MOLD CONTAMINATION OF BUFFALO AND CATTLE MEAT AND OFFAL: A COMPARATIVE STUDY

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ABSTRACT

Bovine (buffalo and cattle) meat and edible offal are considered as essential sources of the red meat worldwide. This study aimed at investigation of the mold contamination of the buffalo and cattle meat (round), and their edible offal including neck muscles, masseter muscles, liver, and kidney in a comparative way. Identification of the prevalent mold genera was followed. Besides, identification of the *Aspergillus* spp. to the species level was also conducted. The obtained results revealed higher mold contamination of the cattle samples compared with the buffaloes. In both species, neck muscles had the highest contamination rates, followed by kidney, liver, masseter muscles, and round, respectively. *Aspergillus* spp. was the most prevalent mold genera in all examined samples. *Aspergillus niger (A. niger)*, *A. flavus*, *A. fumigatus. A. ochraceous*, *A. parasiticus*, and *A. terreus* were the identified Aspergilli. In conclusion, this study demonstrates isolation and identification of different molds from the retailed buffalo and cattle meat and edible offal. Therefore, strict hygienic measures should be adopted during all steps of preparation of such valuable protein sources.

Keywords: *Bubalus bubalis*, buffaloes, cattle, edible offal, mold

INTRODUCTION

Buffalo meat industry is a growing industry worldwide, particularly in the central Asia, Italy, Iraq, and Egypt. Buffalo meats share many similarities with cattle meat (beef) in terms of the high-quality protein rich in essential amino acids, vitamins such as Vitamin B group, minerals such as selenium, zinc, and iron. Buffaloes and beef may differ in their texture, rearing method, and leanness (Cockrill, 1981; Preiato, 2020). Bovine edible offal such as neck muscles, masseter muscles, liver, and kidney) is considered as ethnic and popular foods in many parts of the world, particularly in the Middle East because of its specific flavor, low price, and their high nutritive value (Tang *et al*., 2020). One major task of the food safety sector is to ensure microbial safety and wholesomeness of such food products introduced to the public.

Fungal contamination of meat, particularly with different mold genera, is a significant problem

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for foods with high moisture contents that served at the temperate zones (Darwish *et al*., 2016). In addition, mold contamination of meat and edible offal is affected by the hygienic status and procedures followed during handling of such food products starting from the slaughtering of the source animals, dressing, evisceration, transportation, and storage. Contamination of meat and offal with the animal excreta, unclean water, equipment, and operators' hands might increase the chance of mold contamination of meat and offal. Mold contamination of such food sources might affect both safety and quality of the final products (Aberle *et al*., 2001; Darwish *et al*., 2016). However, the available information about mold contamination in both buffalo and cattle meats and their edible offal is scarce.

The objectives of the present study were first to investigate the mold contamination of buffalo and cattle meats (round muscle), and edible offal (neck muscles, masseter muscles, liver, and kidney) in a comparative way. Identification of the prevalent mold genera and further identification of Aspergillus to the species level was followed. The public health significance of the identified molds was further discussed.

MATERIALS AND METHODS

All experiments were conducted according to the guidelines of both King Faisal University, Saudi Arabia, and Zagazig University, Egypt.

Collection of samples

Two hundred samples (100 from each of buffalo and cattle meats and edible offal) including round muscle, neck muscles, masseter muscles, liver, and kidney (n = 20, 100 g per each sample), were collected from butchery shops at Al-Ahsa Governorate, Saudi Arabia (cattle samples), and from Zagazig city, Egypt (buffalo samples). Samples were collected during the period of July to December 2020. The collected samples were transferred cooled directly without delay to the laboratory for mycological examination.

Organoleptical examinations

Organoleptical examination of the examined meat and offal samples was conducted according to Varnam and Sutherland (1995). All selected samples were fresh in odor, firm in consistency, and brick red color for muscles, dark brownish for liver, and rosy-red for kidney.

Preparation of samples for mycological examination

Samples were prepared according to Vanderzant and Splittstroesser (2001). In brief, 25 grams of each sample were homogenized in 225 ml of sterile buffered peptone water 0.1% (LAB104, LAB M, UK). The homogenization process was conducted in a sterile meat homogenizer (type M-p3-302, mechanic, precyzina, Poland), and lasted 2 minutes at 2000 rpm. Then, ten-fold decimal serial dilutions were prepared.

