CONTAMINATION OF BEEF CARCASSES DURING SLAUGHTERING IN TWO EGYPTIAN SLAUGHTERHOUSES

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ABSTRACT

Slaughtering of animals (especially; cattle and buffaloes) in Egypt challenged by severe hygienic problems which results in heavy bacterial loads on the produced meat through cross contamination. The present study highlighted the improper practices such as slaughtering on the ground and then skinning and evisceration in the same place under poor hygienic practices which considered a threat to food safety and consumers health and have been implicated in many cases of foodborne illness. Visual inspection of both slaughterhouses was performed to carefully define the operation description and reporting observation. Thirty carcasses were sampled at different processing steps over a 6-months period at two Egyptian slaughterhouses. For each sample, total aerobic count, anaerobic count, *Staphylococcus aureus* count, coliform count, as well as isolation of *Salmonella* and *Escherichia coli O157:H7,* were conducted. Finally the main sources of infection were determined based on visuals observation of slaughter procedures and microbial examination. Sever limitations in hygienic practices were observed which require a serious attention from all relevant authorities to apply and maintain the basic hygienic slaughtering practices to prevent hazards which may affect the public health.

INTRODUCTION

Beef may be the vehicle of foodborne diseases as a result of deficient sanitary conditions during animal slaughter **(Loretz *et al.*, 2011).** Pathogenic micro-organisms are found in the digestive tract of healthy cattle and sheep. These micro-organisms are excreted in the feces and can be found on the hides and fleeces of the live animal. Bacterial contamination of the fleece/hide can then be transferred onto previously sterile meat surfaces during slaughtering

and dressing especially when slaughtering performed on the floor with absence of a carcass suspension system with careless evisceration that spreads intestinal content onto the meat surface. Bovine carcasses can be contaminated during the slaughter process through the contact with the animal's skin and hair, limbs, blood, stomach, gut contents, bile and other excretions, facilities, equipment, and hands and worker's clothes **(Sofos, 2008)**. After becoming contaminated, the meat provides an excellent environment for growth of bacteria. Slaughterhouses in Egypt are suffering from many administrative limitations. There is no full control on butchers working in the slaughterhouse by the managers as they administratively to nongovernmental organization (Division of Butchers). There is no penalty enforced by law in case of fault operations during meat processing that could affect the quality or safety of produced meat. The hanging too closely together both un-skinned and skinned carcasses in the slaughter line, resulting in contact of hair or skin to already-skinned carcass parts in presence of malpractices of butchers (untrained workers on proper sanitary measures) considered unhygienic practices leading to spread of microorganism and affecting safety and quality of meat. During moving and handling animals; violent acts to move animals, such as crushing or breaking tails of animals, grasping their eyes or pulling them by the ears. Animal forced to walk without proper securing over slippery floors or a sudden drop in floor levels which increase the possibilities of harm, distress or injury **(OIE, 2010)**. The present study involved a comparison of microbiological contamination between two slaughterhouses in Egypt to identify and evaluate the biological hazards created at work that may effect on public health aiming to improvement.

MATERIAL AND METHODS

Slaughterhouses selection:

In this study, two slaughterhouses (large and small) were selected based on numbers of animal slaughtered per weeks. The slaughter operations in both visually observed and monitored step by step to identify gaps and limitations during processing which may contribute in contamination of raw meat and increasing the microbial load.

The large selected slaughterhouse is governmental livestock and considered one of the largest slaughterhouses in Egypt. Slaughter around 1,500 heads of cattle per week. They have facilities for slaughtering cattle, sheep and camels. Its construction from holding pens, four

slaughtering hall, and free yards inside the slaughterhouses but it is not properly used; Animal pathways between halls are existed but not used. The small selected slaughterhouse is also governmental livestock. Slaughter around 250 heads of cattle per week. They have facilities for slaughtering cattle, sheep and camels. The slaughter operations were started early morning usually at 6:00 am and lasted in 10:00 to 12:00 according to the availabilities of animals indented to be slaughtered. The operations are the same in both slaughterhouses. The main differences are in number of cattle slaughtered per week. In the small slaughterhouse, there are to some extent control on the slaughtering area, and usually slaughtering area cleaned on daily base at the end of the working day.

