

Incidence of Fungal Infections and Mycotoxicosis in Pork Meat and Pork By-Products in Egyptian Markets

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Abstract—The consumption of food contaminated with molds (microscopic filamentous fungi) and their toxic metabolites results in the development of food-borne mycotoxicosis. The spores of molds are ubiquitously spread in the environment and can be detected everywhere. Ochratoxin A is a toxic and potentially carcinogenic fungal toxin found in a variety of food commodities. In this study, the mycological quality of various ready-to-eat local and imported pork meat and meat byproducts sold in Egyptian markets were assessed and the presence of various molds was determined in pork used as a raw material, edible organs as liver and kidney as well as in fermented raw meat by-products. The study assessed the mycological quality of pork raw meat and their by-products sold in commercial shops in Cairo, Egypt. Mycological analysis was conducted on (n=110) samples which included pig's livers and kidneys from Egyptian Bassatin slaughter house; local and imported processed pork meat by-products from Egyptian pork markets. The isolates were identified using traditional mycological and biochemical tests. All kidney and liver samples were positive to molds growth while all byproducts were negative. Ochratoxin A levels were quantitatively analyzed using the high performance liquid chromatography (HPLC) and the highest results were present in kidney 7.51 part per billion (ppb) followed by minced meat 6.19 ppb generally the local samples showed higher levels than the imported ones. To the best of our knowledge, this is the first report on mycotoxins detection and quantification from pork by-products in Egypt.

Keywords—Egypt, imported pork by-products, local, mycotoxins.

I. INTRODUCTION

IN recent decades, the question of molds toxicity has attracted attention, especially in the fields of agriculture and food industry. Microscopic filamentous fungi often contaminate vegetal and animal products, becoming a source of diseases in man and slaughter animals [1]. Mycotoxins Production is favored by both intrinsic and extrinsic factors influence fungal growth and mycotoxins production on a given substrate. The intrinsic factors include water activity and pH whereas extrinsic factors which influence mycotoxins production are relative humidity, temperature and availability of oxygen, they can grow on feeds containing more than 12 – 15 % moisture. In wet feeds such as silage, higher moisture levels allow molds growth if oxygen is available. The conditions suitable for molds growth and for mycotoxins formation are not necessarily the same as there are generally associated with dry conditions in midseason followed by wet

weather [2]. Conditions that favor production of one type of mycotoxins may not be favorable for production of another type. For example, aflatoxin production by *Aspergillus* is dependent on concentrations of O₂, CO₂, zinc, and copper, as well as physical location, while production of ochratoxin relates to air exhaustion [3].

Exposure to mycotoxins can occur through ingestion, contact or inhalation of airborne particulates containing the toxins, including dust and molds components such as spores and mycelial fragments. In agricultural settings, mycotoxicosis (mycotoxins poisoning) in both farm animals and humans can result from oral, dermal, or exposure through inhalation of mycotoxins-contaminated grain or dust [2]. Feeds and foods are often contaminated with various molds, when the temperature and relative humidity are optimal after contamination; there is also a risk of mycotoxin production [4]. Relatively low water activity ($aw < 0.9$) and low pH-values ($pH < 6.0$) are particularly favorable for molds development [5].

Ochratoxin A is a moderately stable molecule and is able to survive most food processing to some extent and may thus occur in consumer products. The lack of hygienic measures during slaughtering, processing and storage of meat and meat products as well as the added spices and some food additives were considered as the main source of toxigenic molds and mycotoxins which lead to either food spoilage or food borne mycotoxicosis [6]-[8].

Processing may involve boiling, baking, roasting or fermentation, and the degree to which it is destroyed will further depend on other parameters such as pH, temperature and the other ingredients present. Ochratoxin A is only partly destroyed during cooking and bread making. Baking and roasting have been reported to reduce the toxin content by a mere 20%. [9], [10]. OTA can be produced by molds growing on pork meat products during ripening (direct contamination): *Penicillium nordicum*, a high OTA producer, has been proven to be able to grow on meat [11]. OTA can also occur in meat and meat products as a result of indirect contamination from animals fed contaminated diets. Pigs are known to be particularly sensitive to OTA accumulation, with a tissue distribution following the pattern: kidney > liver > muscle > fat [12]; while in farm animals, the risk is limited to monogastric species, because ruminants can hydrolyze the amidic bond of OTA into phenylalanine and ochratoxin a, which is generally considered to be non-toxic [13].

