

# Aflatoxins Aggravate the Incidence of Salmonellosis Outbreak in Fattening Calves: A Case Study

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**Abstract**—Fever, bloody diarrhea and high mortality rate were the main clinical finding in a group of fattening calves. Analysis of corn silage revealed presence of aflatoxins at level of 370 ppb. This level of aflatoxins in the feed of cattle is somewhat low to be the main cause of reported signs. Leukocytopenia, anemia, decreased lymphocytic activity and lowered phagocytic index are the main hematological and immunological alterations in diseased calves. Bacteriological investigation revealed isolation of pathogenic *Salmonella typhimurium* from the rectal swabs of diseased calves. Our results suggested that, long duration of exposure to aflatoxins even at small concentrations may be considered as predisposing factor for the incidence of natural infectious outbreaks as salmonellosis due to its immunosuppressive effect. We can conclude that the veterinarians and owners must be given an attention to the relation between aflatoxicosis and salmonellosis under field condition. We are recommended that the treatment program during similar outbreaks must be including anti-aflatoxins preparations beside the antimicrobial therapy.

**Keywords**—Aflatoxins, Calves, Salmonellosis.

## I. INTRODUCTION

AFLATOXINS constitute a group of fungal metabolites that have varied toxic and carcinogenic properties, depending on dose and duration of exposure. Aflatoxins contamination can occur in a wide variety of feedstuffs including corn, sorghum, barley, rye, wheat, peanuts, soya, rice, cottonseed and various derivative products made from these primary feedstuffs [1]. All animal species are susceptible to aflatoxicosis, but outbreaks occur mostly in pigs, sheep and cattle [18]. Beef and dairy cattle are more susceptible to aflatoxicosis than sheep or horses. Young animals of all species are more susceptible than mature animals to the effects of aflatoxin [5]. Chronic, low level exposure to aflatoxins has been shown to be associated with a range of more insidious effects, such as reduced weight gain, suppression of the immune system, interference with reproductive function and neoplasia [16], [22], [24]. The possible interaction between aflatoxicosis and infectious diseases has been a question since the discovery of aflatoxins. Many scientific researches described the interaction between aflatoxins and the infection with various *Salmonella spp.* in poultry. The results of these experiments and field cases indicate presence of evidences to an interaction between them on body weight and mortality [3],

[4], [23]. Also, chickens receiving aflatoxin-contaminated diets showed higher susceptibility to salmonellosis than chickens receiving aflatoxin free diet [26].

In animals, salmonellosis is a collective description of a group of diseases caused by bacteria of the genus *Salmonella* with signs that vary from severe enteric fever to mild food poisoning and is a common cause of neonatal morbidity and mortality. Cattle are probably infected with salmonellas by the oral route, although respiratory and conjunctival infection may also occur. The dose required to initiate infection is thought to be high and will be dependent on the age, immunity and dietary status of the animal and virulence of the infecting strain. The disease in cattle associated primarily with *S. dublin* and *S. typhimurium* [9]. Under field condition, there is little available data about the interaction between aflatoxicosis and salmonellosis in cattle, so the objective of this case report was to describe the findings related with an outbreak of suspected aflatoxicosis and salmonellosis in calves.

## II. MATERIAL AND METHODS

### A. Circumstantial Evidence, Clinical Finding, and Treatment

In February 2012, fifty-five 8-11 months-old Holstein Friesian calves from a fattening farm in El-Bahiera Governorate, Egypt were suddenly showed signs of: off food, depression, fever (41°C) and severe bloody diarrhea. About 17 calves died after 3 days from appearance of signs. History revealed feeding of animals on 1 year old corn silage for 2 months before the appearance of signs. Diseased animals were treated by using Alamycine L.A containing 200 mg/ml of Oxytetracycline base (Norbrook Laboratories Limited- Ireland) and Finadyne, containing 50mg/ml flunixin meglumine (Schering- Plough Animal Health, Germany). In another part of the same farm there was 15 calves doesn't suffered from any clinical signs and not fed on corn silage. We used these animals as a control for our study.