Determination of total mold count (TMC)

Both malt extract agar and Czapeck-Dox agar with 5% NaCl (Oxoid, Basingstoke, UK) were used as culture media to obtain total mold counts (TMC), followed by incubation of the cultured plates in dark at 25ºC for 5 to 7 days. During the incubation time, the plates were examined daily for mold growth. Total mold counts were obtained by direct counting of the cultured plates using the colony counter (Vanderzant and Splittstroesser, 2001).

TMC/g = Average No. of colonies × reciprocal of the dilution

Counted colonies were expressed as log 10 cfu/g.

Identification of the isolated molds

Molds were identified using their macroscopical and microscopical characteristics according to Pitt and Hocking (2009). Both the surface and backside of the mold cultures were examined. Mold colonies were examined daily for the rate and pattern of growth during the incubation period. The consistency of the surface growth and folding, the colony margins, and the surface and reverse pigmentation were observed. The prevalence rate and relative density (RD %) were calculated according to Pacin *et al.* (2003).

$$RD\ \% = \frac{\text{number of samples with genus or species}}{\text{Total number of isolates x 100}}$$

Molecular identification of the isolated molds

To avoid any uncertainty in mold identification, sequencing of the ITS regions of fungal DNA was performed. Fungal DNA was extracted by Cetyl trimethylammonium bromide (CTAB) DNA extraction protocol (Murray and Thompson, 1980). The fungus-specific universal primers ITS1 (5′-TCCGTAGGT-GAACCTGCGG-3′) and ITS4 (5′-GCATATCAATAAGCGGAGGA-3′) were used to amplify genes encoding the ITS region according to White *et al.* (1990). Sequencing was performed using an ABI Prism automated DNA sequencer (Applied Biosystems, Foster city, CA). Big Dye Terminator cycle sequencing kit (Applied Biosystems, Foster city, CA) was used following the manufacturer's manual. Sequence comparisons were performed using the basic local alignment search tool (BLAST) in GenBank (www. ncbi.nlm. nih.gov/blast).

Statistical analysis

Mold counts were converted into log 10 colony forming units per g (log 10 cfu/g). All values are expressed as means ± SE. Statistical significance was evaluated using one way analysis of variance (ANOVA), followed by the Tukey-Kramer HSD, where $P<0.05$ indicated statistical significance.

RESULTS AND DISCUSSIONS

Sensory evaluation of all examined samples revealed normal organoleptical characters, in terms of fresh odor, firm consistency, and brick red color for muscle, and red to blue color in case of the liver (Data are not shown). Fungal examination of the examined samples revealed mold contamination of the examined samples at variable rates, where neck muscles had the highest contamination rates in both buffalo and cattle with 75%, and 95%, respectively, followed by the kidney at 60%, and 70%, respectively, the liver at 55%, and 60%, respectively, masseter muscles at 30%, and 40%, respectively, and the round at 20%, and 30%, in buffalo and cattle, respectively (Figure 1).

Likely, neck muscles had the highest TMC among the examined samples in both examined animal species. The average total mold counts were 3.27±0.15, 3.18±0.09, 2.76±0.11, 2.51±0.09, and 2.31±0.05-log 10 cfu/g in the examined buffalo neck muscles, kidney, liver, masseter muscles, and round, respectively. These values were 3.51±0.44, 3.43±0.04, 3.32±0.05, 2.96±0.09, and 2.88±0.12-log 10 cfu/g in the examined cattle neck muscles, kidney, liver, masseter muscles, and round, respectively (Figure 2). It was clear that cattle

