Isolation and Identification of Escherichia coli and Salmonella typhimurium from Sheep in Baghdad city

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Summary
One hundred fifty fecal samples of sheep were collected from September, 2017-March, 2018 in Baghdad city areas (Abu-Grab zone, Dora zone, Saydyia zone, Arab Jbur zone, Al-Usfia zone, Al-Fudhalia zone and College Veterinary medicine –University of Baghdad). Samples were cultured on MacConkey and Eosine Methylene Blue agar for E.coli isolation, Xylose Lysine Deoxycholate and Salmonella –Shigella agar for S. typhimurium isolation. Results showed high percent (78.57%) of infection in female of E.coli than 68.42% in Salmonella typhimurium. E.coli was recovered with an infection rate 93.33% in Al-Fudhalia area, and S. typhimurium with an infection rate 12.66% in Arab Jbur. Also, the results showed an infection rate of E.coli 78.75% (110/140) and 21.43% (30/140), and S. typhimurium was recovered in 68.42% (13/19) and 31.58% (6/19) in females and males respectively.

Keywords: E. coli, Salmonella typhimurium, Sheep.

Introduction
Escherichia coli is a normal flora of the intestine of most animals and humans. Some E.coli strains can cause a wide variety of intestinal and extra–intestinal diseases, such as diarrhea, urinary tract infections, sepsis, mastitis and neonatal meningitis (1). The formation of bacterial biofilms of E.coli in a host in general seems to be based on current evidence to a large extent an intracellular event (2). The illnesses caused by a particular strain of E. coli depend on spreading and expression of many virulence determinants such as biofilm formation, adhesion, production of hemolysin, enterotoxin, Shiga toxin, endotoxin and capsules formation (3). Diarrheal diseases were major problem in third world countries which are responsible for death of millions of people and animals each year (4). It can be either acute or chronic (5). Diarrhea causing of 46% of calves and lambs mortality (6). The most common causes of acute diarrhea are bacterial and viral infections (7). Infections with E. coli being one of the major causative agents (8). E. coli is a pathogen responsible for numerous infections outbreaks worldwide. It is resident commensals bacterium commonly found in the intestinal tract of ruminant such as cattle, sheep, goat and deer; human exposure to this microbial pathogen is classically associated with the ingestion of undercooked beef (9). Persons working direct or indirect with animals may have E.coli and fecal material of these animals may contaminate meat during slaughtering; in addition to that; the fecal material may reach the lakes, rivers or any other water sources. These organisms may also adhere on fruits or vegetables and may even are transported by air, water or even animal movement. Person to person spread of E.coli has also been recorded to be the primary mode of infection in many outbreaks especially in hospitals (10). The unchlorinated water was highly found to be contaminated with other organism (11). Salmonella is a rod-shaped, Gram-negative, oxidase negative, non-spore forming, and facultative anaerobic (12). Most of the members of this genus are motile by peritrichous flagella except S. Pullorum and S. gallinarum. Salmonellae are frequently facultative intracellular parasites. Salmonella are non-capsulated except S.typhi, S. paratyphi C and some strains of S. dublin. These bacteria can resist dehydration for a very long time (13). Although primarily intestinal bacteria, Salmonella are widespread in the environment and commonly found in farm effluents, human sewage, and in any material subject to fecal
contamination. Salmonellosis has been recognized in all countries but appears to be most prevalent in areas of intensive animal husbandry (14). The aim of study was to isolate and identification of E.coli and Salmonella typhimurium from fecal sample of sheep in different areas of Baghdad city.

Materials and Methods

One hundred fifty fecal samples were collected from sheep suffering from diarrhea from different areas of Baghdad city (Abu-Grab, Dora, Saydyia, Arab Jbure, Al-Usfia zone, Al-Fudhalia and field of College Veterinary Medicine, University of Baghdad. All samples collected were used for the diagnosis to the isolation in laboratory of Central Public Health (15).

Results and Discussion

Escherichia coli (93.33%) was recovered 140 out of 150, while 19 fecal samples showed positive results for the presence of Salmonella typhimurium 19/150 (12.66%). Also 15 fecal samples from Abu-Grhab showed positive results for E.coli (100%) and negative result for S. typhimurium (0.0%), while 25 fecal samples from Dora were positive 100% for E.coli and 5 fecal samples (20.0%) were positive for S. typhimurium, 30 fecal samples 25 (83.33%) were positive for E.coli, while 4 fecal samples (13.33%) were positive for S. typhimurium in Saydyia, also 15 fecal samples 10 were positive for E.coli (66.66%) and 6 (40.00%) of S. typhimurium in Arab Jburn. 20 fecal samples 20 were positive for E.coli (100%) ; 3 samples (15%) S. typhimurium, in Al-Usfia. 30 fecal samples 30 (100%) were positive results for E.coli ,while 1 sample (3.33%) were positive for S. typhimurium in Al-Fudhali; Finally 15 fecal samples15 (100%) were positive results for E.coli, while 0.0% for S. typhimurium in College of Veterinary Medicine, University of Baghdad (Table 1).