The symptoms of a mycotoxicosis depend on the type of mycotoxins; the amount and duration of the exposure; the age, health, and sex of the exposed individual; and many poorly

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understood synergistic effects involving genetics, dietary status, and interactions with other toxic insults [14].

At present, acute toxicity is rarely reported in humans, while the sub-chronic and chronic effects of ochratoxin A are of greatest concern. Ochratoxin A has been shown to be nephrotoxic, hepatotoxic, teratogenic and immunotoxic to several species of animals and carcinogenic in mice and rats causing tumors of the kidney and liver [15].

Ochratoxin A has been reported to be an immunosuppressor and affects the immune system in a number of mammalian species. It was able to cause inhibition of protein biosynthesis and inhibition of macrophage migration. Ochratoxin A has been shown to induce DNA damage, DNA repair, and chromosomal aberrations in mammalian cells *in vitro* as well as DNA damage and chromosomal aberrations in mice treated *in vivo*. However, the mechanism for genotoxicity is unclear and there was no evidence that it is mediated by direct interaction with DNA [16].

Awareness of mycotoxins has grown mainly in the last fifty years since the discovery of aflatoxins in the 1960s, although they have accompanied mankind from the very beginnings and are probably associated with several mysterious diseases known from history [15]. Moreover, there is also a growing awareness of the mycotoxins present in the living and working environment and associated disease [17]-[20].

In Egypt, in spite of presence of a considerable swine population and consumers, a very limited research work concerning epidemiological studies has implicated foods of porcine origin as an important vehicle associated with illnesses caused by molds and mycotoxins, which lead to the development of public health hazards [21].

The aim of this study was to determine the occurrence of molds and mycotoxins in collected pork and pork byproducts from Egyptian slaughter house and pork markets, to the best of our knowledge, this is the first trial on mycotoxins detection and quantification from pork by-products in Egypt.

II. MATERIALS AND METHODS

A. Samples

The samples were taken from meat (imported salami, local salami, local mortadella, local minced pork meat, Bavarian sausages, Canadian bacon, smoked bacon, local sausages, imported mortadella, fresh local pigs' kidney and liver).

Ten grams were taken from each sample with the help of a sterile scalpel, and 90 ml of diluent (physiological saline) were added to the sample in a sterile homogenizing vessel. The propeller homogenizer with 10 000 rev. was used for homogenization; the homogenization time was 2.5 min.

B. Isolation of Molds

Samples were prepared and examined for isolation of fungi (molds) according to the technique recommended by [22]; five grams of finely ground meat (or tissues) were added to 45 ml of Sabouraud's Dextrose Broth in stomacher jar (original suspension). One ml from this suspension was transferred to a test tube containing 9 ml of sterile Sabouraud's Dextrose Broth

and thoroughly mixed to have a dilution of 1/100. Ten - fold dilutions were prepared and 1 ml of each dilution was poured in sterile Petri dish, then the melted and tempered Sabouraud's dextrose agar was added. The plates were left to solidify at room temperature then incubated at 25°C for 5 – 10 days. After the end of incubation period, the isolated molds were identified [23].

C. Analysis of Ochratoxin A

1. Extraction and Clean-up for Ochratoxin Analyses

Meat and meat products analyses were performed [24], which briefly includes a double extraction with acidic ethyl acetate. The organic phase was removed and extracted with 0.5M NaHCO₃, pH 8.4. The aqueous extract was acidified to pH-2.5 with 7M H₃PO₄ OTA was finally back extracted into ethyl acetate (3 mL). The organic phase was evaporated to dryness under N₂ steam, reconstituted in 150 µL mobile phase and a 20 µL aliquot injected. The detection limit for OTA in organs was 0.01 ng/g with a 61% (C.V. =14.5%) mean recovery from artificially contaminated samples at 3 ng/g (n = 3).

