Hygienic Studies On Veal Meat Marketed On Sharkia Governorate

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Abstract:

One hundred samples of buffalo veal meat obtained from thigh and shoulder muscle (50 of each) were randomly collected from butcher shops at Sharkia Governorate.The aerobic plate count, Psychrotrophic count, Enterobacteriaceae count and Staphylococci count was 5.26 ± 0.18 and 5.59 ± 0.26, 3.66 ± 0.19 and 3.74 ± 0.21, 2.85 ± 0.12 and 3.78 ± 0.17, 3.21 ± 0.09 and 3.64 ± 0.15 log_{10}CFU/g in examined thigh and shoulder meat, respectively.*Staphylococcus aureus* detected in 14 (28%) and 9 (18%) in examined thigh and shoulder of veal meat with all over percentage 23%.The prevalence of *E.coli* is 10% and 18% in both thigh and shoulder veal meat samples.The serotypes in veal meat the more prominent serotypes were O_{26} (21.42%) , O_{44} (7.14%) , O_{55}(14.28%), O_{86} (7.14%), O_{111} (7.14%), O_{119} (21.42%), O_{124}(4.14%) negative and O_{128} (14.28%).

Key words:

Staphylococcus aureus- E. coli – Veal meat - Aerobic plate count- Enterobacteriaceae.

1- Introduction:

Veal meat in Egypt obtained from buffalo calves slaughtered between 5 to 6 weeks, delicate in flavour, pale grayish white in colour, firm and fine-grained, with velvety texture. It has no marbling, and the small amount of fat covering is firm and white. The veal price was affected by season of calving and veal demand. The price decreased in winter by 20-40% compared to summer season, where most of buffaloes calved in winter season. In summer, a high demand of grill meat was reported by some butchers due to low fat content in veal meat compared to alternative grilled meat like lamb's meat or beef that prepared by restaurants. The veal carcasses are sold to consumers and/or restaurants. Grading veal carcasses were done based on local standards. Carcasses are classified into grade “A” and grade “B”. Hind quarters and fore ribs were classified as “A”, while fore-quarters are classified as “B”. Restaurants pay 21% higher prices in grade “A”. All butchers interviewed reported that, the demand was higher in summer compared to winter season **(El-Asheeri et al., 2017)**. Veal meat during it's long chain of slaughtering, transporting and marketing is subjected to many risks of contamination from different sources till it reach to the consumers. Such contamination may render the product unfit for human consumption or even harmful to consumers and resulting in economic losses and may also constitute a public health hazard. *E.coli* is a member of the family Enterobacteriaceae and constitutes a part of the normal intestinal flora of man and animals, however some pathogenic *E.coli* strains can cause diseases that differ in severity from diarrhea to meningitis, septicemia and hemolytic uremic syndrome (HUS)

(Sussman, 1985). Also, Staphylococcus *aureus* is considered to be one of the most important foodborne pathogens worldwide due to its ability to produce a wide arrays of toxins **(Argudin, et al., 2010**). It is an important cause of food poisoning associated with nausea, vomition, abdominal cramps and prostration as the most common symptoms of the case **(Sea and Bohach, 2007).** So that, contamination of meat has promoted consumer fear and global concern, threatened trade and economic profit and stimulated ideas in developing new process control measures. Owing to the aforementioned points, the present study will planned out to determine some bacteriological parameters of veal meat.

2- Materials and Methods:

One hundred samples of buffalo veal meat obtained from thigh and shoulder muscle (50 of each) were randomly collected from butcher shops at Sharkia Governorate. The collected samples were transferred in an insulated ice box under complete aseptic conditions, without undue delay to the laboratory of meat hygiene, Faculty of Veterinary Medicine, Zagazig University where they were investigated and examined bacteriologically.