Bacteriologic culture of fecal samples on selective media recovered E.coli. The red color on MacConkey agar occurred by utilizing of lactose in the agar with surrounding areas of precipitated bile salts, while Eosin Methylene Blue (EMB) agar was used for isolation and identification, and was considered as a rapid and accurate method for distinguishing E.coli from other gram-negative pathogens. The visible colonies appeared as green metallic sheen, which indicated vigorous fermentation of lactose and acid production which precipitated and appeared as green metallic pigment. The results agreed with (15 and 16). Lysine of E.coli was positive and growth on TSI slant with a (A/A/g+/H2S-) profile, IMVIC was (+) for E.coli, indole positive (red ring), methyl red-positive (bright red), but VP was negative (no change - colorless) and citrate was negative (no change green color). Optimum growth of E. coli occurred at 37 °C - 98.6 °F (17).

The results of this study concerning recovery of E.coli from different areas of Bagdad city found was 93.33% fecal samples that may be referred to the high infection rate in the present study. Previous researches reported that the colonization of the gastrointestinal tract of both large and small ruminants with E.coli (18 and 19) found 36 out of 41 fecal samples of sheep and goats in Duhok governorate were positive for E.coli 36/41(87.80%); (20) found that 35 out of 102 and 22 out of 102 fecal samples of sheep in Abu-Grhaib and Dora zone respectively. Also (21) reported that 16 samples out of 53 (30.20%) rectal swabs collected from diarrheic sheep and this is disagreed with our results ,but current study agreed with those of (22) that isolated E. coli from diarrheic goats (27.3%) and sheep (9.1%) and the strains isolated were 100% hemolytic nonverotoxic .

Recovery rate of E.coli was 110/140 (78.57%) in females, while 30/140 (21.43%) in males. In less than one year showed high infection rate 80.00% (40/50) in females, while 10/50 (20.00%) in males; between one to two years of age showed high infection rate 45/60 (75.00%) in females, while 25.00% (15/60) in males and in the age more than
two years the infection rate 25/110 (83.33%) in females, while 5/30 (16.67%) in males (Table, 2).

Table,1: Incidences *E. coli* and *S. typhimurium* isolated from sheep in different areas of Baghdad city.

<table>
<thead>
<tr>
<th>Areas</th>
<th>No. of sample examine</th>
<th><em>E. coli</em></th>
<th><em>S. typhimurium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MacConkey agar (%)</td>
<td>EMB agar (%)</td>
</tr>
<tr>
<td>Abu-Ghraib</td>
<td>15</td>
<td>15(100)</td>
<td>15(100)</td>
</tr>
<tr>
<td>Dora</td>
<td>25</td>
<td>25(100)</td>
<td>25(100)</td>
</tr>
<tr>
<td>Saydyia</td>
<td>30</td>
<td>25(83.33)</td>
<td>25(83.33)</td>
</tr>
<tr>
<td>Arab Jbur</td>
<td>15</td>
<td>10(66.66)</td>
<td>10(66.66)</td>
</tr>
<tr>
<td>AL-Usfia</td>
<td>20</td>
<td>20(100)</td>
<td>20(100)</td>
</tr>
<tr>
<td>AL-Fudhalia</td>
<td>30</td>
<td>30(100)</td>
<td>30(100)</td>
</tr>
<tr>
<td>Coll. Vet. Med. Univ. Baghdad</td>
<td>15</td>
<td>15(100)</td>
<td>15(100)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>150</td>
<td>140(93.33)</td>
<td>140(93.33)</td>
</tr>
</tbody>
</table>

Table, 2: Infection rate of *E. coli* isolated according to the age and sexes of the sheep.

<table>
<thead>
<tr>
<th>Age(year)</th>
<th>No. of sample</th>
<th>Males (%)</th>
<th>Females (%)**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>&lt; 1</strong>*</td>
<td>50</td>
<td>10 (20.00)</td>
<td>40 (80.00)</td>
</tr>
<tr>
<td><strong>1 - 2</strong></td>
<td>60</td>
<td>15 (25.00)</td>
<td>45 (75.00)</td>
</tr>
<tr>
<td><strong>&gt; 2</strong></td>
<td>30</td>
<td>5 (16.67)</td>
<td>25 (83.33)</td>
</tr>
<tr>
<td><strong>Total No.</strong></td>
<td>140</td>
<td>30 (21.43)</td>
<td>110 (78.57)</td>
</tr>
</tbody>
</table>