Visual observation:

Sources of contamination were visually identified based on level of contamination observed and possibilities of cross-contamination in both slaughterhouses. For visually recording and identifying the degree of contamination in dirty area and the possibilities of cross-contamination in clean area, 50 animals were observed in 10 days of work in each slaughterhouse. The degree of classification was ranged from 1-5 degree based on cleanliness classification of livestock **(FSA, 2004)**. Each animal were scored in each processing steps according to the following criteria. The total points were collected and percentage of contamination was calculated for each processing steps (Tables 1, 2).

Classification Criteria of Cleanliness classification of livestock (FSA, 2004).

Category	*Classification*	*Description*
1-1.5	**Clean and dry**	**Dry, clean with regard to dung/dirt. Very minor amounts of loosely adherent straw/bedding.**
1.5 -2.5	**Slightly dirty (SD)**	**Dry/damp, light contamination with dirt/dung, small amounts of loosely adherent straw/bedding.**
2.5 - 3.5	**Dirty (D)**	**Dry/damp, significant contamination with dirt/dung and/or significant amounts of adherent straw/bedding.**
3.5 - 4.5	**Very dirty (VD)**	**Dry/damp, heavily contaminated with dirty / dung, heavily clogged and/or significant amounts of adherent bedding.**
4.5 - 5	**Filthy and wet (F)**	**Very wet, very heavily contaminated with dirt/dung and/or very heavily clogged and/or a lot of bedding adherent to the coat.**

Classification Criteria of Possibilities of cross contamination (GOVS 2006)

Category	*Classification*	*Description*
1-1.5	**Very low (VL)**	**Carcass completely separated, equipment and utensil clean**
1.5 -2.5	**Low (L)**	**Carcass separated, equipment and utensil slightly clean**
2.5 - 3.5	**Moderate (M)**	**Carcass partially separated, equipment and utensil contaminated**
3.5 - 4.5	**High (H)**	**Carcass partially separated, equipment and utensil heavily contaminated**
4.5 - 5	**Very high (VH)**	**Carcass are not separated , equipment and utensil very heavily contaminated**

Samples collection:

Sampling plan is based on random samples taken throughout the plant. It is also based upon the hypothesis that, any defect that is to be analyzed is distributed randomly throughout. For more accuracy in identifying the microbiological counts and isolation of selected microorganism,Fifteen samples were collected from each processing steps:1) Cattle receiving 2) Slaughtering 3) Bleeding 4) Fore shank and head removal 5) Hind shank removal 6) Hide removal 7) Carcass Wash 8) Eviscerations 9) Splitting 10) Final wash and Weighting 11) Meat cutting and loading. A sterile metal wire guide with an area 10 cm^2 was placed firmly against the surface of the left side of the carcass behind forelimb to unify the area of sampling. Then by rolling sterile cotton swab over the surface of limited area, swabs were taken after each of the following processing steps which represent to one sample.

Microbiological examination:

All samples were directly transferred to the laboratory in a cooling box with a minimum of delay. In the laboratory, from each swab which immersed in sterile peptone water, one ml was transferred to test tube containing nine ml of sterile peptone water (0.1%) to provide the original dilution (10^{-1}). From which further ten-fold decimal dilutions were prepared up to (10^{-6}). Nine ml of Selenite-F-broth were added to the second swabs which collected in sterile tube. The following bacteriological examinations were done in this study.

Determination of Total Aerobic Count (TAC):

Spread 0.1 ml of each dilution onto the surface of duplicated nutrient agar plates then incubated inverted at 37°C for 24 hours. The average number of colonies per countable plate was enumerated and the total aerobic count for each dilution was calculated and recorded as described by **ICMSF (1978)** as the following: The TAC = the arithmetic overage of the two counts × dilution factor.

Determination of anaerobic bacterial count:

Pipette aseptically 0.1 ml of each dilution of swabs collected onto duplicated plates with pre-poured, solidified and dried Reinforced Clostridial Medium agar. The inoculum was spread over the entire surface with a sterile bent glass rod by using a back and forth motion and let to dry for 5 - 10 minutes. After the agar has been dried, all plates were incubated anaerobically by placing the plates in an upright position in mackintosh jar provided with anaerobic kits and incubated inverted at 37°C for 24 hours. Counting and calculation was adopted according to **(Gudkove and Sharpe, 1966).**

Determination of *Staphylococcus aureus* count:

Pipette aseptically 0.1 ml of each dilution of swabs collected onto the surfaces of separate Baird-Parkers plates. Count plates showing typical egg-yolk reaction on plates (black shiny colonies with white clear halo zone) and showing coagulase positive reaction. Calculate the number of *Staph. aureus* per gram of the original sample by using the arithmetic overage of the two counts multiplied by dilution factor.

