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

Alaa Taha Hanoun and Ikram Abbas Aboud Al-Samrraae
Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq
E-mail: alaath725@gmail.com , samrrikram@gmail.com

Received: 03/03/2019
Accepted: 02/07/2019
Publishing: 04/08/2019

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 jbure 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, septicemia, 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 cultured on pre-enrichment broth (peptone water) for isolation of MacConkey and Eosin Methylene Blue agar were used for growth and isolation of *E.coli* and also cultured on enrichment (Selenite broth) for *Salmonella typhimurium* at 37°C for 24 hrs. *Salmonella-Shigella* agar and XLD agar were used for growth and isolation of *Salmonella typhimurium* .Morphological, cultural and biochemical tests in addition to API20 E system 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, also15 fecal samples 10 were positive for *E.coli* (66.66%) and 6 (40.00%) of *S. typhimurium* in Arab Jbur. 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-Fdhalia; 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-Ghraib 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.

Areas	No. of sample examine	*E.coli*		*S. typhimurium*	
		MacConkey agar (%)	EMB agar (%)	S.S agar (%)	XLD agar (%)
Abu-Ghraib	15	15(100)	15(100)	0(0.0)	0(0.0)
Dora	25	25(100)	25(100)	5(20.0)	5(20.0)
Saydyia	30	25(83.33)	25(83.33)	4 (13.33)	4(13.0)
Arab Jbur	15	10(66.66)	10(66.66)	6(40.0)	6(40.0)
AL-Usfia	20	20(100)	20(100)	3(15.0)	3(15.0)
AL-Fudhalia	30	30(100)	30(100)	1(3.33)	1(3.33)
Coll. Vet. Med. Univ. Baghdad	15	15(100)	15(100)	0(0.00)	0(0.0)
Total	150	140(93.33)	140(93.33)	19(12.66)	19(12.66)

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

Age(year)	No. of sample	Males (%)	Females (%)**
< 1*	50	10 (20.00)	40 (80.00)
1 -2	60	15 (25.00)	45 (75.00)
>2	30	5 (16.67)	25 (83.33)
Total No.	140	30 (21.43)	110 (78.57)

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

Age (year)	No.	Males (%)	Females (%)
<1*	12	4 (33.33)	8 (66.67)
1-2	7	2 (28.57)	5 (71.43)
Total	19	6 (31.58)	13 (68.42)

Recovery rates *E.coli* was110/140 (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 4: Infection rate of *E.coli* and *S. typhimurium* isolated from sheep according to sex in Baghdad city.

Species of bacteria	Total.	Males (%)	Females (%)
E. coli*	140	30 (21.42)	110 (78.57)
S typhimurium	19	6 (31.57)	13 (68.42)

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.

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عزل وتشخيص الاشريشيا القولونية و السالمونيلا تايفيموريم من الاغنام في مدينة بغداد

الاء طه حنون و اكرام عباس عبود السامرائي
فرع الاحياء المجهرية ، كلية الطب البيطري ، جامعة بغداد ،بغداد ، العراق .
E-mail:alaath725@gmail.com , samrrikram@gmail.com

الخلاصة

جمعت مائة و خمسون عينة من براز الاغنام خلال المدة من بداية ايلول / 2017 الى نهاية اذار /2018 لمناطق مختلفة في مدينة بغداد (ابو غريب- الدورة ، السيدية ،عرب جبور، اليوسفية ، الفضيلية وحقل كلية الطب البيطري). وزرعت هذه النماذج على الاوساط الزرعية صبغة المثيل الزرقاء ، الماكونكي ، SS وX LD . واجريت الاختبارات المجهرية والكيماحيوية. فاظهرت النتائج وجود اصابه عاليه (78.57%) لجرثومة الاشريشيا القولونية و(68.42% **للسالمونيلا** تايفيموريم وان نسبه الاصابه 93.33% في منطقة الفضيلية و 19 عزلة ظهرت نتيجة ايجابية السالمونيلا تايفيموريم وبنسبة بلغت 12.66% في منطقة عرب جبور واظهرت النتائج ان نسبة الاصابة بجرثومة : الاشريشيا القولونية 78,75% و 43,21% وكانت نسبة الاصابة بجرثومة السالمونيلا تايفيموريم 42,68% و31.58% في الاناث والذكور على التوالي .

الكلمات المفتاحية: الاشريشيا القولونية ،السالمونيلا تايفيموريم، اغنام .