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## Study the prevalence of *Escherichia coli* O157:H7 isolated from humans and sheep with histopathological Study

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### Abstract

In order to study the prevalence of *Escherichia coli* O157:H7, (228) urine samples were collected from young and adult patients from both sexes suffering from Urinary Tract Infection, in addition to collected (75) urine samples from apparently healthy sheep from both genders. The results showed that among (228) human urine samples, 8 (3.50%) samples out of 228 were *E.coli* O157:H7 positive isolates (6 (5.17%) samples out of (116) in females and 2 (1.7%) samples out of (112) in males). In sheep the results revealed that 13 (17.33%) out of (75) urine samples were *E.coli* O157:H7 positive isolates. also the current study showed that 11(18.03 %) out of (61) ram's urine samples were positive for *E.coli* O157:H7 isolates and 2 (14.28 %) out of (14) ewe's urine samples were positive for *E.coli* O157:H7. The second goal, in this study is determined histopathological lesions in certain internal organs to experimentally infected with *E. coli* O157:H7. The results revealed that *E. coli* O 157:H7 cause thrombus, necrosis, apoptosis suppurative inflammation in the examined organs as well as granulomatous reaction in the liver.

**Keywords:** *Escherichia coli* O157:H7, prevalence in human and sheep

### Introduction

*E. coli* are considered a normal flora of gastrointestinal tract of humans and animals but some strains of these microorganisms become pathogen including enterohemorrhagic *E.coli* (EHEC) [1], these pathogens are characterized by producing Shiga toxins that induced hemorrhagic colitis in which its sequel is hemolytic uremic syndrome (HUS) [2]. The importance serotype of EHEC is O157:H7 worldwide [3], estimated that *E. coli* O157:H7 cause 73,000 infections with sixty deaths per year in the USA, investigated high economic losses cause by these bacteria in USA [4]. Cattle were considered the principle reservoir of *E.coli* O157:H7 which continuously shaded these pathogens in their feces [5] and cattle meats can contaminated through slaughter, therefore, the beef products may considered a main way for transmission of *E.coli* O157:H7 for humans in addition to consumption of contaminated water [6], these bacteria can survive in different environmental condition as in soil, water or low temperature [7, 8]. Urinary tract infection caused by EHEC is one of the most important disease in infants and children, in addition, there is no protective vaccine against *Escherichia coli* O157:H7, therefore these organism must be rapidly diagnosed in order to treat and prevent renal damage which lead to renal failure specially in infants and young children [9].

In Iraq, several studies were detected the *E.coli* O157: H7 in milk or feces of human and animals [10, 11], but in urine were few, only at 2013 the *E.coli* O157:H7 in children with Urinary Tract Infection showed a percentage (1.5%) in children with age (first month - 5 years) but in age more than five years there is no evidence of isolation [12]. So the aim of the current study are to determine the percentage of urinary tract infection of humans and sheep in addition to study the pathological lesions induced by experimentally infection by these pathogens in mice.

### Materials and methods

In these study, were used general media (blood and MacConkey agar) and selective media (Cefixime Tellurite - Sorbitol MacConkey agar (CT-SMAC) and CHROME agar O157) to isolate *E.coli*O157:H7 with pure culture [13], that prepared according to the manufacturer requirements.

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**Collection of human urine samples:** 228 urine samples from young and adult patients for both gender and different ages suffering from (UTIs) were collected from three Hospitals (Al Yarmouk Teaching Hospital, Al karama Hospital and Abu Ghraib Hospital), during the period that extended from December 2012 to the end of March 2013. Collected midstream urine (A few milliliters of urine were allowed to pass, then the sterile container was passed into the urine stream and removed before the void ends) and transported immediately to the laboratory in iceboxes packed with ice. After 2 hrs. post collection urine samples were cultured directly in blood and MacConkey agar.

**Collection of Sheep urine samples:** Seventy five sheep urine samples were randomly collected from apparent healthy sheep of both sexes that were slaughtered in AL Shoela and AL end Rahmana abattoirs and College of Veterinary Medicine animal field in Baghdad during the of February to half of April 2013. The sheep bladder was brought from animals slaughtered to the laboratory in with a cooler box and after sterilization alcohol withdrawn of urine by a clean and sterile syringe, and after that put the urine in clean container and directly cultured on blood and MacConkey agar.