2. Chromatographic Analysis (HPLC)

OTA determination was carried out in organs (liver and kidney), tissues (local fresh minced meat) and ripened pork products [25]. Analysis was performed using an HPLC instrument consisting of Water Binary Pump model 1525, a model Waters 1500 Rheodyne manual injector; Water 2475 multi wave length fluorescence detector and a data workstation with software Breeze. A phenomex C18 (250 X 4,6 mm I,d), particle size 5 µm from Waters Cooperation USA. Water was purified through a Milli- Q treatment system (Millipore, London, U.K.) and Phosphate Buffered Saline (PBS) was prepared as per Vacuum (NaCl 8 g l⁻¹, KCl 0.2 g l⁻¹, Na₂HPO₄ 1.15 g l⁻¹, KH₂PO₄ 0.2 g l⁻¹; pH 7.4).

3. Statistical Analysis

Descriptive statistics of the data set were performed with a standard programmed and included arithmetic mean, standard deviation, coefficient of variation, minimum, 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). Significance was set at p<0.05.

III. RESULTS

All the examined kidneys showed gross pathological abnormalities, some were pale, swollen, tan color and some showed grey – white foci on their surface. Minor pathological changes were observed on liver, they were limited to paleness in color not more. For the rest of the samples (local and imported), there was not any pathological changes observed on the collected samples.

TABLE I
MOLDS ISOLATION IN MEAT AND MEAT PRODUCTS SAMPLES

Types of Examined Samples	Number of Examined Samples	Number of Positive Samples	
		Number	%
Fresh Local Liver	N= 10	10	100
Fresh Local Kidney	N= 10	10	100
Local Processed Meat Products	N= 10	0	0
Imported Processed Meat Products	N= 10	0	0
Total	110	20	8.18

TABLE II
LEVELS OF OTA RESIDUES IN FRESH KIDNEY, LIVER, LOCAL AND IMPORTED PROCESSED MEAT PRODUCTS (PPB)

Sample	Result
1 Liver (N= 10)	3.78 PPB
2 Kidney (N= 10)	7.51 PPB
3 Local Salami (N= 10)	4.05 PPB
4 Imported Salami (N= 10)	1.26 PPB
5 Local Mortadella (N= 5)	3.57 PPB
6 Local Mortadella (N= 5)	3.21 PPB
7 Local Minced Meat (N= 10)	6.19 PPB
8 Bavarian Sausage (N= 10)	1.08 PPB
9 Canadian Bacon (N= 10)	1.13 PPB
10 Local Sausages (N= 10)	3.32 PPB
11 Imported Mortadella (N= 10)	1.16 PPB
12 Smoked Bacon (N= 5)	2.67 PPB
13 Smoked Bacon (N= 5)	3.01 PPB

IV. DISCUSSION

Ochratoxins are a group of mycotoxins produced as secondary metabolites by several fungi of the *Aspergillus* or *Penicillium* families and are weak organic acids consisting of a derivative of an isocoumarin. The family of ochratoxins consists of three members, A, B, and C which differ slightly from each other in chemical structures. These differences, however, have marked effects on their respective toxic potentials. Ochratoxin A is the most abundant and hence the most commonly detected member but is also the most toxic of the three [26], [27]. It is a potent toxin affecting mainly the kidney. As in other mycotoxins, ochratoxin A can contaminate a wide variety of foods as a result of fungal infection incrops, in the field during growth, at harvest, in storage and in shipment 4 under favourable environmental conditions especially when they are not properly dried. Ochratoxin A may be present in a foodstuff even when the visible molds is not seen [28]. Molds contamination was identified in the current study and the result was shown in Table I. As seen from the table; various molds were detected in raw pork materials (liver and kidney) and the most frequently detected strains were *Aspergillus* spp., *Mucor* spp. and *Penicillium* spp; while processed meat – both local and imported – were clear from molds contamination.

The production of mycotoxins in meat and meat products is enabled by the following factors, for example, the presence of oxygen, temperature between 4°C and 40°C, pH-value between 2.5 and 8 (with an optimum between 5 and 8), minimum water activity of 0.80, maximum salt concentration of 14% [29].

