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Microbial Assessment for Camel and Mutton Carcasses Slaughtered at Al-Ahsaa Abattoir, Saudi Arabia

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Abstract: The study was not only carried out to assess surface microbial contamination of camel and mutton carcasses but also to evaluate thepersonnel hygiene. Twenty camel and mutton carcasses were swabbed and surface excised within a period of 5 months in Al-Ahsaa central abattoir located ateastern region of Saudi Arabia. Further, 10 personnel hands were also swabbed from the persons who have direct contact with carcasses. Microbial assessment includedtotal aerobic bacterial, total enterobacteriaceae, *S. aureus* (identified by VITEK 2 technique) and total yeast and mould were enumerated as means±SE. Results revealed an increase ofenterobacteriaceae counts on mutton carcasses. Regarding the microbial counts in mutton carcasses, there was no significant difference ($p<0.05$) between swabbing and surface excision methods.In camel carcasses, significant differences between the two methods in total bacterial count ($p\leq0.02$), enterobacteriaceae count ($p\leq0.001$), total yeast and mould count ($p\leq0.001$). No *salmonella* sp. was detected from all examined samples. Furthermore, the members of enterobacteriaceae recovered from the examined samples using VITEK 2 technique were *E. coli*, *Enterobacter aerogenes*, *Klebsiella oxytoca*, *Sphingomonas paucimobilis*, *Pseudomonas luteola*, *Methylobacterium* sp., *Proteus mirabilis*, *Raultella ornithinolytica*, *Serratia liquefaciens* and *Enterobacter hormaechei* with various percentages ranged from 3.33-23.33% in camel carcasses and from 6.67-20.00% in mutton carcasses. Personnel hands were highly contaminated. The results proved that Al-Ahsaa abattoir pose a medium risk through, the poor quality of the prepared carcasses due to the increased microbial load. This study clarified the public health importance of existed microorganisms and recommended the feasible methods for improving the sanitary status of carcasses.

Key words: Abattoir, camel, mutton, carcass, microbial, assessment

INTRODUCTION

Abattoirs are an important industry in Kingdom of Saudi Arabia. They play a major role in meat supply as well as employment opportunities to many people. Developed countries established requirements for slaughterhouses which represented in the selection of appropriate location and facilities necessary for safe operation as clean water and uncongested roads, safe disposal of wastes and provide spaces to create pens to receive animals and another pens to isolate diseased and suspected to be diseased animals (Sofos, 2008). There is a need to evaluate the effectiveness of current hygiene management systems used during animal slaughter in preventing or reducing the risk of food pathogens entering and proliferating in the food chain. Enterobacteriaceae has been used as indicator of possible post-processing contamination and its presence is indicator of fecal contamination in food (Dogan-Halkman *et al*., 2003). A display process for raw meat can be assessed with respect to microbiological safety by estimation of the *E. coli* growth (Greer *et al*., 1994). During preparation of camel and mutton carcasses, enterobacteriaceae can increase or decrease during processing depending on factors such as the level of contamination of livinganimal, efficiency of evisceration, personnel hygiene and hygienic practice adopted in the abattoir (Rigobelo *et al*., 2006). *E. coli* is usually used to determine the level of abattoir hygiene and carcass contamination in the EU Decision (2001/471/EC) which required that the Total Viable Counts (TVC) and enterobacteriaceae countbe recorded by swab samples