Isolation and biochemical confirmation were done in the laboratory of zoonotic unit College of Veterinary Medicine. All urine samples were incubated aerobically at 37 °C for 24-48 hrs on blood agar, MacConkey agar<sup>[13]</sup>, as well as special media (CefiximeTellurite-Sorbitol MacConkey agar

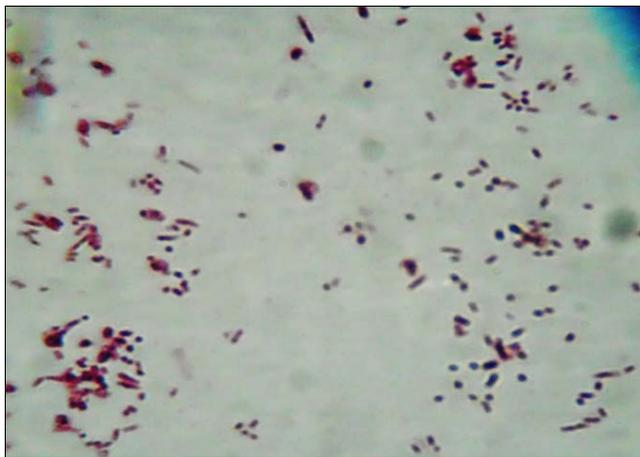
and Chrom agar *E. coli* O157)<sup>[14]</sup>. CHROM agar O157 used as a selective medium for *E. coli* O157. The isolates were identified by biochemical tests like IMViC (Indole, Methyl Red, Voges Proskaur and Citrate utilization) test<sup>[13]</sup>.

Twenty white mice from both gender, average age 7 to 8 weeks were randomly divided into two equal groups and treatment as following:

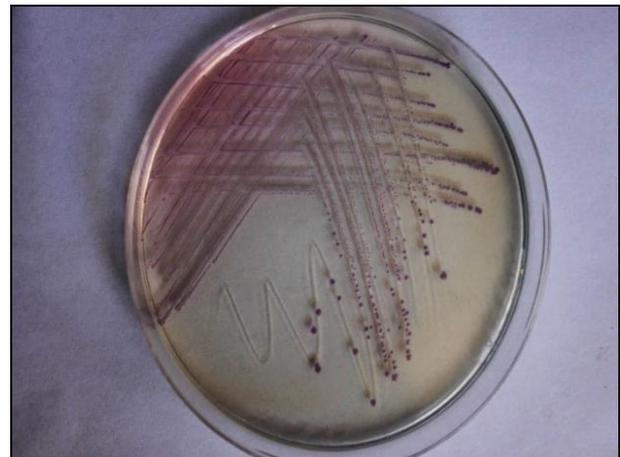
1<sup>st</sup> group was inoculated I/P with 0.2 ml of bacterial suspension containing (15×10<sup>8</sup> CFU/ml). 2<sup>nd</sup> group was inoculated I/P with 0.2 ml from PBS and was considered as a control negative group, all animals were sacrificed at 72hrs post infection and small pieces were taken from kidney, liver, lung and heart, and these samples were fixed in 10 percentage of neutral buffer formalin for 72hr. for histopathological examination<sup>[15]</sup>.

## Results and Discussion

Bacterial colonies appeared rosy pink color during 24-48 hr post-incubation on MacConkey media at 37 °C and a metallic green sheen color colonies on Eosin methylene blue agar. The bacterial isolates were gram-negative (Fig 1), motile bacilli According to microscopic appearance and biochemical tests (Table. 1), it was indicated that the bacterial isolates were *E. coli*. Smooth colorless colonies appeared on selective media Cefixime Tellurite-Sorbitol MacConkey agar and mauve color on Chrom agar O157 (Fig 2), it was indicated that the bacterial isolates were *E. coli* O157.