Determination of Coliform (Most Probable Number "MPN"):

The three tubes fermentation method was applied. Pipette one ml of the decimal dilutions previously prepared to each three separate tubes of MacConkey broth supplemented with inverted Durham's tubes. Inoculated and control tubes were incubated at 37°C for 24 - 48 hours. Positive tubes which showing gas formation were recorded and confirmed by Eosin Methylene Blue agar (EMB) (see point 3.3.5). Then the Most Probable Number (MPN) of coliforms per cm^2 sample of each swap was estimated according to the tables recommended by **FAO (1991).**

Isolation of Salmonella organisms:

Enrichment:

Nine ml of Selenite-F-broth was transferred aseptically to each swab which collected in sterile tube. Then inoculated enrichment broth was incubated at 43°C for 18 hours.

Selective plating:

A loopful of selective enrichment broth was streaked on Salmonella Shigellae (SS) medium in a manner to obtain isolated colonies. The inoculated plates were incubated at 37°C for 24 hours. Suspected colonies (non lactose fermenters, red or pink in color with or without black centers) were picked up from plate for further identification.

Identification of suspected isolates:

Suspected colonies were purified on (SS) agar plates and incubated at 37°C for 24 hours. Then each purified suspected colony was streaked onto nutrient agar slope for further investigation. The obtained purified isolates were identified by biochemical examination.

Biochemical identification:

Suspected isolates were identified by biochemical examination and applied as recommended by **ICMSF (1978).**

Isolation *E-coli* O157:H7 micro-organisms:

Streak a loopful from each gas positive MacConkey broth tube which was previously incubated at 37°C for 48 hours on Eosin Methylene Blue agar (EMB) in a manner to obtain separate isolate. Incubate plates inverted at 37°C for 24 hours. The formation of nucleated colonies with or without bluish metallic shin confirms the presence of *E-coli* organism. The strains of Escherichia coli serotype O157:H7 were isolated by using selective agar media (Rainbow Agar O157) which has both selective and chromogenic properties that make it particularly useful for isolating pathogenic *E. coli* strains. The medium inoculated by streaking or spreading a sample suspected of containing *E. coli* on the surface of the medium. Incubate the plates for 20 to 24 hours, or longer, at 35°C without elevated CO2 and observe for the presence of colored colonies. The distinctive black or gray coloration of *E. coli* O157:H7 colonies is easily viewed by laying the petri plate against a white background. When O157 is surrounded by pink or magenta non-toxigenic colonies, it may have a bluish hue.

Statistical Analysis:

The symmetrical data in all phases of the study were compiled in excel database, and organized for statistical analysis. The analysis was done using IBM SPSS version 21 **(Coakes, 2005),** a computer-based statistical software package. Different statistical approaches were used for comparing between means which include One Way ANOVA and

Paired T test as well as linear logistic regression to estimate the coefficients of the linear equation. Data represented by mean (standard deviation "SD") of 15 samples collected per each processing steps per each slaughterhouse (Table 3). For comparing counts between slaughterhouses per each processing step: $^{a-b}$ data with different superscripts are significantly difference at P value<0.05 (using One Way ANOVA). For comparing between successive processing steps per each slaughterhouse: $^{q\text{-to-}z}$ data with different superscripts are significantly difference at P value<0.05 (using paired samples T test).

RESULTS

Table (1): Degree of cleanliness for the dirty area (FSA 2004)

Processing steps	Large Slaughterhouse			Small Slaughterhouse		
	Degree of classification		% of contamination	Degree of classification		% of contamination
Cattle receiving	**3.93**	**VD**	**79%**	**3.00**	**D**	**60%**
Slaughtering	**3.67**	**VD**	**73%**	**3.00**	**D**	**60%**
Bleeding	**3.73**	**VD**	**75%**	**3.20**	**D**	**64%**
Fore shank and head removal	**3.13**	**D**	**63%**	**2.93**	**D**	**59%**
Hind shank removal	**3.20**	**D**	**64%**	**2.87**	**D**	**57%**
Hide removal	**3.60**	**D**	**72%**	**3.07**	**D**	**61%**

