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Determination of Hygienic Condition of Municipal Slaughterhouse and Its Microbial Effect on the Meat Quality

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ABSTRACT

Beef meat considers one of the major and expensive sources of animal protein. It is an excellent media for bacterial growth. To ensure production of good keeping quality meat, slaughtering should be in slaughterhouses under veterinary supervision and complete hygienic measures as the main sources of meat contamination occur during slaughtering processes. This study aimed to evaluate the hygienic conditions of Elkharga municipal slaughterhouse, New Valley governorate, Egypt as well as the meat quality. A total of 200 different samples include; meat, air, tank water, tap water and floor/wall swabs (40 samples/each) were randomly collected from the slaughterhouse. Samples were examined bacteriologically for determination of the total aerobic, total anaerobic count, *Escherichia coli* (*E. coli*), *Staphylococcus aureus (S. aureus)*, count as well as detection of *Salmonellae* was attempted. The results revealed that the means values of total aerobic counts, total anaerobic counts, *E. coli*, *S. aureus* counts for meat samples were: $3.796 \times 10^5 \pm (93.07 \times 10^3)$, $1.468 \times 10^3 \pm (10.70 \times 10)$, $2.466 \times 10^3 \pm (15.54 \times 10)$, $9.94 \times 10^2 \pm (15.7 \times 10)$ CFU/g, respectively while Salmonellae spp. Could not be detected. Also, it was noticed that tap water had higher microbial load than tank water and floor was more contaminated than walls samples. On contrary, the lowest microbial contamination was recorded in air samples. On comparing the obtained results of meat evaluation with the Egyptian Standard Specification (ESS), it was found that 47.5% of samples exceeded the permissible limit for total aerobic count (10^6), 15% for total anaerobic count (10^2), 35% for *E. coli* (10^2) and 27.5% for *S. aureus* (10^2). In conclusion, more governmental efforts are still needed to control the microbial contamination and improve the environmental quality and infrastructure of Elkharga slaughterhouse in New Valley, Egypt.

1. INTRODUCTION

One of the major and expensive sources of animal protein is beef meat. Its high nutritive values make it an excellent media for bacterial growth. To ensure production of meat of good keeping quality, slaughtering should be in slaughterhouses under veterinary supervision and complete hygienic measures (Serda et. al., 2015 and Zailani et. al., 2016). The main contamination sources of meat occurred during slaughtering processes such as hides and gastro-intestinal tract contents of the slaughtered animals, the staff and the work environment. Additionally, carcasses can be contaminated during the slaughter process through the contact with the animal's skin, blood, hair, limbs, bile and stomach, gut contents, or/and facilities, equipment, water supplies, air pollution and worker's hands and clothes (Koffi-Nevry et. al., 2011 and Muhammad et. al., 2012). The routine veterinary

inspection in the slaughterhouses is not included a microbiological examination. Therefore, microbial contamination of meat may affect its quality with a potential of food poisoning or spoilage due to microbial feeding on meat nutrients such as sugars and free amino acids, which liberate undesired volatile metabolites (Muhammad et. al., 2011 and Bogere and Baluka, 2014).

Elkharga slaughterhouse, like many Egyptian slaughterhouses, is suffering from many administrative limitations. There is no penalty enforced the veterinary service authorities in case of fault operations during meat processing that could affect the quality or safety of produced meat. The presence of both un-skinned and skinned carcasses in the same area might be a source of meat contamination by many pathogenic agents (Hemmat et. al., 2014). The aims of this study were to evaluate the health hazards of microbial contamination of the meat carcasses and assessment of slaughterhouses hygienic measures by microbiological examination of Elkharga slaughterhouses in New Valley, Egypt.

2.MATERIAL AND METHODS

2.1. Study area:

This study was conducted in the municipal slaughterhouse in Elkharga city which is the capital of New Valley Governorate. The New Valley is a part of the oasis which is located to the west of the Nile Valley. New Valley Governorate located 232 km to the South of Cairo and represented about 45% from the total Egypt area.

Elkharga slaughterhouse is the municipal slaughterhouse in the New Valley Governorate. It is the manually operated slaughterhouse. It is well constructed with a fence and consisted of a slaughtering hall, two quarantine partitions, two eviscerated rooms, emergency slaughtering room and condemnation room. Slaughtering capacity is around 150 heads of cattle per week with fewer slaughtering rate of sheep and camels. The slaughter operations were started early morning usually at 6:00 am and lasted in 10:00 to 12:00 based on the number of heads admitted for slaughtering. The slaughtering area routinely cleaned at the end of working day.

