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SURVEILLANCE OF BUFFALO CALF CARCASSES FOR THEIR SURFACE FUNGAL CONTAMINATION AT OLD-FASHIONED ABATTOIRS

BY

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ABSTRACT

A fifth milliliter inoculums – from the original dilution of each triple swab sample – spread onto each of duplicate enumeration plates of dichloran rose bengal chloramphenicol agar, revealed the presence of fungal contaminants (moulds + yeasts) on the all 152 tested surfaces (100%) of 38 freshly dressed buffalo calf carcasses at old – fashioned abattoirs in Dakahlia province (one triple swab sample from every of thigh, abdomen, thorax and neck surface per each carcass. Statistical analytical results of the counts of mould and yeast contaminants found on investigated surface were indicated that the minimum – maximum and mean ± S.E levels of moulds were 10 – 32.5 and 21.58 ± 0.99 cfu/cm^2 on thigh, 12.5 – 45 and 24.14 ± 1.15 cfu/cm^2 on abdomen, 10 – 40 and 22.7 ± 0.99 cfu/cm^2 on thorax, besides 12.5 - 30 and 22.3 ± 0.72 cfu/cm^2 on neck surfaces. On the other hand, these levels for yeasts onto the same surfaces were 7.5 -75 and 29.74 ± 2.89, 7.5 – 87.5 and 31.05 ±2.97, 10 – 75 and 27.11 ± 2.45, plus 5-82.5 and 25.66 ± 2.33 cfu/cm^2, respectively. A total of 2466 (100%) mould strains can be harvested from the duplicate enumeration plates, distributed as 955 (38.73%) *Penicillium*, 502 (20.36%) *Aspergillus*, 332 (13.46%) *Cladosporium*, 294 (11.92%) *Alternaria*, 104 (4.22%) *Fusarium*, 68 (2.76%) *Acremonium strictum*, 62. (2.51%) *Mucor*, 50 (2.03%) *Trichoderma harzianum*, 28 (1.14%) *Thermoascus aurintiacus*, 11 (0.45%) *Rhizopus*, 9 (0.36%) *Aureobasidium pullulans*, 6 (0.24%) *Stemphylium*, 4(0.16%) *Moniliella acetoabutans*, 3 (0.12%) *Thamndium elegans* and 38 (1.54%) unidentified mould strains. The aforementioned mould strains were distributed as 583 (23.64%) from thigh, 665 (26.97%) from abdomen, 610 (24.74%) from thorax and 608 (24.65%) from neck surface samples. As

concerns the numbers and their percentages of tested samples which contaminated with each type of the obtained mould genera, a sum of 118 (77.63%) samples contained *Penicilium*, followed by 65 (42.76%) samples were contaminated with *Aspergillus*, then almost equal total samples 50 (32.89%) & 49 (32.24%) harboured *Cladosporium* and *Alternaria*, 23 (15.13%) total samples possessed *Fusarium*, 16 (10.53%) & 12 (7.89%) collective samples showed contamination with *Acremonium strictum* and *Mucor*, 8 (5.26%) whole samples had *Trichoderma harzianum*, 5 (3.29%) sum samples revealed contamination with *Thermoascus aurintiacus*, total 4 (2.63%) samples exhibited *Rhizopus*, 3(1.97%) samples were contaminated with *Aureobasidium pullulans*, an exclusive one (0.66%) total sample for each of *Stemphylium*, *Moniliella acetoabutans*, and *Thamnidium elegans*; in addition to 11 (7.24%) sum samples contained unidentified moulds. A sum of 502 (100%) *Apergillus* strains were obtained in this work; 95 (18.92%) of them were found on thigh, 154 (30.68%) strains on abdomen, 124 (24.7%) strains on thorax and 129 (25.7%) strains on neck surfaces. Detailed characterization of these *Aspergilli* into groups resulted in 173 (34.46%) *Aspergillus ochraceus*, 118 (23.51%). *Aspergillus niger*, 65 (12.95%) *Aspergillus flavus*, 59 (11.75%) *Aspergillus fumigatus*, 55 (10.96%) *Aspergillus parasiticus*, 18 (3.59%) *Apergillus versicolor*, 6(1.2%) *Aspergillus amstelodami*, 5 (1%) *Aspergillus ustus* and 3 (0.6%) *Aspergillus aculeatus* strains. The numbers and their percentages of samples contaminated with different *Aspergillus* groups were; collectively, *A. ochraceus* and *A. niger* were detected in 30 (19.74%) and 21 (13.82%), respectively out of total 152 samples, while each of *A. flavus* and *A. fumigtus* was represented by 12 (7.89%) of total samples, *A. parasiticus* and *A. versicolor* were found in 8 (5.26%) and 4 (2.63%) of these samples, successively in addition to every of *A. amstelodami, A. ustus* and *A. aculeatus* groups was contained in 1 (0.66%) of such samples. The public health and economic impacts of the fungi – obtained in this work - were also discussed.

INTRODUCTION

Since ancient times, the Egyptian farmers doing a stupid practice by selling most of born buffalo calves on the mid-term of their suckling period (about 6 – 7 weeks-old), at the beginning of winter season. These calves are considered mature enough and suitable for slaughtering-from Meat Hygiene viewpoint- as they are weighing about 100kg live body weight and yielding 50kg- dressed carcass approximately, in addition to their flesh termed as

veal is possessing desirable features by a lot of Egyptians. Unfortunately, such unweaned calves are usually slaughtered in Egypt, either wrongly inside municipal old-fashioned abattoirs or illegally outside such abattoirs although severe beef scarcity in our country that necessitate 1 – 2 years fattening of these calves for obtaining more beef by about 6-fold more than veal harvested. Therefore, new strict regulations are necessary to prohibit calf slaughtering on the future, for saving animal proteins in Egypt.