Average degree of 50 observed animal in 10 days of work in each slaughterhouse

Table (2): Possibilities of cross contamination in the clean area

Processing steps	Large Slaughterhouse			Small Slaughterhouse		
	Degree of classification		% of Cross-contamination	Degree of classification		% of Cross-contamination
Carcass Wash	**4.67**	**VH**	**93%**	**4.53**	**VH**	**91%**
Eviscerations	**4.60**	**VH**	**92%**	**4.20**	**H**	**84%**
Splitting	**3.27**	**M**	**65%**	**4.07**	**H**	**81%**
Final wash & Weighting	**3.60**	**H**	**72%**	**4.53**	**VH**	**91%**
Meat cutting & loading	**3.07**	**M**	**61%**	**4.00**	**H**	**80%**

Average degree of 50 observed animal in 10 days of work in each slaughterhouse

Table (3): Micro-bacterial count of different processing steps of slaughtering of cattle under normal circumstance

Processing steps	Total Aerobic Count *		Total Anaerobic Count*		Staphylococcus aureus Count*		Coliform Count*	
	Large	Small	Large	Small	Large	Small	Large	Small
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Cattle receiving	5.31 (.25) $^{a\,q}$	5.31 (.20) $^{a\,q}$	3.19 (.13) $^{a\,q}$	2.89 (.18) $^{b\,q}$	3.00 (.26) $^{a\,q}$	2.77 (.25) $^{b\,q}$	2.22 (.75) $^{a\,q}$	2.59 (.58) $^{a\,q}$
Slaughtering	5.23 (.26) $^{a\,r}$	5.20 (.23) $^{a\,r}$	3.17 (.16) $^{a\,q}$	2.62 (.29) $^{b\,r}$	2.91 (.40) $^{a\,q}$	2.75 (.28) $^{a\,q}$	2.23 (.75) $^{a\,q}$	2.57 (.52) $^{a\,q}$
Bleeding	5.12 (.34) $^{a\,s}$	5.15 (.30) $^{a\,r}$	3.06 (.18) $^{a\,r}$	2.54 (.48) $^{b\,r}$	2.83 (.36) $^{a\,r}$	2.73 (.32) $^{a\,q}$	2.28 (.43) $^{a\,q}$	2.48 (.63) $^{a\,q}$
Fore shank and head removal	5.08 (.45) $^{a\,s}$	5.07 (.50) $^{a\,r}$	3.18 (.14) $^{a\,s}$	2.72 (.43) $^{b\,s}$	2.86 (.36) $^{a\,r}$	2.56 (.35) $^{b\,r}$	2.45 (.74) $^{a\,q}$	2.66 (.77) $^{a\,q}$
Hind shank removal	5.14 (.45) $^{a\,s}$	5.09 (.55) $^{a\,r}$	3.34 (.12) $^{a\,t}$	2.33 (.59) $^{b\,t}$	2.91 (.29) $^{a\,r}$	2.76 (.30) $^{a\,s}$	2.16 (.35) $^{a\,q}$	2.66 (.58) $^{b\,q}$
Hide removal	5.44 (.27) $^{a\,t}$	5.33 (.29) $^{a\,s}$	3.29 (.13) $^{a\,u}$	2.96 (.26) $^{b\,u}$	3.32 (.35) $^{a\,s}$	2.90 (.21) $^{b\,t}$	2.85 (.44) $^{a\,r}$	2.96 (.73) $^{a\,q}$
Carcass Wash	5.32 (.34) $^{a\,t}$	5.23 (.29) $^{a\,t}$	3.09 (.15) $^{a\,v}$	2.64 (.29) $^{b\,v}$	3.05 (.23) $^{a\,t}$	2.79 (.25) $^{b\,u}$	2.96 (.30) $^{a\,r}$	3.23 (.82) $^{a\,q}$
Eviscerations	5.43 (.24) $^{a\,u}$	5.29 (.30) $^{a\,t}$	3.50 (.14) $^{a\,w}$	3.24 (.11) $^{b\,w}$	3.36 (.30) $^{a\,u}$	3.07 (.19) $^{b\,v}$	5.42 (2.35) $^{a\,s}$	4.19 (2.15) $^{a\,q}$
Splitting	5.27 (.22) $^{a\,v}$	5.20 (.33) $^{a\,u}$	3.41 (.13) $^{a\,x}$	3.12 (.19) $^{b\,x}$	3.34 (.17) $^{a\,u}$	2.86 (.18) $^{b\,w}$	5.43 (3.09) $^{a\,s}$	4.07 (1.83) $^{a\,q}$
Final wash & Weighting	5.31 (.17) $^{a\,v}$	5.28 (.21) $^{a\,v}$	3.37 (.12) $^{a\,x}$	2.93 (.25) $^{b\,y}$	3.31 (.18) $^{a\,u}$	2.91 (.19) $^{b\,x}$	5.25 (3.12) $^{a\,s}$	4.49 (2.58) $^{a\,q}$
Meat cutting & loading	5.45 (.14) $^{a\,w}$	5.23 (.34) $^{b\,v}$	3.51 (.31) $^{a\,x}$	2.74 (.39) $^{b\,z}$	3.33 (.24) $^{a\,u}$	2.87 (.24) $^{b\,x}$	5.37 (3.14) $^{a\,s}$	4.67 (2.51) $^{a\,q}$

