Occurrence and Contamination Level of Ochratoxin A in Tissues of Slaughtered Pigs in Greece

Mikela VLACHOU, Andreana PEXARA, Nikolaos SOLOMAKOS, Alexander GOVARIS

Laboratory of Hygiene of Foods of Animal Origin, University of Thessaly, Faculty of Veterinary Science, Karditsa


ORCID iDs of the authors: M.V. 0000-0001-5700-6372, A.P. 0000-0002-4819-2705, N.S. 0000-0002-7578-4984, A.G. 0000-0002-9412-034X

Abstract

The aim of this work was to investigate the occurrence and contamination levels of ochratoxin A in slaughtered pigs in Greece. Samples were obtained from 1260 randomly collected healthy slaughtered pigs originating from 84 different farms located in four different geographic major swine farm regions of Greece (Western Macedonia, Thessaly, Central Greece, Central Macedonia). The ochratoxin A presence in blood serum, kidney, liver, muscle, and fatty tissues in samples of slaughtered pigs was examined by using an enzyme-linked immunosorbent assay method. None of all kidney, liver, muscle, and fat samples were found ochratoxin A contaminated. A total of 579 (45.95%) of the 1260 analyzed serum samples were ochratoxin A contaminated in a range from 0.21 to 5.39 μg/mL (mean level 0.66 μg/mL) and ochratoxin A was present in serum in 65 (77.38%) of the 84 examined farms. The mean levels of ochratoxin A serum contamination in regions of Western Macedonia, Central Greece, and Central Macedonia were similar (0.29-0.32 μg/mL). The highest ochratoxin A value (mean 1.20 μg/L, median 0.53 μg/L) with the highest coefficient of variation (1.12) was found in the Thessaly region. Although ochratoxin A was not found in pork meat in Greece, the presence of ochratoxin A in pig serum requires continuous ochratoxin A monitoring in the swine industry and pork meat.

Keywords: Enzyme-linked immunosorbent assay (ELISA), ochratoxin A, slaughtered pigs, tissues

Introduction

Ochratoxin A (OTA) is a mycotoxin, produced by several fungal species of the genera Aspergillus and Penicillium, in a wide variety of agricultural commodities [European Food Safety Authority (EFSA), 2020; Kure & Skaar, 2019; Vlachou et al., 2022]. The major toxigenic OTA species are Penicillium verrucosum and Penicillium nigrum in the Penicillium family, and Aspergillus ochraceus, Aspergillus niger, and Aspergillus carbonarius in Aspergillus family (Gallo et al., 2017; Wang et al., 2016).

Ochratoxin A was found to possess nephrotoxic, immunosuppressive, genotoxic, and teratogenic properties (EFSA 2020; Stoev, 2020). In humans, dietary exposure to OTA represents a serious health issue, including nephropathies and urinary tract tumors (Heussner & Bingle, 2015). Ochratoxin A has been classified by the International Agency for Research on Cancer as possible carcinogenic to human “Group 2B” (IARC, 1993).

Among food-producing animals, pigs are the most susceptible animals to OTA exposure (Duarte et al., 2011). High OTA levels have been found in swine feed in various countries (Ganesan et al., 2021; Leiva et al., 2019; Li et al., 2014; Pozzo et al., 2010; Rosa et al., 2009), highlighting the pig exposure to the toxin. Exposure of pigs to OTA via contaminated feed can lead to OTA presence in high levels in blood followed by low levels in kidneys and liver, and even lower levels in muscles and fat (Altavini et al. 2017; Battacone et al., 2010; Hort et al., 2018; Persi et al., 2014).