**Fig 1:** *E. coli* under microscopic appear as gram negative bacilli



**Fig 2:** *E. coli* O157:H7 mauve color

**Table 1:** Biochemical test positive and negative result

Test	Result
Indol	(+)ve
MR	(+)ve
VP	(-)ve
Citrate	(-)ve
Catalase	(+)ve
Urease	(-)ve
T.S.I	Acidic/Acidic

(+)ve= Positive, (-)ve =Negative, MR= Methyl red, VP= Voges proskauer, T.S.I = Triple Sugar Iron

The result of bacterial isolation and identification revealed that 16 (7%) isolates out of 228 human urine samples were

*E. coli* positive and 8 (3.50%) isolates out of 228 urine samples showed *E. coli* O157:H7 positive Table (2). These result was supported by results of Masoumeh and other in 2012, who recorded that among (12572) urine samples that were collected from children admitted to Mofid hospital, 378 isolates were *E. coli* and only 9 isolates were EHEC. Also the results showed that the number of serotype O157:H7 isolates were equal in young children (4 isolates) as compared to adult patients (4 isolates)<sup>[16]</sup>, These results supported the result that mentioned by Robert Koch Institute in (2011) who found that the *E. coli* O157:H7 was the main etiology of UTIs, mainly in young children and in elderly people<sup>[17]</sup>, but Paton and Paton in 1998 found that the children were more susceptible to *E. coli* O157:H7 infection

than adults [18]. in addition females showed higher percentage of serotype O157:H7 isolates (5.17%) as compared to males (1.78%). These observations indicated that the females were more susceptible than males to UTIs particularly with serotype O157:H7, such result was

coincidence with previous observation that the prevalence of UTIs were more in the women than men because the urethra in females is much shorter and closer to the anus as well as estrogen levels decrease with menopause, in addition due to loss of protective vaginal flora [19]

**Table 2:** The prevalence level of *E.coli*O157:H7 in human urine samples according to age and sex:

Average age	Total samples	+ve <i>E.coli</i>	%	+ve <i>O157:H7</i>	%	NO. of female's samples	+ve <i>O157:H7</i> in female's	NO. of male's samples	+ve <i>O157:H7</i> in male's
Less one year	11	-	-	-		5	-	6	-
1-14	51	5	9.80	4	7.8	27	3	24	1
15-25	20	1	5	-	-	11	-	9	-
26-36	17	2	11.7	-	-	10	-	7	-
37-47	28	1	3.5	-	-	20	-	8	-
48-58	30	2	6.6	2	6.6	16	2	4	-
59-69	20	5	25	2	10	11	1	9	1
70-80	51	-	-	-	-	16	-	35	-
Total	228	16	7	8	3.5	116	6	112	2

The *E. coli* that isolated from patients in this present study who showed urinary tracts problem such as cystitis, stone, these condition are considered a risk factor for UTIs, may be due to urinary catheterization these results were agreement with Dielubanza and Schaeffer in 2011 [19], also the urine samples were collected from patients suffering from diabetes that considered a risk factor for UTIs, these results were agreement with Nicolle in 2008 [20]. The present study showed that the bacterial strains isolated from children were not associated with renal failure and such result was in agreement with Jian and others in 2006 who explained that not all *E. coli* O157:H7 induced HUS [21], also Thorpe in 2004, reported that only approximately 2–15% of people with STEC infection developed HUS, and of which 10% die or had permanent renal failure [22]. In the current study, it was recorded that *E.coli* O157 isolates were found in adult patients whom suffering from renal failure, which indicated that this strain was an important etiological agent of renal

failure, these idea was supported result of Banatvala and others who believed that *E.coli*O157:H7 caused more than 80% of the STEC infection that led to HUS [23]. Renal failure occurred due to the damage of endothelial cells that became swollen and detached from the basement membrane in the renal glomerula that contributed to kidney damage and renal failure in addition to the development of fibrin thrombi that led to narrowing or occlusion of the capillary lumen for kidney [24].

