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ENTEROBACTERIACEAE IN SLAUGHTERED ANIMALS WITH PARTICULAR REFERENCE TO PATHOGENIC STRAINS

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ABSTRACT

A total of 75 random swab samples collected from cattle, camel and sheep carcasses at Cairo and Qalyubia abattoirs to determine the contamination level of such carcasses with Enterobacteriaceae either quantitatively or qualitatively. The obtained results indicated that the mean values of these bacterial counts in the examined swab samples of sheep, cattle and camel were $2.54\pm.44\times10^3$, $1.33\pm0.26\times10^3$ and $5.91\pm1.02\times10^2/cm^2$ for the total Enterobacteriaceae count and $2.97\pm0.51\times10^3$, $8.54\pm1.67\times10^2$ and $2.28\pm 0.75\times10^2/cm^2$ for the total coliform count, respectively. The differences associated with the examined swab samples as a result of total Enterobacteriaceae and coliform counts were significant. On the other hand, Salmonella, E. coli, Citrobacter, Enterobacter, Klebsiella and Proteus species were isolated from the examined swab samples with varying percentages. Accurately, 16%, 4% and 16% of sheep, cattle and camel swab samples were contaminated with E. coli, however, the identified serovars were O86: k61(B7), O124:k72(B17), O55:k59(B5),O128:k67(B12) and O26:k60(B6). Referring to Salmonellae; S. enteritidis and S. typhimurium were detected only in cattle surface swab samples (4% of each).

KEY WORDS: Camel, Cattle, Enterobacteriaceae, Sheep, Slaughtered animals.

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1. I N T R O D U C T I O N

Fresh meat is highly perishable due to its biological composition. Microbial contamination of the carcass during the slaughtering process results in spoilage of meat, reduced shelf-life of meat and public health hazards [18, 19]. Many food borne diseases are related to consumption of meat containing pathogenic microorganisms. External contamination of raw meat is a constant possibility from the moment of bleeding until consumption. The microbial load of meat is directly related to good manufacturing practices during slaughter. There are large numbers of potential sources for contamination by microorganisms. These include contact with the hide, skin or feet, content of gastrointestinal tract, aqueous sources, instruments used for dressing (knives, saws, cleavers or hooks), and even air borne areas [14].

The family Enterobacteriaceae comprises a large number of organisms, not all of faecal origin, and are more useful as an indicator of overall process hygiene in the abattoir. E. coli are considered to be a more suitable choice of indicator as they are a single species more specifically associated with feces [7, 10, 17]. Enteric organisms, such as coliforms were frequently isolated from meat indicating

that the gut is a common source of contamination [12].
Therefore, the objective of the current study was to determine the level of Enterobacteriaceae contamination of sheep, cattle and camel carcasses during slaughtering and to identify their pathogenic strains.

2. MATERIALS AND METHODS

2.1. Collection of samples:
A total of 75 random swab samples collected from slaughtered cattle, camel and sheep carcasses at Cairo and Qalyubia abattoirs. The swab samples were taken after complete dressing of slaughtered animals into ice box and transferred immediately to the laboratory without undue delay for evaluation of their contamination with Enterobacteriaceae.

2.2. Preparation of swab samples according to ICMSF [13]:
The sterilized template placed firmly against the surface of the meat to limit the examined area. The sterile cotton swab drawn from screw capped plastic tubes and moistened in rinsing fluid solutions (0.1% buffered peptone water), then rolled over the limited area of the carcass. The template was rolled in one direction and perpendicular to this direction to represent all area. Finally, the cotton swab was aseptically retained into the rinsing fluid tubes containing 10 ml buffered peptone water. One ml of the original dilution was transferred to another sterile tube containing 9 ml of sterile peptone water and mixed well to make the next dilution from which further decimal serial dilutions were prepared.

2.3. Determination of Enterobacteriaceae count:
The purple colonies on Violet Red Bile Glucose agar plates were counted and the average number per cm² of the sample was calculated and recorded as total Enterobacteriaceae count.

2.4. Determination of coliform count according to ICMSF [13]:
All dark red colonies on Violet Red Bile agar plates were enumerated and the average number of coliforms per cm² of the sample was recorded.