samples had higher mold contamination rates and counts compared with that of the buffalo. This could be attributed to the large numbers of slaughtered cattle compared with the buffalo, and therefore cattle samples received less hygienic measures compared with the buffalo. Lack of proper hygienic measures and sanitary precautions were considered as possible sources of mold contamination of meat and edible offal (Darwish *et al*., 2016). In agreement with the obtained results, Nasser (2015) demonstrated mold contamination (70% of the examined samples) of the retailed meat products in Riyadh city, Saudi Arabia were contaminated with molds. In Egypt, Tawakkol and Khafaga (2007) reported mold contamination of retailed meat and their surroundings (floors, tools, and operator's hands) at high rates. Besides, Darwish *et al.* (2016) recorded high mold counts ranged between 2.69 to 3.60 log cfu/g in frozen chicken meat and giblets including liver and kidney. Furthermore, Tegegne *et al.* (2019) recorded a mold contamination rate of 8.57% in camel meat samples collected from Jigjiga municipal abattoir, Ethiopia. In addition, water buffalo meat was found to be contaminated with mold during all stages of production (Cruz-Monterrosa *et al*., 2020). The high mold contamination of the neck muscles, and edible offal reflects unsatisfactory hygienic measures adopted during slaughtering, skinning, and evisceration (Mizakova *et al*., 2002). In addition, neck muscles had high residual blood from the act of slaughtering, and it is well known that blood is an ideal medium for growth and multiplication of microorganisms as it is rich in nutrients, high pH (~6.0), and high-water activity (~0.97) (Pereira *et al*., 2015). Similar unsatisfactory hygienic measures associated with high mold contamination reaching 100% was recorded in luncheon samples retailed in Assiut city, Egypt (Ismail and Zaki, 1999).

The prevalent mold genera in the present study were *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Cladosporium* spp., *Mucor* spp., *Rhizopus* spp., *Fusarium* spp., and *Eurotium* Spp. at variable rates **(**Figure 3 and 4**)**. *Aspergillus* spp., and *Penicillium* spp. were isolated at the highest rates in all examined buffalo and cattle samples. For instances, *Aspergillus* spp. could be isolated from the examined buffalos' round, liver, kidney, neck muscles, and masseter muscles at 15%, 35%, 40%, 55%, and 15%, respectively; while in the cattle samples, these percentages were 20%, 40%, 65%, 75%, and 20%, respectively. In case of *Penicillium* spp., such prevalence rates were 15%, 35%, 30%, 55%, and 15%, respectively in the examined buffalos' round, liver, kidney, neck muscles, and masseter muscles, respectively. While in cattle samples, these rates were 15%, 30%, 55%, 75%, and 20%, respectively. The calculated relative densities of the identified mold genera among the examined buffalo samples were 40.35%, 27.19%, 10.53%, 4.38%, 10.53%, 3.51%, 2.63%, and 0.88% for *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Cladosporium* spp., *Mucor* spp., *Fusarium* spp., *Eurotium* spp., and *Rhizopus* spp., respectively. While these rates in the case of cattle were 42.48%, 26.14%, 7.19%, 5.88%, 6.54%, 5.88%, 5.23%, and 0.65% for *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Cladosporium* spp., *Mucor* spp., *Fusarium* spp., *Eurotium* spp., and *Rhizopus* spp. respectively.

Further identification of the isolated *Aspergillus* spp. revealed detection of *A. niger*, *A. flavus*, *A. fumigatus*, *A. ochraceous*, *A. parasiticus*, *and A. terreus* at variable rates among the examined samples. In case of buffalo samples, *A. niger*, was the most dominant Aspergilli. (18.42%) followed by *A. flavus* (16.66%), *A. fumigatus* (1.75%), *A. parasiticus* (1.75%), *A. ochraceous* (0.87%), and