$^{a\text{-}b}$ **data with different superscripts are significantly difference at P value<0.05 (using One Way ANOVA).**

$^{q\text{-to-}z}$ **data with different superscripts are significantly difference at P value<0.05 (using paired samples T test).**

Table (4): Comparison between the incidences of Salmonella and *E. Coli* O157:H7 isolated in both slaughterhouses per selected processing step

Processing steps	Salmonella				*E.coli* O157:H7			
	Large		Small		Large		Small	
	No	%	No	%	No	%	No	%
Eviscerations	5	33%	2	13%	1	7%	1	7%
Splitting	7	47%	2	13%	4	27%	3	20%
Final wash & Weighting	6	40%	2	13%	5	33%	3	20%
Meat cutting & loading	6	40%	2	13%	5	33%	3	20%
Total	24	40 %	8	13 %	15	25 %	10	16 %

Table (5): Comparing the impact of slaughterhouse on bacterial count before and after correction using of Logistic regression (stepwise) to estimate R^2 at P value < 0.05

Bacterial count	R^2	*Sig.*
Total Anaerobic Count	.580	.000[b]
Staphylococcus aureus Count	.303	.002[b]

b. Significant effect of slaughterhouse on bacterial count (stepwise used for excluding the insignificant effect)

DISCUSSION

Main gaps and limitations identified during visual inspection in both slaughterhouses were in large slaughterhouse located within residential area while small slaughterhouse directly located on the high way but approximately 300 meter away from residential area. Gates of slaughterhouses are always opened without any restriction on personal movement to go inside and out of slaughterhouses. There is no area for pre-slaughter rest which hinders ante-mortem inspection. There is no separation between dirty and clean area (slaughter and post mortem operation conducted in the same place) that lead to high possibilities of cross-contamination during meat processing. Any visual contaminations on the carcasses were removed by washing. No intension for personnel hygiene even official veterinarians are not interested to

wash their hand or knife. Clothes of workers are dirty throughout the working days and their hands were not cleaned. There are no written minimum sanitary measures to be followed. No training provided for workers for such measures. Data recorded are very limited to number of slaughtered animals, species, rejected parts and reasons for rejections. There is no data on infectious or contagious diseases observed during slaughtering or records for animal trace-back. In the large slaughterhouse, the slaughter halls (floor and wall) are not cleaned at the end of the working and may be left for 2 to 4 weeks or even months while in small one it could be done daily. In large slaughterhouse, the degree of classification was 3.93 (Very Dirty) with a percentage of 79 % in cattle receiving as most of animal arrived at the slaughterhouse in dirty trucks. After that, the degree of contamination start to decrease during slaughter (3.67 degree; 73%) and bleeding (3.73 degree; 75%) but it remain (Very Dirty) category. While in fore and hind shank removal and hide removal steps the degrees was decreased and classified in (Dirty) category ranged from 3.13 to 3.60 with percentage ranged from (63 % to 72 %). In small slaughterhouse, the degree of classification was starting from (Dirty) category in cattle receiving as most of animal coming from adjacent area with short period of transportation which reflect on the cleanliness of the trucks. The level of contamination was remaining in (Dirty) category staring from cattle receiving up to hide removal. Its range was from 2.87 - 3.20 with percentage ranged from (57 % to 64%). The cross-contamination was very high in large slaughterhouse during carcass wash (4.67; 93%) and eviscerating (4.60; 92%). During evisceration, nicking of the rectum, leakage from the anus or bursting of the visceral contents will cause contamination of the anus, rump and brisket areas, respectively **(Gill *et al.*, 1995)**. In splitting step it was decreased to be high (3.27; 65%) as carcass moved to area partially separated from other steps. During final wash and weighting the cross-contamination re-increased to high (3.60; 72%) due to heavily contaminated equipment and utensil then decreased again to moderate (3.07; 61%) in meat cutting and loading as less contaminated utensil observed. In small slaughterhouse, the cross-contamination was very high in carcass wash (4.53; 93%) after hide removal. De-hiding operations were identified as the primary source of fecal contamination on the bovine carcasses. This is in agreement with similar studies reported in the scientific literature **(Bell, 1997; Gill and McGinnis, 1999; Mackey and Roberts, 1993)**. The cross-contamination decreased to be high in evisceration (4.20; 84%) and splitting step (4.07; 81%). During final