The healthy inner flesh of dressed carcasses has been reported to contain few or no microorganisms, although they have been found in lymph nodes, bone marrow, and even flesh. The important contamination, however, comes from external sources during bleeding, handling, and processing. During bleeding, skinning, and cutting, the main sources of microorganisms are the exterior of animal (hide, hooves, and hair) and the intestinal tract. The exterior of animal harbors large numbers and many kinds of microorganisms from soil, water, feed, and manure, as well as its natural surface flora, and the intestinal contents contain the intestinal organisms. Knives, cloths, air, alongside hands and clothing of the workers can serve as intermediate sources of contaminants. During the handling of meat thereafter, contamination can come from carts, boxes, or other containers; other contaminated meat; air; and personnel. Growth of microorganisms on surfaces touching the meat and on the meats themselves increases their numbers. In the retail market and in the home additional contamination usually takes place. In the market knives, saws, cleavers, slicers, grinders, chopping blocks, and containers as well as the market operators may be sources of organisms. In the home refrigerator containers used previously to store meat can serve as sources of spoilage organisms.

Because of the varied sources, the kinds of microorganisms likely to contaminate meat are many. At the same time, there is no fungus-free environment in our life **(Chao et al., 2002)**. Therefore, moulds of many genera could reach the meat surfaces and grow there; *Cladosporium*, *Sporotrichum*, *Geotrichum*, *Thamnidium*, *Mucor*, *Penicillium*, *Alternaria* and *Monilia* are prevalent species. The mycological state of carcasses is very dependent on the conditions under which the animals are reared, slaughtered, and dressed. Fresh meat is an ideal environment for the growth of many mycoflora and contamination can therefore soon result in spoilage or be danger to the health of the consumer.

Along with moulds, yeasts belong to the class Mycota or fungi, which are primitive plant-like structures lacking in chlorophyll. The yeasts are microscopic, single-celled organisms

generally larger than the bacteria. Yeasts are mostly saprophytic, while few species are pathogenic. They occur almost everywhere in the environment as well as on skin and in alimentary tract of mammals. A mould consists of a mycelium of branched filaments (hyphae) which bear spores or conidia. In contrast to the yeasts, moulds can be seen with the naked eye as fluffy growths on foods; coloured black, white, or other pigment. Like yeasts, they are primarily saprophytic organisms, breaking down complex organic materials into simpler substances, thus contributing to the decomposition of meats **(Gracey and Collins, 1992)**.

Meat is usually spoiled by bacteria, which grow more rapidly than moulds. It is usually suggested that mould spoilage occurs mainly with frozen meat when the temperature permits growth of moulds but not bacteria. Moulds were thought to much grow at -10°C or even lower temperatures, while bacteria cannot grow much below -2°C, the freezing point of meat. It has also been suggested that the reduced water activity of frozen media rather than the temperature per se may determine the minimum growth temperatures for moulds. Examination of meat spoilage moulds showed most to be moderately xerotolerant with minimum growth temperatures of -5°C or higher. In only one species was growth limited by reduced water activity rather than temperature. During prolonged storage at -5°C, meat developed a flora dominated by yeasts. Visible mould colonies did not appear until the eighth month of storage. It therefore seems that most cases of mould spoilage arise when the temperature of meat surfaces approaches 0°C with surface drying inhibiting bacterial growth **(Lowry and Gill, 1984)**. The moulds and yeasts contaminating cattle carcasses in slaughterhouses have been studied by many researchers **(Refai and Loot, 1969; Eldaly et al., 1988; Mansour et al., 1990; and Elgazzar, 1992).**

The aim of present work, therefore, was to evaluate the mycological condition of the freshly dressed veal carcasses at old-fashioned abattoirs of Dakahlia Province through fulfilling these points:

(1) Estimation of both mould and yeast populations on each square centimeter of the outside surface onto different sites of freshly dressed veal carcasses.

(2) Generic identification of isolated moulds with further group characterization of the obtained aspergilli.

MATERIALS AND METHODS

(I) COLLECTION AND PREPARATION OF THE SAMPLES:

The outside (subcutaneous) surface of different sites (thigh, abdomen, thorax and neck of 38 freshly dressed buffalo calf carcasses, at old - fashioned abattoir in Dakhlia province was swabbed (triple swab sample) and tested, mycologically. All sampled carcasses were from animals had a relatively clean skin and processed under similar conditions, where they have been slaughtered by Islamic method, after being lain on a dirty floor, through severing both carotid arteries, and jugular veins, trachea, and oesophagus then left 2 min for efficient bleeding followed by floor-dressing.

A limited area (20 cm^2) over each surface sample inside a sterilized metal template (4×5 cm) was rubbed repeatedly and successively by 3 sterilized gauze cotton swabs (having a size of about 3.5×1.5 cm and attached to flat wooden stick of about 10 cm length); the first swab was moistened with a 0.1% peptone water (the diluent used) while the other 2 swabs were dry. The 3 swab sticks were broken off below the contaminated handled area into a sterile test tube containing 10 ml of the used diluent 0.1% peptone water to give an original dilution of 2:1 after thorough homogenization of the triple swabs. Each swab sample was then marked and subjected to prompt mycological examination.

(II) MYCOLOGICAL TESTS:

(1) Enumeration of yeast and mould populations in the samples (King et al., 1979):

One fifth ml (0.2) amount from the previously prepared original dilution (2: 1) was delivered and spread onto the dried surface each of sterilized duplicate plates of dichloran rose bengal chloramphenicol agar (DRCA). The inoculated plates as well as the control one were incubated at 25°C for 5 days. After the incubation period, the average of mould and yeast colonies were enumerated over the duplicate plates and the total yeast count/cm^2 plus the total mould count/cm^2 of the tested surface were then calculated and recorded. Each mould growth was picked up and transferred either onto a slope of Czapek yeast extract agar (CYA) (for hydrophilic moulds) or onto a slope of Czapek yeast extract agar with 20%

sucrose (CY20S) (for osmophilic moulds) which incubated at 25˚C for 1-2 weeks and subjected for identification of the obtained moulds.

