MOULDY LUCERNE HAY SUSPECTED TO CAUSE BOVINE ABORTION

(Sospecha de aborto bovino por heno enmohecido)

L. Carrillo, L.M. Campero, F.E. Labarta
Facultad de Ciencias Agrarias, Alberdi 47, 4600 Jujuy, Argentina
Telephone and fax: +54-0388-422-1547
Casilla de Correos 576, 4600 Jujuy, Argentina. Email: "lcarrillo@arnet.com.ar"

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Key word: mycotoxicosis – bovine – lucerne hay

SUMMARY

In a farm at the province of Jujuy (Argentina), a cattle ate some mouldy bales of lucerne hay and some cows aborted immediately after. Considering that there could be mycotoxicosis present, some samples of lucerne hay were collected and the following fungi were isolated: Myrothecium verrucaria (29.2%), Eurotium amstelodami (28.8%), Aspergillus versicolor (19.5%), Alternaria alternata (10.1%), Aspergillus ochraceus (5.28%), Penicillum aurantiogriseum (1.64%), Aspergillus flavus (0.70%), Aspergillus niger (0.47%), and others (4.33%). Ochratoxin A and aflatoxin B<sub>1</sub> were detected in the fodder yet there was absence of macrocyclic trichotheccenes. When the animals stopped eating the contaminated fodder, ochratoxin α was detected in the milk of one cow.

INTRODUCTION

During the growing season farm animals can graze on the living crop, but in semiarid regions they depend on winter, on grass or leguminous crops conserved as hay. After harvest, the development of the microbiota is controlled by storage conditions. Aspergillus and Penicillium spp. are characteristic on stored hay and conserved forage crops have been involved in mycotoxicoses (Lacey, 1991).

Aspergillus ochraceus in mouldy lucerne hay was involved in bovine abortion (Lacey, 1991). Ruminants are reported to be very resistant to the acutely toxic effects of ochratoxin A, because it was degraded to ochratoxin α and phenylalanine by ruminal microbiota (Raisbeck et al., 1991). A cow given a single ochratoxin A dose had ochratoxins A and α in the milk the following day. After that, only ochratoxin α could be detected (Ribelin et al., 1978).

During the dry season in Jujuy at northwest of Argentina, a herd of cows was being fed with mouldy Medicago sativa hay. The animals became ill and some of them aborted, but they did not suffer any infectious illness. The search of fungi and mycotoxins detection in the remnant fodder was carried out. Milk mycotoxins were analysed.

MATERIALS AND METHODS

Screening and enumeration of fungi

Samples of remaining fodder were collected from a cattle farm in the month of August. For the general screening of the moulds rose Bengal chloramphenicol oil was used (Jarvis, 1973). Each 25 g sub-sample was treated with a blender. Dilutions were made in aqueous 0.1% w/v
RESULTS AND DISCUSSION

Outbreak

The dairy farm was placed in San Antonio, province of Jujuy, Argentina. Holstein breed animals grazed on natural pasture and supplemental balanced feeding was supplied during the milking time. In dry season, cows were fed with bales of *Medicago sativa* hay. As cattle aborted before the 5th month of gestation the veterinarian was consulted. The laboratory analysis for brucellosis, leptospirosis and neosporosis was negative.

Fungi and toxins in fodder

Three samples of the remaining fodder pool were serially diluted and plated in triplicate. The mean enumeration was 2.96 ± 0.49 x 105 cfu g⁻¹. Table 1 lists the fungal species isolated from fodder and mycotoxins detected.

<table>
<thead>
<tr>
<th>MICROORGANISMS</th>
<th>% CFU</th>
<th>MYCOTOXINS DETECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Myrothecium verrucaria</em></td>
<td>29.2</td>
<td><em>in vitro</em> verrucarins, roridins</td>
</tr>
<tr>
<td><em>(Alb. &amp; Schw.) Dttm-Stoeckel</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eurotium hermaphroditum</em></td>
<td>28.7</td>
<td></td>
</tr>
<tr>
<td><em>Mangin</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus versicolor</em></td>
<td>19.5</td>
<td></td>
</tr>
<tr>
<td><em>(Vuill.) Tiranobochi</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Alternaria alternata</em> complex*</td>
<td>10.1</td>
<td></td>
</tr>
<tr>
<td><em>(Fr.) Keissler</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus ochraceus</em></td>
<td>5.28</td>
<td><em>in fodder</em> ochratoxin A</td>
</tr>
<tr>
<td>Wilhelm</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acreosporium pullulans</em></td>
<td>3.28</td>
<td></td>
</tr>
<tr>
<td><em>(de Bary) Amaud</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Penicillium aurantiogriseum</em></td>
<td>1.64</td>
<td></td>
</tr>
<tr>
<td>Dierckx</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>0.70</td>
<td><em>in fodder</em> aflatoxin B₁</td>
</tr>
<tr>
<td>Link ex Gray</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>van Tieghem</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Epichloë nigra</em></td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Link</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chaetomium sp.</em></td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td><em>Drechslera sp.</em></td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td><em>Curvularia sp.</em></td>
<td>0.12</td>
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Aflatoxin B₁ and ochratoxin A were detected in fodder samples but macrocyclic trichotheccenes were not found. The TLC agar plug method revealed macrocyclic