Veal meat samples were prepared according to the technique recommended by **APHA (2001)**.The enumeration of Aerobic plate count was performed according to **(ISO, 2002)** on standard plate count agar Oxiod (CM325). After solidification the inoculated plates as well as control one was inverted and incubated promptly for 2 days at 30^{O} C. The plates with 30-300 colonies were counted and total colony count per g. was calculated and recorded. Total psychrotrophic count the same technique as in the aerobic plate count was carried out except that the incubation at 7^{o} C for 10 days**(APHA, 2002)**. The *Staphylococcous aureus* counted on Baired-Parker agar medium plates Oxoid (CM 275) according to **(ISO 6888-1, 1999)** and biochemical identification according to C**ruickshank et al. (1975)**. Enumeration of Enterobactericeae was carried out on a violet red bile glucose agar (VRBG) agar. The agar was inoculated by spreading 0.1 ml of the decimal dilution onto the surface according to **(ISO 21528-2, 2004)**

Isolation of *Escherichia coli* according to **(APHA, 1992)** One ml of original dilution was used to inoculate separately into a test tubes containing MacConkey broth with inverted Durham's tubes. The inoculated tubes were incubated at 44 ± 0.5 °C for 48 hrs. Positive tubes showing acid (yellow color) and gas production in inverted Durham's tubes were recorded. A loopfull from each positive tube (acid and gas) of MacConkey broth which firstly incubated at 44 ± 0.5 °C for 48 hrs was streaked onto Eosin Methylene blue (EMB) agar. The inoculated plates were incubated at 37 °C for 24 hrs. Typical colonies of *E. coli* appear greenish, metallic with dark purple center. Suspected colonies were purified and subcultured onto nutrient agar slope and incubated for further investigations.

Serological identification of *E.coli*

The isolates were serologically identified according to **Kok *et al.* (1996)** by using rapid diagnostic *E.coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic types.

Detection of Toxin producing genes in isolated *E.coli* strains using Multiplex PCR:

Application of PCR for identification of shiga toxins (stx1 & stx2) and Intimin (eaeA) as virulence genes of *E.coli* was performed essentially by using primers (Pharmacia Biotech) as shown in table(1) :

Dhanashree and Mallya (2008)	614	**5′** ACACTGGATGATCTCAGTGG **′3**	stx1 (F)
		5′CTGAATCCCCCTCCATTATG **′3**	Stx1(R)
Dhanashree and Mallya (2008)	779	**5′** CCATGACAACGGACAGCAGTT **′3**	Stx2 (F)
		5′CCTGTCAACTGAGCAGCACTTTG **′3**	Stx2(R)
Mazaheri *et al.* (2014)	890	**5′** GTGGCGAATACTGGCGAGACT **′3**	eaeA(F)
		5′ CCCCATTCTTTTTCACCGTCG **′3**	eaeA(R)

Amplification reaction of *E. coli*:

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). PCR assays were carried out in 1 ml of nucleic acid template prepared by using reference EHEC isolates (approximately 30 ng of DNA), 10 mM Tris-HCl (pH 8.4), 10 mM KCl, 3 mM MgCl2; 2 mM concentrations of each primer, 0.2 mM concentrations of each 29-deoxynucleoside 59-triphosphate, and 4 U of AmpliTaq DNA polymerase (Perkin-Elmer). Amplification conditions consisted of an initial 95°C denaturation step for 3 min. followed by 35 cycles of 95°C for 20 sec, 58°C for 40 s, and 72°C for 90 sec. The final cycle was followed by 72 °C incubation for 5 min. The reference strains were *E. coli* O157:H7 Sakai (positive for stx1, stx2, eaeA and hylA) and *E. coli* K12DH5α (a nonpathogenic negative control strain) that does not possess any virulence gene according to **(Fagan *et al.*, 1999)**

DNA amplification was performed using the following conditions:

Initial denaturation for 5 min. at 95 °C followed by 30 cycles of denaturation (94 °C for 2 min.), annealing (55 °C for 1 min.), and extension (72 °C for 2 min.). A final extension step (72 °C for 5 min.) was performed after the completion of the cycles. Amplified products were analyzed by 3% of agarose gel electrophoresis (Applichem, Germany, GmbH) in 1x TBE buffer stained with Ethedium bromide and captured as well as visualized on UV transilluminator at 254 nm. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes.