wash and weighting the cross-contamination re-increased to very high (4.53; 91%) due to heavily contaminated equipment and utensil without separation from other steps then decreased again to high (4.00; 80 %) in meat cutting and loading as this area are partially separated (Table 2). Generally, it was concluded that, the degree of contamination is larger in large slaughterhouse while the possibilities of cross-contamination is higher in small slaughterhouse. It might be preferable for visible contamination to be controlled largely by superior skinning and eviscerating practices rather than by animal or carcass cleaning treatments, which may not prevent the depositing of bacteria **(Gill, 2004)**. The results obtained of mean values of Total Aerobic Count (TAC) on beef carcass in large slaughterhouse (Table 3) are higher than what is reported by **(McEvoy, 2004)**. By statistical analysis using paired samples T test; it was revealed in the large slaughterhouse that there is significant difference in TAC at P Value 0.05 between slaughtering and bleeding; hind shank removal and hide removal; carcass wash and evisceration; evisceration and splitting; and final wash and meat cutting. That means TAC after slaughtering significantly decreased in bleeding and fore shank removal then significantly increased in hide removal, evisceration and during meat cutting where improper handling of meat observed. De-hiding and evisceration have been identified as causative operations in the microbial contamination of beef carcasses **(Bell, 1997; Gill, McGinnis, and Badoni, 1995; Sheridan, 1998)**. In the small slaughterhouse, there is significant difference in TAC at P Value 0.05 between cattle receiving and slaughtering, hind shank removal and hide removal hide removal and carcass wash, evisceration and splitting, splitting and final wash. That means TAC after cattle receiving significantly decreased in slaughtering and bleeding then significantly increased in hide removal and during final wash and weighting. In large slaughterhouse it was revealed that, there is significant difference in anaerobic count at P Value 0.05 starting from slaughtering up to splitting by using paired samples T test (Table 3). That means anaerobic count significantly gradually increased after bleeding till hind shank removal then gradual significant decreased during hide removal and carcass wash then re-increased significantly at evisceration. After that, the anaerobic count decreased significantly in splitting without any significant changes in final wash and weighting and meat cutting and loading. In the small slaughterhouse, there is significant difference in anaerobic count at P Value 0.05 between cattle receiving and slaughtering, bleeding and then starting from bleeding up to meat cutting

and loading by using paired samples T test. That means anaerobic count after cattle receiving significantly decreased in slaughtering and bleeding then significantly fluctuated up to down till to the final step. The anaerobic count significantly increased in fore shank removal, hide removal and evisceration. In large slaughterhouse, there is significant difference in Staph count at P Value 0.05 between slaughtering and bleeding, hind shank removal and hide removal, hide removal and carcass wash, and finauy carcass wash and evisceration. That means Staph count after slaughtering significantly decreased in bleeding then significantly increased in hide removal and evisceration and maintain in successive processing steps at this level without any significant changes. While, in the small slaughterhouse, there is significant difference in Staph count at P Value 0.05 starting from bleeding up to final wash. Meaning that Staph count after bleeding significantly fluctuated up to down till to final wash step. The Staph Count significantly increased during hide removal, evisceration and final wash. Statistical significant difference were identified in large slaughterhouse by using paired samples T test at P Value 0.05 between hind shank removal and hid removal, and carcass wash and evisceration. That means Coliform count during hide removal significantly increased then continued at this level. Moreover, the Coliform count significantly marked increased again during evisceration from 2.96 to 5.42 $\log_{10}$ CFU/cm^2 and maintain in successive processing steps at this level without significant changes. Despite the decreased rates of carcass fecal contamination after splitting and final wash, the coliform count remained constant after evisceration; this result suggested that, there were other important sources of bacterial contamination on the carcasses. **Schnell *et al.* (1995)** found a similar finding and suggested that aerosols, humans and equipment may also play a role in carcass contamination. In small slaughterhouse, there is no significant difference in Coliform count between any processing steps. That means coliform count is gradually insignificantly increased starting from cattle receiving up to meat cutting and loading. By comparing between the risks of microbial count in each processing steps in the two slaughterhouses using **One Way ANOVA at P Value 0.05**, it was revealed that, the meat cutting and loading step in the large slaughterhouse is significantly larger than TAC in small slaughterhouse while the remaining processing steps there is no significant difference between the TAC in the two slaughterhouses. Despite the risks associated in large in TAC in the final step only but it should be considered as fresh meat directly distributed to consumers without any further

