Natural exposure of Martina Franca jennies and their foals to Ochratoxin A

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Introduction

Ochratoxin A (OTA) is a worldwide occurring mycotoxin produced by *Penicillium* and *Aspergillus* fungi during unfavourable storage of cereals, with nephrotoxic, immunotoxic, teratogenic and reprotoxic activity. Monogastric animals are very susceptible to OTA, and swine and horses have been shown to be naturally exposed to it (Pozzo et al, 2010; Minervini et al, 2013). To date, no data are available on natural exposure of Martina Franca jennies and their foals to this mycotoxin during late pregnancy and after delivery.

Materials & Methods

Sampling of feed, blood and milk samples

Feed (n= 53) and blood (n= 101) samples were collected from January to September, 2018. Feed was made by oats, flaked barley, corn, bran, soy and mineral vitamin components, stored in 25-kg bags and sampled at the opening of each bag. Sampling was performed according to the sampling procedures of the EU Regulation (CE) N. 152/2009.

Blood samples (n= 67) were collected from 7 jennies every 15 days, two months before and three months after delivery. Blood samples (n= 34) from foals and milk samples (n= 33) were sampled, every 15 days, for three months after delivery.

Determination of OTA in feed samples

Feed samples were comminuted/homogenized with Ultra-Turrax T50 (slurry sample) and analysed by the AOAC Official method No. 2000.03 (Entwisle et al, 2000) for the determination of OTA in barley based on immunoaffinity column (IAC) clean up of extracts and HPLC/FLD detection, with minor modifications.

Determination of OTA in blood samples

OTA determination in blood samples was performed by ELISA (RIDASCREEN® Ochratoxin A) according to the protocol provided by the manufacturer (R-Biopharm AG, Darmstadt, Germany). A portion of samples (n= 23) was also confirmed by an optimized HPLC method based on immunoaffinity column (IAC) clean up.

Determination of OTA in milk samples

OTA determination in milk samples was performed by using the HPLC method based IAC clean up reported by Bascaran et al (2007) with minor modifications.

Results

OTA in feed samples

The OTA concentrations found in feed samples ranged from 0.3 to 2.7 ng/g and were far below the guidance OTA values in feed materials (250 ng/g) and feeds for pig poultry, cats and dogs (10-100 ng/g) reported by the EU Recommendation No. 2016/1319.

The analysis of each cereal component present in the feed showed similar low OTA levels (0.15-0.18 ng/g) in bran, corn flake and soy, whereas barley and oat resulted uncontaminated (LOQ of 0.3 ng/g).

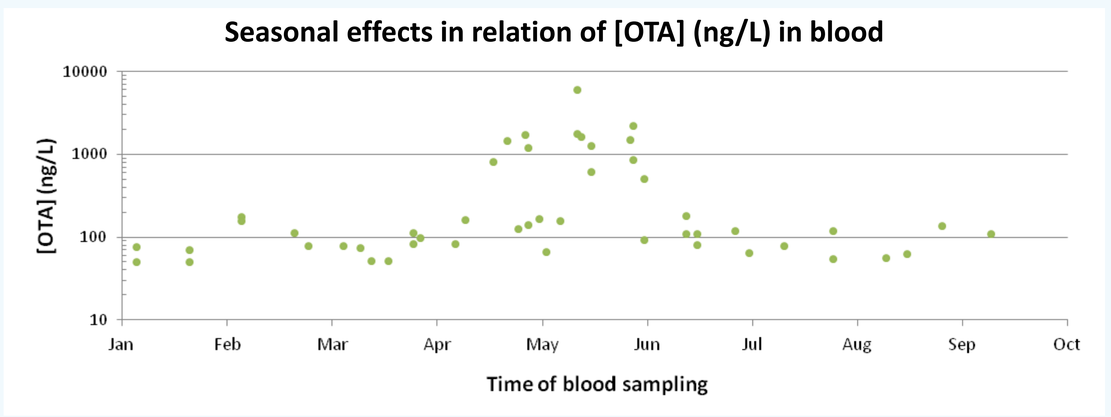
Ochratoxin A occurrence in feed samples

Feed samples	OTA occurrence		
Contamination ranges (ng/g)	< 0.3	from 0.3 to 1	from 1 to 3
N = 53	36	14	3
Incidence of contamination	68%	26%	6%
Range of OTA level (ng/g)	-	0.3-0.7	1.4-2.7

Limit of Quantification (LOQ): 0.3 ng/g

OTA in blood and milk samples

In **jennies,** the OTA incidence rate of positive blood samples (with OTA levels higher than the LOD) was 73%, with median value of 114 ng/L and range from 51 to 6,000 ng/L. No significant differences of OTA serum levels were found among jennies, but a season-effect on the levels of OTA in the blood was found with significant increases from 10 to 60 times in 46% of the positive ones collected from April to June, probably due to possible contamination of the hay.