(2) Identification of the isolated moulds:

Mould genera were identified according to **Raper and Thom (1949)**, **Arx (1967)**, **Zycha et al. (1969)**, **Barnett and Hunter (1972)**, **Samson et al. (1976)**, **Schipper (1978)**, and **Pitt and Hocking (1985)**, whereas

Aspergillus groups were characterized owing to **Raper and Fennell (1965)**, and **Samson (1979)**.

The isolated mould colonies were picked up from the agar slopes and subcultured on plates of Czapek yeast extract agar (CYA) and Czapek yeast extract agar with 20% sucrose (CY20S) by the three points inoculation technique. The inoculated plates were incubated at 25˚C for 1-2 weeks or more, if necessary.

The identification of both mould genera and *Aspergillus* groups was carried out by careful observation and measurements of the macro-and microscopical characteristics of their colonies, as described in data sheet below:

The data obtained in this study were statistically analysed according to methods described by **Snedecor (1971)**. The mean value ($\overline{X}$) was obtained from the sum of individuals (X) divided on number of samples (N).

RESULTS

Table (1): Prevalence of mould and yeast contaminants on the external (subcutaneous) surfaces of buffalo calf carcasses at old – fashioned abattoirs in Dakahlia province (n = 38 swab samples for each site surfaces)

Tested sites and numbers of contaminated samples of each site / Types of contaminated samples	Thigh	Abdomen	Thorax	Neck
Mould – contaminated samples	38 (100%)	38 (100%)	38 (100%)	38 (100%)
Yeast – contaminated samples	38 (100%)	38 (100%)	38 (100%)	38 (100%)

n = number of tested samples.

Table (2): Statistical analytical results of the counts of mould and yeast contaminants on the external (subcutaneous) surfaces of buffalo calf carcasses (cfu/cm^2) at old - fashioned abattoirs in Dakahlia province (n = 38 swab samples for each site surfaces)

Tested sites and statistical analytical results / Types of contaminated samples	Thigh			Abdomen			Thorax			Neck		
	Min	Max	Mean ± S.E.	Min	Max	Mean ± S.E.	Min	Max	Mean ± S.E.	Min	Max	Mean ± S.E.
Mould – contaminated samples	10	32.5	21.58 ± 0.99	12.5	45	24.14 ± 1.15	10	40	22.7 ± 0.99	12.5	30	22.3 ± 0.72
Yeast – contaminated samples	7.5	75	29.74 ± 2.89	7.5	87.5	31.05 ± 2.97	10	75	27.11 ± 2.45	5	82.5	25.66 ± 2.33

n = number of tested samples. Min. = Minimum.

Max. = Maximum. S.E. = standard error of the sample data.

Table (3): Numbers and percentages of mould strains obtained from duplicate enumeration plates* of the correspondent original dilutions of swab samples-taken from external (subcutaneous) surfaces of buffalo calf carcasses at old–fashioned abattoirs in Dakahlia province (n = 38 swab samples for each site surfaces)

Tested sites and numbers & percentages of obtained mould strains / Types of mould strains	Thigh	Abdomen	Thorax	Neck	Total
	No. & %	No. & %	No. & %	No. & %	No. & %
Penicillium spp	230 (39.45%)	202 (30.38%)	269 (44.1%)	254 (41.78%)	955 (38.73%)
Aspergillus spp	95 (16.30%)	154 (23.16%)	124 (20.33%)	129 (21.22%)	502 (20.36%)
Cladosporium spp	77 (13.21%)	104 (15.64%)	77 (12.62%)	74 (12.17%)	332 (13.46%)
Alternaria spp	92 (15.78%)	67 (10.08%)	68 (11.15%)	67 (11.02%)	294 (11.92%)
Fusarium spp	20 (3.43%)	44 (6.62%)	13 (2.13%)	27 (4.44%)	104 (4.22%)
Acremonium strictum	18 (3.09%)	25 (3.76%)	18 (2.95%)	7 (1.15%)	68 (2.76%)
Mucor spp	14 (2.4%)	23 (3.46%)	16 (2.62%)	9 (1.48%)	62 (2.51%)
Trichoderma harzianum	21 (3.6%)	18 (2.71%)	-	11 (1.81%)	50 (2.03%)
Thermoascus aurintiacus	6 (1.03%)	6 (0.9%)	6 (0.98%)	10 (1.64%)	28 (1.14%)
Rhizopus spp	-	-	7 (1.15%)	4 (0.66%)	11 (0.45%)
Aureobasidium pullulans	-	3 (0.45%)	1 (0.16%)	5 (0.82%)	9 (0.36%)
Stemphylium spp	-	-	-	6 (0.99%)	6 (0.24%)
Moniliella acetoabutans	-	-	4 (0.66%)	-	4 (0.16%)
Thamnidium elegans	-	3 (0.45%)	-	-	3 (0.12%)
Unidentified moulds	10 (1.72%)	16 (2.41%)	7 (1.15%)	5 (0.82%)	38 (1.54%)
Total	**583 (23.64%)**	**665 (26.97%)**	**610 (24.74%)**	**608 (24.65%)**	**2466 (100%)**

n = number of tested samples.

*Each sample was represented by one set of duplicate enumeration plates.

