ABSTRACT: In order to find information on the occurrence of mycotoxic porcine nephropathy in Serbia, during a six month period (2006/2007) samples of kidney from individual healthy slaughtered pigs were collected (n=90) and analyzed by HPLC for ochratoxin A. In addition, histological examinations were carried out. The incidence of OTA in kidney was 33.3% and varied between 0.17—52.5 ng/g. Histopathological examination of kidneys confirmed tubulopathies with oedema and cell vacuolization. In addition, hemorrhages and necrosis of proximal kidney tubules cells were found. These findings indicate that it is likely that most of the kidney injury is related to ochratoxin A and other nephrotoxic compounds which enhance the toxicity of OTA.

KEY WORDS: Ochratoxin A, pig, nephropathy

INTRODUCTION

Ochratoxin A (OTA) is a nephrotoxic mycotoxin produced by several species in the Aspergillus and Penicillium genera. It is detected widely as a contaminant of agricultural commodities, especially cereals (20). As cereals are widely used in animal feed, and because OTA is relatively stable in vivo, whence it is further transmitted to animal edible tissues (8), especially in kidney and liver. Major target for the toxicity of ochratoxin A in mammalian species is kidney, where the toxin primarily affects the proximal convoluted
tubules (2, 14, 17, 18). OTA has been causally associated with nephropathy in pigs and poultry. Among farmed animals, pigs are particularly sensitive to OTA. This mycotoxin plays a special role in the genesis of swine mycotoxic nephropathy, a common disease in Scandinavia (10, 16, 23). It exhibits also immunosuppressive, teratogenic, nephrotoxic and genotoxic properties in several animal species (12).

Occurrence of OTA has been recognized as a potential human health hazard and OTA contamination is of public health significance since it is associated with Balkan nephropathy, a kidney disease in humans (4) described in several rural regions of Bulgaria, Romania, Serbia, Croatia, and Bosnia, and associated with an increased incidence of tumors of the upper urinary tract. However, causality has not yet been established. Special attention has been paid to OTA since 1993, when the International Agency for Research on Cancer classified this toxin as a possible human carcinogen (group 2B) (24). Human exposure to OTA can occur directly by consumption of food containing the toxin, or indirectly by consumption of animal tissue exposed to contaminated materials (8).

Up to now, there has been little published information on the occurrence of porcine nephropathy and content of ochratoxin A in kidneys of Serbian slaughtered pigs. Therefore, the aim of this paper was to evaluate the natural occurrence of porcine nephropathy and OTA content in kidneys from healthy slaughtered pigs originating from different regions of Serbia. Also, the purpose of this paper is to briefly review risk assessments of OTA in order to highlight the critical issues that these assessments have identified.

MATERIALS AND METHODS

Reagents

OTA were purchased from Sigma-Aldrich Chemie GmbH. A working standard OTA for HPLC containing 25 ng/mL methanol was prepared daily just before starting the injection of a series of samples. Other reagents were HPLC grade. All other chemicals were reagent grade or chemically pure.

Sample collection

During six month period (September 2006/February 2007), sample of kidney per animal was collected from healthy slaughtered pigs (n = 90) originating from three different regions of Serbia. Slaughtered pigs were randomly sampled in the slaughterhouse during meat inspection, and whole kidneys were sampled from each pig. The samples were homogenized and stored at –18°C before analysis. No preservatives were added.

Microscopical examination: Kidney samples were fixed in 10% neutral buffered formalin and absolute alcohol for 5 to 7 days, processed by routine
methods, sectioned at 5—8 µm, and stained with hematoxylin and eosin (HE) for light microscopy.

**Extraction and clean-up for ochratoxin analyses**

**Kidney** Analyses were performed by the method of Matrell et al (2006) (21), which briefly includes double extraction with acidic ethyl acetate. The organic phase was removed and extracted with 0.5M NaHCO₃ pH 8.4. The organic phase was evaporated to dryness under N₂ steam, reconstituted in 150 µl mobile phase and a 20 µl aliquot was injected. The detection limit for OTA in organs was 0.01 ng/g with a 61% mean recovery from artificially contaminated samples at 3 ng/g.

**Chromatographic conditions (HPLC)**

An aliquot of 50 µl for kidneys samples were injected into Waters Symmetry Shield RP 18, high pressure liquid chromatography column (length and inner diameter 150x4,6 mm, particle size 5 µm, of the HPLC system (Waters Alliance). The column was eluted with 4% acetic acid and acetonitrile (32:68 v/v) at 25°C and a flow rate of 1 mL/min. Measurements were performed by fluorescence detection at wavelengths of 334 nm (excitation) and 460 nm (emission) gain 10.