Statistical analysis:

The results were reported as mean values ± standard error of mean (S.E.). Statistical analysis of data was done by using the statistical package for social sciences (SPSS Inc.; Chicago, IL, USA) software. Independent samples T test at 95% level of confidence was done to determine significant differences (Statistical analysis was done using SPSS-16 statistical software and Independent samples T test). $P<0.05$ was considered as significant).

3- Results and Discussion:

The aerobic plate count is the most widely used bacteriological tests for foods. Its purpose is to determine the number of living micro-organisms unit in food and it provide information about the general degree of microbial contamination of the product, raw material and working surfaces of equipment, so it considered as an essential parameter for efficiency of hygiene. Total bacterial count is a commonly recommended microbiological method for estimating the food shelf-life, the microbiological quality and the overall degree of microbial contamination of red meat.

Table (1): Microbial counts log_{10} CFU/g of veal meat samples (N=50 for each).

Sample		Minimum	Maximum	Mean±SE
Thigh meat	APC	4.23	5.81	5.26 ± 0.18
Shoulder meat		4.82	6.11	5.59 ± 0.26
Thigh meat	Psychrotrophic count	3.4	4.15	3.66 ± 0.19
Shoulder meat		3.35	4.28	3.74 ± 0.21
Thigh meat	Enterobacteriaceae count	2.21	3.24	2.85 ± 0.12
Shoulder meat		2.94	4.13	3.78 ± 0.17
Thigh meat	Staphylococci	2	3.82	3.21 ± 0.09
Shoulder meat		2	3.95	3.64 ± 0.15

The results achieved in table (1) showed that APC ranged from 4.33 to 5.81 log_{10}CFU/g with a mean value of 5.26 ± 0.18 log_{10}CFU/g for examined thigh while, the count ranged from 4.82 to 6.11 log_{10}CFU/g with a mean value of 5.59 ± 0.26 log_{10}CFU/g for examined shoulder meat.

The results of aerobic plate count in meat samples were nearly similar with **Cohen et al. (2006), Tharwat (2008)**, **El-Abbasy (2010)** and **Nevry et al. (2011)**. Higher counts obtained by **Cohen et al. (2006)**, **Khalafalla et al. (2010)**.Lower counts obtained by **Badawi (2008).**

Aerobic Plate Count of any food article is not a sure indicative of its safety for consumption, but also, it is very important in judging the hygienic conditions under which the meat has been produced,

handled and stored **(Jay, 1997).** Hundred percentage of examined thigh meat accepted and 94% of examined shoulder meat accepted according to the legal requirement of **(ES 2005**), the permissible limit of APC in fresh meat (< 10^6CFU/g).

Psychrotrophs are these bacteria that grow well at or below 7°C and have their optimum temperature for growth between 20-30°C. Some psychrotrophic pathogens can grow in the refrigerated food with little or no obvious change of sensory characteristics **(Berrong *et al.*, 1989).**

The factors which make the psychrotrophic microorganisms important in food are their ubiquitous in the atmosphere in which the meat is handled and stored **(Mousa *et al.*, 1988)**, their ability to use nitrogenous foods, their proteolytic and lipolytic activity, their aerobic tendency that enabling them to grow rapidly and produce oxidized products and slime formation at the surface of foods where heavy contamination is most likely, their ability to grow at low temperature, and pigment production by some species **(Frazier and Westhoff, 1984)**

The results achieved in table (1) showed that Psychrotrophic ranged from 3.4 to 4.15 $\log_{10}$CFU/g with a mean value of 3.66 ± 0.19 $\log_{10}$CFU/g for examined thigh while, the count ranged from 3.35 to 4.28 $\log_{10}$CFU/g with a mean value of 3.74 ± 0.21 $\log_{10}$CFU/g for examined shoulder meat. The results of Psychrotrophic count in meat samples were nearly similar with **Badawi (2008)** and lower than **Lefebure et al. (1992)** and **Mohamed (1996)**.