Concerning **foals**, no OTA was detected in blood samples collected at the day of delivery showing no placental transfer. On the contrary, in horses the placental transfer was observed in 50% of newborns and this disagreement should be linked to the different placental structure of the jenny and the mare (Minervini et al, 2013). The incidence rate of positive blood samples was 50% with median value of 136 ng/L and concentrations ranged from 79 to 4034 ng/L. The exposure of foals to OTA was due to the intake of milk whose incidence of positive samples was 36% with OTA levels ranging from 17 to 82 ng/L.

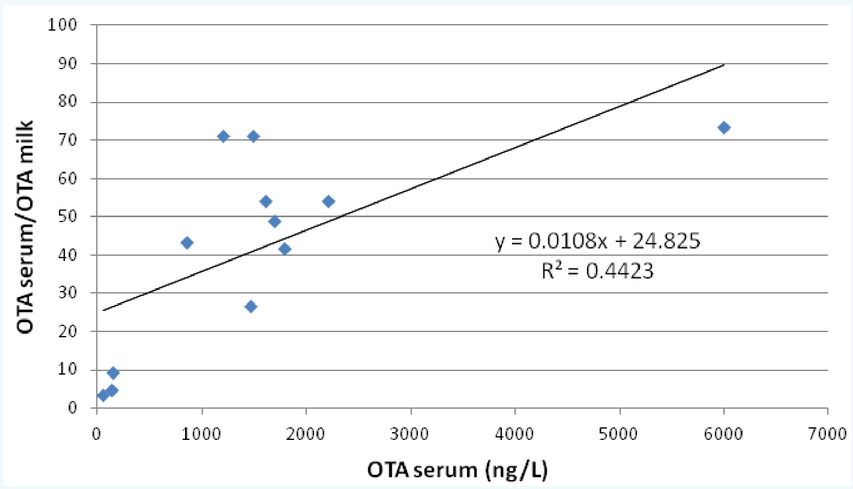
Occurrence of OTA concentration in blood and milk collected after delivery from jennies and foals.

Jennies (date of delivery)	Jenny's blood samples: Positive/total samples (%)	Jenny's blood samples: Median *Range* (ng/L)	Milk samples: Positive/total (%)	Milk samples: Median *Range* (ng/L)	Foal's blood samples: Positive/total (%)	Foal's blood samples: Median *Range* (ng/L)
Adelaide (17th March)	4/6 (67 %)	632 *52-1796*	2/5 (40 %)	30 *17-43*	3/5 (60 %)	133 *109-4034*
Francisca (31st March)	4/5 (80 %)	1596 *97-6000*	3/5 (60 %)	35 *21-82*	4/5 (80 %)	150 *136-594*
Gaia (18th June)	2/5 (40 %)	86 *63-109*	0/5 (0 %)	0 -	0/5 (0 %)	0 -
Etiopia (1st May)	5/5 (100 %)	181 *118-1620*	2/5 (40 %)	25 *20-30*	4/5 (80 %)	94 *79-108*
Antiqua (11th March)	3/4 (75 %)	157 *82-1467*	2/4 (50 %)	36 *17-55*	3/5 (60 %)	192 *87-306*
Falaria (31st May)	2/4 (50 %)	1162 *110-2215*	1/4 (25 %)	41 -	0/4 (0 %)	0 -
Eritrea (13th July)	5/5 (100 %)	109 *57-138*	2/5 (40 %)	23 *17-30*	3/5 (60 %)	140 *123-367*

Limit of Detections (LODs) of 50 ng/L for blood samples and of 15 ng/L for milk samples.

Relationship between serum OTA levels and the ratio serum/milk OTA levels in sample from jennies

A positive relationship (r= 0.67) between serum OTA level in jennies and the ratio serum/milk OTA levels was found in agreement with results on human milk reported by Biasucci et al (2011), suggesting that OTA carry-over to milk increased less than proportionally, probably for saturation of the transport system.



Conclusions

The occurrence of OTA in blood and milk samples showed a natural exposure of jennies and foals to this mycotoxin. In addition, the presence of OTA in jenny milk could pose a risk for human newborns considering its well-known nutritional and health properties.

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References

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