**Statistical analysis**

Descriptive statistics of the data set were performed with a standard programmed and included arithmetic mean, standard deviation, coefficient of variation, minimum and maximum. Statistical differences in the mean levels of OTA contamination across the three groups of positive samples were determined by one-way ANOVA (p < 0.05).

**RESULTS AND DISCUSSION**

The incidences and mean values of ochratoxin A in swine kidney and the results of pathomorphology examination are summarized in Table 1 and Figure 1 and 2.

**Ochratoxin A in Kidney**

The incidence of OTA in kidneys was 33.3% in the range 0.17—52.5 with the mean level 1.26 ng/g. The majority of samples (16.6%) contained OTA at low levels (0.01—1.0 ng/g). The concentrations in ten samples (11.1%) ranged between 1—5 ng/g, while ochratoxin A in five (5.5%) samples was
greater than 5 ng/g. In regard to regional distribution of OTA, the occurrence of OTA among the three regions whence samples were collected are almost similar and varied between 26.6% (region Vladimirci) and 36.6% (region Senta and Bogatić) (Table 1), but the mean level of contamination is different. The highest OTA level (52.5 ng/g, mean 2.20 ng/g), with the highest coefficient of variation (4.33) was found in the samples originating from region Bogatić. In 2.2% samples of kidneys, OTA levels were considerably higher and greatly exceeded the permissible levels for this toxin established in Serbia, including those proposed by the SCF (1998) (27), and JECFA (2001) (10 ng/g) (13).

Tab. 1 — Incidence of Ochratoxin A in kidney of slaughtered pigs

<table>
<thead>
<tr>
<th>Region</th>
<th>N</th>
<th>n (%)</th>
<th>ng/g</th>
<th>C.V. %</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Vladimirci</td>
<td>30</td>
<td>8 (26.6)</td>
<td>0.42±1.2</td>
<td>2.96</td>
<td>0.18—6.5</td>
</tr>
<tr>
<td>Senta</td>
<td>30</td>
<td>11 (36.6)</td>
<td>1.14±3.3</td>
<td>2.89</td>
<td>0.17—17.0</td>
</tr>
<tr>
<td>Bogatić</td>
<td>30</td>
<td>11 (36.6)</td>
<td>2.2±9.54</td>
<td>4.33</td>
<td>0.26—52.5</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>30 (33.3)</td>
<td>1.26±5.85</td>
<td>4.64</td>
<td>0.17—52.5</td>
</tr>
</tbody>
</table>

N — total number of analyzed samples, n — number of positive samples, X — arithmetic mean (conc. below LOD are regarded as zero), C.V. — coeff. of variation.

Comparison of data obtained in this trial, along with the other recently published data for the occurrence of OTA in pig edible tissues, shows that the found levels are comparable with levels in other European countries or Canada (5, 7, 9, 23). The incidence of OTA in kidneys was 33.3% and almost comparable with the incidence reported by Koller (1991) in Austria (41.9%) or by Gareis and Scheuer (1999) in Germany (41.9%), and in Serbia (41.6%) (22). The mean level (1.26 ± 5.85 ng/g) was lower than those reported in Austria (2.5 ng/g) (15) and recently published data in Serbia (3.12 ng/g) (22), but higher then the mean level found in Germany (0.43 ng/g) (8).

**Patomorphology Examination**

**Gross pathology**

All 90 pigs were slaughtered during the study period. Kidneys submitted to the laboratory were pale, swollen and enlarged and changed in colour from normal mahogany to tan, as follows: 43 (47.7%) had “mottled or pale kidneys”, 27 (30%) had enlarged kidneys and 11 (12.2%) were smaller than normal. The only macroscopic lesions observed in few cases were small grey-white foci on the kidney surface. No obvious difference was observed between the right and left kidney. No significant changes were seen in other organs.
Histological examination

Histological findings of renal tissues and incidence of ochratoxin A in kidneys from slaughtered pigs are summarized in Figure 1 and 2. Histological examination showed two types of changes: degenerative — affecting epithelial cells in some proximal tubules of pigs, and proliferative changes in the interstitium. The major renal histopathological changes were in the epithelium of proximal tubules (Fig. 2). Dystrophy, (moderate to obvious degenerative changes, Fig. 1A), swelling, vacuolization and lipophilic degeneration, were the main changes in the tubular epithelial cells. The majority of glomeruli exhibited mild or moderate exudates in the Bowman’s capsular spaces as well as hypercellularity of vascular loops. In addition vascular changes expressed as a hyperaemia of blood vessels, moderate to marked hemorrhages of some renal cortical regions occurred occasionally (Fig. 1D). In the interstitium of the renal cortical regions, there was limited proliferation of connective tissue (Fig. 1B) and focal infiltration of mononuclear inflammatory cells which was sometimes