Enterobacteriaceae are widely distributed. Although strains of some species are harmless commensals, some members of *Enterobacteriaceae* constitute a great public health hazard if present in large numbers as well as they play an important role in meat spoilage **(Frazier and Westhoff, 1978).**

Determination of *Enterobacteriaceae* count indicates enteric contamination and declares the hygienic quality of raw food in addition it was reported that they cause health hazard to food consumers as well as spoilage and deterioration of meat accordingly, the presence of a considerable number of *Enterobacteriaceae* indicates inadequate processing and or post processing recontamination **(Mercuri and Cox, 1979 and ICMSF, 1980).**

The results in table (1showed that *Enterobacteriaceae* ranged from 2.21 to 3.24 $\log_{10}$CFU/g with a mean value of 2.85 ± 0.12 $\log_{10}$CFU/g for examined thigh while, the count ranged from 2.94 to 4.13 $\log_{10}$CFU/g with a mean value of 3.78 ± 0.17 $\log_{10}$CFU/g for examined shoulder meat. The results of *Enterobacteriaceae* count in meat samples were nearly similar with **Crowley et al. (2004)**, **El-Abbasy (2010)** and **Nouh (2012)**. Higher counts obtained by **Nel et al. (2004)** and **Kilonzo-Nthenge et al. (2013)**.Lowere counts obtained by **Tharwat (2008)** and **El-Sheikh (2014).**

Raw foods may be contaminated by Staphylococci from contaminated hide and food handlers as a result of their coughing, sneezing or contact with arms and hands lesions **(APHA, 1992).**

The results in table (1) showed that Staphylococci ranged from 2 to 3.82$\log_{10}$CFU/g with a mean value of 3.21 ± 0.09 $\log_{10}$CFU/g for examined thigh while, the count ranged from 2 to 3.95 $\log_{10}$CFU/g with a mean value of 3.64 ± 0.15 $\log_{10}$CFU/g for examined shoulder meat. The results of *Staphylococci* count in meat samples were nearly similar with **Çetin *et al.* (2010)** and **Attala and kassem (2011)**.Higher counts obtained by **Moussa et al. (1992)**, **Gonulalan and Kose (2003)** and **El-Gendy *et al.* (2014**).Lower counts obtained by **Cohen et al. (2006)**, **Nevry et al. (2011)** and **Shaltout *et al.* (2016).**

Staphylococci are commonly found in the skin and upper respiratory tract of man and animals and can easily contaminate the carcass. The presence of *Staphylococci* on carcass surface may be due to

contamination during dressing and evisceration in slaughter house, contaminated equipment, butcher's hand with abrasions and wounds, slaughter of animal beside dressed one in the same area in the slaughter hall and contamination of air from crowdness of workers and their aerosols, poor personal hygiene of the workers **(Lasts et al., 1992).**

Staphylococcus aureus detected in 14 (28%) and 9 (18%) in examined thigh and shoulder of veal meat with all over percentage 23% as shown in table 12 and figure (2). Although, *S..aureus* contamination can be readily avoided by heat treatment of food, it remains a major cause of food borne disease; the presence of *S.aureus* in heat treated food may be also due to its contamination from food handlers, inadequately cleaned equipment or post- processing contamination (**Duffy *et al.*, 2000)**.*S.aureus* is a true food poisoning organism; it produces heat stable toxins when allowed to grow for several hours in food. This bacterial growth may not cause any off odor, flavor or abnormal color or texture, but the toxins will be secreted into the food (**Reynolds *et al.*, 2003).** *S.aureus* toxins are not markedly affected by heating or freezing because they are heat stable. Even if the food is heated before eating, the poison in the food will cause illness , although the heat has killed the bacterial cells **(Estes *et al.*, 2007).**

E.coli is a natural inhabitant of the intestinal tracts of humans and warm-blooded animals, its presence reliably reflects fecal contamination. Moreover, it indicates a possible contamination by enteric pathogens. Undercooked or raw foodstuffs gets contamination either during primary production as slaughtering or further processing and handling e.g. cross contamination during processing, human-to-food contamination by food handlers **(Adeyanju and Ishola, 2014)**.

Escherichia coli is commonly used as surrogate indicator, its presence in food generally indicates direct and indirect fecal contamination **(Clarence *et al.*, 2009)**.