In Egypt, feedstuff used for nourishing swine is considered the most serious source of molds growth and mycotoxins production, whereas in Egypt, especially after eradication of all swine's farms in order to eliminate swine influenza, rearing and nourishing of swine became very difficult and depend mainly on: spoiled food, garbage, human's residues and sometimes dead animals or organs....etc. Beside, in Egyptian's slaughter houses, there aren't any hygienic measures to be taken in considerations. On the other hand, no moldy growth was observed in processed pork meat. Reference [30] stated a contrary result as they found *A. flavus* and *A. oryzae* in luncheon meat. Reference [31] also reported a 20% average occurrence of *A. flavus* and *A. parasiticus* in smoked meat products, pork salami and sausage, bacon and ham. These may be resulted from bad hygienic conditions during storage or due to the presence of the moldy growth on the primary processed meat. Beside, many studies explained the isolation of aflatoxins – producing strains and other molds from different kinds of spices used in processing of meat such as aniseed, black pepper corns, caraway seeds, black cumin seeds, fennel seeds, peppermint leaves, paprika, white pepper, marjoram, nutmeg and thyme [32]-[34].

In the present study, OTA was detected in all fresh local kidneys, liver and minced meat samples as shown in Table II, with regards to a consumer safety point of view, the literature contains several studies investigating the ratio between the levels of OTA in pig's kidneys and pig's meat [35]-[39].

A contamination level for the entire carcass at 25 ng/g pig's kidney should secure that the level in meat do not exceed 10 ng/g, based on the estimation that the OTA level in pork meat is approximately 40% of the level in pig's kidney. The content of OTA in meat: content in ratio varies between 10 – 90% and depends on many factors such as the content of OTA in feed, feeding period, feeding in relation to time of slaughtering. The effects of OTA appeared to be longer – lasting than those of other mycotoxins and possessed cumulative feature [40].

V. CONCLUSION

The results reported the presence of mycotoxins in edible organs, pork and pork byproducts especially the local samples while the molds isolation was negative in pork byproducts may be due to the processing but the addition of spices especially in local byproducts which may be contaminated with mycotoxins enhance the problem.

VI. RECOMMENDATION

From the data depicted in this study, a great concern should be raised for the hygienic measures taken from the beginning of the process of pigs rearing till the process of slaughtering and meat processing. These measures should include prevention of the admixture between swine and other animal species, prevention of mold growth on the swine ration, good ventilation of their feed, avoiding their feeding on garbage and spoiled food. The hygienic measures taken during animal slaughtering and standard measures during meat processing such as using clean microbial free meat and spices, limiting

the humidity during processing and storage, destructing of any insects and following all the available hygienic measures.

Control of mycotoxin is a matter of importance not only for health implications, but also for improvement of economy in the affected countries. Since ochratoxin A is stable and generally resistant to heat and processing, control of ochratoxin A contamination lies in the control of the growth of the toxin-producing fungi. Effective prevention of ochratoxin A contamination therefore depends on good farming and agricultural practices. Good Agricultural Practices (GAP) including methods to reduce fungal infection and growth during harvest, storage, transport and processing provide the primary line of defense against contamination with ochratoxin A in addition to periodical qualitative and quantitative evaluation and measuring of mycotoxins.