Table (4): Distribution of the mould strains obtained from duplicate enumeration plates* of the correspondent original dilutions of swab sample – taken fromexternal (subcutaneous) surfaces of buffalo calf carcasses at old–fashioned abattoirs in Dakahlia province (n= 38 swab samples for each site surfaces)

Numbers and percentages of samples contaminated with different mould strains / Types of mould strains	Thigh	Abdomen	Thorax	Neck	Total
	No. & %	No. & %	No. & %	No. & %	No. & %
Penicillium spp	29 (76.32%)	27 (71.05%)	32 (84.21%)	30 (78.95%)	118 (77.63%)
Aspergillus spp	14 (36.84%)	19 (50%)	17 (44.74%)	15 (39.47%)	65 (42.76%)
Cladosporium spp	9 (23.68%)	14 (36.84%)	15 (39.47%)	12 (31.58%)	50 (32.89%)
Alternaria spp	14 (36.84%)	12 (31.58%)	11 (28.95%)	12 (31.58%)	49 (32.24%)
Fusarium spp	5 (13.16%)	9 (23.68%)	3 (7.89%)	6 (15.79%)	23 (15.13%)
Acremonium strictum	5 (13.16%)	4 (10.53%)	4 (10.53%)	3 (7.89%)	16 (10.53%)
Mucor spp	2 (5.26%)	5 (13.16%)	3 (7.89%)	2 (5.26%)	12 (7.89%)
Trichoderma harzianum	5 (13.16%)	2 (5.26%)	-	1 (2.63%)	8 (5.26%)
Thermoascus aurintiacus	1 (2.63%)	1 (2.63%)	1 (2.63%)	2 (5.26%)	5 (3.29%)
Rhizopus spp	-	-	2 (5.26%)	2 (5.26%)	4 (2.63%)
Aureobasidium pullulans	-	1 (2.63%)	1 (2.63%)	1 (2.63%)	3 (1.97%)
Stemphylium spp	-	-	-	1 (2.63%)	1 (0.66%)
Moniliella acetoabutans	-	-	1 (2.63%)	-	1 (0.66%)
Thamnidium elegans	-	1 (2.63%)	-	-	1 (0.66%)
Unidentified moulds	3 (7.89%)	4 (10.53%)	2 (5.26%)	2 (5.26%)	11 (7.24%)

n = number of tested samples *Each sample was represented by one set of duplicate enumeration plates.

Table (5): Numbers and percentages of *Aspergillus* groups' strains obtained from duplicate enumeration plates* of the correspondent original dilutions of swab samples taken from external (subcutaneous) surfaces of buffalo calf carcasses at old-fashioned abattoirs in Dakahlia province (n = 38 swab samples for each sit surfaces)

Tested sites and numbers & percentages of obtained *Aspergillus* strains / Types of *Aspergillus* groups	Thigh	Abdomen	Thorax	Neck	Total
	No. & %	No. & %	No. & %	No. & %	No. & %
Aspergillus ochraceus	19 (20%)	58 (37.66%)	50(40.32%)	46(35.66%)	173(34.46%)
Aspergillus niger	36(37.89%)	45 (29.22%)	19(15.32%)	18(13.95%)	118(23.51%)
Aspergillus flavus	11(11.58%)	12 (7.79%)	14(11.29%)	28(21.71%)	65 (12.95%)
Aspergillus fumigatus	7 (7.37%)	28 (18.18%)	13(10.48%)	11 (8.53%)	59 (11.75%)
Aspergillus parasiticus	17 (17.89%)	-	24(19.35%)	14(10.85%)	55 (10.96%)
Aspergillus versicolor	5 (5.26%)	3 (1.95%)	4 (3.23%)	6 (4.65%)	18 (3.59%)
Aspergillus amstelodami	-	-	-	6 (4.65%)	6 (1.2%)
Aspergillus ustus	-	5 (3.25%)	-	-	5 (1%)
Aspergillus aculeatus	-	3 (1.95%)	-	-	3 (0.6%)
Total	95 (18.92%)	154(30.68%)	124(24.7%)	129(25.7%)	502 (100%)

n = number of tested samples

*Each sample was represented by one set of duplicate enumeration plates.

Table (6): Distribution of the *Aspergillus* groups strains obtained from duplicate enumeration plates* of the correspondent original dilutions of swab samples taken from external (subcutaneous) surfaces of buffalo calf carcasses at old- fashioned abattoirs in Dakahlia province (n = 38 swab samples for each site surfaces)

Numbers and percentages of samples contaminated with different *Aspergillus* groups strains / Types of *Aspergillus* groups	Thigh	Abdomen	Thorax	Neck	Total
	No. & %	No. & %	No. & %	No. & %	No. & %
Aspergillus ochraceus	4(10.53%)	10(26.32%)	9 (23.68%)	7 (18.42%)	30(19.74%)
Aspergillus niger	7(18.42%)	7 (18.42%)	4 (10.53%)	3 (7.89%)	21(13.82%)
Aspergillus flavus	2 (5.26%)	2 (5.26%)	3 (7.89%)	5 (13.16%)	12 (7.89%)
Aspergillus fumigatus	2 (5.26%)	5 (13.16%)	3(7.89%)	2 (5.26%)	12 (7.89%)
Aspergillus parasiticus	2 (5.26%)	-	4 (10.53%)	2 (5.26%)	8 (5.26%)
Aspergillus versicolor	1 (2.63%)	1 (2.63%)	1 (2.63%)	1(2.63%)	4 (2.63%)
Aspergillus amstelodami	-	-	-	1 (2.63%)	1 (0.66%)
Aspergillus ustus	-	1 (2.63%)	-	-	1 (0.66%)
Aspergillus aculeatus	-	1(2.63%)	-	-	1 (0.66%)

n = number of tested samples

*Each sample was represented by one set of duplicate enumeration plates.

DISCUSSION

A fifth milliliter inoculum – from the original dilution of each triple swab sample – spread onto each of duplicate enumeration plates of dichloran rose bengal chloramphenicol agar, revealed the presence of fungal contaminants (moulds + yeasts) on the all 152 tested surfaces (100%) of 38 freshly dressed calf carcasses at old – fashioned abattoirs in Dakahlia Province (one triple swab sample from every of thigh, abdomen, thorax and neck surface per each carcass) (Table 1). Identical omnipresence of both moulds and yeasts was obtained by **Elgazzar (1992)** and **Abd–Allah (2005)** on bovine carcasses, while a relatively decreased incidence of mould – (90%) and yeast – contamination (80%) was found on chilled beef surfaces by **Elmetwally (2013)**, whereas an extremely lower occurrence of moulds (<4%) and moderately decreased yeasts (63%) out of 420 brisket swab samples taken from chilled beef carcasses by **Murray et al. (2001)**. These variations may be explained by differences in timing (season), and method of sampling as well as the level of hygienic practices applied in various abattoirs (**Patterson, 1971; Kitchell et al., 1973; Catsaras et al., 1974; Banwart, 1989 and Murray et al., 2001**).