### B. Sampling

For the collection of a representative sample from corn silage and concentrates, small samples were collected from different places of food stores at the time of problem. Samples (totally about 5kg) were combined and mixed thoroughly then stored at -20 in cloth bag until analysis [15]. Blood for hematological and immunological analysis was collected into sterile tubes with anticoagulant from the jugular vein of the diseased animals. For the detection of pathogenic salmonella spp., rectal swabs were taken from diarrheic calves using

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sterile cotton swabs and kept on ice before storage in the laboratory.

#### C. Analysis of Aflatoxins

Aflatoxins B<sub>1</sub> and B<sub>2</sub> were analyzed in corn silage and concentrates using fluorometric assay [27]. Food samples were extracted by 80% methanol water extraction solution. Filtrate was cleaned-up using immunoaffinity column (Aflatest™ column). Fluorometric measurement for the toxins fluorescence signal was detected by fluorometer series – 4EX.

#### D. Bacteriological Investigation

Isolation and identification of *salmonella* species was carried out according to [7], [13], [17]. Triple sugar iron agar medium (TSI) was used for the detection of H<sub>2</sub>S production and types of sugar fermentation. Also this medium was used for propagation of isolated *salmonella* before serological typing. For serological typing a loopful of the slope positive TSI was suspended in a drop of saline in a clean slide then add drop of (O) or (H) or (Vi) antisera with gentle rotation then examined for agglutination (Difco salmonella O antisera, O poly group A, B, C, D, E, F, G, Difco salmonella H antisera and Difco salmonella Vi antisera, Becton, Dickinson and company sparks pendix limited shanon, Ireland).

#### E. Hematological and Immunological Tests

Hematological parameters were investigated according to [21]. Lymphocyte transformation test and phagocytic index were assayed according to [12]-[19].

#### F. Statistical Analysis

Data were subjected to analysis using T test procedures to assess the hematological and immunological differences between control and diseased animals by the aid of [20].

### III. RESULTS

#### A. Postmortem Finding

Liver and gall bladder enlargement associated with petechial hemorrhages and severe congestion in the intestinal mucosa were the main gross pathological lesions observed after the postmortem examination of dead animals.

#### B. Aflatoxins Level and Bacteriological Investigation

A negligible amount of aflatoxins was detected in concentrates, while in corn silage the aflatoxins level was 370 ppb. Bacteriological investigation revealed isolation of *salmonella typhimurium*, serogroup B with somatic (O) antigen type 4 and flagellar (H) antigen (phase 1= i; phase 2= 1, 2) according to Kauffmann–White scheme.

#### C. Hematological and Immunological Parameters

As shown in Table I hematological analysis revealed a significant decrease in RBCs, WBCs count and hemoglobin concentration in affected calves as compared with unaffected animals. Differential leukocytic count showed significant lymphocytosis and neutropenia in diseased animals. We also detected a significant decrease in lymphocyte activity and phagocytic index.

TABLE I  
HEMATOLOGICAL AND IMMUNOLOGICAL PARAMETERS OF DISEASED AND CONTROL ANIMALS

Parameter	Diseased	Control
RBCs count ( $\times 10^6 / \mu\text{L}$ )	9.17 $\pm$ 0.23**	12.61 $\pm$ 0.31
Hemoglobin (g/dL)	8.88 $\pm$ 0.20**	12.65 $\pm$ 0.18
WBCs count ( $10^3 / \mu\text{L}$ )	3.2 $\pm$ 23.26***	4.1 $\pm$ 60.10
Neutrophils (%)	32.00 $\pm$ 0.58**	42.00 $\pm$ 0.58
Lymphocytes (%)	63.33 $\pm$ 0.33**	54.00 $\pm$ 0.58
Monocytes (%)	2.00 $\pm$ 0.00	1.33 $\pm$ 0.33
Basophils (%)	0.50 $\pm$ 0.03	0.40 $\pm$ 0.01
Eosinophils (%)	1.51 $\pm$ 0.01	1.61 $\pm$ 0.02
Lymphocyte activity	22.67 $\pm$ 1.20*	35.00 $\pm$ 0.58
Phagocytic index	1.23 $\pm$ 0.05*	2.43 $\pm$ 0.14

Values are means  $\pm$  SE; \* P<0.01; \*\* P<0.001; \*\*\* P<0.0001

### IV. DISCUSSION

Under field condition interactions between different causes of the diseases in farm animals are frequently observed. In this case we suspected presence of interaction between aflatoxins and the pathogenic *Salmonella* spp. in a group of fattening calves.