interventions. This is slightly comply with **Sumner (2003),** that was found a little difference in mean log TAC/cm2 of beef carcass produced at four large abattoirs and 13 very small plants, 1.72 versus 1.81, respectively while contradict with **Hansson and Ingrid (2001)** that was found a significantly greater amount of aerobic microorganisms in beef carcasses slaughtered at low-capacity slaughterhouses than high-capacity slaughterhouses. The risks associated in anaerobic count in large slaughterhouse is significantly larger than the anaerobic count in small slaughterhouses in all processing steps starting from slaughtering up to meat cutting and loading. The risk associated in Staph count in large slaughterhouse is significantly larger than the Staph count in small slaughterhouses in cattle receiving, fore shank removal, hide removal, carcass wash, eviscerations, splitting, final wash and weighting, and meat cutting and loading. This in agreement with **Hansson and Ingrid (2001)** that was found staphylococci significantly higher amounts in beef carcasses from the high-capacity slaughterhouses than the low capacity slaughterhouses. There is no significant difference in risk associated in Coliform count in large slaughterhouse than the Coliform count in small slaughterhouses. This is contradicting with **Hansson and Ingrid (2001)** who mentioned that, coliform count was significantly higher amounts from the high-capacity slaughterhouses than the low capacity slaughterhouses. Salmonella is known to colonize the gastrointestinal tract of animals without producing any clinical signs. There for, carcasses can become contaminated with Salmonella at the time of slaughter, **(Meyer, 2010)**. In large slaughterhouse, it was noticed that, only one carcass was recovered from Salmonella during final wash and meat cutting. The lower incidence reported in this study may be attributed to the other sources of variation as a result of the swabbing technique, such as carcass surface variation **(Gill *et al.*, 2001)**. Although every effort was made to ensure variation in the swabbing data was minimized by using the same operator, working with a single animal species, using the same sampling materials, ensuring the absence of inhibitory substances and sampling at the same point along the slaughter line. The incidence of Salmonella in small slaughterhouse are contradict with **Arthur (2008)** who was detected salmonella prior to loading cattle, over 70 % of the samples from each lairage environment area (Table 4). Prior to loading cattle, also **Arthur (2008)** found E. coli O157:H7 in 9 (64%) of 14 tractor trailers from each lairage environment area which is not complying with the current study finding. Hide is the main source of carcass contamination during cattle processing; therefore, it is

crucial to minimize the amount of Escherichia coli O157:H7 on cattle hides before slaughter **Arthur (2007)**. In this study, *E. coli* O157:H7 in both slaughterhouses were isolated from the carcasses after removal of hide. Several potential sources of *E. coli* O157: H7 are encountered during transportation and in the lairage environment at beef-processing facilities that could increase the prevalence and numbers of *E. coli* O157:H7 on the hides of cattle. Contaminated hides have been identified also by **Mather (2007)** as one of the major sources of Escherichia coli O157 carcass contamination. By comparing incidence of isolation of Salmonella and *E-coli* O157:H7 between both slaughterhouses starting from evisceration (where the first isolation defined) it was revealed that. the incidence of isolation of Salmonella in large slaughterhouse was higher than in Small slaughterhouse in all processing steps (Table 4). In large slaughterhouse, out of 165 swabs samples collected from 15 carcasses throughout the 11 processing steps, 24 Salmonella isolates were detected from 7 carcasses in 4 processing steps starting from evisceration (Table 4). Salmonella were found to be distributed in evisceration, splitting, final wash and meat cutting in percentage of 5/15 (33 %), 7/15 (46 %), 6/15 (40 %) and 6/15 (40 %) respectively. In small slaughterhouse, out of 165 swabs samples collected from 15 carcasses throughout the 11 processing steps, 8 Salmonella microorganism were isolated from 2 carcasses in 4 processing steps starting from evisceration (Table 4). Salmonella were isolated from evisceration, splitting, final wash and meat cutting in the same percentage of 2/15 (13%) in each of them. Cattle feces and hides might be considered as important sources of Salmonella for carcass contamination at different slaughter stages. The presence of potentially pathogenic Salmonella serotypes at the slaughtering stages is an evidence of the circulation of this pathogen in the food environment; its presence could increase consumers' risks of infection if proper food handling and preparation techniques are not followed **(Narváez-Bravo *et al.*, 2013)**. These data should serve as a baseline for future comparisons in Salmonella prevalence on beef carcasses to be used by the government and industry in order to establish preventive measures and to better address the risks of Salmonella contamination. The incidence of isolation of *E-coli* O157:H7 in Large slaughterhouse was the same or slightly higher than in small slaughterhouse in all processing steps (Table 4). Out of 165 swabs samples collected from 15 carcasses throughout the 11 processing steps, 15 *E.coli* O157:H7 isolates were detected from 5 carcasses in 4 processing steps starting from evisceration (Table 4). *E.coli* O157:H7 were found to be distributed in

