

## Forensic Findings on Acute Mortality of Piglets after Ingestion of Aflatoxin

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### Abstract

Aflatoxin (AF) is one of the most significant mycotoxins in pigs and results in a significant decrease of production performance. This paper describes the first case of acute effects of AF in suckling piglets in Serbia. Within 7 days, 420 suckling piglets aged 20±3 days died on a farm. Forensic findings included a medical history, clinical presentation with pathomorphological and histopathological confirmation of intoxication of the piglets with AF. Detection of AF in sows' milk (870 µg/kg), which was the primary nutriment for the piglets, confirmed the suspicion of AF intoxication. The piglets at that age also consumed solid feed, which was found to be contaminated with AF (960 µg/kg), and which accelerated the process of intoxication. After removal of the contaminated feed, the number of deaths began to reduce, as well as the clinical symptoms typical for AF.

**Keywords:** Aflatoxin, Forensic finding, Intoxication, Piglet

## Aflatoksin Sindirilmesi Sonucu Akut Mortalite Şekillenen Domuz Yavrularında Adli Bulgular

### Özet

Aflatoksin (AF) domuzlarda rastlanan en önemli mikotoksinlerden biri olup üretim performansında önemli azalmalara sebebiyet verir. Bu vaka takdimi süt emme dönemindeki domuz yavrularında AF'nin akut etkilerini gösteren Sırbistan'daki ilk çalışmadır. Bir çiftlikte 7 günlük bir süreçte yaşları 20±3 gün arasında değişen 420 adet domuz eniği ölü bulundu. Anamnez, klinik bulgular, patomorfolojik ve histopatolojik bulgular AF toksikasyonunu şüphelendirdi. Temel besin kaynağı olarak annelerin sütünde AF belirlenmesi (870 µg/kg) AF toksikasyonunu teşhisini doğruladı. Katı yem de tüketen yavruların bu yemlerinde de AF belirlenmesi (960 µg/kg) toksikasyonun hızlı seyrini açıklar nitelikteydi. Kontamine yemin uzaklaştırılmasından sonra ölümlerde ve AF'nin tipik klinik bulgularında azalma tespit edildi.

**Anahtar sözcükler:** Aflatoksin, Adli bulgular, İntoksikasyon, Domuz eniği

### INTRODUCTION

Aflatoxins (AF) are toxic metabolites of fungi (*Aspergillus flavus*, *A. parasiticus* and *A. nomius*) commonly found on cereal grains, widespread contaminants of foods and feeds [1,2]. Ingestion of AF can harm the health of pigs. Depending on the ingested quantity and the age of the pig, changes can manifest themselves in acute, subacute or chronic forms. In the acute form, the liver is a major target-organ and widespread hemorrhage and jaundice and enlargement of gallbladder occur. Fatty buildup, centrilobular necrosis and hemorrhages can be observed microscopically on the liver. The immunotoxic

potential of AF was detected in several animal species, including pigs [3], in which it decreases the blastogenesis response to mitogen, reduces the complement titers, decreases macrophage activation, and depresses delayed hypersensitivity [4]. The acute course of the disease occurs more frequently in younger age groups of pigs and the younger the pigs, the greater the risk of a lethal outcome.

### CASE HISTORY

This paper describes forensic findings leading to confirmation of AF ingestion as the cause of death in



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piglets. On a commercial pig farm with the capacity to hold 2.000 sows, 420 suckling piglets aged  $20\pm 3$  days died within seven days. The piglets were apathetic, depressed, moved reluctantly, cachectic and they were not febrile (Fig. 1). Deaths occurred after a short period of inappetence, weakness and anorexia. The farm conducted regular immunoprophylaxis of sows and piglets (Aujeszky's disease (AD)), *E.coli* enterotoxaemia, necrotic enteritis in piglets (*Clostridium perfringens* (type C)), *Mycoplasma hyopneumoniae*, Porcine circovirus (PCV2), Classical swine fever (CSF) and porcine parvovirus (PPV)).