In the animal samples the morphological appearance of bacterial colonies as well as the microscopical finding of bacterial isolates were similar to those described in humans samples. The result revealed that 13 (17.33%) out of (75) urine samples were *E. coli* O157:H7 positive isolates and also the current study showed that 11(18.03 %) out of (61) ram's urine samples were positive for *E.coli* O157:H7 isolates and 2 (14.28 %) out of (14) ewe's urine samples were positive for *E.coli* O157:H7. Table (3).

**Table 3:** The prevalence level of *E.coli*O157:H7 in male and female sheep's urine samples

sex	No. of samples	+ve <i>E.coli</i>	%	+ve serotype O157:H7	% of O157:H7 isolates
Males	61	16	26.2	11	18.03
females	14	5	35.7	2	14.28
Total	75	21	28	13	17.33

The present finding showed that a high percentage of *E.coli*O157:H7 isolates from sheep urine samples and these results indicated that the sheep served as an important natural source of humans infection by *E. coli* O157:H7 through the contamination of carcasses during slaughtering, particularly these animals were carried these organism without any symptoms, such result was in agreement with Hussein and others in 2000, who explained that sheep did not carry specific receptor of shiga-toxin and they might harbor *E. coli* O157:H7 without any symptoms and they shaded these organisms in their feces which may spread to humans [25], also in 1993 the Beutin and others mentioned that *E.coli* O157 lived naturally in the intestine of sheep and cattle [26], these animals were considered as a main natural reservoir that contaminated meat during slaughtering [27].

The present study revealed that there were a high percentage of bacterial isolates from urine samples of sheep as compared with results of Ebrahim in 2012, who found that the prevalence of *E.coli*O157 were 4.8% and 1.7% in the meat of sheep and goat respectively [28], in other study,

Heuveliak and others recorded that the prevalence of *E.coli* O157:H7, in faeces of slaughtered sheep was varied from 3.8% to 4.1% of animals depending on the age of the animal sampled [29]. The present study explained that the healthy sheep might carried these organisms in their urinary tract and considered as a natural reservoir of *E.coli*O157:H7 in Iraq, such evidence was in agreement with Rey and others 2003, who suggested that *E. coli*O157 might be present in healthy domestic ruminants such as sheep and goats and they isolated this organism in rate 1% out of 697 health lambs in Spain [30], also in Egypt, Hiko and others in 2008 recorded that the prevalence of *E.coli* O157:H7 in raw meat products was 2,5% and 2% in sheep and goats respectively [31].

The present study showed that the percentage of *E. coli* O157:H7 (18%) isolated from rams were higher than those (14.2%) isolated from ewes, these result could be attributed to the low number of urine samples that were collected from ewes or could be due to the contamination of ram's penis by fecal materials or contamination of female vagina during

fertility season. The present result was inconsistent with Gulhan who isolated *E.coli O157:H7* from 26% of ewes and 20% of lambs sampled at the Van slaughter house in the east of Turkey [32].

The present study showed that the percentage *E.coli O157:H7* isolated from human (3.50%) was lower than isolated from animal (17.33%), these results may indicate that the sheep are the main source of bacterial infection for human. Table (4).

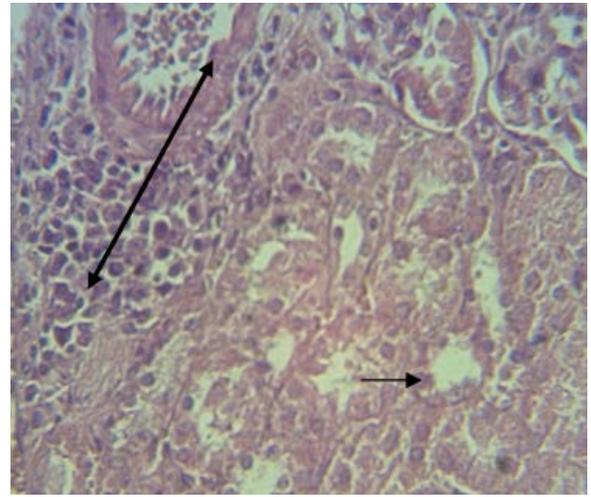
**Table 4:** The percentage of *E.coli O157:H7* isolated from human and sheep

	No. of samples	+ve serotype <i>O157:H7</i>	% of <i>O157:H7</i> isolates
Human	228	8	3.50
Animal	75	13	17.33