From table (2) it is indicated that the prevalence of *E.coli* is 10% and 18% in both (thigh and shoulder) veal meat samples.

The current results nearly agreed with **(Mousa *et al.*, 1993), (Fathi *et al.*, 1994),** (**Sayed *et al.*, 2001)** and **(Abd El Tawab *et al.*, 2015)** who isolated *E.coli* from 34% of examined meat, **Abdel - Rahman *et al.* (2014)** isolated *E.coli* from frozen ground beef with an incidence of 35% and **Rabie (2014)** who isolated *E.coli* from meat with rates of 28%. *E. coli* incidence much higher than the results obtained by **Fahmi (1993), Habeel (1999), Phillips *et al.* (2001), Phillips *et al.* (2006**) who reported *E. coli* 1.8% of boneless beef, and on the other hand**, Hassan (2012)** failed to isolate *E.coli* from frozen meat and isolated it in 7.1% from meat in thawed state.

Table (2): prevalence of *S. aureus and E.coli* in veal meat samples (N=50 for each)

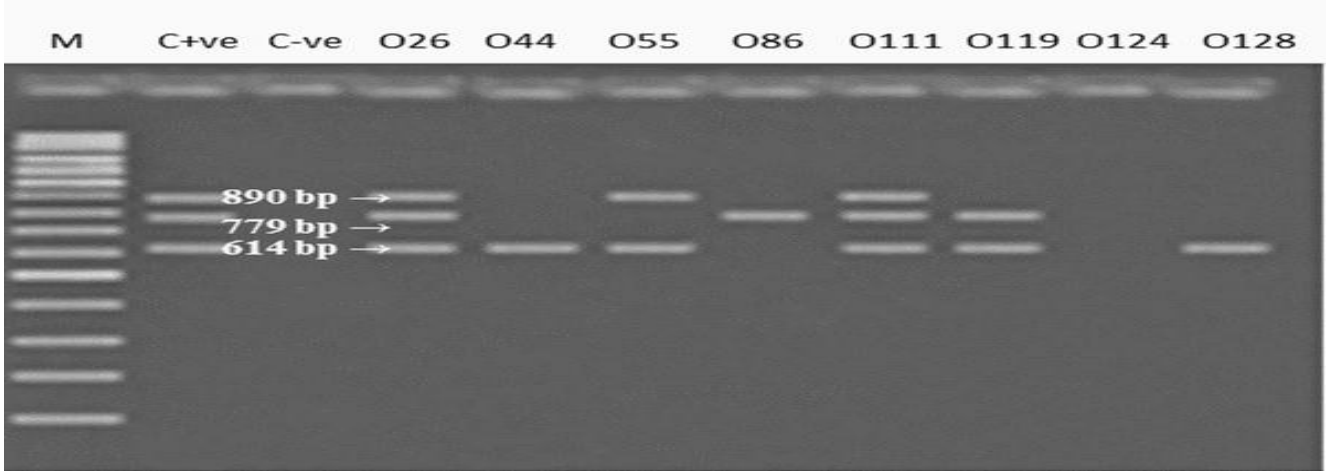
Samples	*S. aureus*	*E.coli*
Thigh meat	14 (28%)	5 (10%)
Shoulder meat	9 (18%)	9 (18%)
Total	23 (23%)	14 (14%)

The recorded data in table (3) and photograph (1) it is concluded that serotypes in veal meat the more prominent serotypes were O_{26} (21.42%) contain *stx1, stx2 and eaeA* genes , O_{44} (7.14%) contain *stx1* gene O_{55}(14.28%) contain *stx1and eaeA* genes , O_{86} (7.14%) contain *stx2* genes , O_{111} (7.14%) contain *stx1, stx2 and eaeA* genes, O_{119} (21.42%) contain *stx1 and stx2* genes , O_{124}(4.14%) negative and O_{128} (14.28%) contain *stx1* genes.

The present results are near that of (**Lee *et al.*, 2009**) who reported the occurrence of 39 pathogenic *E. coli* isolates recovered from different meat types. **Murphy *et al.* (2005)** who detected *E. coli* O_{26} in 0.25% (2/800) of samples but not *E. coli* O_{111}**.**

Table (3): Serotypes of *E. coli* isolates from examined veal meat.