evisceration, splitting, final wash and meat cutting in percentage of 1 (6.6 %), 4 (26.6 %), 5 (33.3%) and 5 (33.3%), respectively. In small slaughterhouse, out of 165 swabs samples collected from 15 carcasses throughout the 11 processing steps, 10 *E-coli* O157:H7 isolates were found from 3 carcasses in 4 processing steps starting from evisceration. *E-coli* O157:H7 were isolated from evisceration, splitting, final wash and meat cutting in percentage of 1 (6.6%), 3 (20%), 3 (20%) and 3 (20%), respectively. This is comply with **Sumner (2003),** who found the prevalence of *E. coli* for beef was lower at small plants 4.7% than in the large abattoirs (28.4%). Interestingly, by statistical comparing between the two slaughterhouses using ***logistic regression and estimating the*** R^2 (Table 5), it was found that, the proportion of variability in the microbiological count explained by the type of slaughterhouse at 95% CI, as the following: 1) 58% of the variation of Total Anaerobic count is affected by the type of slaughterhouse, 2) 30.3% of the variation of Staph. Aureus count is affected by the type of slaughterhouse. That means there is effect on the anaerobic and staph. Aureus count by the type of slaughterhouse by the above mentioned percentage, while the remaining percentage due to other factors. By another way the slaughtering of animal in Large slaughterhouse are significantly increasing the proportion of getting contaminated than Small slaughterhouse regarding the anaerobic and staph aureus count, while in TAC and coliform count there is no significant difference. By comparing incidence of isolation of Salmonella and *E.coli* O157:H7 between both slaughterhouses starting from evisceration (where the first isolation defined) it was revealed that, the incidence of isolation of Salmonella in large slaughterhouse was 24/60 (40%) higher than in small slaughterhouse 8/60 (13%). Even in comparing between the processing steps in both slaughterhouses, the incidence of Salmonella infection was higher in evisceration, splitting, final wash and meat cutting in large slaughterhouse than in small one. Cattle feces and hides might be considered as important sources of Salmonella for carcass contamination at different slaughter stages. The presence of potentially pathogenic Salmonella serotypes at the slaughtering stages is an evidence of the circulation of this pathogen in the food environment; its presence could increase consumers' risks of infection if proper food handling and preparation techniques are not followed, **(Narváez-Bravo *et al.*, 2013).** These data should serve as a baseline for future comparisons in Salmonella prevalence on beef carcasses to be used by the government and industry in order to establish preventive measures and to better address the risks of Salmonella

contamination. The incidence of isolation of *E-coli* O157:H7 in large slaughterhouse was 15/60 (25%) higher than in small slaughterhouse 10/60 (16%). Even in comparing between the processing steps in both slaughterhouses, the incidence of *E.coli* O157:H7 infection was the same in evisceration and higher in splitting, final wash and meat cutting in large than in small slaughterhouse. This is comply with **Sumner (2003)** who was found the prevalence of *E. coli* for beef was lower at small plants 4.7% than in the large abattoirs (28.4%).

CONCLUSION

According to the visual observations and microbiological finding, it was recommended that corrective measures for reducing risks associated in slaughterhouses should be identified and addressed. The relevant authorities should develop minimum guidelines on basic hygienic practices in Egypt and ensure enforcement. This requires securing adequate resources and the legal power for implementation. Short and long action plan should be in place regarding the restructure of slaughterhouse to enable proper hygienic practices with efficient monitoring.

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