ACTA VET. BRNO 2022, 91: 409–415; https://doi.org/10.2754/avb202291040409

A survey on occurrence of ochratoxin A in the kidneys of slaughtered pigs in the Czech Republic during the years 2012–2021

Zuzana Široká[1], Petr Linhart[1], Alena Honzlová[2], Veronika Vlasáková[3], Jan Rosmus[4], Pavla Macharáčková[5], Zdeňka Svobodová[1], Martin Svoboda[6]

[1]University of Veterinary Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Department of Animal Protection and Welfare and Veterinary Public Health, Brno, Czech Republic
[2]State Veterinary Institute Jihlava, Jihlava, Czech Republic
[3]State Veterinary Administration of the Czech Republic, Prague, Czech Republic
[4]State Veterinary Institute Praha, Prague, Czech Republic
[5]State Veterinary Institute Olomouc, Olomouc, Czech Republic
[6]University of Veterinary Sciences Brno, Faculty of Veterinary Medicine, Ruminant and Swine Clinic, Brno, Czech Republic

Received July 22, 2022
Accepted October 10, 2022

Abstract

Ochratoxin A (OTA) is a mycotoxin produced by several moulds of *Aspergillus* and *Penicillium* genera on many agricultural commodities used for feed production. It is primarily a nephrotoxic substance, but it can also cause immunosuppression in pigs and increase their susceptibility to infections. Porcine mycotoxic nephropathy (PMN) is a chronic poisoning associated with OTA exposure. The aim of this paper was to evaluate the content of OTA in pig kidneys which were examined during the regular monitoring of the safety and quality of food of animal origin in the Czech Republic during the years 2012–2021. Results revealed no differences between the monitored years or age categories and no differences between the year seasons, which could have an influence on the storage conditions and thus feed contamination by moulds. The values found in this study are low and similar to those assessed in several studies performed in other European countries and do not exceed the limits set in their legislation, which means these organs do not pose a risk for consumers and do not indicate PMN in monitored pigs. Czech Republic as well as EU legislation do not regulate OTA levels in the food of animal origin yet.

Mycotoxin, nephrotoxicity, OTA

Ochratoxin A (OTA) is a ubiquitous mycotoxin produced by several genera of *Aspergillus* and *Penicillium* moulds in a broad range of commodities such as cereal grains, legumes, and peanuts. Especially improper storage of crops contaminated with *Penicillium* spores in humid conditions can lead to mycotoxin production at a range of temperatures between 4 and 30 °C (Marin et al. 2009). These crops are then fed directly or used for commercial feed production and thus are a source of OTA exposure to animals. Humans are probably less affected as OTA levels in human food are lower compared to those in animal feed and moreover is slightly destroyed during cooking over 180 °C and other processing of human food depending on the commodity in contrary to the usually raw feed (Raters and Matissek 2008; Kabak 2009; Gupta et al. 2018).

Ochratoxin A found in feed can adversely affect health of especially farm animals due to its mechanisms of action – proteosynthesis inhibition and oxidative stress induction (Marin et al. 2009). In low doses (concentrations in feed around 200 ng/kg) it can reduce their productivity, but it can also increase their sensitivity to bacterial infections and cause immunosuppression (Marin et al. 2009). Moreover, OTA is mutagenic, genotoxic, teratogenic, neurotoxic (Gupta et al. 2018) and has been classified by the International Agency for Research on Cancer as probably carcinogenic to humans (group 2B) (IARC 1993).

Address for correspondence:
Zuzana Široká
Department of Animal Protection and Welfare and Veterinary Public Health
Faculty of Veterinary Hygiene and Ecology
University of Veterinary Sciences Brno
Palackého třída 1946/1, 61242 Brno, Czech Republic

Phone: +420 541 562 779
E-mail: sirokaz@vfu.cz

Pigs are particularly sensitive to the effects of OTA, with the kidney being the target organ (Vlachou et al. 2022). Porcine mycotoxic nephropathy (PMN) is a chronic disease/poisoning associated mainly with OTA exposure, but other mycotoxins (e.g. citrinin) may also be involved (Marin et al. 2009; Gupta et al. 2018). The severity of the disease is determined by the amount of ochratoxin ingested and the duration of its action. Lower concentrations of OTA in feed may not cause overt clinical signs, but can still be sufficient to induce histological and morphological changes in the kidney (paler colour, glomerular fibrosis, tubular degeneration). Changes detected at necropsy/slaughter at higher doses are enlargement (up to several times), changes in texture, distinct discolouration, and advanced histological changes. Typical clinical signs of PMN nephrotoxicity (excess water intake, diarrhoea, polyuria) and also vomiting, anorexia, serious diarrhoeas, and even the death of pigs are observed at higher dietary concentrations (1400 ng/kg). During laboratory analysis the highest concentrations of OTA are detected in the blood, followed by the kidneys, liver, muscles and fat (Marin et al. 2009).

