The occurrence of ochratoxin A in kidneys of healthy pigs from Vojvodina province, Serbia

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Abstract. Ochratoxin A is a potential contaminant of feed and consequently meat and meat products. Residues of this mycotoxin in meat can pose a food safety issue. Swine production in Serbia and the northern province of Vojvodina is highly developed, since household consumers in Serbia frequently purchase pork. The occurrence of OTA in pig’s kidney taken from the slaughter line is a good indicator of the presence of this mycotoxin in meat and meat products. A total of 95 pig’s kidneys from Vojvodina, Serbia, were analyzed for ochratoxin A. The results from 19 farms (5 samples per farm from Bačka, Banat and Srem districts of Vojvodina) showed the presence of OTA in 14.74% (14/95 kidneys). The average OTA content was 1.36 µg/kg, median 0.99 µg/kg, and range from 0.10 to 3.97 µg/kg. Results from our research do not suggest any serious problems with OTA contamination in pig’s kidneys, but continuous monitoring is needed to avoid any possible future problems.

1. Introduction

Ochratoxin A (OTA)-producing fungi are from the genus Penicillium and Aspergillus. In tropical regions, the most prominent OTA-producing mold is Aspergillus ochraceus, while in temperate climate regions, Penicillium verrucosum is mostly responsible for OTA [1]. In feeds, OTA is mainly produced by the storage fungi [2] so the management on the farm can have a huge effect on the occurrence of OTA and other mycotoxins.

The Balkan Peninsula region is associated with Balkan Endemic Nephropathy, chronic tubulointerstitial disease associated with the occurrence of OTA in food [3]. The population is directly exposed to OTA through cereals, cereal products, nuts, spices, grape juice, coffee, beer and wine [4]. During a 30-year study of the occurrence of OTA and zearalenone in cereals and feed in neighboring Croatia, a few years with high incidences of these two mycotoxins in cereals were observed. Years with a lot of rain and lower air temperatures produced the highest levels of OTA, up to 68,900 µg/kg. During the rest of the 30 year-period, OTA levels were mostly between 0.26 and 220 µg/kg. However, in the last few years, OTA levels were lower due to dry season droughts [5].

Populations can be also exposed to this mycotoxin indirectly through consuming meat or milk containing OTA. Sub-chronic pig exposure to OTA leads to its accumulation in meat and consequently in meat product [6]. Products of animal origin can contribute up to 3% of the overall human intake of OTA. However, in some cases, animal products might contribute up to 10% of overall OTA, depending on the nutritional preferences of the population [7]. These preferences can
play a vital role in OTA intake, especially if people consume traditional animal products made from animal blood.

In Serbia, the largest meat production sector is pork meat production. In 2017, 307,000 tons of pork was produced [8]. In Vojvodina, the northern province of Serbia, pork is mostly produced at the intensive industrial scale. However, there are small household producers who raise pigs for their own consumption and cure the meat in the traditional way.

In Serbia, average annual household consumption of pork (fresh and frozen) was 45.4 kg, while in Vojvodina, average consumption was slightly higher (47.8 kg) [9]. However, total pork consumption does not just include fresh meat, as other meats are surveyed separately; average annual household consumption of cured meat (Serbia 14.9 kg, Vojvodina 14.8 kg) and processed meats (Serbia 38.9 kg, Vojvodina 40.7 kg) was also reported [9]. A large proportion of these other categories (cured meat, processed meat) are made from pork.

The European Commission regulates the maximum limit (ML) in the European Union for OTA content, 0.05 mg/kg, in complementary and complete feedingstuffs for pigs [10], while in Serbia, the ML of OTA in complementary and complete feedingstuffs for pigs is 0.1 mg/kg [11].

Regulatory limits for OTA in meat and entrails differs among countries. Legislation in Romania and Slovakia for ML of OTA in pig kidney is 5µg/kg [12,13], while in Denmark, the ML is 10 µg/kg [14] (Jørgensen and Petersen, 2002). About 40% of the level of OTA in kidneys can be found in the meat of the same animal [15]. The Italian regulation for OTA in meat allows a ML of 1 µg/kg [16].

It should be mentioned that OTA in meat products can have differing origins. Some of this mycotoxin can originate from the consumed mycotoxin in feed and this results in OTA residues in the meat. The other origins are from the added spices used in meat processing or from mold growth during the curing process, which can also lead to contamination or production with OTA and/or with other mycotoxins.