REFERENCES

- [1] A. Laciakova, and V. Laciak, "Možnosti eliminácie mikroskopických vláknitých húb dezinfekčnými prostriedkami". Vet. Med. (Czech), 39:723–731, 1994.
- [2] CAST, "Mycotoxins: Risks in plants, animals and humans". Task Force Report No.139. Council for Agriculture Science and Technology (CAST), Ames, Iowa, USA, 2003.
- [3] S. Homdork, H. Fehrmannand, and R. Beck "Influence of different storage conditions on the mycotoxin production and quality of Fusarium- infected wheat grain". J. of phytopathology, 148(1): 7-15, 2000.
- [4] I. Štyriak, E. Čonkova, A. Lacikova, and J. Bohm, "Prevention of fumonisin production by microorganisms". Czech J. Anim. Sci., 43: 449–452, 1998.
- [5] H. P. Hanssen, "Molds control in the meat processing industry". Fleischwirtschaft, 75: 52, 1995.
- [6] A. A. Hassan and R. El- Sharnouby, "Antimicrobial effect of some chemicals and plant extracts on Egyptian food". J. Egypt. Vet. Med. Assoc., 57 (4): 1331– 1349, 1997.
- [7] A. A. Hassan, "Detection of some mycotoxins and mycotoxins producing fungi in both macro- and microenvironment of diseased animals". 7th Sci. Cong. Egyptian Society for Cattle Diseases, pp. 112 – 119, 7-9, 2003.
- [8] M. A. Abdel-Wahhab, and A. M. Kholif, "Mycotoxins in Animal Feeds and Prevention Strategies: A Review". Asian Journal of Animal Sciences, 2: 7-25, 2008.
- [9] L. Berry, "The pathology of mycotoxins". Journal of Pathology 154: 301, 1988.
- [10] D. Puntaric, J. Bosnir, Z., Smit, and Z. Baklaic, "Ochratoxin A in corn and wheat: Geographical association with endemic nephropathy". Croatian Medical Journal. 42(2):175, 2001.
- [11] A. Rodríguez, M. Rodríguez, A. Martín, F. Nuñez, and J.J. Córdoba, "Evaluation of hazard of aflatoxin B1, ochratoxin A and patulin production in dry-cured ham and early detection of producing molds by qPCR". Food Control 27 (1), 118-126, 2012.
- [12] L. Malagutti, M. Zannotti, A. Scampini, and F. Sciaraffia, "Effects of Ochratoxin A on heavy pig production". Anim. Res.54 (3): 179-184, 2005.
- [13] J. Fink-Gremmels "The role of mycotoxins in the health and performance of dairy cows". Vet J. 176(1):84-92, 2008.
- [14] J.W. Bennett and M. Klich, "Mycotoxins". Clinical Microbiology Reviews, 16(3): 497-516, 2003.
- [15] J.L. Richard, "Some major mycotoxins and their mycotoxicoses--an overview". Int J Food Microbiol. 20; 119(1-2):3-10, 2007.
- [16] Risk Assessment Studies Report No. 23, Ochratoxin A in Food", 2006.
- [17] A. Tarin, M.G. Rosell, and X. Guardino, "Use of high-performance liquid chromatography to assess airborne mycotoxins. Aflatoxins and ochratoxin A. J Chromatography. 27; 1047(2):235-40, 2004.
- [18] R.C.Shoemaker and D.E. House, "A time-series study of sick building syndrome: chronic, biotoxin-associated illness from exposure to water-damaged buildings". Neurotoxicol. Teratol., 27:29-46, 2005.
- [19] B. Gutarowska, and M. Piotrowska, "Methods of mycological analysis in building". Build. Environ., 42, 1843-1850, 2007.
- [20] Y. Wang, T. Chai, G. Lu, C. Quan, H. Duan, M. Yao, B.A. Zaucker and G. Schlenker, "Simultaneous detection of airborne Aflatoxin, Ochratoxin and Zearalenone in a poultry house by immune-affinity clean-up and high-performance liquid chromatography". Environ. Res., 107, 139-144, 2008.
- [21] A.S. Hakim, S. M. Azza Abuelnaga, M. Afaf Ezz-Eldeen., M.A. Bakry, and A. Seham Ismail, "Prevalence of some food poisoning bacteria in local and imported retail pork by-products in Egyptian markets". Afr. Journal of Microbiol. Res., 9(22):1492-1498, 2015.