Huge populations and diverse genera of moulds and yeasts determined in environment of abattoirs, particularly the old – fashioned ones, surrounding the carcasses of slaughtered animals (air – water – floors – walls) as well as those detected in intestinal and ruminal contents plus dirts and hairs of both hides and feet of carcasses, in addition to fungi found on knife blades and hands, arms, and clothing of workers – all of them may explain the ubiquity every of mould – and yeast – contamination on the four sites's surfaces examined in the present work (**Klare, 1970; Gill et al., 1978; Farghaly, 1985; Sobih and Hefnawy, 1987; Yassien et al., 1989; Mansour et al., 1990; Refai et al., 1993; Lawrie and Ledward, 2006; Obrie et al., 2008; Veskovic – Morcanin, 2009 and Hedayati et al., 2011**).

Statistical analytical results of the counts of mould and yeast contaminants found on investigated surfaces were arranged in both Table (2) and Figure (2) indicated that the minimum – maximum and mean ± S.E. levels of moulds were 10 – 32.5 and 21.58 ± 0.99 cfu/cm^2 on thigh, 12.5 – 45 and 24.14 ± 1.15 cfu/cm^2 on abdomen, 10 – 40 and 22.7 ± 0.99 cfu/cm^2 on thorax, besides 12.5 – 30 and 22.3 ± 0.72 cfu/cm^2 on neck surfaces. On the other hand, these levels for yeasts onto the same surfaces were 7.5 – 75 and 29.74 ± 2.89, 7.5 – 87.5 and 31.05 ± 2.97, 10 – 75 and 27.11 ± 2. 45, plus 5 – 82.5 and 25.66 ± 2.33 cfu/cm^2, respectively. In view of the mould populations on the surfaces of four sites, they showed

nearly similar levels, the same thing in relating to the yeast ones although were somewhat higher than those of mould counts. An extremely higher mould counts were estimated by **Edreis (1986)** as 560 cfu/cm^2 on frozen beef; **Eldaly et al. (1988)** as 200 and 400 cfu/cm^2 on thigh and thorax surfaces of 50 cattle carcasses at Zagazig abattoir, successively; **Elgazzar (1992)** as 221 cfu/cm^2 on thigh, 195 cfu/cm^2 on abdomen, 251 cfu/cm^2 on thorax and 266 cfu/cm^2 on neck of 20 cattle carcasses at Zagazig abattoir; **Hamdy et al. (1993)** as 940 cfu/cm^2 on fresh meat and 8900 cfu/cm^2 on cold – stored meat at butchers' shops; **Hassan (2004)** as 5.67 × 10^4 cfu/cm^2 on surfaces of 30 sheep carcasses. While a moderately higher mould counts were estimated as 120 cfu/cm^2 on shoulder and 56 cfu/cm^2 on thigh surface of 25 cattle carcasses by **Yassien et al. (1989)**; as 83.5, 64, 66 and 53 cfu/cm^2 on round, flank, shoulder and neck surfaces, consecutively of 10 bovine carcasses at Mansoura abattoir by **Abd-Allah (2005)**; as well as 43 cfu/cm^2 on chilled beef surfaces by **Elmetwally (2013)**. On the contrary, lower fungal (moulds + yeasts) counts were determined on the surfaces of 30 lamb carcasses by **Martineli et al. (2011)** as a range of 5.62 – 16.98 cfu/cm^2 onto forequarters and 7.94 – 11.48 cfu/cm^2 onto hindquarters. As regards the yeast counts obtained by other researchers in relation to those found in present work, a considerably higher counts were estimated by **Eldaly et al. (1988)** as 4 x 10^3 cfu/cm^2 on thigh and 2 x 10^4 cfu/cm^2 on thorax surfaces of 50 cattle carcasses, while a moderately higher yeast populations were calculated by **Abd-Allah (2005)** as 72.5, 41.43, 72 and 100 cfu/cm^2 on round, flank, shoulder and neck surfaces, respectively of 10 bovine carcasses besides **Elmetwally (2013)** as 52.88 cfu/cm^2 on chilled beef surface, meanwhile fewer yeast counts were estimated by **Murray et al. (2001)** as a mean of 13.18 cfu/cm^2 on brisket surfaces of chilled beef carcasses. By comparison, our findings of fungal intensities on tested surfaces of freshly dressed carcasses of unweaned buffalo calves were lower or even exceedingly lower than those obtained by many worker onto similar surfaces, this may by attributed to the nature of their relatively cleaner skins and fewer fungal populations of their ruminal and intestinal contents.

A total of 2466 (100%) mould strains can be harvested from the duplicate enumeration plates, distributed as 955 (38.73%) *Penicillium*, 502 (20.36%) *Aspergillus*, 332 (13.46%) *Cladosporium*, 294 (11.92%) *Alternaria*, 104 (4.22%) *Fusarium*, 68 (2.76%) *Acremonium strictum*, 62 (2.51%) *Mucor*, 50 (2.03%) *Trichoderma harzianum*, 28 (1.14%) *Thermoascus aurintiacus*, 11 (0.45%) *Rhizopus*, 9 (0.36%) *Aureobasidium pullulans*, 6 (0.24%) *Stemphylium*, 4 (0.16%) *Moniliella acetoabutans*, 3 (0.12%) *Thamnidium elegans* and 38 (1.54%) unidentified mould strains (Table 3). The aforementioned mould strains were

distributed as 583 (23.64%) from thigh, 665 (26.79%) from abdomen, 610 (24.74%) from thorax and 608 (24.65%) from neck surface samples.