	Thigh muscle	Shoulder muscle	Total
O_{26}	1	2	3/14 (21.42%)
O_{44}	0	1	1/14 (7.14%)
O_{55}	1	1	2/14 (14.28%)
O_{86}	0	1	1/14 (7.14%)
O_{111}	1	0	1/14 (7.14%)
O_{119}	1	2	3/14 (21.42%)
O_{124}	0	1	1/14 (7.14%)
O_{128}	1	1	2/14 (14.28%)



Photograph (1):DNA expression of virulent genes of Shiga Toxin producing *E.coli* strains by using Multiplex PCR.Agarose gel electrophoresis of multiplex PCR of stx1 (614 bp), stx2 (779 bp) and eaeA (890 bp). Lane M: 100 bp ladder as molecular size DNA marker. C+ve: Control positive *E. coli* for stx1, stx2 and eaeA genes. C-ve: Control negative.

Regarding to virulence genes nearly similar genes obtained by **Shawish, (2015)** who examined different *E.coli* serovars isolated from different meat products by multiplex PCR with specific primers for stx_1, stx_2 and eaeA genes. He found that *E.coli* serovars **O_{111}** proved to have the three genes *(*stx_1, stx_2 and eaeA genes).Moreover, **(Farhan *et al.*, 2014)** used the multiplex PCR for the detection of stx_1, stx_2 and eaeA virulence genes; they found that 8.7% from 115 isolates of *E. coli* were positive for the target genes of shiga toxin production. On the other hand **(El Gamel *et al.*, 2015)** detected O_{111}:H_{21} strain which was non producer of any of shiga toxins (STs). **El-Aboudy (2018)** who found that **O_{26}** hundred% of it contain stx_1, 66.67% of it contain stx_2 and 66.67% contain eaeA virulent genes, **O_{55}** hundred % of it contain stx_1 virulant gene**, O_{111}** hundred % of it contain stx_1, stx_2 and eaeA virulent genes, **O_{119}** hundred % 0f it contain stx_1 and stx_2 virulant genes, and **O_{128}** hundred % of it contain stx_1 virulent gene.

The polymerase chain reaction (PCR) based diagnostic assays have been developed to target these three genes (stx_1, stx_2 *and* eaeA genes).

4- References:

Abdelrahman H.; Meawad,A and Shaheen,H . (2014): Quantitative and Qualitative studies on *Enterobacteriaceae* in ground beef .SCVMJ, XIX (2) 77 -88.

American Public Health Association "APHA" (1992): Compendium of Methods for the Microbiological examination of Foods. 3rd Ed. (Carl, v), APHA, Washington, DC., USA

American Public Health Association "APHA" (2001): Compendium of methods for the microbiological examination of foods (4th Ed.). APHA technical committee on microbiological methods for foods. Washington, D.C., USA.

Argudín, M. Á.; Mendoza, M. C. and Rodicio, M. R. (2010): Food Poisoning and Staphylococcus aureus enterotoxins.Toxins.2(7). 1751:1773.

Berrong, M. E.; Brachett, R. E. and Beuchat, L. R. (1989): Growth of *Listeria monocytogens* on fresh vegetables stored under a controlled atmosphere. *J. Food Prot.* (62): 702-705.

CDC "Center for disease control and prevention" (2016): Staphylococcal Food Poisoning WWW.cdc.gov › Food Safety Homepage ›Foodborne Illness A-Z.

Cetin, O.;Bingol, E. B.; Colak, H.;Ergun, O. andDemir, C.(2010): The microbiological, serological and chemical qualities of mincemeat marketed in İstanbul. *Turkish Journal Veterinary Animal Sci*ence 34(4).407:412.

Clarence, S.Y.; Obinna, C.N. and Shalom, N.C. (2009): Assessment of bacteriological quality of ready to eat food (Meat pie) in Benin City metropolis, Nigeria. Afr. J. Microbiol. Res. 3: 390-395.