After ingestion of feed contaminated with the OTA, 65% is absorbed by the pigs. In the blood, OTA binds to albumin and other macromolecules. The serum half-life of OTA after oral administration is 72-120 h [17]. During the process of metabolism, OTA is accumulated in the tubules of the kidney [18], so the amount of OTA in kidneys can be good indicator of overall exposure of animals to this mycotoxin.

Aside from the problems related to human exposure to OTA residues in meat, OTA can have affect the production parameters in live pigs. OTA added to pig feed at the level of 25 µg/kg reduced the final body weight, average daily gain and feed efficiency, while food intake was not affected at the end of the study [1].

In food and feed production, it is a rare situation that only one mycotoxin is found. This is indeed the situation with OTA, as usually several mycotoxins, the effects of which can be synergistic, co-exist in the food/feed [19]. Very intensive production processes in industrial pig production means animals are at the edge of their biological limits, so even the relatively small amounts of mycotoxins in feed can have dramatic effects on producers’ productivity and profitability in the difficult market conditions in Serbia.

2. Materials and methods

2.1. Sample collection and preparation

Pig’s kidneys (five from each farm) were collected from 19 pig farms in Vojvodina in the period October-December, 2018 at the two slaughterhouses. The 95 pig’s kidneys originated from all three districts in Vojvodina: Banat (4 farms, 20 kidneys), Bačka (10 farms, 50 kidneys) and Srem (5 farms, 25 kidneys). Kidneys originated only from healthy pigs, and on inspection, there were no visual signs of changes on the kidneys. After collection, each kidney was put in a plastic bag, labeled and frozen to -20 °C.

2.2. Extraction
Each entire kidney was blended and a potion of 25 g was extracted with 100 ml of acetonitrile:water (84:16, v/v) using Ultra Turrax T18 homogenizer (IKA, Germany) for 3 min at 11,000 rpm. Crude extract was then filtered through quantitative slow filtration filter paper (Filtros Anoia, Spain). Prior to HPLC analysis, filtered crude extract was cleaned up on MycoSep® 229 SPE columns (Romer Labs, USA).

2.3. HPLC analysis
OTA was determined on an Agilent Technologies 1260 Infinity LC system, using an ODS Hypersil column (150 x 4.6 mm, 5µm) (Agilent Technologies, USA). Detection was conducted using a FLD detector at excitation wavelength $\lambda_{ex}=333$ nm and emission $\lambda_{em}=470$ nm. The mobile phase was acetonitrile:water (50:50, v/v) with 1% of acetic acid at a flow rate of 1 ml/min. Injection volume was 20 µl, run time was set to 8 minutes, and retention time for OTA was 5.4 minutes. The limit of quantification was 0.10 µg/kg.

3. Results and Discussion
The OTA incidences and levels in pig’s kidneys originating from 19 farms in Vojvodina are shown in Table 1.

| District | Number of samples | >LOQ | % | Average µg/kg | Median µg/kg | Range µg/kg |
|----------|------------------|------|---|--------------|-------------|------------|
| Bačka    | 50               | 10   | 20.0 | 1.43         | 1.42        | 0.10-3.97 |
| Banat    | 20               | 1    | 5.0  | 3.93         | 3.93        | -          |
| Srem     | 25               | 3    | 12.0 | 0.31         | 0.30        | 0.10-0.54 |
| Vojvodina| 95               | 14   | 14.7 | 1.36         | 0.99        | 0.10-3.97 |

In total, 95 kidneys originating from Vojvodina were analyzed for the occurrence of OTA. The highest number of kidneys was taken from Bačka district, which is the region of Vojvodina with the most intensive agriculture and swine production. The average value in all kidneys in which OTA was above the limit of quantification was 1.36 µg/kg, while median value was 0.99 µg/kg. The overall incidence of OTA in pig’s kidneys from Vojvodina was 14.74%. The highest content of OTA in one kidney was 3.97 µg/kg, in a pig from Bačka district. In Banat district, out of 20 kidneys taken from 4 different farms, OTA was found in only one kidney at a level of 3.93 µg/kg.