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comprising four sites of carcass taken before chilling (McEvoy *et al*., 2004). Enterobacteriaceae are an indicator of fecal contamination (Cartier, 1990). Total aerobic mesophilic flora is an indicator of overall contamination of carcasses (Roberts, 1980). The main sources of contamination of carcasses along the chain of slaughter are: the animal (leather and dung), equipment (machines and cutting tools), the environment (building, air, dust, water and harmful), method of work (non-compliance with the rules of slaughter) and labor (lack of personal hygiene; Sheridan, 1998). The level of surface contamination of carcasses varies depending on the conditions of hygiene and the procedure of slaughter (Widders *et al*., 1995). The slaughter process for meat-producing animals involves the removal of bact eria-free meat from between two contaminated surfaces, the hide and gastrointestinal tract (Buege and Ingham, 2003). The bacterial load recovered on beef and sheep carcasses is a constant fraction, 0.3% of those on hides. Other factors affecting the microbiological quality of carcasses are the design of abattoirs, equipment which does not always allow thorough cleaning and disinfection, aerosols or condensation forming on equipment and ceilings (Shale *et al*., 2006). From a hygiene and food safety point of view, the goal of the slaughter process is to minimize bacterial contamination of the carcass and effectively remove contamination (Buege and Ingham, 2003). The degree of superficial bacterial contamination of ovine and bovine carcasses at El-Harrach (Algeria) slaughterhouse; quantitatively by counting the total viable and fecal coliform counts, and qualitatively by the search for *Salmonella* sp. at ovine carcasses and at 3 different bovine carcasses sites; results reflect poor conditions of slaughtering and handling of carcasses, as well as hygiene deficiencies at El-Harrach slaughterhouse. The high prevalence of Salmonella spp. represents a real danger for the consumer (Nouichi and Hamdi, 2009). The assessment of Jeddah northern slaughterhouse showed that the percentage of the total score of application of GHP is 63% reflecting medium hazard which directly affects the hygienic quality and microbiological specifications of the produced meats and such slaughterhouse does not apply any quality or safety standards required for the production of good quality meat conform to Saudi and international specifications (El-Tawila *et al*., 2010). Beef carcasses contamination were assessed against *E.coli* O157 before and after washing with water at abattoirs in Nigeria (Bello *et al*., 2011). Ovine slaughterhouses hygiene of Algiers region were surveyed by bacteriological analysis of carcasses, they sampled ten carcasses in each slaughterhouse during 4 consecutive weeks. The amount of total aerobic mesophilic flora and of enterobacteriaceae was calculated on the 80 carcasses. The results were evaluated regarding European Regulation 2001/471/EC. For both slaughterhouses, the total aerobic mesophilic bacteria counts 4.84 log10 CFUscm^{-2} were close to the upper limit (5 log10 CFUscm^{-2}). The enterobacteriaceae counts forthe two slaughterhouses were 4.38 log10 and 3.30 log10 CFUscm^{-2}, respectively. These values were above the upper acceptable limit (2.5 log10 CFUscm^{-2}) (Harhoura *et al*., 2012). A few studies highlighted the hygienic assessment of camel and mutton in Saudi Arabia.

The method of slaughtering and floor dressing forcamel and mutton carcasses in most Saudian traditional abattoirs, raise the importance of the microbial quality and evaluation of thepersonnel hygiene.

MATERIALS AND METHODS

Sampling site: Samples were collected from the slaughter hall of Al-Ahsaa traditional abattoir, situated in the Estern Province, Saudi Arabia.

Sampling protocol: The abattoir wasvisited four times during the research period. During the visits, camel and mutton carcasseswere selected randomly after final wash. Both sides (right and left) of each of carcass was sampled. Another set of 10 swab samples was collected from the personnel hands. On each visit, the right sides of camel and mutton carcasses were sampled at three sites immediately after final wash. The sites were the rump, flank and brisket according to Roberts *et al*. (1980). At each site, an area of 50 cm^2 was swab sampled, using the wet and dry swab technique described by Kitchell *et al*. (1973). In addition to suface tissue excision using sterile scalpel and forceps.Ten personnel hands were swabbed (an area of 10 cm^2).

Microbiological assessment of carcasses: The swabs and surface excised tissueswere placed in 10 mL of Maximum Recovery Diluent (Oxoid CM733), transported to Meat Hygiene lab., College of Vet. Med., KFU on ice in an insulated container, stored overnight at 0°C and10-fold serial dilution in sterile 0.1% (wt/vol) peptone water (PW, Oxoid CM9) were preparedand then examined fortotal bacterial counts, enterobacteriaceae count, *S. aureus* count and total yeast and mould count. All plates were incubated under aerobic conditions. After incubation the colonies were enumerated by means of a colony counter (Gerber Instruments AG, Effretikon, Switzerland). The results were expressed as log10 colony forming units (CFU)/cm^2 and as logarithmic means. The microbiological quality of carcasses assessed following the technical indications of European Commission.

Fig. 1: VITEK 2-compact

Aerobic plate counts and enterobacteriaceae: For the enumeration of aerobic plate counts, plate count agar (PCA, Oxoid CM325)plates, incubated at 25°C for 3 days, whilst for enterobacteriaceae overlayed Violet Red Bile Glucose Agar (VRBG, Oxoid CM0485) were incubated for 24-48 h at 37°C. In the case of the latter, typical colonies were purple surrounded bypurple haloes. The dominant microorganisms of enterobacteriaceae isolates were identified with VITEK 2-compact technique (Pincus, 2006; BioMerieux, Rev 03, 2004; Fig. 1).

Staphylococcus aureus: Baird-Parker (BP agar base, CM027 and Egg yolk-Tellurite emulsion, SR0054C) agar plates were incubated for 24-48 h at 37°C. Typical *S. aureus* colonies (black colonies with white margins surrounded byclear zones) were enumerated. The colonies were confirmed using VITEK 2-compact (BioMerieux, Rev 03, 2004).

Total yeast and mould count: Sabouraud Dextrose (Oxoid, CM0041) agar plates were incubated for 5-7 days at 25°C.