Exposure of food animals to OTA through feed consumption can consequently lead to undesirable OTA residues in food products of animal origin and thus contribute to human intake of OTA through indirect transfer. Due to long half-life of OTA in tissues (around 1 month because of plasma protein binding, enterohepatic cycle and tubular reabsorption), serious concerns have been raised about pig health and consequent human exposure, in whom it can probably also cause immunosuppression and renal damage (Marin et al. 2009; Gupta et al. 2018). Thus, based on the valid legislation, it is obligatory to monitor OTA (as well as other nephrotoxic substances, e.g. mercury and cadmium) in certain foods and feeds (Commission Recommendation 2006; Commission Regulation 2006; Svoboda et al. 2020; Svoboda et al. 2021).

The aim of this study was to evaluate the content of OTA in pig kidneys for which the monitoring is not obligatory yet, and which were examined during the regular monitoring of the safety and quality of food of animal origin in the Czech Republic during the years 2012–2021. Different age and breeding categories, which were fed different diets, were compared and the influence of the season on the OTA content in kidneys was evaluated, as the storage conditions for feed affecting the risk of mould growth vary in different seasons.

Materials and Methods

Samples

The samples used were whole kidneys of fattening pigs and culled sows taken during slaughter in the last 10 years, i.e. 2012–2021, within the regular control plan of residues and contaminants according to Council Directive 96/23/EC. The samples were withdrawn by veterinary inspectors of the State Veterinary Administration of the Czech Republic and analysed in State Veterinary Institutes in the Czech Republic (Praha, Jihlava, Olomouc).

Determination of OTA in pig kidney

Ochratoxin A was determined after purification of the sample on a Neocolumn immunoaffinity column (Neogen Europe Ltd., Ayr, Scotland, UK) by high-performance liquid chromatography with fluorescence detection (Waters Corporation, Milford, MA, USA). The chromatographic analysis was performed on Nova-Pak C18 column (250 mm × 4.6 mm, 4 µm) (Waters Corporation), mobile phase acetonitrile (Chromasolv gradient grade for HPLC, Honeywell, Charlotte, North Carolina, USA) + redistilled water (Milli-Q plus, 5.5 .10-8 Scm^{-1} , Merck KGaA, Darmstadt, Germany) + acetic acid (p.a., Lach-Ner, s.r.o., Neratovice, Czech Republic) (99+99+2), flow rate 0.8 ml/min, injection volume 20 µl, excitation wavelength 333 nm, emission wavelength 477 nm, column temperature 35 °C. The limit of quantification is 0.1 µg/kg; repeatability of this method is 15% and recovery 70–95%.

Statistical analysis

The Microsoft Excel was used for data arrangements into tables and for the descriptive statistics calculations. The STATISTICA software (StatSoft, Inc. 2014, version 12) was used for statistical analyses. Normality was assessed with Shapiro Wilks test. Because of nonparametric data, nonparametric tests were used consequently. Kruskal-Wallis one-factor ANOVA was used for the assessment of the differences between individual years and differences between periods of year. Mann-Whitney U Test was used to evaluate differences between fattening pigs and culled sows. A P value < 0.05 was considered as a significant difference.

Results

The total number of the pig kidneys analysed between 2012 and 2021 were 156. None of them had gross morphological changes. Descriptive statistics of OTA content in these samples (differentiated for fattening pigs and culled sows) for individual years are presented in Table 1.