Cohen, N.; Ennaji, H.; Hassar, M.; Karib, H. (2006): The bacterial quality of red meat and offal in Casablanca (Morocco). *Mol. Nutr. Food Res.;* 50(6):557- 562.

Cruickshank, R.; Duguid, J. P.; Marmion, B. D. and Swain, R. H. A. (1975): Medical Microbiology. The practice of medical microbiology. VII, 12th Ed. Churchill Living Stone, Edinburgh.

Egyptian Organization for Standardization "EOS" (2005): Egyptian Standard Specification of frozen meat No. 1552. Cairo. Egypt.

El-Abbasy, M. T. M. (2010): Assessment of microbiological aspects of meat served at the university hospital with relation to the public health. Ph. D. Thesis (Meat Hygiene). Fac. Vet. Med. Zag. Univ. Egypt.

El-Aboudy, S. G. A. (2016): Hygienic status of marketed imported frozen meat. M.V.Sc. Thesis (Meat Hygiene). Fac. Vet. Med. Zagazig. Univ. Egypt.

El-Asheeri, A. K., Abdelghany, S., Alary, V., Corniaux, C. and Radwan, M. A. (2017): Characterization of Buffalo veal supply chain in villages around Cairo. Egyptian J. Anim. Prod, 54(2), 105-109.

Fagan, P.; Hornitzky, M.; Bettelheim, K. and Djordjevic, S. (1999): Detection of Shiga-Like Toxin (stx1 and stx2), Intimin (eaeA), and Enterohemorrhagic *Escherichia coli* (EHEC) Hemolysin (EHEC hlyA) Genes in Animal Feces by Multiplex PCR. Appl. Environ. Microbiol., 65 (2): 868–872.

Fahmi, A. A. (1993): Handling of imported frozen meat and its microbiological quality M.V.SC .thesis, meat hygiene .fac.Vet.Med., Cairo univ.

Frazier, W. C. and Westhoff, D. C. (1984): Food Microbiology. 3rdEd. TATA.McGraw Hill public.Comp. Ltd-New Delhi.

ISO (1974): Meat and Meat products: measurement of pH (refrence method): 1917-1974.

ISO (2002): Horizontal method for the enumeration of microorganisms, colony count technique at 30 ˚C.

ISO 21528-2 (2004): Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of Enterobacteriacae, part 2: colony count method. Enumeration. EN ISO, Geneva.

ISO 6888-1(1999): Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive Staphylococci (*Staphylococcus aureus* and other species). Part 1: Technique using Baird-Parker agar medium.

Jay J., Vilai J., and Hughes M. (2003): "Profile and activity of the bacterial biota of ground beef held from freshness to spoilage at 5-7ºC". *International J. of Food Microbiology*, Volume 81. p. 105-111.

Khalafalla F.A., Nagwa S.S., Ata, Mona Elshabrauy A.E., Azza S.M., Abu ElnagaDorgham S.M. and Khairy A.E.(2010): Effect of packing on extension of self-life of retail meat, *J. of American Science*, 6(12), 1049-1058.

Kok, T.; Worswich, D. and Gowans, E. (1996): Some serologicaltechniques for microbial and viral infections. In Practical Medical Microbiology (Collee, J.; Fraser, A.; Marmion, B. and Simmons, A., eds.), 14th ed., Edinburgh, Churchill livingstone, UK.

Mohamed, T. M. (1996): quality evaluation of imported brisket and flank cuts. M.V.Sc. Thesis (Meat Hygiene). Fac. Vet. Med. Benha Univ. Egypt.

Mohamed, T. M. (1996): quality evaluation of imported brisket and flank cuts. M.V.Sc. Thesis (Meat Hygiene). Fac. Vet. Med. Benha Univ. Egypt.

Mousa, M. M.; Abd El-Galil, H. and Radwan, M. (2011): Microbiological evaluation of frozen meat. Alex. J. Vet. Sci., 34(1): 235-241.

Moussa, M.M.; Samaha, I.; Eldaly, E. and Saleh, E. (1992): Occurrence of certain food poisoning organisms in some ready-to-eat foods. *Egypt. J. Appl. Science*. 7(2): 21.

