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±0.44×10³, 1.33±0.26×10³ and 5.91±0.10×10²/cm² for the total Enterobacteriaceae count and 2.97±0.51×10³, 8.54±1.67×10² and 2.28±0.75×10²/cm² 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.

1. INTRODUCTION

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 8×10³ with an average of 2.54±0.44×10³/cm² for sheep, 10 to 2.9×10⁴ with an average of 1.33±0.26×10³/cm² for cattle and 2 to 1.8×10³ with an average of 5.91±1.02×10²/cm² 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 \times 10^4, 7.7±2.8 \times 10^3, 1.6±0.68 \times 10^4 and 1.8±0.6x10^3/cm^2 for Enterobacteriaceae and 3.9±1.3 \times 10^2, 2.81±0.62x10^2, 2.1±0.67 \times 10^3 and 1.40±0.38 \times 10^2/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\times 10^5, 8.2\times 10^2 and 6.2\times 10^4 CFU/cm^2. In case of coliforms (MPN) were 6.3\times 10^5, 3.1\times 10^2 and 5.8\times 10^4 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\times 10^1 and 1.0 \times 10^2 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].

The summarized result given in table (2) showed that the total coliform count in the examined swab samples were 10 to 7.2\times 10^3 with an average of 2.97\times 10^3± 0.51\times 10^3/cm^2 for sheep, 10 to 1.68\times 10^4 with an average of 8.54±1.67\times 10^2/cm^2 for cattle and 10 to 1.36\times 10^3 with an average of 2.28±0.75\times 10^2 /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\times 10^2 to 2\times 10^3 CFU/cm^2 for total coliforms, from 4\times 10 to 2\times 10^2 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.

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

<table>
<thead>
<tr>
<th>Carcasses</th>
<th>Positive samples</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean ± SE (x10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>23</td>
<td>92</td>
<td>2 \times 10^3</td>
<td>8 \times 10^7</td>
</tr>
<tr>
<td>Cattle</td>
<td>11</td>
<td>44</td>
<td>10</td>
<td>2.9 \times 10^4</td>
</tr>
<tr>
<td>Camel</td>
<td>11</td>
<td>44</td>
<td>2 \times 10^3</td>
<td>1.8 \times 10^3</td>
</tr>
</tbody>
</table>

** Significant difference (P < 0.05).

** High Significant difference (P ≤ 0.01).
The incidence and sero-typing of enterobacteriaceae isolated from the examined swab samples of different animal carcasses (n=25).

<table>
<thead>
<tr>
<th>Isolated Bacteria</th>
<th>Sheep</th>
<th>Cattle</th>
<th>Camel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Citerobacter</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E.coli</td>
<td>4</td>
<td>16%</td>
<td>2</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>2</td>
<td>8%</td>
<td>-</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>2</td>
<td>8%</td>
<td>-</td>
</tr>
<tr>
<td>Proteus Spp.</td>
<td>3</td>
<td>12%</td>
<td>5</td>
</tr>
<tr>
<td>Salmonella</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
</tbody>
</table>

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 O55:K59 in 4%, E. coli O124:K72 in 7.66%, E. coli O86:K61 in 2.66%, E. coli O26:K59 K58 in 2.66%, E. coli O124:K72 K58 in 2%, E. coli O44:K74 K58 in 1.33% from the total examined swab samples. Higher results were obtained by Hamdy et al. [11].
Table 5 Incidence of Salmonella isolated from the examined swab samples of different animal carcasses (n=25).

<table>
<thead>
<tr>
<th>Salmonella strains</th>
<th>Sheep</th>
<th>Cattle</th>
<th>Camel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella enteritidis</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

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).

5. REFERENCE

البكتريا المعوية في الحيوانات المذبوحة وبالخص العترات الممرضة

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قسم الرقابة الصحية على الأغذية - كلية الطب البيطري - جامعتها بنيـها

الملخص العربي

أجريت هذه الدراسة للتعرف على مدى تواجد الميكروبات المعوية المختلفة على سطوح الغنم والأبل والجمال المذبوحة بمجازر القميوبية والقاهرة حيث تم أخذ 75 مسحة من سطوح الغنم، الماشية، والجمال (بمعدل 25 من كل نوع) حيث أجريت الفحوص البيئولوجية عليها لتحديد العدد الكلي للميكروبات المعوية والميكروبات القولونية، وكذلك محاولة عزل الأشريشيا كولاي والسالمونيلا. أظهرت النتائج أن متوسط العدد الكلي للميكروبات المعوية في الغنم، الأبل، والجمال لكل سم² من سطح الحيوان كانت 4.4±2.5×10⁴، 4.5±0.3×10⁴، و 0.45±7.0×10⁴ عملي الترتيب.

النتائج عل فيما كميروبات القولونية في الغنم، الأبل، والجمال لكل سم² من سطح الحيوان كانت 4.7±5.5×10⁴، 0.05±5.7×10⁴، و 4.57±5.5×10⁴ عملي الترتيب. كان العدد الكلي للميكروبات القولونية في الغنم، الأبل، والجمال لكل سم² من سطح الحيوان 5.1±2.97×10⁴، 1.67±5.4×10⁴، و 0.75±2.8×10⁴ عملي الترتيب. تم عزل ميكروب السالمونيلا الإشريشيا كولاي، الاستروباكتر، الاستروباكتر، الكليسيلا، والبروتيس بنسبة مختلفة وكذلك تم تصنيفهم باستخدام الطرق البيئولوجية حيث تم عزل ميكروب الإشريشيا كولاي الممرضة في الغنم، الأبل، والجمال لكل سم² من سطح الحيوان بنسبة 16%، 8%، و16% على الترتيب من مجموع العيوب. تم عزل ميكروب السالمونيلا تيفيموريوم، والسالمونيلا انتربرديس بنسبة 8% من الحيوان بينما لم يتم عزل ميكروب السالمونيلا من أي من الغنم أو الجمال.