Table 1. Content of ochratoxin A in kidneys of fattening pigs and culled sows for individual years (in μg/kg fresh weight)

	Fattening pigs						Culled sows					
Year	n	Median	Mean	SEM	Min	Max	n	Median	Mean	SEM	Min	Max
2012	15	0.200	0.211	0.054	0.10	0.56	3	0.200	0.203	0.117	0.10	0.21
2013	15	0.160	0.274	0.071	0.10	1.82	3	0.200	0.167	0.096	0.10	0.20
2014	15	0.200	0.183	0.047	0.10	0.52	0	-	-	-	-	-
2015	14	0.200	0.411	0.110	0.10	2.90	1	0.340	0.340	-	-	-
2016	13	0.200	0.409	0.113	0.10	2.68	2	0.200	0.200	0.141	0.20	0.20
2017	14	0.170	0.154	0.041	0.10	0.20	1	0.200	0.200	-	-	-
2018	14	0.175	0.205	0.055	0.10	0.85	1	0.100	0.100	-	-	-
2019	14	0.150	0.418	0.112	0.10	1.97	1	0.100	0.100	-	-	-
2020	11	0.100	0.388	0.117	0.10	2.87	4	0.150	0.158	0.079	0.10	0.23
2021	14	0.100	0.140	0.037	0.10	0.20	1	0.100	0.100	-	-	-

SEM – standard error of the mean; Min – minimum; Max – maximum

Statistical analysis performed to evaluate differences in OTA contents in all pigs between the monitored years revealed no significant differences (P = 0.37751 to 1.0; Fig. 1).

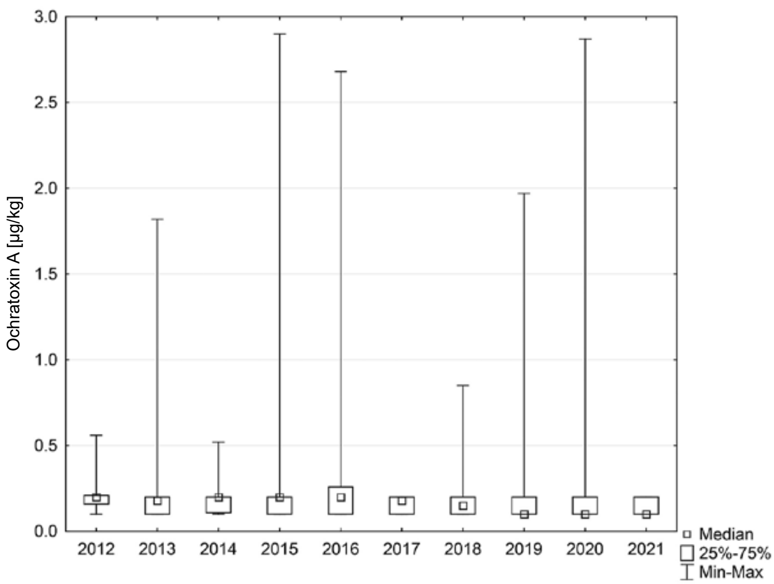

Figure 1: Comparison of ochratoxin A content in μg/kg fresh weight in all pig kidneys during the monitored years

Table 2. Content of ochratoxin A in kidneys of all pigs during year seasons (in µg/kg fresh weight).

Season	n	Median	Mean	SEM	Min	Max
spring	55	0.200	0.273	0.058	0.100	2.68
summer	36	0.200	0.298	0.088	0.100	2.90
autumn	44	0.105	0.211	0.046	0.100	1.97
winter	21	0.200	0.298	0.133	0.100	2.87

SEM – standard error of the mean; Min – minimum; Max – maximum

Table 3. Content of ochratoxin A in kidneys of fattening pigs and culled sows during whole the monitored period (in µg/kg fresh weight).

Category	n	Median	Mean	SEM	Min	Max
fattening pigs	139	0.200	0.275	0.040	0.100	2.90
culled sows	17	0.200	0.175	0.016	0.100	0.34

SEM – standard error of the mean; Min – minimum; Max – maximum

Similar findings were obtained when the seasons of the year were compared in all pigs (P = 0.70484 to 1.0; Table 2) and the age categories (younger fattening pigs and culled sows excluded from breeding due to age or health reasons) were compared (P = 0.62677; Table 3). This indicates a stable quality of breeding conditions and feed quality and storage conditions for all categories compared in this study.

Discussion

Of all farm animals, pigs are the most sensitive species to OTA effects (Marin et al. 2009). Frequent occurrence of OTA in feed components and feeds for pigs and other animals has been reported in various countries, including the Czech Republic (Pozzo et al. 2010; Almeida et al. 2011; Pleadin et al. 2012; Svoboda et al. 2019; Mikula et al. 2020).