2.2. Sampling:

Samples were collected during twice visits per week for 10 weeks (from the 1st week of February to the end of 2nd week of April 2017). A total of 200 samples as following: 40 slaughtered meat samples each about 100 ±10gm, 40 air samples each of one liter in sterile buffered peptone water, 40 water samples (20 tank water and 20 tap water samples) each sample about 0.5 liter, 40-floor swabs and 40 wall swabs. Samples were labeled and transferred in an ice-box to the laboratory to the Central Laboratory of the Faculty of Veterinary Medicine, (New Valley Branch), Assuit University for bacteriological analyses.

2.3. Samples Preparation:

2.3.1. Meat samples:

Upon received to the laboratory, 25 g from meat sample was aseptically incised with sterile scalpel, and diluted with 225 ml of sterile 1% peptone water (Merck) (w/v) in sterile stomacher bag and homogenized in a Stomacher (Lab-blender, 400) for 1 min providing 10-1 dilution. Tenfold serial dilution was prepared up to 10^{-6}.

2.3.2. Water samples:

Water samples were collected from the identified functional tanks and water taps. Samples were labeled and transported in coolers to the laboratory with minimal delay.

2.3.3. Air samples:

Air samples were collected using impinge filled impinger with 225-259 ml peptone water. The outlet of the impinge was connected to the inlet (top) of the trap whereas the outlet (side arm) of the trap to the inlet of the pump. The impinger inlet was connected to the external calibrator and calibrate.

2.3.4. Floor and Wall swabs:

Swabs were collected from floors and walls using sterilized cotton swabs by swabbing on the surface of floor and walls in approximately 1 cm2 surface area, and then insert the swabs in sterile peptone water (Merck) and transport under the chilling condition to the laboratory.

2.4. Bacteriological evaluation:

Bacteriological evaluation was performed according to (APHA, 2002). Appropriate diluents of each tube were placed on the following media in duplicate as follows: (1) total aerobic count cultured in nutrient agar (Merck, Darmstadt, Germany) by surface plating of 0.1 ml of the serial dilutions from each sample. Plates were incubated at 37°C/24 hr. Plates with distinct colonies counted 30-300 were enumerated as Colony Forming Units (CFU). (2) total anaerobic count using Reinforced Clostridial Medium (Oxoid, CM151) by placing 1 ml of homogenized samples spread onto duplicate plates of double layers of Reinforced Clostridial medium agar and incubated in an anaerobic jar (Gaspak plus anaerobic system) at 37°C/48 hr. (3) for *E. coli* isolation, Levine's Eosin Methylene Blue (EMB) agar by streaking of 1 ml of

homogenized samples in 5 ml MacKonkey broth (Merck, Darmstadt, Germany) with an inverted Durham tubes followed by incubation at 37°C/24 hr. 4) Staphylococcus aureus count was determined by streaking the samples on Baird-Parker Agar (Oxoid, CM 275) with Egg Yolk-Tellurite Emulsion (Oxoid, SR 54) inverted incubation at 37°C/24 hr. & 48 hr. Countable suspected colonies. 5) The isolation of Salmonella performed by initial enrichment in 9 ml of Selenite-F-broth, then incubated at 43°C/18 hr, followed by streaking a loopful of enriched broth on Salmonella Shigella (SS) medium incubated at 37°C/24 hr. suspected colonies were picked up from the plate for further biochemical identification.

2.5. Statistical Analysis (GraphPad Instant, 2009):

GraphPad Instant version 3 for the window was used for determination of means the analysis of variance between the different data in this study were determined using standard error and analysis of variance.

3. RESULTS and Discussion

Food-borne illnesses resulted from contaminated meat consumption with pathogenic bacteria such as *Salmonella* spp., *S. aureus* and *E. coli* which adversely affects shelf-life and renders the meat unfit for human consumption to avoid its several human health hazardous which ranging from mild illness to death (Saleh et. al., 2013 and Bogere and Baluka, 2014).

3.1. Microbial quality of meat:

The mean microbial load of meat samples was shown in the table (1) as follows: $3.796 \times 10^{5a} \pm (93.07 \times 10^3)$, $1.468 \times 10^{3b} \pm (10.70 \times 10)$, $2.466 \times 10^{3c} \pm (15.54 \times 10)$, 3.79×10^5, 1.47×10^3, 2.47×10^3, 9.94×10^2 for the total aerobic count, total anaerobic count, *E. coli*, *S. aureus* count, respectively. Salmonellae were not detected. The differences between these results were highly significant ($p < 0.0001$). Table (2) and fig. (1), showed the number and percent of the microbial load in examined meat samples matched to the Egyptian Standard Specification (ESS, 2004). Results revealed that out of 40 meat samples, 19 (47.5%) was $> 10^6$ in the total aerobic count, 6 (15%) was $>10^2$ in the total anaerobic count. There was 14 (35%) of examined meat counted 10^2 *E. coli*, whereas 11 (27.5%) was 10^2 *S. aureus*.