2.5. Screening of Enteropathogenic
2.5.1. Escherichia coli:
The technique recommended by ICMSF [13] was carried out using MacConkey broth and Eosin Methylene Blue plates. The metallic green colonies were picked up and identified biochemically and serologically. Antisera used for typing of E. coli were coli test sera poly1, coli test sera poly11 and Bacto E. coli antisera (Difco).

2.5.2. Screening of Salmonellae:
Rappaport-Vassiliadis Salmonella Enrichment Broth tubes were used as enrichment broth and incubated at 43°C for 24 hours, while Desoxycholate agar plates were used as plating media. Pure cultures were serologically identified using rapid diagnostic antisera sets (Welcome Diagnostic A Division, Dartford, England DA 15 AH).

3. RESULTS AND DISCUSSION

The obtained results in table (1) indicated that the total Enterobacteriaceae count in the examined swab samples were varied from 2 to 8×10^3 with an average of $2.54\pm0.44\times10^3/cm^2$ for sheep, 10 to 2.9×10^4 with an average of $1.33\pm0.26\times10^3/cm^2$ for cattle and 2 to 1.8×10^3 with an average of $5.91\pm1.02\times10^2/cm^2$ for camel. Significant differences were detected among different species of carcasses after washing in this study at ($P < 0.05$). Nearly similar results were obtained by Hamdy [11], Samaha and Draz [21], and Ahmed [2] who reported that the mean values of

the bacterial groups in the examined camel shoulder, thigh, outer thorax and inner thorax samples Enterobacteriaceae were 2.5±0.86 $\times10^4$, 7.7±2.8$\times10^3$, 1.6±0.68$\times10^4$ and 1.8±0.6$\times10^3$/cm^2 for Entero-bacteriaceae and 3.9±1.3$\times10^2$, 2.81±0.62$\times10^2$, 2.1±0.67 $\times10^3$ and 1.40±0.38$\times10^2$/cm^2 for coliform counts per surface area (cm^2), respectively. Higher results were obtained by Khalifa [15] and Al-Dughaym and Yassien [4] who found that the mean values of Enterobacteriaceae count were 6.6$\times10^5$, 8.2$\times10^2$ and 6.2$\times10^4$ CFU/cm^2. In case of coliforms (MPN) were 6.3$\times10^5$, 3.1$\times10^2$ and 5.8$\times10^4$ bacteria/cm^2 on the surface of camel carcasses before skinning, after skinning and after preparation and stamping. While, lower results were obtained by Pearce and Bolton [20] who found counts ranging from 9.8$\times10^1$ and 1.0 $\times10^2$ CFU/cm^2 for Enterobacteriaceae in samples collected from thorax, shoulder/neck, breast/brisket and flank.

The comparatively high Enterobacteriaceae count in the examined sheep samples is an indication of inadequate sanitation during stages of slaughtering, evisceration, transportation, non-cleaned equipment or improper handling. In general, the Enterobacteriaceae were regularly detected on meat surface [7].

Table 1 statistical analytical result of total Enterobacteriaceae counts in the examined swab samples of different animal carcasses.

Carcasses	Positive samples N	%	Min.	Max.	Mean ± SE ($\times10^3$)
Sheep	23	92	2×10	8$\times10^3$	2.54±0.44*
Cattle	11	44	10	2.9$\times10^4$	1.33±0.26
Camel	11	44	2×10	1.8$\times10^3$	0.591±0.102

*Significant difference (P < 0.05)

The summarized result given in table (2) showed that the total coliform count in the examined swab samples were 10 to 7.2$\times10^3$ with an average of 2.97$\times10^3$± 0.51$\times10^3$/cm^2 for sheep, 10 to 1.68$\times10^4$ with an average of 8.54±1.67$\times10^2$ /cm^2 for cattle and 10 to 1.36$\times10^3$ with an average of 2.28±0.75$\times10^2$ /cm^2 for camel. Statistically, high significant differences were detected among different species of carcasses after washing in this study at (P< 0.01). Nearly similar results were obtained by Fliss et al. [8] found that all meat surface samples were analyzed for total coliforms, faecal *coliforms* and *E. coli* as an indicator of faecal contamination. Regardless to animal species, counts were relatively higher for freshly prepared meat. The mean level of contamination of all meat samples varied from 2$\times10^2$ to 2$\times10^5$ CFU/cm^2 for total coliforms, from 4×10 to 2$\times10^2$ for faecal coliforms and from 10 to 10^2 for *E. coli*. Higher results were obtained by Khalifa [15], Al-Dughaym and Yassien [4]. Moreover, Lower results were obtained by Vanderlinde et al. [22] and Yalçin et al. [25] who found the mean values of fecal coliform counts on the rump of beef carcasses were 0.75, 0.41, 0.23 $\log_{10}$/cm^2 after dressing, after evisceration, after washing and not detected after chilling, respectively, while on brisket were 0.95, 0.16, 1.72 and 0.15 $\log_{10}$ /cm^2 after dressing, after evisceration, after washing and after chilling, respectively.