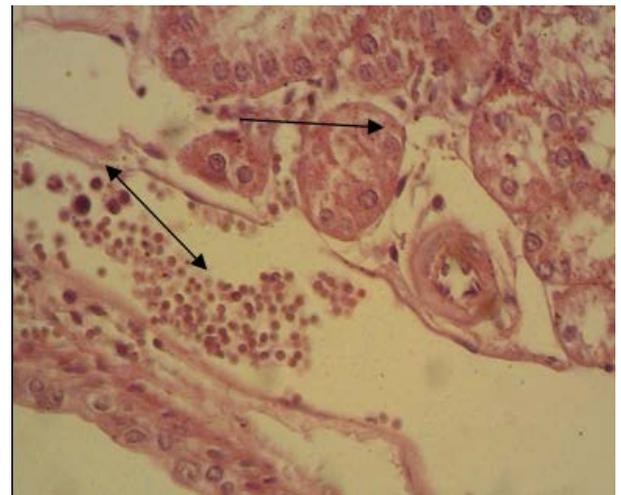
Histopathological examination at 72 hrs post infection of the kidney showed neutrophils and mononuclear cells aggregation around congested blood vessels with acute cellular degeneration of epithelial cells of renal tubules (Fig 3), in other animals, the kidney revealed neutrophils attachment to endothelial cells of congested bloodvessels in addition to enlargement of epithelial lining cells of renal tubules (Fig 4). The main lesions in the liver characterized by thrombus formation and fragmentation of RBCs in the portal blood vessels as well as single apoptotic cells (Fig 5), in other section, it was recorded focal necrosis of hepatocytes, vacuolar degeneration and granulomatous lesion in liver parenchyma (Fig 6). Microscopic section in lung expressed thrombosis, fragmented RBCs, emphysema and inflammatory cells particularly neutrophils and macrophages infiltration in the interstitial tissues (Fig 7) as well as in the bronchioles (Fig 8). The intestinal lesions characterized by neutrophils and mononuclear cells infiltration in the lamina propria of villi that become round and adhesion to each other (Fig 9). Also pathological lesion was seen in cardiac muscle fiber (Fig 10).

The present of thrombus may be due to effects of bacterial Stxs on platelet, these ideas were consistent with Stahl and others in 2006, who suggested that absorption of LPS of *E.coli O157:H7* in early stage of infection may lead to bind and direct activation of platelets or binding to endothelial cells [33].

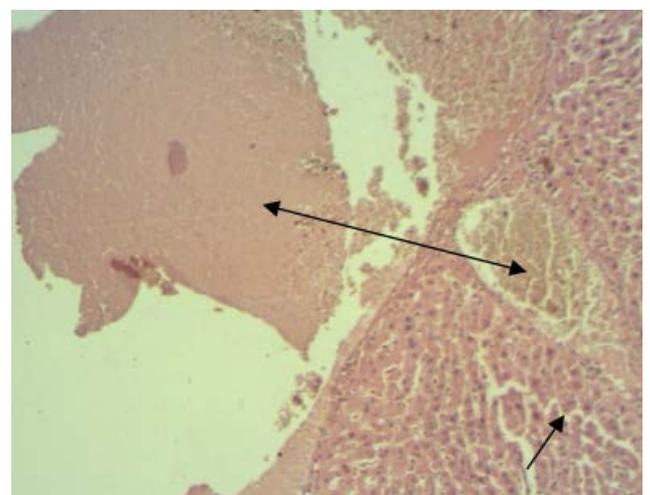
The pathological lesions in the examined organs may be due to cytotoxic effects of Stxs on vascular endothelial cells as present their receptors on these cells [34]. RBCs fragmentation in the present study may direct cytotoxic effects of Stxs on RBCs, these ideas were agreement with Alexis, who recorded RBC fragmentation in the kidney of rabbits infected with *E.coli O157:H7* [35]. The present study showed that most infected animals were died during 72hr post infection with necrosis and severe suppurative reaction in the liver and lung, these results may be indicated that these pathogens cause microvascular, occluded blood stream, ischemia and necrosis, these ideas were supported by observation of Jian-Gue, who reported fatally infected experimental animals due bacterial embolism in the kidney [36], severe suppurative reaction may be due to Stxs that stimulated phagocytic cells to produce large amount of pro inflammatory cytokines particularly IL 8 that active attractor factor for neutrophils [37], granulomatous lesion development in the current study may indicate that the body adapted to localized and control the infection and these pathogens became intracellular pathogens in order to avoid host immunity [38].