Table (1): Microbiological quality of meat samples from municipal slaughterhouse in New Valley.

Microorganisms	Minimum	Maximum	Mean ± (SE)
Total Aerobic Count	>100	1.6×10^6	$3.796 \times 10^{5a} \pm (93.07 \times 10^3)$
Total Anaerobic Count	> 100	2.1×10^2	$1.468 \times 10^{3b} \pm (10.70 \times 10)$
E. coli	> 100	3.5×10^3	$2.466 \times 10^{3c} \pm (15.54 \times 10)$
***Staphylococcus* spp.**	> 100	2.9×10^3	$9.94 \times 10^{2d} \pm (15.7 \times 10)$
Salmonellae spp.	ND	ND	ND

P value is <0.0001 considered exteremly significant. ND: Not Detected.

Table (2): Prevalence of microbiological quality of 40 meat samples collected from municipal slaughterhouse in New Valley matched to EOS.

Microorganisms	No. of samples within EOS	No. of samples exceeding EOS
Total aerobic count	21 (52.5%)	19 (47.5%)
Total anaerobic count	34 (85%)	6 (15%)
E. coli	26 (65%)	14 (35%)
Staphylococcus aureus	29 (72.5)	11 (27.5%)

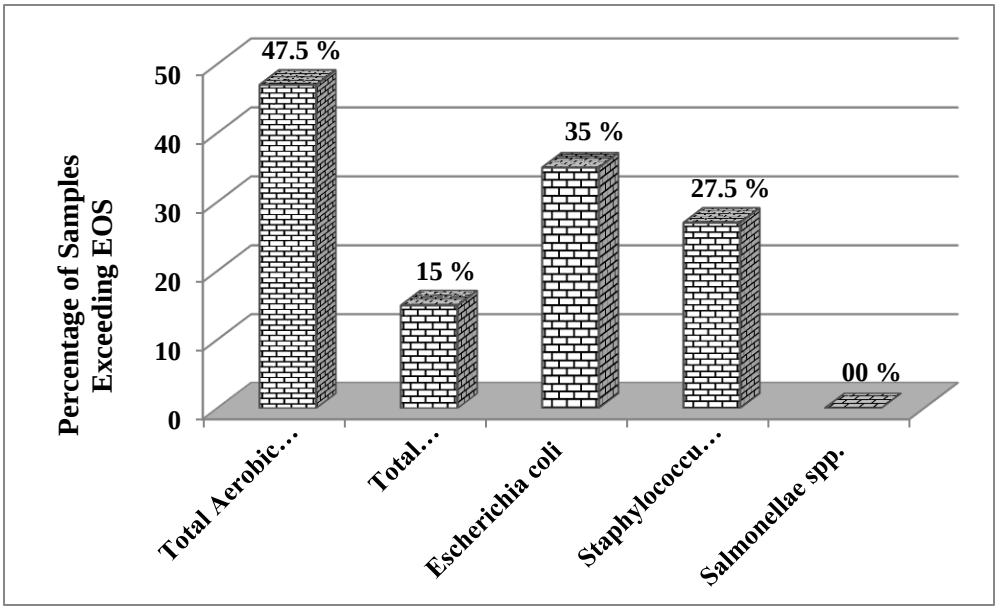

Fig. 1. Percentage of samples exceeding the limits of the Egyptian Standard of total aerobic count, total anaerobic count, *E. coli*, *Staphylococcus areus*, and *Salmonella spp.* in meat samples collected from the municipal slaughterhouse in New Valley.

All these results exceed the standard levels of the Egyptian Organization for Standardization and Quality Control "EOS". The total aerobic count was the highest level exceeding the permissible limits, followed by *E. coli, S. aureus*, and anaerobic count, consequently.

Obtained results similar to Holeckova et. al., (2002) and Olayinka & Sani, (2014), whereas, higher results were found by Hemmat et al., (2014) who tested quality of beef and edible offal at abattoir level in governorate, they reported 2.36×10^5, 10.8×10^4, 44×10^3, cfu/g for total aerobic bacteria, E. coli, S. aureus, respectively. Furthermore, Aftab et al. (2012) and Bogere & Baluka, (2014) recorded higher results in Uganda Abattoir meat as following; were 1.64×10^9, 8.4×10^4, 2.7×10^3 CFU/g of the total bacterial count, E. coli, and S. aureus respectively. Hughes et. al., (2015) recorded higher results in Ghana slaughtered meat; 8.32, 5.97, 5.50 (log10cfu/g), Total viable mean counts, E. coli, S. aureus respectively.