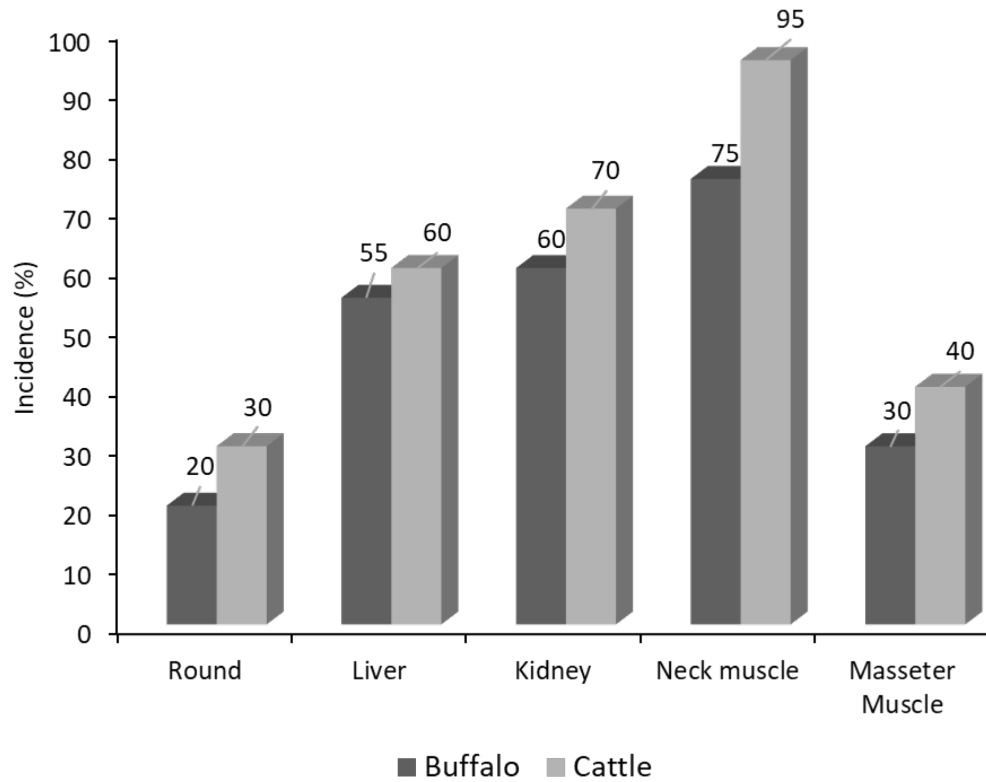

Figure 1. Mold contamination of buffalo and cattle meat and edible offal (n = 20 from each sample per each species).