Salmonella Isolation: The research method of Salmonella recommended by the French routine Standard (NF V 08-52) was used. Briefly, each sample was pre-enriched in 100 mL of BPW at 37°C for 16-20 h, aliquots from each pre-enriched culture were subjected to a 24 h selective enrichment in Rappaport-Vassiliadis broth (RV) at 42°C and in Selenite Cystine broth (SC) at 37°C. Afterwards, samples were spread, in duplicate, onto Hektoen agar and Brilliant Green Agar (BGA) and incubated at 37°C for 24 h. Suspected colonies were confirmed by using VITEK 2-compact (BioMerieux, Rev 03, 2004).

Statistical analysis: Statistical analysis was performed using SPSS for Windows version 16. Comparison between groups was made by independent Mann Whitney test. The Kolmogorov-Smirnov test was used to test the normal distribution of the data. Results are presented as means±Standard Errors (SE). Values of $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Aerobic plate counts are widely used to determine the general degree of microbial contamination (Aberle *et al*., 2001). The results of this work showed that there was an increase in the mean log of total bacterial count in samples from rump and flank sites in camel and mutton carcasses by excision method. The difference between excision and swab method in camel carcass was significant ($p<0.05$) (Table 1). However in mutton carcasses, there was no difference between excision and swabbing method in any of microbial load detected. It is evident from the results recorded in (Table 2) that the Mean log±SE of enterobacteriaceae in the examined surface samples of camel and mutton carcasseswere rangedbetween1.76±0.57-2.01±0.69,1.703±0.645-3.07±1.05 for excision and 2.56±0.82-3.338±1.159, 2.97±1.075-4.58±1.56 for swabbing method, respectively. Such count was mostly higher in flank swab samples of mutton carcasses which may be due to contamination during the evisceration process. Lower values were declared by El-Mossalami (1988) and Madden *et al*. (2004). There was a significant difference between excision and swabbing methods for enterobacteriaceae count in camel carcasses. The higher rate of enterobacteriaceae may be attributed to the lack of standard operating procedures and poor hygienic practices such as flaying, evisceration and splitting of carcass on the floor as well as the poor quality of water used for washing carcasses in the abattoir (Bello *et al*., 2011). Enterobacteriaceae counts indicate inadequate sanitation during production and handling of raw material, meat contact surfaces and employees. Meanwhile, the occurrence of large numbers of them on carcass surfaces are highly undesirable and suggests faecal contamination. The same tables revealed that the surface tissue excision samples gave higher microbial counts than that of swab samples. The higher counts of enterobacteriaceae may be attributed to the contamination of carcass surface from gastrointestinal tract during dressing, evisceration faults and mishandling. A significant higher enterobacteriaceae count on carcass surfaces may be related to poor sanitary conditions prevailing at the abattoir. Therefore, the presence of enterobacteriaceae bacteria in great numbers may be responsible for the inferior quality of meat resulting in economic losses and the possibility of the presence of enteric pathogens which constitute public health hazards.

Results in Table 3, showed that the mean value of *S.aureus* count on outer surface of camel carcass was 2.02±0.66 (rump), 1.93±0.63 (flank) and 2.007±0.60 (brisket) $CFUcm^{-2}$ by tissue surface excision method. The main source of *S. aureus* contamination is human being. The occurrence of *Staphylococcus aureus* on raw meat

Table 1: Mean log±SE of Total Bacterial Count (TBC) on examined carcasses (N = 30 for each)

	Excision (10 for each)			Swab (10 for each)			
Meats	A	B	C	A	B	C	p-value
Camel	4.11±1.34	4.25±1.4000	3.870±1.216	3.87±1.29	3.73±1.21	3.494±1.156	0.02
Mutton	4.29±1.40	4.312±1.392	4.42±1.3900	3.38±1.11	4.69±1.57	4.20±1.4000	0.30

A = Rump; B= Flank; C = Brisket; SE = Standard Error of Mean

Table 2: Mean log±SE of total Enterobacteriaceae Count (EC) on examined carcasses (N = 30 for each)

	Excision (10 for each)			Swab (10 for each)			
Meats	A	B	C	A	B	C	p-value
Camel	1.76±0.57	2.01±0.69	2.007±0.600	2.56±0.82	3.26±1.01	3.338±1.159	
Mutton	3.07±1.05	1.703±0.645	2.64±0.92	2.97±1.075	4.58±1.56	3.39±1.15	0.39

Table 3: Mean log±SE of *S. aureus* count on examined carcasses (N= 30 for each)

	Excision (10 for each)			Swab (10 for each)			
Meats	A	B	C	A	B	C	p-value
Camel	2.02±0.66	1.93±0.63	2.007±0.60	1.86±0.65	1.74±0.54	1.82±0.637	
Mutton	1.38±0.50	1.69±0.59	2.94±1.06	1.66±0.538	2.11±0.718	2.02±0.68	0.27