When the first symptoms of disease occurred in the piglets, they were treated with antibiotics, but treatment had no visible effect. Subsequently, bacterial, viral and serological testing of the sick piglets was conducted.

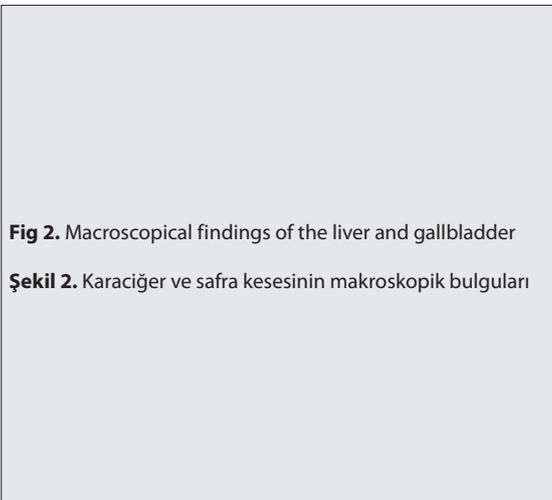
None of the bacteria or viruses tested for were detected, while the presence of antibodies specific for AD, *E. coli* and *Clostridium*, CSF and PPV was established serologically. Considering the vaccination of sows, this finding suggests that stated specific antibodies in piglets of sows are the consequence of immunoprophylaxis sows.

Macroscopical examination revealed bright, yellowish color of the liver with subserous petechial bleedings and enlarged gallbladder (Fig. 2). The tissues were fixed in 10% formalin and processed by routine paraffin technique. Microtome sections of 5  $\mu$ m thickness, after deparaffinization, were stained using standard hematoxylin and eosin method. Vacuolization of hepatocytes, necrosis and fatty degeneration of the liver were determined histopathologically (Fig. 3).



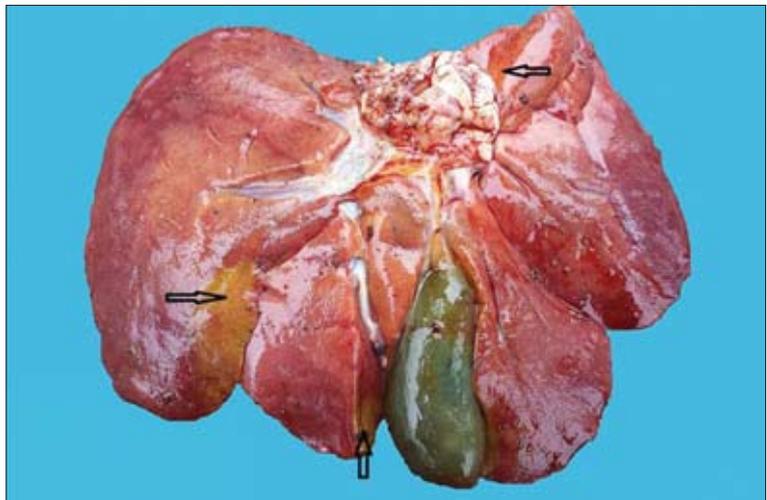
**Fig 1.** The clinical picture of piglets aged  $20\pm 3$  days, after AF intoxication