A lot of mould genera, identical to those obtained in the present work, were recovered from many sources of mould contamination at abattoirs by different researchers, where **Klare (1970); Nakae et al. (1976); Farghaly (1985); Mansour et al. (1990); Manuel et al. (2006)** and **Obrie et al. (2008)** could isolate *Penicillium*, *Aspergillus*, *Cladosporium*, *Alternaria*, *Fusarium*, *Acremonium*, *Mucor*, *Rhizopus* and *Thamnidium* species from the intestinal contents (faeces) of slaughtered cattle and camel carcasses as well as from buffalo and cow hair samples – meanwhile, **Ayres et al. (1980); Hill et al. (1984); Jay (1986); Sobih and Hefnawy (1987); Mansour et al. (1990); Hamdy et al. (1991); Ismail et al. (1995); Hageskal et al. (2006); Veskovic-Morcanin (2009)** and **Hedayati et al. (2011)** detected and reported the same aforementioned mould genera except *Thamnidium*, in addition to *Trichoderma* and *Aureobasidium* species in abattoir environments (air – dust – soil – municipal water – walls – floors). Moreover, all the mould genera – obtained in our study with an exception of *Thermoascus aurintiacus* – could be isolated from the surfaces of fresh (warm), chilled and frozen meat as well as of cattle and sheep carcasses by **Refai and Loot (1969); Kamel et al. (1976); Edreis (1986); Mansour (1986); Eldaly et al. (1988); Yassien et al. (1989); El – Naggar (1990); Salem (1991); Elgazzar (1992); Refai et al. (1993); Ismail et al. (1995); Shabanh (1995); Hassan (2004); Abd–Allah (2005) and Elmetwally (2013**). Also, diverse mould genera identical to those harvested in this work – except *Thermoascus aurintiacus* and *Thamnidium elegans* species – were determined by **Hadlok (1972); Hadlok et al. (1976); Hefnawy (1980); Osipain and Davtain (1980); Lotfi et al. (1983); Adel-Rahman et al. (1984); El–Khateib** and **Abdel–Rahman (1989); Mansour et al. (1991); Elgazzar (1995); Hussien et al. (1997); Shaltout** and **Salem (2000); Ouf (2004)** and **Samaha (2013)** in tissues of goat flesh, meat products, frozen minced meat, frozen meat, frozen hamburger, frozen fresh sausage, dry sausage, frozen "Kofta", frozen camel meat products, beef luncheon and basterma.

The aforementioned variations among the percentages of harvested mould strains were undoubtedly attributed to the differences in geographical area, and season, sampling and cultural techniques, as well as the sanitation levels of sampled parts and or surfaces.

Table (4) described the numbers and percentages of samples (collectively) which contaminated with each type of the obtained mould genera, where a sum of 118 (77.63%)

samples contained *Penicillium*, followed by 65 (42.76%) samples were contaminated with *Aspergillus* neck samples; then almost equal total samples 50 (32.89%) and 49 (32.24%) harboured *Cladosporium* and *Alternaria*, 23 (15.13%) total samples possessed *Fusarium*, 16 (10.53%) & 12 (7.89%) collective samples showed contamination with *Acremonium strictum* and *Mucor*, 8 (5.26%) whole samples had *Trichoderma harzianum*, 5 (3.29%) sum samples revealed contamination with *Thermoascus aurintiacus*, total 4 (2.36%) samples exhibited *Rhizopus*, 3 (1.97%) samples were contaminated with *Aureobasidium pullulans*, an exclusive one (0.66%) total sample for each of *Stemphylium*, *Moniliella acetoabutans* – recognized in 1 (2.63%) sample, and *Thamnidium elegans* – harvested from 1 (2.63%) sample; in addition to 11 (7.24%) sum samples contained unidentified moulds. By comparison, an almost equal percentage (8%) of shoulder surface samples of cattle carcasses, at Assiut old-fashioned abattoirs, harboured *Fusarium* **(Ismail et al., 1995)** – as well as 13 & 34.8% out of 100 frozen beef samples contained *Fusarium* and *Alternaria* species, respectively **(Samaha, 2013)**. On the other hand, higher percentages of shoulder surface samples (24%) exhibited *Acremonium strictum* by **Ismail et al. (1995)**, besides 73.9%, 51.1%, 39.1%, 44.6% and 26.1% of frozen beef samples showed *Aspergillus, Cladosporium, Mucor, Rhizopus* and *Trichoderma* species, consecutively by **Samaha (2013)**. However, lower percentages of samples; 24% shoulder surface samples, 36% thigh surface samples and 56.5% frozen beef samples contained *Penicillium* – in addition to 28 % & 16% and 12%&4% of shoulder & thigh samples were contaminated with *Cladosporium* and *Alternaria* species, successively, besides presence of *Acremonium strictum* in 8% thigh samples **(Ismail et al., 1995** and **Samaha, 2013)**.

A sum of 502 (100%) *Aspergillus* strains were obtained in this work; 95 (18.92%) of them were found on thigh, 154 (30.68%) strains on abdomen, 124 (24.7%) strains on thorax and 129 (25.7%) strains on neck surfaces. Detailed characterization of these aspergilli into groups resulted in 173 (34.46%) *Aspergillus ochraceus*, 118 (23.51%) *Aspergillus niger*, 65 (12.95%) *Aspergillus flavus*, 59 (11.75%) *Aspergillus fimigatus*, 55 (10.96%) *Apergillus parasiticus*, 18 (3.59%) *Aspergillus versicolor*, 6 (1.2%) *Aspergillus amstelodami*, 5 (1%) *Aspergillus ustus* and 3 (0.6%) *Aspergillus aculeatus* strains.