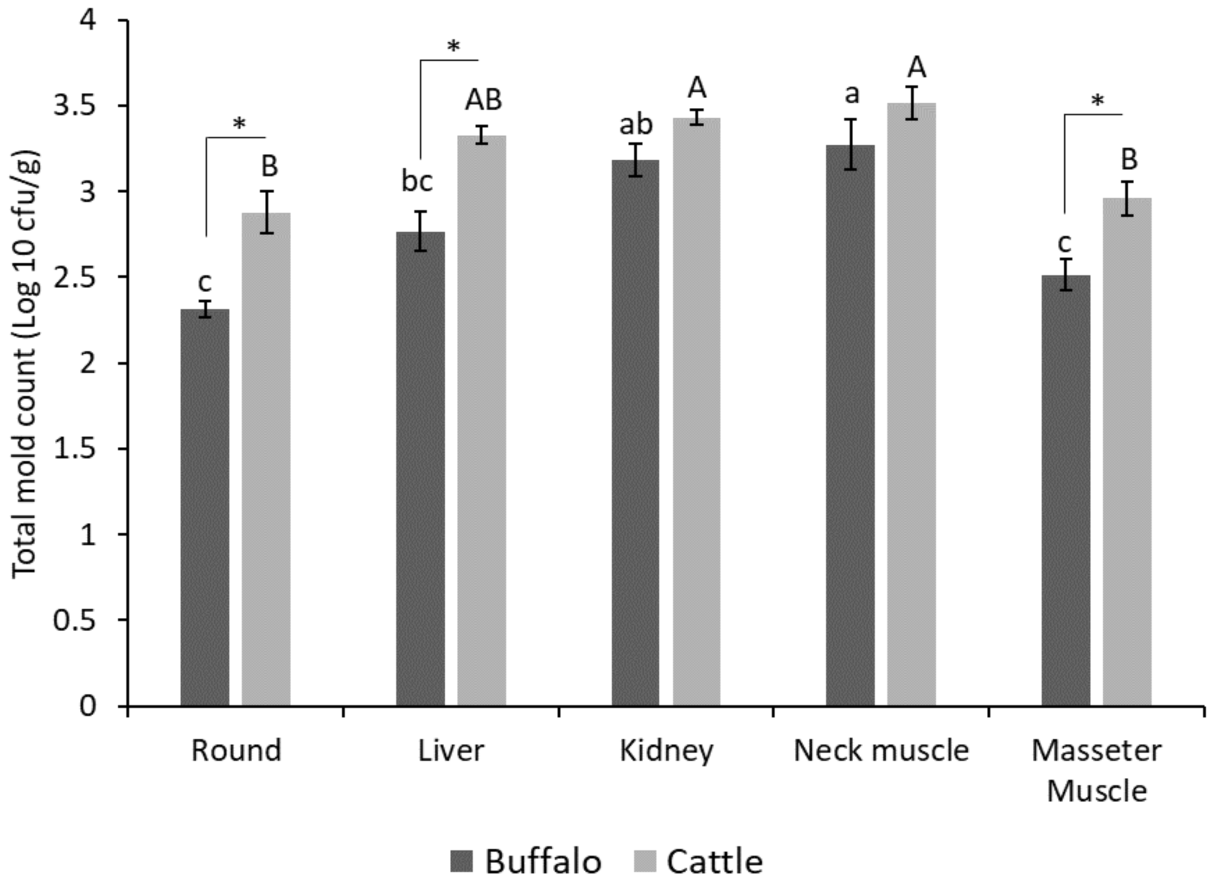

Figure 2. Total mold counts (TMC) in buffalo and cattle meat and edible offal (n = 20 from each sample per each species). Columns with different letter are significantly different at P<0.05. Where, a, b, c refers to the statistical variation among buffalo's samples, while, A, B, C refers to the statistical variation among the cattle's samples. Star marks refer to statistical variation among the cattle's and buffalos' samples.