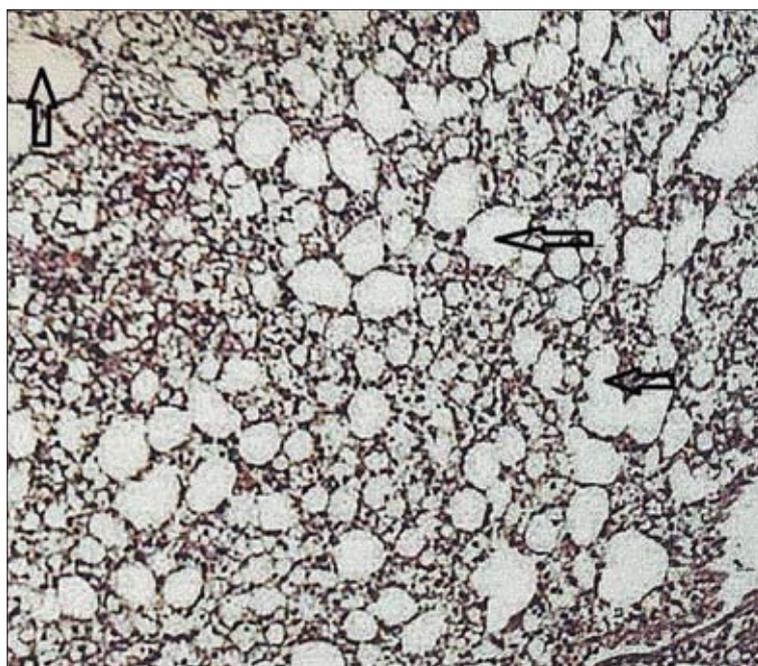
**Şekil 1.** AF toksikasyonu sonrası  $20\pm 3$  günlük domuz yavrularının klinik tablosu



**Fig 2.** Macroscopical findings of the liver and gallbladder

**Şekil 2.** Karaciğer ve safra kesesinin makroskopik bulguları



**Fig 3.** Microscopical findings of the liver**Şekil 3.** Karaciğerde mikroskopik bulgular**Table 1.** Level of mycotoxins ( $\mu\text{g}/\text{kg}$ ) in sows' milk and feeds on the farm**Tablo 1.** Çiftlikteki domuz süt ve yemlerindeki mikotoksin düzeyleri ( $\mu\text{g}/\text{kg}$ )

Mycotoxin	Milk	Sows' Feed	Piglets' Feed
Aflatoxin	870	1710	960
Deoxynivalenol	52	201	74
Fumonisin	<50	<50	<50
Ochratoxin	<5	<5	<5
Zearalenone	<25	29	<25

The macroscopical examination of the stomachs of dead piglets revealed the presence of small amounts of milk and feed. After that, samples were taken from the sows' milk, sows' feed and piglets' feed for mycotoxin analysis (aflatoxin, deoxynivalenol, fumonisin, ochratoxin and zearalenone). Mycotoxins analysis were conducted using validated analytical method, enzyme-linked immunosorbent assay (ELISA) with good sensitivity and precision. Results are shown in [Table 1](#).

After removal of the contaminated feed, the number of deaths began to reduce, as well as the clinical symptoms typical for AF.

## DISCUSSION

Based on medical history, clinical, macroscopical and microscopical findings, the deaths of the suckling piglets were believed to likely be due to AF intoxication. Many infectious organisms (bacteria and viruses) can cause similar changes in piglets [5-7], but the forensic analysis excluded them. The final diagnosis was confirmed by the finding of AF in the sows' milk (870  $\mu\text{g}/\text{kg}$ ), which was a consequence of the presence of AF in the sows' feed. This

is the first report of AF intoxication of piglets through the milk of sows, which is the primary nutriment of piglets of this age. Many other authors have reported the adverse effect of AF in pigs, and this effect is primarily reflected in the reduction of the production performance (average daily gain, feed conversion ratio and morbidity) and immunosuppressive effect depending on the ingested quantity of AF and the age of the individual piglets [8-11]. Their studies were conducted on older piglets exhibiting chronic effects [12,13], while in suckling piglets, much lower AF quantities can produce a lethal effect, as in our current report. Detection of 870  $\mu\text{g}/\text{kg}$  of AF in sows' milk suggests that the level of AF in the feed was even higher than the determined level of 1710  $\mu\text{g}/\text{kg}$ , because AF is catabolized in the body, and is excreted through the milk in lower dosage than that ingested. The piglets were aged  $20 \pm 3$  days and already consuming solid feed which was also found to be contaminated with AF (960  $\mu\text{g}/\text{kg}$ ); this only served to accelerate the process of intoxication.

After confirmation that the cause of death of piglets was intoxication with AF, the contaminated feed was withdrawn from use. Over the next few days the number of deaths among the piglets decreased, no new forms of clinical cases occurred and production performance began to increase.

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