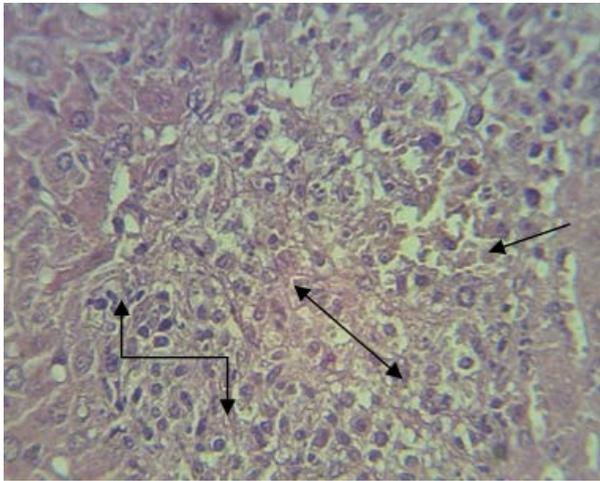
**Fig 3:** Histopathological section in kidney of animal at 72hr post-infection shows mononuclear cells aggregation around congestion blood vessels  $\longleftrightarrow$  and acute cellular degeneration of epithelial cells of renal tubules  $\rightarrow$  (H&E stain 40X).



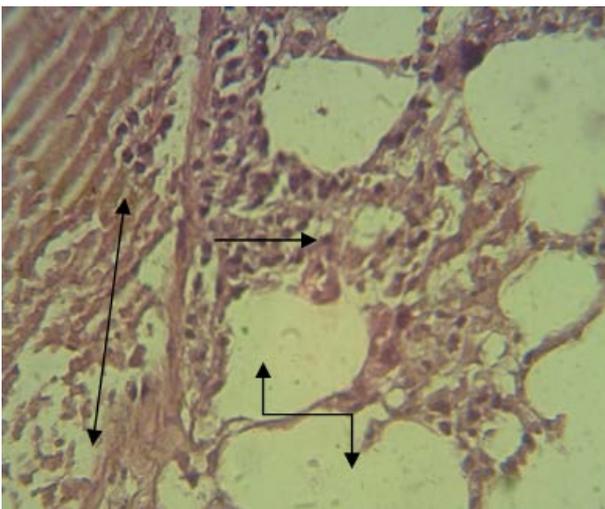
**Fig 4:** Histopathological section in the kidney liver of animal at 72hr post infection shows congested blood vessels with inflammatory cells particularly neutrophils and mononuclear cells in their lumen,  $\longleftrightarrow$  in addition to vacuolation of cytoplasm of enlargement epithelial lining cell of renal tubules  $\rightarrow$  (H&E stain 40X).



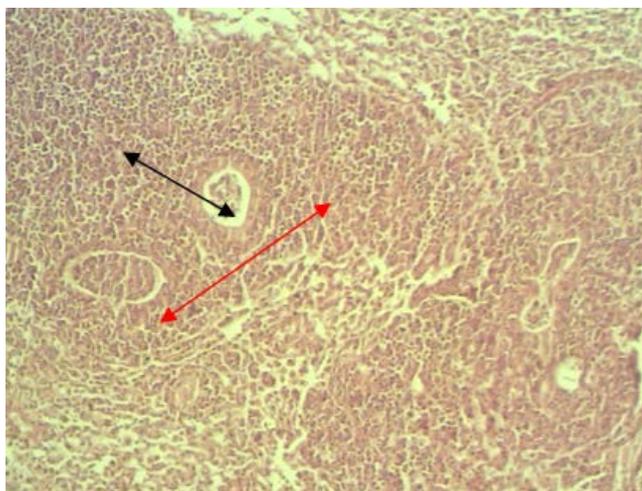
**Fig 5:** Histopathological section in the liver of animal at 72hr post-infection shows thrombus  $\longleftrightarrow$  and fragmentation of RBCs in the portal blood vessels as well as single apoptotic cells (H&E stain 40 X).