Table 2 Statistical analytical results of total coliform counts in the examined swab samples of different animal carcasses (n=25).

Carcasses	Positive samples N	%	Min.	Max.	Mean ± SE ($\times10^3$)
Sheep	20	80	10	7.2$\times10^3$	2.97±0.51**
Cattle	13	52	10	1.7$\times10^4$	0.854±0.167
Camel	17	68	10	1.4$\times10^3$	0.228±0.075

** High Significant difference (P ≤ 0.01).

The incidence of enteric bacteria isolated from the examined swab samples of different animal carcasses was outlined in table (3), where Citrobacter freundii was isolated from 4% of examined camel samples but not isolated from sheep and

cattle samples. Moreover, the incidence of *Enterobacter aerogenes, Enterobacter agglomerans, Enterobacter cloacae* and *Enterobacter hafniae* declared that it was isolated from sheep, cattle and camel at rate of 8% , 0% and 20% respectively, from the examined samples. While, *Klebsiella ozaenae and Klebsiella pneumonae* were isolated from sheep, cattle and camel at rate of 8%, 0% and 4%, from the examined samples, respectively. Lastly, *Proteus mirabilis, Proteus rettgri and Proteus vulgaris* were isolated from sheep and cattle at rates of 12% and 20%, respectively.

Table 3 Incidence of Enterobacteriaceae isolated from the examined swab samples of different animal carcasses (n=25).

Isolated Bacteria	Sheep		Cattle		Camel	
	n	%	n	%	n	%
Citerobacter	-	-	-	-	1	4%
E.coli	4	16%	2	8%	4	16%
Enterobacter	2	8%	-	-	5	20%
Klebsiella	2	8%	-	-	1	4%
Proteus Spp.	3	12%	5	20%	-	-
Salmonella	-	-	2	8	-	-

The incidence and sero-typing of E. coli in the different animals' species were illustrated in Table (4). The serotypes of *E. coli* were O55:K59 (1.33%), O26:K60 (2.66%), O86:K61 (2.66%), O124:K72 (2.66%), O128:K67 (1.33%), and untypable (2.66%), from the total examined swab samples. The obtained results agree with those of Wassef [23] who stated that E. coli was the most predominant microorganisms present on the surface. Similarly Leung et al. [16], Arthur et al. [5], and Ahmed [3] found that serological isolates of *E. coli* were *E. coli* O_{55}:K_{59} in 4%, *E. coli* O_{111}:K_{58} in 3.33%, *E. coli* O_{26}:K_{66} K_{58} in 2.33%, *E. coli* O_{119}:K_{69} K_{58} in 2%, *E. coli* O_{44}:K_{74} K_{58} in 1.33% from the total examined swab samples. Higher results were obtained by Hamdy et al. [11].

Table 4 Incidence of *E. coli* isolated from the examined swab samples of different animal carcasses (n=25).

E.coli strain	Sheep		Cattle		Camel		Strain
	n	%	n	%	n	%	
O86:k61 (B7)	-	-	1	4	1	4	EPEC
O124:k72 (B17)	1	4	1	4	-	-	EIEC
O55:k59 (B5)	1	4	-	-	-	-	EPEC
O128:k67 (B12)	1	4	-	-	-	-	ETEC
O26:k60 (B6)	-	-	-	-	2	8	EHEC
Untypable	1	4	-	-	1	4	untypable

EPEC:Enteropathogenic *E. Coli*,EIEC:Enteroinvasive *E., coli*, ETEC:Enterotoxigenic *E. Coli*, EHEC: Enterohaemorrhagic *E. coli*

The incidence and sero-typing of Salmonella in the different animal species was illustrated in Table (5). In cattle, 2(8%) were recorded positive for incidence of Salmonellae. While, all examined swabs of sheep and camel carcasses were free from Salmonellae. This is agreeing with Abou-Yossef [1] who could not detect Salmonellae from any examined samples of fore and hind quarter of camel.