- [22] American Public Health Association, "Standard Methods for the Examination of Water" Wastewater, 16th Ed. APHA, Washington, D.C, 1985.
- [23] J.I. Pitt, A.D. Hocking, and D.R. Glenn "An improved medium for the detection of *Aspergillus flavus* and *A. parasiticus*". J Appl Bacteriol. 54(1):109-14, 1983.
- [24] L. Monaci, F. Palmisano, R. Matrella, and G. Tantillo "Determination of ochratoxin A at part-per-trillion level in Italian salami by immunoaffinity clean-up and high-performance liquid chromatography with fluorescence detection". J Chromatogr A. 7; 1090(1-2):184-7, 2005.
- [25] D.R. Miličević, V.B. Jurić, S.M. Stefanović, M. Jovanovic, and S.D. Jankovic, "Survey of slaughtered pigs for occurrence of ochratoxin A and porcine nephropathy in Serbia". Int. J. Mol. Sci. 9, 2169–2183, 2008.
- [26] K. J. Merwe, P. S. Steyn, and L. Fourie, Mycotoxins. Part 2. "The constitution of ochratoxin A, B, and C, metabolites of *Aspergillus ochraceus*". Journal of the Chemical Society, 7083–7088, 1965b.
- [27] S. Li, R.R. Marquardt, A.A. Frohlich, T.G. Vitti, and G. Crow "Pharmacokinetics of ochratoxin A and its metabolites in rats". Toxicol Appl Pharmacol., 145(1):82-90, 1997.
- [28] Centre for Food Safety Food and Health Bureau, Food and Environmental Hygiene Department, 2006.
- [29] V. Ostry, J. Ruprich, J. Skarkova, I. Prochazkova, and A. Kubatova, "Occurrence of the toxigenic fungi (producers of aflatoxins and ochratoxin A) in foodstuffs in the Czech Republic 1999-2000". Mycotoxin Res. 17 (2):188-92, 2001.
- [30] Z. M. Zaky, M. A. Ismail, and R. S. Refaie "Aspergillus flavus and aflatoxins residues in luncheon meat". Assiut Vet. Med., 33: 114–118, 1995.
- [31] Z.Cvetnić, and S. Pepelnjak "Aflatoxin-producing potential of *Aspergillus flavus* and *Aspergillus parasiticus* isolated from samples of smoked-dried meat". Nahrung. 39 (4):302-7, 1995.
- [32] I. A. EL-Kady, S. S. M. EL-Maraghy, and E. M. Mostafa, "Natural occurrence of mycotoxins in different spices in Egypt". Folia Microbiol., 40: 297–300, 1995.
- [33] M. Hubner, T. Vrabcheva, and M. Garies, "Simultaneous detection of ochratoxin A and B in spices and herbs by immunoaffinity column clean up and HPLC". Rev. Med. Vet., 149: 507, 1998.
- [34] M. Olsen, T. Moller, A.C. Salomonsson, "Occurrence of aflatoxin producing molds in spices". Rev. Med. Vet., 149: 529, 1998.
- [35] A. A. Frohlich, R. R. Marquardt, and J.R. Clarke, "Enzymatic and immunological approaches for the quantitation and confirmation of ochratoxin A in swine kidneys". J. Food Protect., 60 : 172- 176, 1997.
- [36] V.G. Curtui, M. Gareis, E. Usleber, and E. Martlbauer, "Survey of Romanian slaughtered pigs for the occurrence of mycotoxins ochratoxins A and B, and zearalenone". Food Addit. Contam. 18: 730-738, 2001.
- [37] P. Krogh, N.H. Axelsen, N. Gyrd-Hansen, J. Hald, Hyldgaard-Jensen, A. E. Larsen, A. Madsen, and H.P. Mortensen, "Experimental porcine nephropathy". Acta Path. Microb. Scand. Sect. A, 246: 1- 21, 1974.
- [38] P. Krogh, F. Elling, B. Hald, A. E. Larsen, E. B. Lillehoj, A. Madsen, and H.P. Mortensen "Time- dependent disappearance of ochratoxin A residues in tissues of bacon pigs". Toxicol. 6: 235- 242, 1976.
- [39] H.P. Mortensen, B. Hald, A. E. Larsen, and A. Madsen "Ochratoxin A contaminated barley for sows and piglets. Pig performance and residues in milk and pigs". Acta Agric. Scand., 33: 349-352, 1983.
- [40] D. Milicevic, M. Skrinjar and T. Baltic "Real and perceived risks for mycotoxin contamination in foods and feeds: challenges for food safety control". Toxins 2: 572–592, 2010.