Pork meat and meat products, especially those made from pig blood or organs such as the kidney or liver, are considered important sources of chronic dietary exposure to OTA in humans (EFSA, 2020). Although the EU established maximum limits for OTA in a variety of foods [Commission Regulation (EC) No 1881/2006] (CEC, 2006), OTA limits have been not set yet in foods of animal origin including pork meat and meat products. Some European countries have set limits on OTA concentrations in meat, edible offal, and/or meat products, such as 10 μg/kg in pig kidney and 25 μg/mL in pig blood in...
Denmark, 10 μg/kg in pig liver in Estonia, 5 μg/kg in pig kidney, liver and meat in Romania, 5 μg/kg in pork meat in Slovakia, and 1 μg/kg in pork meat and derived products in Italy (Vila-Donat et al., 2018). No binding limits on OTA in meat and meat products have been set in the United States, Australia, Canada, and Asia (Agriopoulou et al., 2020; Malir et al., 2016).

Several studies have been conducted worldwide on the occurrence of OTA in edible tissues and organs of slaughtered pigs. Results of these studies revealed various levels of OTA contamination in pork of OTA in edible tissues and organs of slaughtered pigs. Results of several studies have been conducted worldwide on the occurrence of OTA in edible tissues and organs of slaughtered pigs. Results of these studies revealed various levels of OTA contamination in pork meat and offal from slaughtered pigs in various countries, and in some research high levels particularly in blood serum and kidney were found (Ganesan et al., 2021; Vlahou et al., 2022). Besides chromatographic methods (e.g., high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC)) which are considered precise OTA examination methods, enzyme-linked immunosorbent assay (ELISA) is considered an invaluable tool and is often used for OTA determination for screening purposes in pork meat and pork products (EFSA, 2020; Matrella et al., 2006; Perši et al., 2014). An ELISA analysis for OTA presence was used in pork raw materials such as blood, liver, and kidney (Matrella et al., 2006; Perši et al., 2014; Pleadin et al., 2016).

To our knowledge, in Greece, no such data are available in the literature. The aim of this study was to determine the occurrence and concentration of OTA in edible tissues of slaughtered pigs in Greece, obtained by ELISA as a screening method.

**Method**

**Sample Collection**

The survey was conducted between November 2018 and April 2021 and involved 1,260 randomly collected healthy slaughtered pigs originating from 84 different farms (15 pigs/farm) located in four different geographic regions of Greece (Western Macedonia, Thessaly, Central Greece, Central Macedonia) (Figure 1). Samples were collected from regions with significant swine industry (data provided by the Ministry of Rural Development and Food, 2017).

Pigs were slaughtered in slaughterhouses under the supervision of a veterinarian and the procedure was carried out in full line with the provisions of the Council Regulation (EC) No. 1099/2009 on the protection of animals at the time of killing (CEC, 2009). None of the slaughtered pigs showed macroscopic lesions of the kidneys or other lesions during the post-mortem inspection that indicate a possible nephropathy or other disease. Samples of 50 mL blood/pig were collected during bleeding and after slaughtering, >100 g of liver, muscle (diaphragm), fatty tissue, and one whole kidney were obtained from each pig in the slaughterhouses. Blood samples were allowed to clot for 30–60 minutes at room temperature. After the separation of serum via blood centrifugation at 2000 × g for 5 minutes, all samples were stored at −20°C until OTA analysis. No preservatives were added. Samples were thawed immediately before analysis as described in the following.

**Sample Preparation for Enzyme-Linked Immunosorbent Assay**

**Extraction of Serum**

Ochratoxin A extraction from serum was carried out according to ELISA kit manufacturer instructions [Application Note RIDASCREEN® OTA 30/15 (R1311) in combination with OCHRAPREP® Immunoaffinity column (RRBP14/P14B)]. Briefly, an appropriate aliquot of serum was centrifuged. About 0.5 mL of clear serum was added with 1 mL methanol (100%) (Scharlab, Barcelona, Spain); the mixture was mixed for 30 seconds and centrifuged (Heraeus Biofuge Stratos High-Speed Centrifuge, Hanau, Germany) for 10 minutes at 3000 × g/10°C; 0.9 mL of the supernatant was added with 10 mL 130 mM NaHCO₃ (Chem-Lab NV, Zedelgem, Belgium).