Contamination of pork meat and edible offal occurs mainly due to the contaminated feed consumption by pigs. In addition, the presence OTA in pork products can be caused by the addition of other OTA-contaminated ingredients, such as spices, into processed products. OTA-producing toxigenic *Aspergillus* and *Penicillium* genera can also grow on the surface of dry cured-meat products due to their tolerance to low pH and high salt concentration (Vlachou et al. 2022). Ochratoxin A is stable to heat treatments and low pH conditions. Typical heat treatments of foods such as boiling, baking, frying and roasting often do not cause significant decrease in OTA concentrations (Pleadin et al. 2014), temperatures of 180 °C and higher and wet heating are required to decrease OTA by more than 10% (Raters and Matissek 2008; Kabak 2009). Pork and meat products thus belong among the possible sources of chronic OTA exposure in humans. According to EFSA, the tolerable weekly intake of OTA in adult humans is 120 ng/kg body weight (EFSA 2006). However, the EU has not yet set a maximum limit of OTA in pork and pork products or in meat generally (Commission Regulation 2006). Pork contributes around 1% to the total estimated intake of OTA in humans (Vlachou et al. 2022). Regular consumption of some pig blood and other pork products may contribute significantly to an increased OTA intake, especially in children who have a relatively lower body weight compared to adults, resulting in a higher intake per kg of body weight (EFSA 2004). Children of the age of 4–6 years are considered the most exposed population (Ostry et al. 2015). The average consumption of pork in Czech Republic for the year 2020 was 43.4 kg carcass weight equivalent/year/person and it has been stable throughout the last decade (Czech Statistical Office 2022) and increased contamination of pork products by OTA is therefore not desirable. The evaluation of the real risk of human exposure to OTA as a result of the consumption of pork and pork products or meat and animal products in general is an important future task.

To protect their citizens, some European countries, such as Denmark, Estonia, Romania, Slovakia, Poland and Italy, have adopted their own regulations or guidelines regarding maximum allowed concentrations of OTA in meat and meat products. Denmark established up to 10 μg/kg in pig kidney as an accepted concentration; in Estonia the limit is set at 10 μg/kg in pig liver; in Romania at 5 μg/kg in pig kidney, liver and meat; in Slovakia at 5 μg/kg in pig meat; in Poland at 5 μg/kg for animal tissues (kidney, liver, and muscle); and in Italy at 1 μg/kg in pork and pork products (Pietruszka et al. 2017; Vlachou et al. 2022). These differences in OTA limits require future harmonized approach within EU countries and the implementation of the limits also in other countries.

In pigs, the highest OTA concentrations are found in the blood, while the distribution within tissues usually follows the pattern kidney > liver > muscle > fat (Vlachou et al. 2022). Altafini et al. (2017) also found high concentrations in the lungs and heart, so these edible organs should be also monitored. Ochratoxin A concentrations in pig tissues are dependent on the amount of OTA in feed and the duration of feeding with OTA-contaminated feed (Vlachou et al. 2022). In several studies carried out in different countries, a high incidence of OTA-positive samples taken from pig tissues was found especially in blood serum and kidneys. This is also true for this study, where all kidney samples analysed were positive for OTA presence, and 6 out of 156 samples exceeded the concentration of 1 μg/kg fresh weight (max. 2.9 μg/kg). Similarly, 98% of pig serum samples tested in Romania by Curtui et al. (2001) were found to be OTA-positive in the range of 0.05–13.4 μg/l. A lower incidence of OTA presence (31%) was found in pig serum in Serbia, however, with OTA concentrations ranging as high as 220.8 μg/l (Milicevic et al. 2008).