The slaughterhouse may be the microbial source of meat contamination in case of bad hygienic conditions. Studying of the microbial quality of the slaughterhouse and meat reflects the hygienic quality in the slaughterhouses and estimates the meat quality and the public health risk of food poisoning bacteria. The slaughterhouses should have adequate clean water (free from chemicals or high microbial load). Abattoir requires about (149,358 liters) water for the cleaning and slaughter process (Gracey et. al., 1999). Unhygienic disposal of abattoir waste may contaminate ground water (Adebowale et. al., 2010).

3.2. Microbial quality of water and air samples:

The data tabulated in table (3) revealed the mean microbial load in tank water samples expressed as were as following: $1.0\times10^2\pm4.7\times10$, 100 ± 0.2115, 100 ± 6.375, $54.0\times10\pm8.226$ CFU/m^3 for total total aerobic, anaerobic count, *E. coli*, *S. aureus*, respectively. The mean microbial load of tap water samples was; $5.2\times10^3\pm2.3\times10^2$, $3.8\times10\pm0.3021$, $2.1\times10^2\pm5.684$, $2.9\times10^2\pm1.05\times10$ (CFU/m^3) for total total aerobic, anaerobic count, *E. coli*, *S. aureus*, respectively. Furthermore, the tables declared the mean microbial load of the slaughterhouse air samples were; $1.2\times10^3\pm3.336$, $3.4\times10\pm2.426$, $1.9\times10^2\pm2.732$, $2.2\times10^2\pm2.2\times10$ (CFU/m^3) for total aerobic count, total anaerobic count, *E. coli, S. aureus* count, respectively. All water and air of samples were free from salmonellae spp. The results considered highly significant ($p< 0.0001$). From the mentioned results, it is clear that the tank water microbial load is very low in comparing with the tap water inside the slaughter hall. The results agreed with Mather et. al., (2007) and Meyer et. al., (2010) while, Adebowale et. al., (2010) recorded lower water *E. coli* counts from Bodija Abattoir, Nigeria, average per 100 ml were 20.8 (CFU/m^3).

Table (3): Microbial quality of water and air samples collected from municipal slaughterhouse in New Valley.

Microorganisms	Water samples (CFU/m^3)						Air samples (CFU/m^3)		
	Tank water			Tap water					
	Min.	Max.	Mean ±SE	Min.	Max.	Mean ±SE	Min.	Max.	Mean ±SE
Total aerobic count	> 100	7.16 × 10	1.0 × 10^2 ±4.7×10	> 100	19.3 × 10^2	5.2 × 10^3 ±2.3×10^2	> 100	1.0 × 10^2	1.2 × 10^3 ±3.336
Total anaerobic count	> 100	4.92 × 10	> 100±0.2115	> 100	3.2 × 10	3.8 × 10 ±0.3021	> 100	3.1 × 10	3.4 × 10 ±2.426
E. coli	> 100	6.79 × 10	> 100±6.375	> 100	1.6 × 10^2	2.1 × 10^2 ±5.684	> 100	1.5 × 10^2	1.9 × 10^2 ±2.732
Staphylococcus aureus	> 100	3.51 × 10^2	54.0 × 10 ±8.226	> 100	1.8 × 10^2	2.9 × 10^2 ±1.05×10	> 100	16.5 × 10	2.2 × 10^2 ±2.2×10
Salmonella spp.	ND	ND	ND	ND	ND	ND	ND	ND	ND

Fig. 2. The tank water placed in clean, outdoor area outside the slaughter building.

In this study, tapwater was used for the cleaning processes was stocked in a large clean tank. Tapwater source was ground water. However, there was a possibility water contamination of post-treatment stage or from water pipes. Therefore, regular check for the water supplies of the slaughterhouse should be regularly performed. Figure (2) showed Elkharga slaughterhouse tank water, which properly closed and fitted in clean, good ventilated, and a sunny area outside the slaughter building (fig. 3) declared slaughtering hall with the network of water pipes, which used for washing the carcasses and easily cleaning the floor through taps, in addition to the picture declared the good ventilated and lighting. Clean air is a basic requirement of well-being health. Abattoirs considered important environment pollutes sources from their processes such as the air pollution of slaughterhouses. Lifting the slaughtered animals' blood following on the ground without proper cleaning results in offensive odor and hazards to the people health living around by respiratory manifestation and spreading the microorganisms, which reach to the meat surface and slaughterhouses water especially in high tempered areas (Magaj and Chup, 2012).