Detection of mycotoxins.

Dry extracts were dissolved in 0.2 ml ClCH₃. Thin layer chromatography on silicalgel G60 plates (Merck) were developed with the solvent systems: acetone - chloroform, benzene - methanol - acetic acid or toluene - ethyl acetate - formic acid (Betina, 1985; Scott, 1996).

After development, plates were exposed to longwave UV light for aflatoxins and ochratoxins (Scott 1996) and to short-wave UV light for macrocyclic trichotheccene detection (Hambermehl et al., 1985). Plates were sprayed with aluminum chloride solution or chromatopar acid solution before exposition to longwave UV and visible light for noncyclic trichotheccenes detection (Baxter et al., 1983).

Ochratoxin α standard was prepared by acid hydrolysis of ochratoxin A according to Xiao et al. (1995).

*Mycrothecium verrucaria* isolates were also analyzed for their ability to produce mycotoxins on yeast extract sucrose agar. The agar plug TLC method was used (Filenborg et al., 1983).
trichothecene production by *M. verrucaria* isolates. One strain was analysed at INAME (Bs.As.) and it produced verrucarins A and J; orridins A, D, and E. Other fungi were not analysed because they were not suspected to produce significant mycotoxins.

*Fusarium* spp. and *A. ochraceus* were isolated in Argentina by Saubois *et al.* (1988), from *Medicago sativa* and *Sorghum halepense* fodder during a mycotoxic outbreak, but *Myrothecium* species were not found. Gaggiotti *et al.* (2001), reported deoxynivalenol producing *Fusarium* in fodders during 1997–1998 season, and toxicogenic *A. flavus* in 1998–1999 season.

*Myrothecium* sp. did not produce trichothecenes in a mixed culture with *Aspergillus* spp. and *Penicillium* spp. (Reddy & Reddy, 1992; Reddy *et al.*, 1998). Some outbreaks of mycotoxicosis in cattle were reported in Asia but the fodder was infected with *Mucorales* besides *Myrothecium* sp. (Prudhvi-Reddy *et al.*, 1996).

Lucerne hay is a variable substrate for the growth and development of *A. flavus* and its production of aflatoxin (Lacey, 1991). Only a minority of *A. ochraceus* isolates are toxigenic and other species closely related also produce ochratoxin A (Pitt & Hocking, 1997). *A. flavus* inhibits the toxins production by *A. versicolor* in mixed cultures (Devi & Polasa, 1987) and *A. alternata* toxins are completely inhibited in cultures co-inoculated with *Aspergillus parasiticus* (Etecheverry *et al.*, 1998).

Production of P-sporidesmides and *E. niger* has not been reported. It is generally assumed that *E. amstelodami* and *A. niger* are benign fungi (Pitt & Hocking, 1997) but some *Chaetomium*, *Curvularia*, and *Drechslera* are suspected to produce toxins (Udagawa, 1983).

**Toxins in milk**

Ten milk samples were taken fifteen days after the end of contaminated fodder feeding. Aflatoxin M1 was not found and one sample had traces of ochratoxin α.

Two weeks after oral administration, ochratoxin was detected in milk (Ribelin *et al.*, 1978). Aflatoxin M1 is detected in cow milk within 72 h of ingestion of naturally contaminated feed (Raisbek *et al.*, 1991). These facts explain the absence of aflatoxin M1 and the finding of ochratoxin α in the analysed milk. Ochratoxin α is 25-fold less toxic than the parent toxin ochratoxin A (Sterner, 1992).

**CONCLUSION**

We concluded that mycotoxicosis occurred as a result of a prolonged ingestion of mouldy fodder, because the outbreak finished when the mouldy fodder was exhausted. It might result from a combination of several mycotoxins, mainly aflatoxin B1 and ochratoxin A. The milk can contain the less toxic ochratoxin (α) and this is a hazard for humans.

**ACKNOWLEDGMENTS**

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**REFERENCES**