Fig. 1 — Dystrophy and vacuolar degeneration in the epithelium of proximal tubules’ cells (A), Focal interstitial fibrosis (B), Necrosis of proximal tubules’ cells (C) and Hemorrhages in cortex (D)
accompanied by small granulomas. OTA analysis of the kidney samples with degenerative changes of moderate to marked cloudy swelling revealed the incidence of OTA in 32.2% samples at concentration levels up to 52.5 ng/g (Fig. 2). Additionally, vascular changes, as well as fatty changes were observed in six kidneys of pigs in which ochratoxin A was detected up to 6.5 ng/g, while focal interstitial fibrosis and necrosis of proximal tubules’ cells were only seen in one pig kidney in which ochratoxin A was detected up to 52.5 ng/g. The lesions produced by higher OTA levels were more severe and widespread, including degeneration, atrophy, glomerular swelling and sclerosis and interstitial nephritis (Fig. 2).

![Fig. 2 — Summary of histological findings (PH) of renal tissues and incidence of OTA in kidney from slaughtered pigs (n=90). Necrosis of proximal tubules’ cells (1), hypercellularity of vascular loop (2), vascular changes (3), exudates in Bowman’s space (4), focal interstitial nephritis (5), dystrophy of proximal tubules’ cells (6), swelling of proximal tubules’ cells (7), renal hemorrhages (8), fatty changes of proximal tubules’ cells](image)

The macroscopic and microscopic changes observed in our study were more similar to those reported by other papers (28, 29, 30). These findings confirm that high affinity of OTA towards serum proteins, allows its accumulation in the organs of animals (11). Kidney is the main target of OTA. This high susceptibility of kidney is, at least partly, the result of OTA-toxicokinetics. Renal blood flow per tissue weight is extremely high, which results in the delivery of relatively large amounts of OTA in comparison to other organs. Furthermore, free OTA is secreted in the proximal tubule and subsequently re-absorbed, mainly in the proximal straight tubule, the thick ascending limb of the loop of Henle and the collecting duct (1, 6, 25, 27). The inhibition of protein synthesis and damaged energy production in the mitochondria could be considered as the most important factors for degenerative changes in the epithelial cells of proximal tubules where ochratoxin A was detected. While agreeing that the most important toxicological target of OTA in a pig is kidney, the principal descriptions of the pathology of MPN vary considerably with respect
to some other details, and according to the dosing regime and the duration of OTA exposure. Enlarged kidneys are indicative of renal inflammation and proliferative lesions following chronic exposure to OTA (28, 30). Therefore, it seems that MPN observed in Serbia may have a multitoxic etiology because it cannot be explained by the concentration of OTA alone. The lack of a strong correlation among histopathological changes and incidence of OTA in kidney (33.3% kidney samples were positive, at levels ranging up to 52.5 ng/g) found in our trial might thus explain the result of OTA-toxicokinetics, as well as possible synergism between OTA and other nephrotoxic mycotoxins or compounds which enhance the toxicity of OTA. Such synergism between OTA and other mycotoxins under field conditions may be responsible for the MPN in Serbia, which is associated with relatively low mean contamination by OTA in feed. The production of multiple toxins by one or several fungi, which is sometimes completely normal, presents a problem that has not been sufficiently investigated.

However, it should be remembered that when comparing data factors such as climate conditions during harvest, practices for grain/feed storage etc have influence on the ochratoxin A level in edible tissues. The data obtained in this trial show that there should be more concern for the livestock industry.

REFERENCES


МИКТОКСИЧНА НЕФРОПАТИЈА СВИЊА И ЗАСТУПЉЕНОСТ РЕЗИДУА ОХРАТОКСИНА А У БУБРЕЗИМА ЗАКЛАННИХ СВИЊА

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Резиме

У циљу стицања сазнања о заступљености миктоксичне нефропатије свиња и утицаја ОТА на врсту и локализацију патоморфолошких промена на бубрезима, на линији клина током ветеринарско-санитарног прегледа редовно закланних свиња пореклом са фарми из Војводине и Србије, узимани су узорци бубрега за анализу. Током шестомесечних испитивања укупно је анализирано 90 узо-
рака бубrega закланих свиња. Присуство резидуа ОТА у узорцима бубrega пореклом из испитиваних региона утврђено је код 33,3% испитаних узорака, у количини од 0,17 до 52,5 нг/г. Заступљеност резидуа ОТА била је највећа у узорцима бубrega пореклом са локалитета Сента и Богатић (36,6%), док је највећи просечан садржај резидуа ОТА (−2.2 μg/kg) утврђен у узорцима бубrega пореклом са локалитета Богатић. Патохистолошким прегледом бубrega најчешће су утврђене тубулопатије са едемом и вакуолизацијом ћелија. Такође, утврђене су хеморагије и некроза ћелија проксималних бubreних тубула.