The mixture was briefly mixed and passed through an OCHRAPREP® column (RRBP14/P14B) by using the hydrostatic pressure; the column was rinsed with 10 mL 130 mM NaHCO₃/5% methanol and subsequently was rinsed with 2 mL deionized water. All the liquid from the column was removed by pressing air. The elution of OTA was achieved with 2 mL methanol (100%) into a new vial. The eluted sample was evaporated completely at 60°C under a mild nitrogen stream, and then the dried residue was solved again with 0.6 mL 130 mM NaHCO₃.

**Extraction of Ochratoxin A From Kidney and Liver**

The extraction of OTA from kidney and liver samples was performed according to the method described by Pleadin et al. (2016) based on the method described by Perši et al. (2014). Briefly, a representative amount of the sample (100 g) was homogenized with Ultra Turrax T25 homogenize; to 1 g of each homogenized sample, 0.5 mL of 1 M H₃PO₄ (Chem-Lab NV, Zedelgem, Belgium) and 3 mL of ethyl acetate (Scharlab, Barcelona, Spain) were added. The samples were then vigorously mixed, centrifuged (1 minute, 2000 rpm) at room temperature (22 ± 2°C), and a supernatant (ethyl acetate) was transferred and supplemented with additional 3 mL of ethyl acetate. After mixing and centrifugation, ethyl acetate layers were combined, supplemented with 3 mL of 0.65 M NaHCO₃, vortexed, and continued to be mixed for another 15 minutes. After the centrifugation (5 minutes, 2000 rpm), 1 mL of the lower aqueous phase was transferred and...
heated in a water bath (100 °C, 3 minutes). Finally, the extract was shaken, cooled to room temperature, and 4 mL of distilled water was added. An aliquot was then diluted with 0.13 M NaHCO₃.

Extraction of Ochratoxin A Muscle and Fatty Tissues
The extraction of muscle and fatty tissues samples was performed according to the method described by Pleadin et al. (2016). Briefly, a representative amount of the sample (100 g) was homogenized with Ultra Turrax T25 homogenizer; 1 g of a homogenized sample was diluted in 6 mL of ethyl acetate; mixed on a shaker for 30 seconds and then samples were shaken for 15 minutes using rotatory movements. About 0.5 mL of 1 mol/L H₃PO₄ was added and first was diluted in 6 mL of ethyl acetate; mixed on a shaker for 30 seconds and then for another 15 minutes using rotatory movements. After centrifugation, the supernatant (the ethyl acetate layer) was transferred using decantation; the extraction procedure was repeated by virtue of the addition of 6 mL of ethyl acetate. A total of 3 mL of 0.26 mol/L NaHCO₃ was added to the combined ethyl acetate layers; were then mixed on a shaker for 15 seconds and stirred for another 15 minutes using rotatory movements. After centrifugation, 0.8 mL of the lower aqueous phase was transferred and heated in a water bath (100 °C, 5 minutes); the sample was then gently shaken, cooled to room temperature, and diluted in 0.2 mL of 0.225 mol/L HCl (Scarlab, Barcelona, Spain) and 1 mL of 0.13 mol/L NaHCO₃.

Enzyme-Linked Immunosorbent Assay
The ELISA immunoassay was carried out using BRIDASCREEN® OTA 30/15 ELISA kit provided by R-Biopharm (Darmstadt, Germany). The kit comprises a micro-titer plate with 96 wells coated with antibodies against OTA, as well as the standard OTA water solution (0, 50, 100, 300, 900, and 1800 ng/mL), a conjugate (peroxidase-conjugated OTA), a substrate/chromogen solution (tetramethylbenzidine), a stop solution (1 mol/L), a dilution buffer, and a washing buffer (10 mM phosphate buffer, pH 7.4). All other chemicals used for the analysis were of an analytical grade. A total of 50 μL of each sample, pre-treated as described above, per well was applied to the assay which was performed in full line with the kit manufacturer’s instructions and made use of an auto-analyzer ChemWell 2910 (Awareness Technology, Inc., Palm City, Florida, USA). The limit of detection (LOD) and recovery value for serum, provided by the ELISA kit manufacturer, was 0.207 ppb and 93%, respectively. The LOD, limit of quantification (LOQ), and recovery value was 1.44 μg/kg, 1.89 μg/kg, 85.1% and 1.54 μg/kg, 2.11 μg/kg, 82.3% for kidney and liver, respectively. The LOD, LOQ, and recovery value was 0.45 μg/kg, 0.61 μg/kg, 88.7%, and 0.66 μg/kg, 1.11 μg/kg, 86.5% for muscle and fatty tissue, respectively (Pleadin et al., 2016).