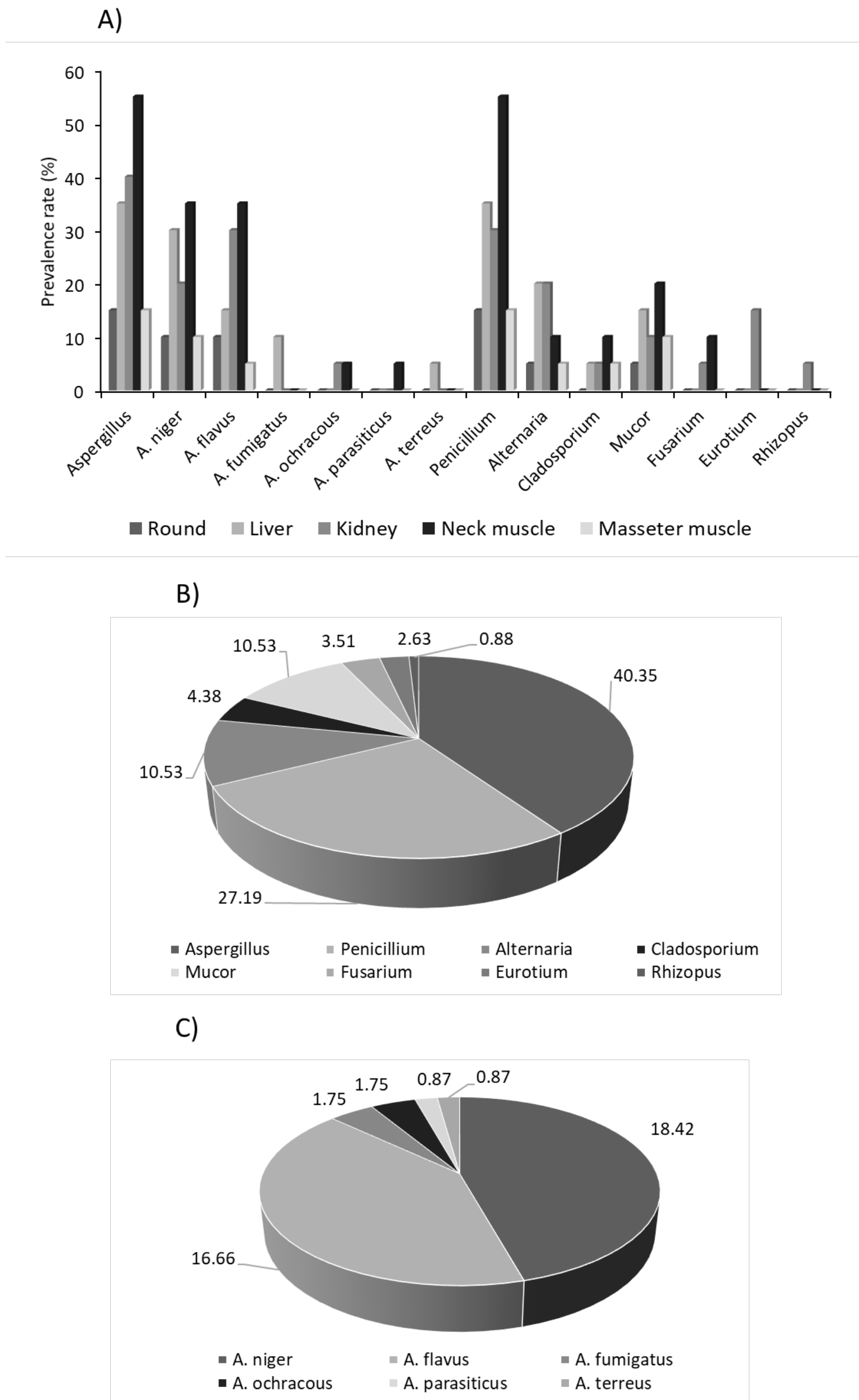

Figure 3. Mold contamination of buffalos' meat and offal samples. A) Prevalence rates (%), B) Relative density (%) of different molds, and C) Relative density (%) of the identified Aspergilli in the examined buffalos' meat and offal samples.

