract obtained after in vitro culture of the *Fusarium verticilloides* strain NRRL 34281 as already described (Oswald et al., 2003; Tran et al., 2003). Briefly, sterilized maize inoculated with the fungal strain was incubated for 4 weeks at 25 °C. The culture was extracted with acetonitrile-water, filtered, and concentrated. The