Statistical Analysis
Statistical data analysis [mean and median values, coefficient of variation (CV)] was performed using Microsoft Excel 2019 software (version 2103, Microsoft Corporation, Redmond, Washington, USA).

Results, Discussion, and Conclusion
The object of the present study was to estimate the occurrence and contamination level of OTA in slaughtered pigs in Greece. Due to the large number of samples, ELISA was implemented as a screening method. Although ELISA tends to slightly underestimate the OTA level compared to chromatographic methods such as HPLC, the literature data have shown that ELISA has become very popular when it comes to the determination of mycotoxins not only due to its relatively low cost and easy application but also due to its high sensitivity and capability to yield a rapid analysis of a large number of samples (EFSA 2020; Matrella et al., 2006; Perši et al., 2014).

Occurrence of Ochratoxin A in Tissues
The occurrence of OTA in pig serum, kidney, liver, muscle, and fat in pigs and farms is summarized in Table 1. In the present study, we used the “positive” for the farm when one OTA contaminated tissue from at least one animal from the farm was found. None of the 1260 samples of pig kidney, liver, muscle, and fat samples analyzed with ELISA were found OTA contaminated; OTA concentrations were below the LOD of the applied methods. In the study of Matrella et al. (2006), all 54 pig kidneys from pigs slaughtered in Italy analyzed with ELISA were found contaminated with a mean value for OTA 0.29 μg/kg, whereas 42 (80.9%) muscle samples were found positive with mean value 0.024 μg/kg. In accordance with the results of our study, Wei et al. (2018) did not find any presence of OTA either in pork meat or in swine liver, kidney, and fat by using an ultraperformance liquid chromatography-tandem mass spectrometry system (UPLC-MS/MS). Conversely, high occurrence in pig tissues, up to 100% particularly in kidney and liver reported in other performed studies using HPLC-FD, ultra-HPLC/MS/MS, or LC-MS/MS analysis (Ceci et al., 2007; Curtui et al., 2001; Gareis & Scheuer, 2000; Giacomo et al., 2016; Hou et al., 2015; Jørgensen & Petersen, 2002; Milčević et al., 2008; Monaci et al., 2004; Polovinski Horvarovic et al., 2019; Skarkova et al., 2013), in which LOD is significantly lower. Various OTA levels in pig kidneys have been reported, but in most studies, low concentrations were recorded, lower than the LOD of the applied method in the present study. In accordance with the results of our study, the LOD is 0.45 μg/kg, whereas 42 (80.9%) muscle samples were found positive with mean value 0.024 μg/kg. In accordance with the results of our study, Wei et al. (2018) did not find any presence of OTA either in pork meat or in swine liver, kidney, and fat by using an ultraperformance liquid chromatography-tandem mass spectrometry system (UPLC-MS/MS). Conversely, high occurrence in pig tissues, up to 100% particularly in kidney and liver reported in other performed studies using HPLC-FD, ultra-HPLC/MS/MS, or LC-MS/MS analysis (Ceci et al., 2007; Curtui et al., 2001; Gareis & Scheuer, 2000; Giacomo et al., 2016; Hou et al., 2015; Jørgensen & Petersen, 2002; Milčević et al., 2008; Monaci et al., 2004; Polovinski Horvarovic et al., 2019; Skarkova et al., 2013), in which LOD is significantly lower. Various OTA levels in pig kidneys have been reported, but in most studies, low concentrations were recorded, lower than the LOD of the applied method in the present study.
study. In Italy and the Czech Republic, low OTA levels in kidneys, in the range of 0.17–0.91 μg/kg (Giacomo et al., 2016) and 0.15–0.46 μg/kg (Skarkova et al., 2013), respectively, were found. In China, the OTA concentrations ranged between 0.03 and 0.323 μg/kg in pig kidneys (Hou et al., 2015). However, Polovinski Horvatovic et al. (2019) found OTA levels in examined pig kidneys as high as 3.97 μg/kg (mean 1.36 μg/kg) in Serbia.