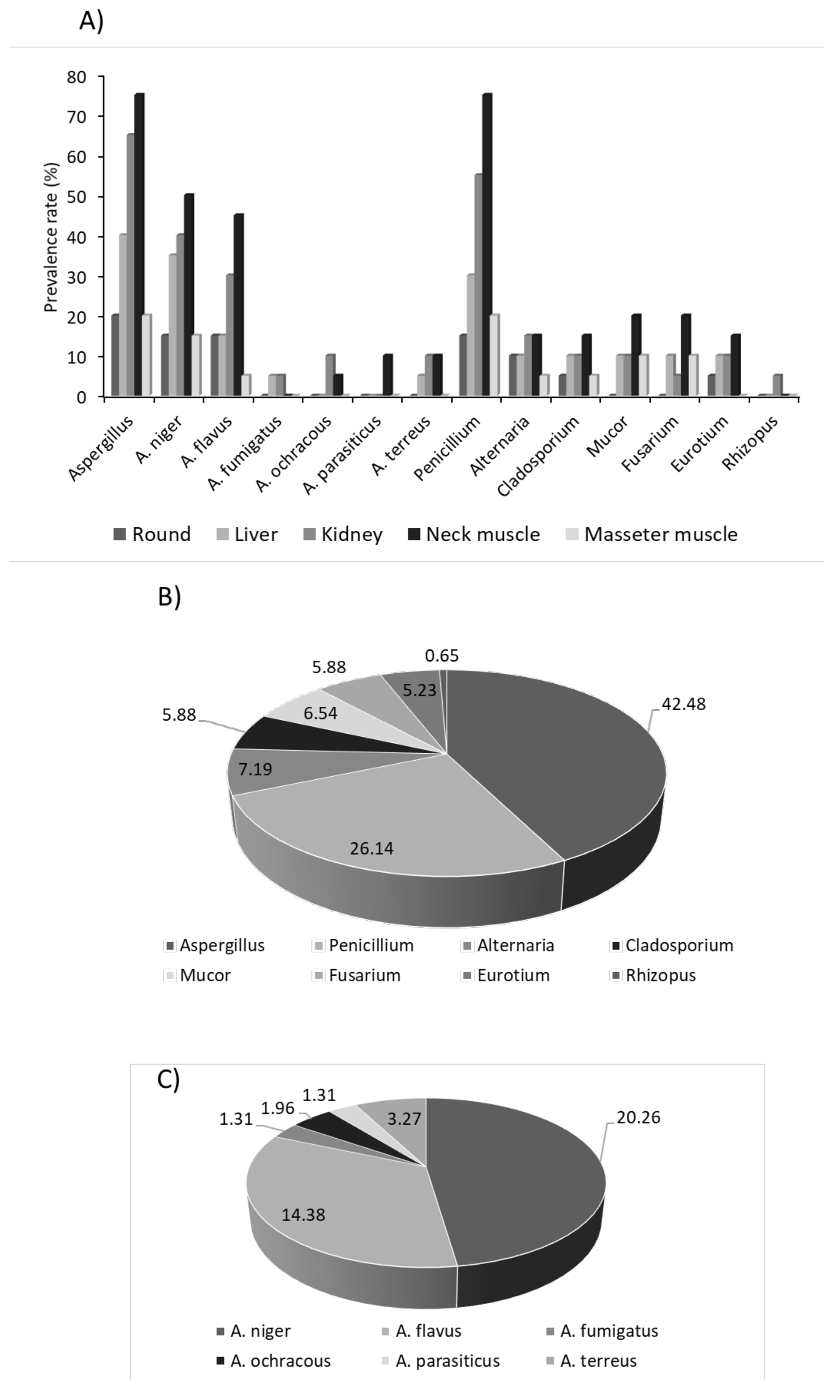

Figure 4. Mold contamination of cattle's' meat and offal samples. A) Prevalence rates (%), B) Relative density (%) of different molds, and C) Relative density (%) of the identified Aspergilli in the examined cattle's' meat and offal samples.

A. terreus (0.87%), respectively. While in case of cattle samples, these rates were 20.26%, 14.38%, 1.31%, 1.96%, 1.31%, and 3.27% in *A. niger*, *A. flavus, A. fumigatus*, *A. ochraceous*, *A. parasiticus*, *and A. terreus,* respectively. The obtained result in the current study goes in agreement with Pitt and Hocking (2009) who demonstrated that *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium*, *Mucor* and *Rhizopus* were the most common isolated mold genera from meats. In addition, Nasser (2015) demonstrated that *Aspergillus* spp., and *Penicillium* spp. were the dominant mold genera isolated from meat products retailed in Saudi Arabia. Besides, *Aspergillus* spp., and *Penicillium* spp. were the dominant molds isolated from offal samples (Darwish *et al*., 2016), and chicken meat products (Habashy *et al*., 2019) retailed in Egypt. The high prevalence of *Aspergillus* spp., and *Penicillium* spp. might be due to their high abilities to grow over a wide range of pH from 2 to 11, water activity (0.62 to 0.99), and temperature from 10 to 60°C (Pitt and Hocking, 2009).

Mold growth in meat and meat products have serious implications on the product safety, quality, marketability, and on public health. Such molds might introduce some secondary metabolites, named mycotoxins, to the contaminated products. These mycotoxins might lead to several toxicological implications such as mutagenesis, teratogenesis, and carcinogenesis (Darwish *et al*., 2014). Therefore, strict hygienic measures should be followed during all steps of preparation and storage of meat and offal.

CONCLUSION

This study revealed mold contamination of meat and edible offal of buffalo, and cattle retailed in both Egypt, and Saudi Arabia. Mold isolation and identification revealed recovery of a vast array of mold genera and species from the examined samples. Therefore, strict hygienic precautions should be followed during all steps of preparation, processing, and distribution of such important meat sources.

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REFERENCES

Aberle, E.D., J.C. Forrest, D.E. Gerrard and E.W. Mills. 2001. *Principles of Meat Science*, 4th ed. Kendall/ Hunt Publishing Co., Dubuque, Iowa, USA.

Cockrill, W.R. 1981. The water buffalo: A review. *Brit. Vet. J*., **137**(1): 8-16. DOI: 10.1016/s0007-1935(17)31782-7

Cruz-Monterrosa, R., D. Mota-Rojas, E. Ramirez-Bribiesca, P. Mora-Medina and I. Guerrero-Legarreta. 2020. Scientific findings on the quality of river buffalo meat and prospects for future studies. *Journal of Buffalo Science*, **9**: 170-180.