When considering kidney samples, Hou et al. (2015) found 87.5% occurrence of OTA in pig kidney samples in a study conducted in China, so again, high incidence of positive samples was revealed. However, concentrations of OTA found in the samples were low, ranging from a trace concentration of 0.03 to 0.323 μg/kg. The incidence of OTA in kidneys found by Curtui et al. (2001) was also high (79%) with but with slightly higher mean concentrations of 0.54 μg/kg. In Belgium, only 37.3% of pig kidney samples were contaminated with OTA with a low mean concentration of 0.22 μg/kg (max. 1.91 μg/kg) (Tangni et al. 2021). Similar mean concentrations were found for OTA in pig kidneys also in this study. In Italy, a small trial in a local slaughterhouse revealed pig kidney OTA concentrations ranging from 0.17 to 0.91 μg/kg with all samples being positive (Luci et al. 2016). A larger survey by Monaci et al. (2004) of pigs slaughtered in southern Italy revealed that 52 out of 54 analysed kidney samples were contaminated, with OTA concentration ranging between 0.26 and 3.05 μg/kg. On the other hand, Pietruszka et al. (2017) revealed OTA contamination only in 23.5% of the animal tissues samples; however, 1% of the sample tissues contained 5–10 μg/kg OTA and 1% contained over 10 μg/kg OTA. In their study, fewer samples were contaminated but several of them reached much higher OTA concentrations than the samples analysed in this study in the Czech Republic (Table 1). Similarly in Serbia, Polovinski-Horvatovic et al. (2019) found detectable OTA levels in only 14.74% of examined pig kidneys, but with a higher average concentration of 1.36 μg/kg, median value 0.99 μg/kg, and maximum 3.97 μg/kg. On the other hand, a Serbian study by Milicevic et al. (2008) found 33.3% of kidney samples to be positive, with a similar mean OTA concentration of 1.26 μg/kg; but the highest OTA concentration was 52.5 μg/kg, and OTA concentrations in 11.1% of samples ranged between 1–5 μg/kg, and in 5.5% samples exceeded 5 μg/kg. The differences between the 2008 and 2019 Serbian studies show a recent decrease in OTA concentrations in pig kidneys in Serbia. In the Czech Republic, a previous study by Skarkova et al. (2013) reported pig kidney OTA concentrations of 0.15–0.46 μg/kg with a median of 0.15 μg/kg. This is in agreement with the findings of this study, where the median concentrations of OTA in pig kidneys was between 0.1–0.2 μg/kg (Table 1).

It can be concluded that values found in this study are low and similar to those assessed in several studies performed in other European countries and do not exceed the limits set in their legislation, which means these organs do not pose a significant risk for consumers and do not indicate porcine mycotoxic nephropathy in the monitored pigs.

References

Almeida I, Martins HM, Santos S, Costa JM, Bernardo F 2011: Co-occurrence of mycotoxins in swine feed produced in Portugal. Mycotoxin Res **27**: 177-181

Altafini A, Armorini S, Zaghini A, Sardi L, Roncada P 2017: Tissue distribution of ochratoxin A in pigs after administration of two-levels contaminated diets. World Mycotox J **10**: 263-272

Commission Recommendation 2006/576/EC of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. Off J Eur Union L **229**: 7-9

Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off J Eur Union L **364**: 5-24

Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC. Off J Eur Union L **125**: 10-32

Curtui VG, Gareis M, Usleber E, Märtlbauer E 2001: Survey of Romanian slaughtered pigs for the occurrence of mycotoxins ochratoxins A and B, and zearalenone. Food Addit Contam **18**: 730-738

Czech Statistical Office 2022: Spotřeba potravin – 2020 (In Czech, Food Consumption in 2020). Accessed September 12, 2022. Available at: https://www.czso.cz/csu/czso/spotreba-potravin

European Food Safety Authority (EFSA) 2004: Opinion of the scientific panel on contaminants in food chain on a request from the commission related to ochratoxin a (OTA) as undesirable substance in animal feed. EFSA J **101**: 1-36

European Food Safety Authority (EFSA) 2006: Opinion of the scientific panel on contaminants in the food chain on request from the commission related to ochratoxin A in food. EFSA J **4**: 365

Gupta RC, Srivastava A, Lall R 2018: Chapter 72 - Ochratoxins and Citrinin. In: Gupta RC (Ed.): Veterinary Toxicology. Third edn. Academic Press, London, 1205 p.