Similarly, in pig liver samples, Giacomo et al. (2016) reported OTA concentrations with a range of 0.07–0.59 μg/kg and a mean value of 0.35 μg/kg in Italy. However, higher OTA contamination levels in pig liver of 1.46 μg/kg (Chen et al., 2012) in China and 0.10–3.65 μg/kg (Hort et al., 2018) in France were also reported.

Pig muscle samples were found contaminated with OTA with a range of 0.15–0.20 μg/kg in the Czech Republic (Skarkova et al., 2013) and 0.09–0.20 μg/kg in Italy (Giacomo et al., 2016) and 0.03–0.23 ng/g (μg/kg) in Canada (Tam et al., 2011). In a study conducted in Portugal 6 out of 15 muscles (40%) were found positive using HPLC-FLD but in all samples, OTA concentrations were below the LOQ of the method (0.9 μg/kg). In Italy, Meucci et al. (2019) found maximum OTA concentration in pig muscles reared in indoor system, ranging between 0.055 and 0.078 μg/kg in indoor and outdoor system, respectively. Analysis by LC-MS/MS showed higher OTA levels in muscles at 1.25 μg/kg in China (Chen et al., 2012) and 0.88 μg/kg in Italy (Cao et al., 2018). Analysis by SIDA–UHPLC–MS/MS showed also high levels of OTA (maximum 1.15 μg/kg) in the muscles of French pigs from organic farming production systems (Hort et al., 2018). The OTA levels in fat tissues of slaughtered pigs in Italy were found low with values of 0.079 and 0.085 μg/kg for indoor and outdoor systems, respectively (Meucci et al., 2019).

Ochratoxin A contamination assessment showed that 579 (45.95%) of the analyzed serum samples (N=1260) were OTA contaminated in a range from 0.21 to 5.39 μg/mL (mean level 0.66 μg/mL) and 65 (77.38 %) of farms (N=84), as presented in Table 1. Compared to other published data for the OTA occurrence and contamination level in pig serum, our results were generally lower than in other countries. For example, 98% and 94% of serum samples tested by HPLC-FLD in Romania in the studies of Curtui et al. (2001) and Curtui and Gareis (2001), respectively were found OTA positive. Thus, OTA ranged from 0.05 to 13.4 μg/L, whereas 85% of positive samples contained OTA under 5 μg/L (Curtui et al., 2001). Curtui & Gareis (2001) reported that the levels of OTA were in the range of 0.1–13.4 μg/L. In Canada, pig serum analysis revealed that all the positive samples had concentrations of OTA between 5.4 and 20 μg/L, in the years 1988–1990 (Frohlich et al., 1991). In comparison to our results, lower incidence of OTA (31.1%) was found in pig serum in Serbia; but contamination levels were higher and ranged between 0.22 and 220.8 μg/L (Miličević et al., 2008).

In our study, the relatively high occurrence of OTA in serum samples as compared to no OTA presence in other examined tissues can be attributed to the lower LOD of applied method (0.207 μg/kg) in serum samples in comparison to the counterparts in other tissue samples. Moreover, the results of this study are consistent with previous reports in terms of OTA tissue distribution proven to follow the pattern: blood > kidney > liver > muscle > fat (Curtui et al., 2001; Dall’Asta et al., 2010; Gareis & Scheuer, 2000, Lusky et al., 1993), hence the low concentration in serum samples (mean level 0.66 μg/mL) seems to lead to the non-detection of the toxin in other tissues. Matrella et al. (2006) reported a ratio “OTA content in muscle/OTA content in kidney” of ca. 4% as obtained by using the ELISA method. However, in other studies with generally higher OTA contamination levels, the above ratio was found to vary between 10% and 90% (Curtui et al., 2001; Jørgensen and Petersen, 2002; Krogh et al., 1976; Madsen et al., 1982). In studies pigs fed with OTA-contaminated feed, the highest average OTA concentration was found by ELISA in the kidneys, followed by blood or liver of the treated animals and lower in muscle and fat (Perši et al., 2014; Pleadin et al., 2016).