(Table 5) showed that the highest aspergilli (numbers and types) were found on abdomen and neck surfaces, while the lowest ones were detected on thigh and thorax surfaces, although the tested samples were uniform equal in number. These results may by explained by the nearby abdomen and neck surfaces to the contamination with intestinal and ruminal contents of slaughted and dressed calf carcasses, while the remaining two surfaces (thigh &

thorax) are apart **(Nickerson and Sinskey, 1972 and Gracey and Collins, 1992).** Also, the obtained data revealed the numbers of harvested *A. ochraceus* strains were higher than *A. niger* ones, such thing disagreed with **Kamel et al. (1976)** and **Osipian and Davtain (1980)** who emphasized that *A. niger* was the dominant mould isolated from meat surfaces and tissues. Identical *Aspergillus* groups – with an exception of *A. aculeatus* – can be isolated from cattle faeces, surrounding air, cow's and buffalo's hairs, plus municipal water & walls & floors of slaughter halls **(Batista et al., 1961; Lacey and Lacey, 1964; Nakae et al., 1976; Farghaly, 1985; Sobih and Hefnawy, 1987; Mansour et al., 1990** and **Manuel et al., 2006)**. Similarly, all *Aspergillus* groups –obtained in this work without *A. aculeatus* – can be detected on a lot of meat surfaces and in tissues of meat products by several researchers. Furthermore, an almost equal percentage each of *A. niger* strains number (14.29%) was found in frozen hamburger by **Hefnawy (1980)**; *A. fumigatus* strains number (11.7%) was determined on cattle carcasses by **Elgazzar (1992)**. Whereas, higher percentages of *A. niger* strains were evaluated by **Refai and Loot (1969)** on meat surfaces and in surrounding environment as 42% of total moulds obtained, **Eldaly et al. (1988)** and **Mansour et al. (1990)** on cattle carcasses as 31.28% and 24.14% of total moulds, respectively, **Mansour et al. (1991)** in frozen beef as 35.29%, **Elgazzar (1992)** onto cattle carcasses as 42.2%, **Hussein et al. (1997)** in tissues of meat products as 35.66% **Abd-Allah (2005)** on cattle carcasses as 49.32%, as well as by **Elmetwally (2013)** on chilled beef as 64.71%. Also, higher percentages of *A. flavus* strains were detected as 28.57% by **Hefnawy (1980)** in tissues of frozen hamburger, as 24.4% by **Abdel-Rahman et al. (1985)** in meat and surrounding environment, as 17.6% by **Elgazzar (1992)** on cattle carcasses, as 24.81% by **Hussein et al. (1997)** in tissues of meat products, and as 19.18% by **Abd-Allah (2005)** on the surfaces of cattle carcasses. Additionally, more percentages of *A. fumigatus* strains were obtained as 42.85% by **Hefnawy (1980)**, 30.7% by **Abdel–Rahman et al. (1985)**, 6.15% of total moulds by **Eldaly et al. (1988)**, 52.94% by **Mansour et al. (1991)**, 16.28% by **Hussein et al. (1997)**, and as 30% by **Elmetwally (2013)** on/in meat and their surrounding environments. Moreover, larger levels of *A. amstelodami* strains were obtained by **Elgazzar (1992)** as 4.5% and **Abd-Allah (2005)** as 13.7% on cattle carcasses, while an exclusive higher percentage of *A. ustus* strains was determined as 2.1% by the former researcher. However, less numbers of *Aspergillus* strains were represented by 3.9% and 4.11% for *A. ochraceus* **(Elgazzar, 1992 and Abd–Allah, 2005)**, 2.23% of total moulds and 2.35% for *A. flavus* **(Eldaly et al., 1988 and**

Elmetwally, 2013), 1.37% for *A. fumigatus* **(Abd-Allah, 2005)** besides 1% for *A. versicolor* **(Elgazzar, 1992)**.

Data arranged in Table (6) declared the numbers and their percentages of samples contaminated with different *Aspergillus* groups; collectively, *A. ochraceus* and *A. niger* were detected in 30 (19.74%) and 21 (13.82%), respectively out of total 152 samples, while each of *A. flavus* and *A. fumigatus* was represented by 12 (7.89%) of total samples, *A. parasiticus* and *A. versicolor* were found in 8 (5.26%) and 4 (2.63%) of total samples, successively in addition to every of *A. amstelodami*, *A. ustus* and *A. aculeatus* groups was contained in 1 (0.66%) of total samples.

Over viewing the obtained results in both tables (5 and 6) revealed the accordance between the numbers of *Aspergillus* strains and those samples harvested from them. By comparison, an approximative percentage (3.17 %) out of 100 frozen beef samples contained *A. versicolor* **(Samaha, 2013)**, while higher percentages of both shoulder and thigh surface samples of cattle carcasses should *A. flavus* strains (24% & 44%) and *A. niger* strains (36% and 44%), respectively **(Ismail et al., 1995)**. Similarly, more percentages (20.63% % & 14.29) out of 100 frozen beef samples exhibited *A. niger* and *A. fumigatus* groups, on the contrary, less percentages (4.76% and 1.59) out of the same samples contained *A. flavus* and *A. parasiticus* groups, consecutively **(Samaha, 2013)**.

In conclusion, the transfer of microfloral contamination from skin and gut to the surface of animal carcass during dressing is inevitable even with suing current slaughterhouse technology. Hence, the goal of modern slaughtering and dressing systems is indicated to reduce such contamination to the lowest practicable level **(Dickson and Anderson, 1992)**. On the other hand, the cutting of oesophagus during the action of Islamic slaughter results in heavy contamination of neck, head and blood with ruminal content **(Gracey and Collins, 1992)**. Moreover, the environment surrounding animal carcasses at slaughter halls, particularly air considered the main source of meat contamination with moulds **(Refai et al., 1993)**. Also, failure to clean and sanitize equipment properly, failure to wash one's hands, poor personal hygiene and lack of care in meat handling are some of carless things that people do which can increase the microfloral load on meat **(Banwart, 1989)**. Finally, the public health and economic impacts of the fungi-obtained in this work – were literatured in a sparate category within review of literature.