Nevry R.K., Marina Koussemon and Seydou O. Coulibaly (2011): Bacteriological Quality of Beef Offered for Retail Sale in Cote d'ivoire. *American J. of Food Technology*, 6: 835-842.

Nouh, S.M.I. (2012): Effect of some antibiotic residues on the microbial quality of meat and meat products. M.V.SC. Thesis, Fac. Vet. Med., Zagazig Univ., Egypt.

Paton, A. W., and Paton, J. C. (2002): Direct detection and characterization of Shiga toxigenic *Escherichia coli* by multiplex PCR for stx1, stx2, eae, ehxA and saa. J. Clin. Microbial., 40: 271- 274.

Paton, A.W., Beutin, L., Paton, J.C., (1995): Heterogenicity of amino acid sequences of Shiga like toxin type-I operons. Genes 153, 71-74.

Phillips, D.; D. Jordan; S. Morris; I. Jenson and J. Sumner (2006): A national survey of the microbiological quality of beef carcasses and frozen boneless beef in Australia. Journal Food Protein,69(5):1113-1117**.**

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Phillips, D.; Sumner, J.; Alexander, J. F. and Dutton, K. M. (2001): Microbiological quality of Australian beef. *J. Food Protect.* 64 (5): 692-696.

Sea, K. and G.A. Bohach, (2007): Staphylococcus aureus. In: Food Microbiology Fundamentals and Frontiers. Eds., M. Doyle and L. Beuehat, Washington, DC: ASM Press ,pp: 493:519.

Sussman, M. (1985): *Escherichia coli* in human and animal disease. In: M. Sussman (Ed.), The Virulence of *Escherichia coll.* Academic Press, London, pp. 7-45.

Tharwat, A.E. (2008): Microbial aspects of some meat products with a trial to improve their quality and shelf life at refrigeration, M.V.Sc. Thesis (Meat Hygiene), Fac. Vet. Med. Zagazig Univ. Egypt.

Vorster, S.M.; Greebe, R.P. and Nortje, G.L. (1994): Incidence of *Staph. aureus and E. coli* in ground beef, broilers and processed meat in Pretoria, South Africa. *J.Food Prot.*, 57(4):305-310.

دراسات صحية على لحوم البتلو المسوقة بمحافظة الشرقية

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فى هذة الدراسة تم تجمع مائة عينة من لحوم البتلو من عضلة الفخذ والكتف (50 من كل منها) عشوائيا من محلات القصابين بمحافظة الشرقية. تم نقل العينات المجمعة في صندوق ثلج معزول تحت ظروف تعقيم كاملة وبدون أي تأخير ل لمعمل الدراسات العليا بقسم مراقبة الأغذية كلية الطب البيطري جامعة الزقازيق حيث تم فحصها بكتيريا. وجد أن العدد الكلى للميكروبات الهوائية ، عدد الميكروبات المحبة للبرودة ، العد الكلى للأمعائيات ، العدد الكلى للمكورات العنقودية كان 5.26 ± 0.18 و 5.59 ± 0.26 ، 3.66 ± 0.19 و 3.74 ± 0.21 ، 2.85 ± 0.12 و 3.78 ± 0.17 ، 3.21 ± 0.09 و 3.64 ± 0.15 مستعمرة بكتيرية لوغاريتم10/ جم في لحم الفخذ والكتف ، على التوالي. ووجد أن تواجد المكورات العنقودية الذهبية في 14 (28 ٪) و 9 (18 ٪) في لحوم الفخذ والكتف للبتلو وبنسبة 23 ٪ كنسبة إجمالية.كان معدل انتشارالإيشيريشيا كولاى هو 10 ٪ و 18 ٪ في كل من عينات لحوم البتلو الفخذ والكتف على الترتيب . وأحتوت الإيشيريشيا كولاى المعزولة على جينات الضراوة مثل الأينتامين والشيجا توكسين 1 و2 بنسب مختلفة. ووجد أن الطراز المصلى للأيشيريشيا كولاى O_{26} و O_{111} تحتوى على الثلاثة جينات المفحوصة عن طريق تفاعل البلمرة المتسلسل.