The reasons of these variability in OTA concentrations in various tissues were ascribed to different factors such as the length of exposure, the OTA dose, the mode of toxicant application, and the exposure in relation to time of slaughtering (Matrella et al., 2006; Pfohl-Leszkowicz & Manderville, 2007; Polovinski Horvatovic et al., 2019). Moreover, pigs fed with naturally contaminated feed showed higher OTA serum/plasma levels compared to studies with pigs fed with artificially supplemented OTA-contaminated feed (Stoev et al., 2002).

In our study, 65 out of 84 tested farms (77.38%) were OTA serum positive. In an earlier study, pig’s blood samples from 47 Sweden farms (16.8% of the total) exhibited OTA in amounts of 0.2 μg/L of blood. In our study, none of the participating farms had any previous history of hygienic problems related to OTA, so this likely explains the no determinable level of OTA in other tissues and especially in the kidneys and is in parallel to Polovinski Horvatovic et al. (2019). In feeds, OTA is mainly produced by fungi during storage, so the management of feed on the farm can have an effect on the occurrence of OTA (Polovinski Horvatovic et al. 2019).

Regional Distribution of Ochratoxin A in Pig Serum

The incidences of OTA in pig serum in the four regions where samples were collected were found high and similar in Western Macedonia and Thessaly, both on farms (100% and 95.45%, respectively) and in pigs (75.69% and 70.91%, respectively). The lower incidence was recorded in Central Greece region (Table 2). The mean level of contamination in three of them (Western Macedonia, Central Greece, Central Macedonia) are very similar; whereas the highest OTA value (mean 1.20 μg/L, median 0.53 μg/L) with the highest CV (1.12) was found in the Thessaly region. The low incidence and OTA contamination level in Central Macedonia (30.00%, mean 0.32 μg/L, range 0.21–0.90), a region situated at the border with Bulgaria (Figure 1) is very interesting.

A very different distribution of OTA serum-positive pigs’ percentage on farms by regions was found (Table 3). In the Thessaly region, in 12 out of 22 (54.55%) farms included in our study, the highest percentage (range 75–100%) of tested pigs were found OTA serum positive, whereas in the Central Greece region in 15 out of 19 (78.95%) farms low incidence (range 0–25%) of OTA serum-positive pigs was recorded. Similarly, to our results in the study of Polovinski Horvatovic et al. (2019), the incidence of OTA in pig kidneys varied from farm to farm in different districts of Serbia, and farms (6 out of 19) with only one positive out of five samples were found. Factor associated with individual variations, the time and the order of magnitude of OTA level in feed seems to affect the percentage of pigs that are positively on a farm.
Table 2.
Presence of OTA in Pig Serum in the Regions Where Samples Were Collected

<table>
<thead>
<tr>
<th>Region</th>
<th>Farms (N = 84)</th>
<th>n (%)</th>
<th>Pigs (N = 1260)</th>
<th>n (%)</th>
<th>Mean±(µg/L)</th>
<th>CV</th>
<th>Median±(µg/mL)</th>
<th>Range±</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Macedonia</td>
<td>17</td>
<td>17 (100)</td>
<td>255</td>
<td>193 (75.69)</td>
<td>0.29</td>
<td>0.16</td>
<td>0.28</td>
<td>0.21–0.44</td>
</tr>
<tr>
<td>Thessaly</td>
<td>22</td>
<td>21 (95.45)</td>
<td>330</td>
<td>234 (70.91)</td>
<td>1.20</td>
<td>1.12</td>
<td>0.53</td>
<td>0.21–5.39</td>
</tr>
<tr>
<td>Central Greece</td>
<td>19</td>
<td>9 (47.37)</td>
<td>285</td>
<td>35 (12.28)</td>
<td>0.26</td>
<td>0.14</td>
<td>0.26</td>
<td>0.21–0.34</td>
</tr>
<tr>
<td>Central Macedonia</td>
<td>26</td>
<td>18 (69.23)</td>
<td>390</td>
<td>117 (30.00)</td>
<td>0.32</td>
<td>0.37</td>
<td>0.29</td>
<td>0.21–0.90</td>
</tr>
</tbody>
</table>