Table 4: Mean log±SE of Total Yeast and Mould count (TY&M) on examined carcasses (N = 30 for each)

	Excision (10 for each)			Swab (10 for each)			
Meats	A	B	C	A	B	C	p-value
Camel	3.52±1.09	2.20±0.60	2.318±0.414	1.76±0.64	2.56±0.88	1.88±0.66	
Mutton	3.85±1.29	4.046±1.414	3.90±1.34	2.79±0.97	3.27±1.06	3.56±1.20	0.12

Table 5: Microbial load on employee hands (by Swabbing Method) (N = 10)

TBC	EC	*S. aureus* count	TY&M
4.452±1.488	4.264±1.274	3.875±1.323	4.626±1.585

Table 6: Frequency distribution of isolated enterobacteriaceae organisms recovered from camel and mutton carcasses surfaces using VITEK 2-compact

	Camel		Mutton	
Isolates	No.	Percentage	No.	Percentage
E. coli	4	13.33	3	10.00
Enterobacter agglomerans	6	20.00	3	10.00
Methylobacterium spp.	3	10.00	2	6.67
Proteus mirabilis	1	3.33	3	10.00
Serratia liquefaciens group	2	6.67	4	13.33
Sphingomonas paucimobilis	5	16.67	4	13.33
Enterobacter hormaechei	7	23.33	6	20.00
Klebsiella oxytoca	2	6.67	2	6.67
Raultella ornithinolytica	0	0.00	3	10.00
Total	30	100.00	30	100.00

would be expected, because it is a principal component of the skin of humans and animals (Adams and Moss, 1997). From Table 4, we noticed high total yeast and mould count revealed by excision method. Yeast and mould are ubiquitous in nature and may cause a public health hazard in meat industry specially mycotoxin producing types. The swab samples from personnel hands who have direct contact with carcasses showed higher levels of enterobacteriaceae (4.264±1.274 CFUcm^{-2}), *S.aureus* (3.875±1.323CFUcm^{-2}) and total yeast and mould count (4.626±1.585 CFUcm^{-2}) (Table 5). One of the most important factors in good hygiene practice is personnel hygiene and cleanliness (Fig. 2). The findings of this research showed that the personnel hands were highly contaminated. Incidence of enteric bacteria isolated from camel and mutton carcasses surfaces were 13.33, 20, 10, 3.33, 6.67, 23.33, 6.67 and 0% and 10, 10, 6.67, 10, 13.33, 13.33, 20, 6.67 and 10% for *E. coli*, *Enterobacter agglomerans*, *Methylobacterium* sp., *Proteus mirabilis*, *Serratia liquefaciens* group, *Sphingomonas paucimobilis*, *Enterobacter hormaechei*, *Klebsiella oxytoca*, *Raultella ornithinolytica*, respectively (Table 6). Moreover, some of these organisms were previously isolated from examined samples by Hamed (1992) and Hussein (2005). The presence of Enterobacter organisms in meat may be responsible for their inferior quality resulting in economic losses. Moreover, some strains of Enterobacter were incriminated in many cases of acute and chronic diarrhea (ICMSF, 1996). Salmonella was not isolated from any of the examined carcasses. The results of the survey carried out by Altabari (2009) on the hygienic regulatory status

Fig. 2: Unhygienic preparation of mutton carcasses

of slaughter animals (before slaughter, after slaughter, skinning, evisceration and after meat inspection) at the slaughterhouses of Sharkia Province in Saudi Arabia was unsatisfactory. In Saudi Arabia, implementing HACCP is not mandatory in abattoirs. It is assumed that there would be more control at abattoir to reduce opportunities for carcasses contamination during the process of slaughtering and resulting in carcasses of superior microbiological quality. High standards of safety and cleanliness are essential in abattoirs. Operatives must follow strict procedures when storing the carcasses, handling the meat and disposing of waste products. The extent of contamination is dependent upon the local environment, the throughput of meat, the temperature and the cleanliness of utensils such as the cutting tables, conveyor belt and knives (Gill and Jones, 1999). The data highlighted the need for more systematic appoach to ensure safe food through implementing the quality control methods to prevent the increased microbial contamination, especially during personnel contact with carcasses. To our knowledge, this study is the first of its type in the study area.

CONCLUSION

Increased contamination of mutton carcasses than camel carcasses during processing and unhygienic personnel practices. Thiswere highly contributed to carcass contamination at the studied abattoir.

RECOMMENDATIONS

Future study should be conducted to assess the good hygienic practices and good manufacturing practice applied at abattoirs in Saudi Arabia.

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