Recovery rate of *S. typhimurium* was 13/19 (68.42%) in females, while 6/19 (31.58%) in males. Less than one year age had high infection rate 8/12 (66.67%) in females, while 4/12 (33.33%) in males. One to two years of age had high infection rate 5/7 (71.43%) in females, while 2/7 (28.57%) in males (Table, 3). *S. typhimurium* colonies were detected on the selective media as smooth, transparent, small and circular, distinguished in their color after their growth which were appeared in the slightly pale colonies on the MacConkey agar, pale with black center on the S-S agar, pink on the Brilliant green agar with conversion of almost dish to the red-pink color, and red color with black spots on the XLD agar. Diagnosis of bacteria by biochemical test, the bacteria showed positive results when inoculated TSI media represented a yellow color in the bottom as an indication for glucose fermentation, the pink color in the slant was appeared as a result of lactose non fermentation, in addition to presence of gas bubbles as indication of CO2 formation. A black color was also formed as a result of H2S production recording the bacterial ability to utilize the citrate when it is grown on the Simmon citrate agar by change the media color from green to blue. The negative results were also recorded for indole and oxidase test as an indication of yellow color formation after adding of Kovacs reagent while unchanged color in oxidase test resulted after adding oxidase reagent (15).

*Salmonella* can survive in the environment, and once established on a farm, contamination can be difficult to be eradicated. It may spread from farm to farm and can disseminate into food-chains as a consequence of further cross-contamination at slaughter houses due to the
ability of *Salmonella* to survive in meat and animal products (23).

Isolation of *S. typhimurium* from 309 different apparently healthy samples were collected from slaughtered sheep in Basra, the results revealed that the incidence rate of *Salmonella* isolation in fecal samples was 7.2%, in bile samples 8.5% and in intestinal content 9.8% (24).

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>No.</th>
<th>Males (%)</th>
<th>Females (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1*</td>
<td>12</td>
<td>4 (33.33)</td>
<td>8 (66.67)</td>
</tr>
<tr>
<td>1-2</td>
<td>7</td>
<td>2 (28.57)</td>
<td>5 (71.43)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>19</td>
<td>6 (31.58)</td>
<td>13 (68.42)</td>
</tr>
</tbody>
</table>

Recovery rates *E. coli* was 110/140 (78.57%) in females, while 30/140 (21.43%) in males. In *S. typhimurium* was found in 13/19 (68.42%) of females, while 6/19 (31.57%) in males. (Table, 4).

Table 3: Infection rate of *S. typhimurium* isolated from sheep according to the ages and sexes.

<table>
<thead>
<tr>
<th>Species of bacteria</th>
<th>Total</th>
<th>Males (%)</th>
<th>Females (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>140</td>
<td>30 (21.42)</td>
<td>110 (78.57)</td>
</tr>
<tr>
<td><em>S typhimurium</em></td>
<td>19</td>
<td>6 (31.57)</td>
<td>13 (68.42)</td>
</tr>
</tbody>
</table>

The infection rate which were higher in females than males. Higher occurrence in sheep might be due to differences in feeding behavior between sexes. Sheep prefer graze while goat browses and rearing taking area. Close contact and holding time during journey and at arrival in market area which predispose to cross contamination through poor hygiene of the market environment.

References

128


عزل وتشخيص الاشريشيا القولونية والسالمونيلا تايفيموريم من الاغنام في مدينة بغداد
الله طه حنون و اكرام عباس عبود السامرائي
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الخلاصة
جمعنا مائة و خمسون عينة من براز الاغنام خلال الفترة من بداية آب 2017 إلى نهاية إيار 2018 في المناطق المختلفة في مدينة بغداد (أبو غريب - الدورة، السيدية، عرب جبور، أبو غريب، الدورة، السيدية، عرب جبور، السيدية، عرب جبور، السيدية). وزرعت هذه النماذج على الأوساط الزراعة صبغة الميثيل الزرقاء، الماكونكي، X LD و SS. وأجرينا الاختبارات المجهرية والكيميائية. فاظهرت النتائج وجود اصابات عالية (78.57%) لجرثومة الاشريشيا القولونية و(68.42%) للسالمونيلا تايفيموريم. انتسب نسبة الإصابة في منطقة الفضيلية (%) 93.3% و 19 عزلة ظهرت نتيجة ايجابية السالمونيلا تايفيموريم ونسبة بلغت 12.66% في منطقة عرب جبور. واظهرت النتائج ان نسبة الإصابة بجرثومة الاشريشيا القولونية 78.75% و 31.58% في الاياث والذكور على التوالي.

الكلمات المفتاحية: الاشريشيا القولونية، السالمونيلا تايفيموريم، اغنام.