Fig.3.
Elkharga slaughter hall. The slaughter hall is 10 × 25 m^2 area. There were water pipes network spreading all over the hall used for washing the carcasses and cleaning processes. The hall is well ventilated, sufficient light. The main hall had many subsidiary rooms for viscera, condemnation, and emergency slaughtering.

In Egypt, most of the butchers and the consumers prefer buying meat on the same day after slaughtering without chilling. Therefore, almost of Egyptian slaughterhouses have not chilled rooms, which used to hanging to get matured which increase the meat quality, tenderness and decrease its microbial load. Humid hot climate areas are a leading cause of the total aerobic counts on meat (Obeng, et. al., 2013). Alkharga area is hot dry weather, which may be unfavorable for microbial growth.

3.2. Microbial quality of floor and walls:

Microbial quality of Elkharga abattoir floor and walls discussed in the table (4). The mean microbial load of the abattoir floor swabs was; $10 \times 10^2 \pm 5.2 \times 10^2$, $> 100 \pm 2.725$, $1.2 \times 10^3 \pm 5.3 \times 10^2$, $> 100 \pm 18$ (CFU/m^3) for the total aerobic count, total anaerobic count, *E. coli, S. aureus* count respectively. Whereas, the mean microbial load of wall swabs was; $10.8 \times 10^3 \pm 43$, $>100 \pm 3.13$, $10 \times 10^3 \pm 22$, 18.7 ± 9.49 (CFU/m^3) for the total aerobic count, total anaerobic count, *E. coli, S. aureus* count, respectively. All floor and walls samples were free from salmonellae spp. The differences between these results were highly significant ($p < 0.0001$).

The results of microbial quality of floor and walls agreed with a study prepared by Gill & McGinnis, (1999) whereas, higher results obtained by Paulsen et al., (2011) who investigated microbial loads on meats and swabs from slaughterhouse and reported the microbial load exceeds 5 logs10 CFU, and become unacceptable on food.

Figure (4) showed the slaughtering steps in Elkharga slaughterhouse hall slaughtering processes, bleeding and skinning which occur on the floor, different species slaughtered in the same area using tap water for cleaning. According to Egyptian regulations, slaughtering must be occurred during complete animal consciousness by cutting the two jaguar vein which called Halal (Islamic) slaughtering, which is aimed to have the meat of good public health and avoid many zoonotic diseases like Salmonellosis, E. coli and Staphylococci infections (Roberts, 2011). Slaughtering, skinning, and evisceration on the ground without separation between dirty and clean area lead to high possibilities of cross-contamination during meat processing which poses hazards of meat consumers of foodborne illness. Other important possibilities for the high microbial load of meat were the dirty hands and clothes of workers and the absence of any written sanitary measures on the slaughterhouse, lack of workers training for these measures. Therefore, all sanitary measures in the slaughterhouse should be applied and regularly evaluated to ensure quality control. There is no data on contagious or infectious diseases detected in the slaughterhouse.

Table (4): Microbiological quality of swabs from walls and floor in municipal slaughterhouse in New Valley

Microorganisms (CFU/gm)	Floor Swabs			Wall Swabs		
	Minimum	**Maximum**	**Mean ±SE**	**Minimum**	**Maximum**	**Mean ±SE**
Total aerobic Count	14.9×10^2	1.8×10^3	$10 \times 10^2 \pm 5.2 \times 10^2$	17.1×10^3	21.3×10^3	$10.8 \times 10^3 \pm 43$
Total anaerobic Count	6.1×10	> 100	$> 100 \pm 2.725$	5.85×10	> 100	$>100 \pm 3.13$
E. coli	15.3×10^2	17.6×10^2	$1.2 \times 10^3 \pm 5.3 \times 10^2$	14.1×10^3	19.6×10^3	$10 \times 10^3 \pm 22$
Staphylococcus aureus	1.9×10^2	2.74×10^2	$> 100 \pm 18$	39.5×10	54.8×10	18.7 ± 9.49
Salmonella spp.	ND	ND	ND	ND	ND	ND

Fig. 4. Slaughtering hall. All slaughtering, processing (bleeding and skinning) is unhygienically manually on the floor, different species slaughtered in the same area using tap water for cleaning.

4. CONCLUSION

The lowest microbial load detected in air samples followed by tank water then taps water followed by walls swabs while the highest microbial loads were in floor swabs. The results revealed the complete absence of Salmonellae spp. in different samples types. More governmental efforts are still needed to improve the environment quality of Elkharga slaughterhouse in New Valley, Egypt.

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