Table 2. The occurrence of OTA on farms in Vojvodina by district (Bačka, Banat and Srem)

| District | No. of farms | Samples per farm | Farms with no OTA | 1 positive sample from farm | 2 positive samples from farm | 3 positive samples from farm | 4 positive samples from farm | 5 positive samples from farm |
|----------|--------------|------------------|-------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Bačka    | 10           | 5                | 5                 | 2                           | 2                           | 0                           | 1                           | 0                           |
| Banat    | 4            | 5                | 3                 | 1                           | 0                           | 0                           | 0                           | 0                           |
| Srem     | 5            | 5                | 2                 | 3                           | 0                           | 0                           | 0                           | 0                           |
| Vojvodina| 19           | 95               | 10                | 6                           | 2                           | 0                           | 1                           | 0                           |

Only on one farm, 4 out of 5 samples contained OTA, and that farm was from Bačka district (Table 2). When farms did stock pigs with OTA in their kidneys, most commonly, OTA was found only in one sample (6 farms in Vojvodina). Ten farms stocked pigs with no determinable level of OTA in their kidneys. None of the participating farms had any previous history of problems related to OTA, so this likely partially explains the relatively low occurrence of OTA. However, on one farm, four out of five
analyzed pig’s kidneys contained OTA, which can be a sign of a possible problem. Since OTA is mainly produced by feed storage fungi [2], the management of feed storage can have huge effect on the occurrence of OTA. Also, since the feed storage conditions can affect the OTA, the incidence of OTA can vary from the farm to farm as can be seen in Table 2.

The maximum level of OTA in our pig’s kidneys, 3.97 µg/kg, was below 5 µg/kg, the ML for OTA in kidneys regulated by some EU countries (Romania, Slovakia) [13]. However, the presence of OTA in kidney means OTA is likely in meat and meat products derived from these pigs as well. It is obvious the incidence of OTA was very low in the analyzed pig’s kidneys. The occurrence of OTA in food and feed depends on climatic conditions and food/feed storage conditions. In Croatia, a few periods with high incidences of OTA and high OTA levels in cereals were reported [5]. It is possible that Serbia, with similar climatic conditions and agricultural production, also has periods with high incidences of OTA, which could lead to OTA in pigs during production or in kidneys or pork.

There are a lot available data on the occurrence of OTA in pig’s kidneys, organs, and in meat and meat products in Europe. Many studies were conducted on different types of animal organs, in different types of pig production and in different meat products. In the French monitoring program for OTA in pig’s kidneys, less than 10% (out of 300) of kidneys were significantly contaminated with this mycotoxin [20]. In 1997, 1% of pig’s kidneys contained from 0.4 to 1.4 µg/kg OTA, while in 1998, 7.6% had the OTA in the range from 0.5 to 5.0 µg/kg [20]. However, in another study from France, out of 70 pig’s livers (from three different production systems), OTA was detected in 67% (range from <0.10 to 3.65 µg/kg) [13]. It is known that the distribution of OTA follows the pattern kidney>liver>serum [21]. In a three-year study from Poland, out of 430 animal tissue samples (pig’s kidneys, poultry liver and fish muscles), 94 samples were contaminated with OTA in the range from 0.2 to 5.0 µg/kg, 4 samples were in the range 5.0 to 10.0 µg/kg and three samples contained above 10 µg/kg OTA [22]. It was not clear how many of the pig’s kidneys contained OTA.

In an Italian report, 31 of 54 analyzed kidneys contained OTA in the range ≤0.05 to ≤0.5 ng/g, while 11 samples had <0.5 to <1 ng/g [16]. Italian guidance for this mycotoxin is in form of the content in meat, so researchers paid attention to those products. A total of 172 salami produced in different regions of Italy were analyzed and OTA was detected in 22 salami, while three salami exceeded the Italian ML for OTA in meat (1 µg/kg) [23]. However, the origin of this OTA contamination was not clear because the mycotoxin can result from the meat, spices or processing. In Croatia, much research was conducted regarding OTA levels in cereals [3, 5, 24] or in finished meat products [25, 26]. In Romania, in a survey on slaughter pigs, the incidence of OTA in kidneys was 79%, with the mean level of 0.54 ng/g, median of 0.40 ng/g and maximum content of 3.18 ng/g [12]. In 2006, 8 out of 10 pig sera taken at the slaughter line from farms in Bulgaria that had problems with nephropathies (enlarged and pale kidneys) contained 28.8 µg/l OTA [27]. In 2007, 9 of 10 samples contained OTA with the mean level of 6.3 µg/l [27]. Previously in Serbia, 33% of pig’s kidneys contained OTA in the range from 0.17 to 52.5 ng/g [28]. Another study detected OTA in 70% of pig’s kidneys, with mean content of 3.97 ng/g (range 1.3 to 22.0 ng/g) [21].

In Serbia, researchers are not currently focused on OTA, since their attention is oriented toward aflatoxin B1 due to its outbreak in 2012 after heat waves and drought. However, cooler weather with a lot of rain was recorded in 2014, i.e., possible favorable conditions for OTA-producing fungi. There is a need for the continuous OTA monitoring, although our results do not suggest any larger problems. Traditional dried pork products made under domestic conditions might be at higher risk of OTA contamination.

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