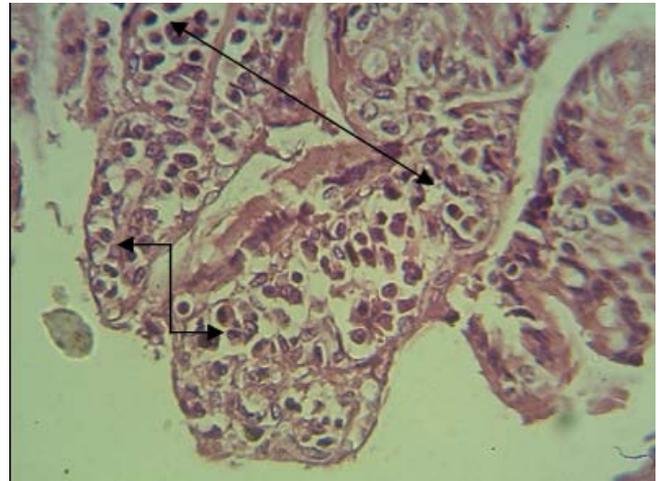
**Fig 6:** Histopathological section in the liver of animal at 72hrs post infection shows focal necrosis of hepatocytes, ↔ vacuolar degeneration, congested blood vessel → and granulomatous lesion ↙ in liver parenchyma (H& E stain 40X).



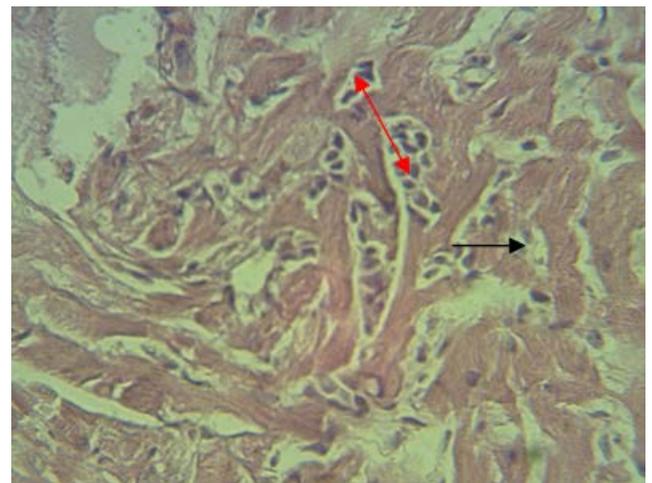
**Fig 7:** Histopathological section in lung of animal at 72hr post-infection shows thrombosis, ↔ fragmented RBCs, → emphysema ↙ and inflammatory cells particularly neutrophils and macrophages infiltration in the interstitial tissues (H&E stain 40X).



**Fig 8:** Histopathological section in the lung of animal at 72hr post-infection shows marked inflammatory cells particularly neutrophils ↔ and mononuclear cells infiltration in the interstitial tissues and in the lumen of bronchioles ↔ (H&E stain 40X).



**Fig 9:** Histopathological section in the intestine of animal at 72 hr post-infection shows neutrophils and mononuclear cells infiltration ↔ in the lamina propria of villi that become round and adhesion to each other (H&Estain 40X).



**Fig 10:** Histopathological section in the heart of animal at 72hrs post-infection shows inflammatory cells particularly neutrophils and ↔ macrophages between fragmented cardiac muscle fiber → (H&E stain 40X).

### Conclusion

In conclusions, *E. coli O157:H7* was the main etiology of UTIS in young children and elderly people. Humans females were more susceptible to the infection with *E. coli O157:H7* than males. Healthy sheep carried these organism in their urinary tract considered as a natural reservoir of *E. coli O157:H7*, and *E. coli O157:H7* can infected most internal organs.

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