Generally, World Health Organization (WHO) [24] recorded that the incidence of Salmonella in the Egyptian raw meat and organs were 10% and 3%, respectively. In Egypt, *Salmonella* is widely recognized as one of the most principal causes of food poisoning outbreaks occurring as a result of consumption of contaminated meat and offal. The isolated Salmonellae strains were *Salmonella typhimurium* and *S. enteritidis*. The predominant strains of Salmonella were *Salmonella typhimurium* and *E. coli* O124:K72, whose incidence 3 isolates (60%) and 4 isolates (28.57%), respectively and this was agreeing with Baumgartner et al. [6] found that *S. typhimurium* and *S. enteritidis* were the most frequent serotypes implicating in cases of human salmonellosis .

Table 5 Incidence of Salmonella isolated from the examined swab samples of different animal carcasses (n=25).

Salmonella strains	Sheep		Cattle		Camel	
	n	%	n	%	n	%
Salmonella enteritidis	-	-	1	4%	-	-
Salmonella typhimurium	-	-	1	4%	-	-

4. CONCLUSIONS

As a conclusion, the data obtained in the present study, for the production of microbiologically clean and safe carcasses The Food Safety and Inspection Service (FSIS) [9] established requirements applicable to meat and poultry establishments designed to reduce the occurrence and numbers of pathogenic microorganisms, reduce the incidence of food-borne illness associated with their consumption and provide a new framework for modernization of the current system of meat and poultry inspection. The new regulations require:

1. Each establishment develops and implements written sanitation standard operating procedures (Sanitation SOP's).
2. Regular microbial testing by slaughter establishments to verify the adequacy of the establishments' process controls for the prevention and removal of fecal contamination and associated bacteria.
3. Establish pathogen reduction performance standards for Salmonella that slaughter establishments and establishments producing raw ground products must meet.
4. All meat and poultry establishments develop and implement a system of preventive controls designed to improve the safety of their products, known as HACCP (Hazard Analysis and Critical Control Points).

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البكتريا المعوية في الحيوانات المذبوحة وبالاخص العترات الممرضة

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الملخص العربى

أُجريت هذه الدراسة للتعرف علي مدي تواجد الميكروبات المعوية المختلفة علي اسطح الغنم والابل والجمال المذبوحة بمجازر القليوبية والقاهرة حيث تم أخذ75 مسحة من أسطح الغنم، الماشية، والجمال(بمعدل 25 من كل نوع) حيث اجريت الفحوص البكتريولوجية عليها لتحديد العدد الكلي للميكروبات المعوية والميكروبات القولونية، وكذلك محاولة عزل الأيشريشيا كولاي والسالمونيلا. اظهرت النتائج ان متوسط العدد الكلي للميكروبات المعوية في الغنم، الابل، والجمال لكل سم^2 من سطح الحيوان كانت 0.44±2.54×10^3، 0.26±1.33×10^3، و1.02±5.91×10^2 علي الترتيب. كان العدد الكلي للميكروبات القولونية في الغنم، الابل، والجمال لكل سم^2 من سطح الحيوان 0.51±2.97×10^3، 1.67±8.54×10^2، و 0.75±2.28×10^2 علي الترتيب. تم عزل ميكروب السالمونيلا، الايشريشيا كولاي، الستروباكتر، الانتروباكتر، الكليبسيلا، والبروتيس بنسب مختلفة وكذلك تم تصنيفهم باستخدام الطرق السيرولوجية حيث تم عزل ميكروب الايشريشيا كولاي الممرضة في الغنم، الابل، والجمال لكل سم^2 من سطح الحيوان بنسبة 16%، 8%، و16% علي الترتيب من مجموع العينات. تم عزل ميكروب السالمونيلا تيفيميوريم، والسالمونيلا انتيريتيديس بنسبة 8% من الماشية بينما لم يتم عزل ميكروب السالمونيلا من اى من الغنم أو الجمال.