Hou Y, Zhou J, Li Y, Xie J, Zhou L, Lv F 2015: Determination of ochratoxin A in pig kidneys by immunoaffinity cleanup and Ultra-High Performance Liquid Chromatography. J AOAC Int **98**: 1566-1570

IARC 1993: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, no. 56. Available at: https://www.ncbi.nlm.nih.gov/books/NBK513574/

Kabak B 2009: The fate of mycotoxins during thermal food processing. J Sci Food Agric **89**: 549-554

Luci G, Vanni M, Ferruzzi G, Mani D, Intorre L, Meucci V 2016: Determination of ochratoxin A in pig tissues using enzymatic digestion coupled with high-performance liquid chromatography with a fluorescence detector. MethodsX **3**: 171-177

Marin DE, Taranu I, Tabuc C, Burgehelea M 2009: Ochratoxin: nature, origin, metabolism and toxic effects in pigs. Arch Zootech **12**: 5-17

Mikula P, Blahová J, Honzlová A, Kalinová J, Macharáčková P, Rosmus J, Svobodová Z, Svoboda M 2020: Occurrence of mycotoxins in complete poultry feeds in the Czech Republic – Multiannual survey (2013–2018). Vet Med Czech **65**: 487-494

Milićević D, Jurić V, Stefanović S, Jovanović M, Janković S 2008: Survey of slaughtered pigs for occurrence of ochratoxin A and porcine nephropathy in Serbia. Int J Mol Sci **9**: 2169-2183

Monaci L, Tantillo G, Palmisano F 2004: Determination of ochratoxin A in pig tissues by liquid–liquid extraction and clean-up and high-performance liquid chromatography. Anal Bioanal Chem **378**: 1777-1782

Ostry V, Malir F, Dofkova M, Skarkova J, Pfohl-Leszkowicz A, Ruprich J 2015: Ochratoxin A dietary exposure of ten population groups in the Czech Republic: Comparison with data over the world. Toxins **7**: 3608-3635

Pietruszka K, Piątkowska M, Jedziniak P 2017: Occurrence of ochratoxin A in animal tissues and feeds in Poland in 2014–2016. J Vet Res **61**: 483-487

Pleadin J, Zadravec M, Perši N, Vulić A, Jaki V, Mitak M 2012: Mould and mycotoxin contamination of pig feed in northwest Croatia. Mycotoxin Res **28**: 157-162

Pleadin J, Perši N, Kovačević D, Vulić A, Frece J, Markov K 2014: Ochratoxin A reduction in meat sausages using processing methods practiced in households. Food Addit Contam B **7**: 239-246

Polovinski-Horvatovic M, Radovic I, Glamocic D, Jajic I, Krstovic S, Mirkov M, Vasiljevic V 2019: The occurrence of ochratoxin A in kidneys of healthy pigs from Vojvodina province, Serbia. In Proceedings of the 60th International Meat Industry Conference MEATCON2019, Kopaonik, Serbia, 22–25 September **333**: 012095

Pozzo L, Cavallarin L, Nucera D, Antoniazzi S, Schiavone A 2010: A survey of ochratoxin A contamination in feeds and sera from organic and standard swine farms in northwest Italy. J Sci Food Agric **90**: 1467-1472

Raters M, Matissek R 2008: Thermal stability of aflatoxin B_1 and ochratoxin A. Mycotoxin Res **24**: 130-134

Skarkova J, Ostry V, Malir F, Roubal T 2013: Determination of ochratoxin A in food by high performance liquid chromatography. Anal Lett **46**: 1495-1504

Svoboda M, Blahová J, Honzlová A, Kalinová J, Macharáčková P, Rosmus J, Mejzlík V, Kúkol P, Vlasáková V, Mikulková K 2019: Multiannual occurrence of mycotoxins in feed ingredients and complete feeds for pigs in the Czech Republic. Acta Vet Brno **88**: 291-301

Svoboda M, Drápal J, Haruštiaková D, Svobodová Z 2020: A multiannual survey of cadmium content in pig tissues collected in the Czech Republic during the years 2015–2019. Acta Vet Brno **89**: 349-355

Svoboda M, Bureš J, Drápal J, Geboliszová K, Haruštiaková D, Nepejchalová L, Skočovská M, Svobodová Z 2021: A survey of mercury content in pig tissues carried out in the Czech Republic during years 2015–2019. Acta Vet Brno **90**: 287-293

Tangni EK, Masquelier J, Van Hoeck E 2021: Determination of ochratoxin A in edible pork offal: Intra-laboratory validation study and estimation of the daily intake via kidney consumption in Belgium. Mycotoxin Res **37**: 79-87

Vlachou M, Pexara A, Solomakos N, Govaris A 2022: Ochratoxin A in slaughtered pigs and pork products. Toxins **14**: 67