Darwish, W.S., Y. Ikenaka, S.M. Nakayama and M. Ishizuka. 2014. An overview on mycotoxin contamination of foods in Africa. *J. Vet. Med. Sci*., **76**(6): 789-797. DOI: 10.1292/jvms.13-0563

Darwish, W., R.M. Bayomi, A.M. El-Moaty and T.M. Gad. 2016. Mould contamination and

aflatoxin residues in frozen chicken meat-cuts and giblets. *Jpn. J. Vet. Res.*, **64** (Suppl. 2): S167-S171.

Habashy, A.H.A., W.S. Darwish, M.A. Hussein and W.M.S. El-Dien. 2019. Prevalence of different mould genera in meat and meat products with some reduction trials using essential oils. *Advances in Animal and Veterinary Sciences*, **7**(s2): 79-85. DOI: 10.17582/journal.aavs/2019/7.s2.79.85

Ismail, M.A. and Z.M. Zaky. 1999. Evaluation of the mycological status of luncheon meat with special reference to aflatoxigenic moulds and aflatoxin residues. *Mycopathologia*, **146**(3): 147-154. DOI: 10.1023/a:1007086930216

Mizakova, A., M. Pipova and P. Turek. 2002. The occurrence of moulds in fermented raw meat products. *Czech J. Food Sci.*, **20**(3): 89-94. DOI: 10.17221/3516-CJFS

Murray, M.G. and W.F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.*, **8**(19): 4321-4325. DOI: 10.1093/nar/8.19.4321

Nasser, L.A. 2015. Molecular identification of isolated fungi, microbial and heavy metal contamination of canned meat products sold in Riyadh, Saudi Arabia. *Saudi J. Biol. Sci.*, **22**(5): 513-520. DOI: 10.1016/j.sjbs.2014.08.003

Pacin, A.M., H.H.L. Gonzalez, M. Etcheverry, S.L. Resnik, L. Vivas and S. Espin. 2003. Fungi associated with food and feed commodities from Ecuador. *Mycopathologia*, **156**(2): 87-92. DOI: 10.1023/a:1022941304447

Pereira, J.A., L. Dionísio, L. Patarata and T.J. Matos. 2015. Effect of packaging technology on microbiological and sensory quality of a cooked blood sausage, Morcela de Arroz, from Monchique region of Portugal. *Meat Sci.*, **101**: 33-41. DOI: 10.1016/j.meatsci.2014.09.147

Pitt, J.I. and A.D. Hocking. 2009. *Fungi and Food Spoilage*, 3rd ed. Published by Blackie Academic and Professional Academic Press New York, London, UK.

Preiato, D. 2020. *All You Need to Know about Bison Meat*. Available on: https://www.healthline.com/nutrition/bison-meat-nutrition.

Tang, H., W.S. Darwish, W.R. El-Ghareeb, N.A. Al-Humam, L. Chen, R.M. Zhong, Z.J. Xiao and J.K. Ma. 2020. Microbial quality and formation of biogenic amines in the meat and edible offal of *Camelus dromedaries* with a protection trial using gingerol and nisin. *Food Science and Nutrition*, **8**(4): 2094-2101. DOI: 10.1002/fsn3.1503

Tawakkol, W. and N.I. Khafaga. 2007. Fungal contamination of meat and its environment with special reference to the strains producing aflatoxins, ochratoxins, proteinase and lipase enzymes. *New Egyptian Journal of Microbiology*, **17**: 1-14. DOI: 10.4314/nejmi.v17i1.40310

Tegegne, H.A., A. Berhanu, Y. Getachew, B. Serda, D. Nölkes, S. Tilahun and B. Sibhat. 2019. Microbiological safety and hygienic quality of camel meat at abattoir and retail houses in Jigjiga city, Ethiopia. *J. Infect. Dev. Countr.*, **13**(3): 188-194. DOI: 10.3855/jidc.9686

Vanderzant, C. and D. Splittstroesser. 2001. *Compendium of Methods for the Microbiological Examination of Foods*, 4th ed. American Public Health Association, Washington, DC, USA.

Varnam, A.H. and J.P. Sutherland. 1995. *Meat and meat products: Technology, chemistry and microbial*, 1st ed. Champman and Hall,

London, UK.

White, T.J., T. Burns, S. Lee and J. Taylor. 1990. *Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics*. Academic Press, New York, USA.