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الملخص العربي

تقييم التلوث الفطرى لأسطح ذبائح العجول الجاموسى الرضيعة بالمجازر القديمة

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قسم الرقابة الصحية على الأغذية – كلية الطب البيطرى – جامعة المنصورة

* طبيب بيطرى – مجزر طنامل – أجا – دقهلية

أجريت الدراسة على ثمانية وثلاثين ذبيحة عجل جاموس بتلو وزنه تقريبا ١٠٠ كجم/وزن حى ذبحت وجهزت بمجازر محافظة الدقهلية التقليدية، وقد تم أخذ عينة المسح الثلاثية من السطح الخارجى "تحت الجلد" لكل ذبيحة من أربعة أماكن (منطقة الفخذ – منطقة البطن – منطقة الصدر – منقطة الرقبة).

٠,٢ سم^{٣} أخذت من المحلول الأصلي لعينة المسح الثلاثية وزرعت على أطباق داى كلوران روذبنجال كلورامفينكول أجار المزدوجة (٢ طبق لكل عينة مسح ثلاثية). وقد أستخلص من النتائج وجود التلوث الفطرى (أعفان +خمائر) لكل الأسطح المختبرة (١٥٢ سطح ١٠٠٪). وقد بينت نتائج التحليل الإحصائى للعد الفطرى الموجود على الأسطح أن أقل وأعلى قيمة ومتوسط والخطأ المعيارى لمستوى الأعفان من ١٠ ـ ٣٢,٥ و ٢١,٥٨ ± ٠,٩٩ وحدة تكوين مستعمرة لكل سم^{٢} لمنطقة الفخذ، ١٢,٥ – ٤٥ و ٢٤,١٤ ± ١,١٥ وحدة تكوين مستعمرة لكل سم^{٢} لمنطقة البطن و ١٠ – ٤٠ و ٢٢,٧ ± ٠,٩٩ وحدة تكوين مستعمرة/ سم^{٢} منطقة الصدر بالإضافة إلى ١٢,٥ – ٣٠ و ٢٢,٣ ± ٠,٧٢ وحدة تكوين مستعمرة سم^{٢} لمنطقة الرقبة.

أما بالنسبة إلى مستوى الخمائر على نفس الأسطح فكانت ٧,٥ – ٧٥ و ٢٩,٧٤ ± ٢,٨٩/ ٧,٥ – ٨٧,٥ و ٣١,٠٥ ± ٢,٩٧ / ١٠ – ٧٥ و ٢٧,١١ ± ٢,٤٥ / ٥ – ٨٢,٥ و ٢٥,٦٦ ± ٢,٣٣ وحدة تكوين مستعمرة لكل سم^{٢} بالتوالى.

فى الدارسة تم الحصول على ٢٤٦٦ عترة عفن من أطباق العد المزدوجة وعددها (٣٠٤ طبق) بعد زراعة تلك العترات المحبة منها للماء على أغار شابك مستخلص الخميرة (CYA) czapek yeast extract والكارهه منها للماء على أغار شابك مستخلص الخميرة يحتوى على ٢٠٪ سكروز Czpak Yeast Extract (CYAs 20%) وبعد تحضينها عند درجة حرارة ٢٥° لمدة ٧ أيام و ١٤ يوما على التوالى وبالفحوص العينية والمجهرية صنفت و وزعت كالتالى: ٩٥٥ (٣٨,٧٣٪) بنسليوم و٥٠٢ (٢٠,٣٦٪) اسبرجلس و ٣٣٢ (١٣,٤٦٪) كلادوسبريوم و ٢٩٤ (١١,٩٢٪) الترناريا و ١٠٤ (٤,٢٢٪) فيوزاريوم و ٦٨ (٢,٧٦٪) اكريمونيوم ستركتم و ٦٢ (٢,٥١٪) مبوكور و ٥٠٠ (٢,٠٣٪) ترابكوديمرما هارزيانم و ٢٨ (١,١٤٪) ثيرموأسكاس أيور ينتياكس و ١١ (٠,٤٥٪) رايزويس و ٩ (٠,٣٦٪) أيوروبازيديوم بوليولنس و٦ (٠,٢٤٪) ستمفايلم و ٤ (٠,١٦٪) مونيليالا استابيوتانس ٣ (٠,١٢٪) ثامنيديام إليجانس و ٣٨ (١,٥٤٪) عترات غير معرفه.

كما سبق ذكر أن مجموع عترات الاسبرجلس التى تم عزلها هو ٥٠٢ (١٠٠٪) فى هذه الدراسة ٩٥ (١٨,٩٢٪) من سطح الفخذ و ١٥٤ (٣٠,٦٨٪) من سطح البطن و ١٢٤ (٢٤,٧٪) من سطح الصدر ١٢٩ (٢٥,٧٪) من سطح الرقبة بالتصنيف المفصل لأعفان الاسبرجلس إلى مجموعات وجد ١٧٣ (٣٤,٤٦٪) اسبرجلس اوكراشيوس و ١١٨ (٢٣,٥١٪) اسبرجلس نيجر ٦٥ (١٢,٩٥٪) اسبرجلس فلافس و ٥٩ (١١,٧٥٪) اسبرجلس فيوميجاتس و ٥٥ (١٠,٩٦٪) اسبرجلس بارازيتكس و ١٨ (٣,٥٩٪) اسبرجلس فيرسيكلور و ٦ (١,٢٪) اسبرجلس اميستيلو دامى و ٥ (١٪) اسبرجلس استس و ٣ (٠,٦٪) اسبرجلس ايوكلاتس.

كما بينت الدراسة الأعداد و النسب المئوية للعينات التى عزل منها مجموعات الاسبرجلس المختلفة وكانت٣٠ (١٩,٧٤٪) و ٢١ (١٣,٨٢٪) عينة لاسبرجلس اوكرا شيوس ونيجر بالتوالى من المجموع الكلى للعينات ١٥٢ عينة بينما اسبرجلس فلافس وفيوميجاتس كانت ١٢ (٧,٨٩٪) لكليهما اما اسبرجلس بارازيتكس وفيرسيكلور فوجدت فى ٨ (٥,٢٦٪) و ٤ (٢,٦٣٪) بالتوالى أما مجموعات اسبرجلس امستيلودامى واستس واكيولاتس فوجدت ١ (٠,٦٦٪) عينة لكل منهم.