The diagnosis of aflatoxicosis is largely depends on the detection and identification of aflatoxins in suspected feed in amounts sufficient to cause a problem. Our results revealed presence of aflatoxins B<sub>1</sub> and B<sub>2</sub> in whole corn silage at level of 370 ppb. Reference [25] found that the frequency of incidence of aflatoxin in corn silage is not different from the frequency of incidence of the toxin in corn grain, but the concentrations are lower and the limiting factor for mold growth in silage is pH. However, if silage is stored insufficiently packed and covered, infiltration of air allows for microbial activity, which depletes silage acids, allowing pH to rise and molds to grow. But, sometimes aflatoxins may occur in corn silage in high concentrations as reported by [10] who detect aflatoxins at concentrations of up to 5 ppm. Recently, the results of some experiments indicated that aflatoxins could increase when silage is exposed to air during conservation or during the feed-out phase [6].

Depending on the interactions with other factors, aflatoxin concentrations as low as 100 ppb may be toxic to beef cattle; however, the toxic level is generally considered to be between 300 and 700 ppb [25]. Also, [2] stated that calves older than 4-6 months and adult non breeding cattle may be fed up to 300 ppb aflatoxins but should associated with a good plane of nutrition.

Several natural outbreaks of acute aflatoxicosis were reported in calves. High mortality rate, anorexia, fever and diarrhea were the most observed signs. Analysis of aflatoxins in food revealed presence of aflatoxins at levels up to 2230 ppb [14] and 1130 ppb [11].

In this outbreak, calves were suddenly suffered from fever, diarrhea and off food while mortality rate was about 30.9%. Detected level of aflatoxins in corn silage was 370 ppb and this concentration is not a high enough to cause these sever signs or the high mortality rate but interpretation of this problem may become easier after the hematological, immunological and bacteriological investigations. Our results

showed a decrease in the total WBCs, RBCs count, haemoglobin level, lymphocyte activity and phagocytic index in diseased calves in comparing with non affected animals (control). Similar hematological alterations associated with experimental aflatoxicosis in rams were reported by [8]. Furthermore, the immunosuppression associated increasing prevalence of endemic infection and inadequate responses to routine vaccination may also be detected in animals affected with aflatoxicosis. This insidious form of aflatoxicosis is often of greater economic significance than acute forms of the disease associated with high concentrations of toxin in the diet [22]. This is in agreement with our immunological results which showed a decrease in the lymphocyte activity and phagocytic index of diseased calves in comparing with non affected animals. Obtained data give us an evidence for the incidence of infectious agent beside aflatoxins. Our bacteriological investigation revealed successful isolation of pathogenic *S. typhimurium* with somatic (O) antigen type 4 and diphasic flagellar (H) antigen from diseased calves which was the responsible for the appearance of clinical signs and mortality.

According to [9] *S. typhimurium* is a common cattle pathogen causing variable symptoms in adult animals or calves. Characteristically, calves become dull and anorexic and develop a fever. Diarrhea follows, which in young calves involves the excretion of faeces with the color and consistency of putty. This may be bloodstained and may contain fibrin and mucus. The calves become very weak and dehydrated and death usually occurs after five to seven days of illness in untreated individuals. Mortality from acute salmonellosis may be as high as 70 % and all calves in a herd may become infected. These signs are similar to those observed by us during outbreak.

We can conclude that the incidence of aflatoxins in animal's food at sub-lethal levels or even at small amounts may become a predisposing factor for the appearance of many infectious diseases as salmonellosis and this may be attributed to the adverse effect of the mycotoxin on immune system. We are recommended that the treatment program during similar outbreaks must be including anti-aflatoxins preparations beside the antimicrobial therapy.

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