Note: a: Calculated for positive samples only. CV = coefficient of variation; N = total number of analyzed samples; n = number of positive samples (>limit of detection, see the “Methods” section); OTA = ochratoxin A.

Table 3.
Distribution of OTA Serum-Positive Pigs’ Percentage on Farms by Regions

<table>
<thead>
<tr>
<th>Region</th>
<th>Serum-Positive Pigs’ Percentage (%) (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–25%</td>
</tr>
<tr>
<td>Western Macedonia (N=17)</td>
<td>1 (5.88)</td>
</tr>
<tr>
<td>Thessaly (N=22)</td>
<td>2 (9.09)</td>
</tr>
<tr>
<td>Central Greece (N=19)</td>
<td>15 (78.95)</td>
</tr>
<tr>
<td>Central Macedonia (N=26)</td>
<td>13 (50.00)</td>
</tr>
</tbody>
</table>

Note: N = total number of tested farms; n = number of farms; OTA = ochratoxin A.

In an earlier study, geographical variation of OTA occurrence in pigs’ blood samples within Sweden was not detected (Hult et al., 1980). However, in other studies, and in accordance with our results, differences in regional distribution of OTA in pig serum have been also reported, such as in Romanian and Serbian slaughtered pigs (Curtui et al., 2001; Milićević et al., 2008). The regional differences have been associated to the concentration differences in the corresponding formulas, and differences in the storage condition of feedstuffs. In addition, fluctuations in mold growth and contamination level of cereals associated with the climatic conditions of each region may result in variations in dietary exposure to OTA (Milićević et al., 2008; Polovinski Horvatovic et al., 2019).

It has been suggested that although differences due to the time of exposure, the order of magnitude of OTA level in feed, individuals, and methods of analyses may always occur, the level of OTA in serum can lead to an estimation of feed contamination with the toxin (Curtui et al., 2001; Lusky et al. 1993; Madsen et al. 1982).

Overall, the finding of our study is in accordance with the previously expressed opinion that OTA determination in serum could serve as a rapid tool for OTA monitoring in slaughtered pigs as well as in pig farms (Curtui et al., 2001; Perši et al., 2014; Pleadin et al., 2016). Moreover, our results reinforce the previously suggested view (Hult et al., 1980) that a larger serum sample size is required for animals’ OTA exposure assessment in farms.

In our study, only serum samples of slaughtered pigs were found OTA contaminated, in contrast to kidney, liver, muscle, and fatty tissues. The presence of OTA in pig serum varied in different regions of Greece. In conclusion, our results do not indicate any large problem with OTA in pork meat in Greece, the incidence of OTA in pig serum demonstrates the need for continuous OTA monitoring in the production of pork and its products.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of the University of Thessaly (Date: 1/2/2018, Number: 154).

Informed Consent: Written informed consent was obtained from Andreana Pexara who participated in this study.

Peer-review: Externally peer-reviewed.


Declaration of Interests: The authors declare that they have no competing interest.

Funding: The research was funded by the Laboratory of Hygiene of Foods of Animal Origin, Faculty of Veterinary Science, University of Thessaly.

References


Vila-Donat, P., Marín, S., Sanchis, V., & Ramos, A. J. (2018). A review of the mycotoxin adsorbing agents, with an emphasis on their multi-binding capacity, for animal feed decontamination. Food and Chemical Toxicology, 114, 246–259. [CrossRef]

