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# Histopathological Study of *Escherichia coli O157:H7* Isolated from Children with Bloody Diarrhea in Mice

Mohamed B. Al-Jobory[1], Amina N. Al-Thwaini[2] and Laith M. Najeeb[1*]
[1] Microbiology Department, College of Science, University of Anbar, Iraq.
[2] Genetic Engineering Department, Institute of Genetic Engineering and Biotechnology for Post graduate Studies, University of Baghdad, Iraq.
[*] Corresponding Author: laith762004@yahoo.com

## Abstract

*Escherichia coli O157:H7* is well documented with Shiga toxin–producing serotypes of *E. coli*. Infection with this type of pathogenic bacteria may lead to hemorrhagic diarrhea and kidney failure. This study was carried out to detect the pathogenicity of *E. coli O157:H7* isolated from patients with bloody diarrhea. A total of 200 bloody diarrhea samples were collected from children of both sexes, with age between 3 to10 years in the period from beginning of September to the end of December 2016 in Al-Eskan Pediatrics Hospital and Children Safe Hospital (Baghdad/Iraq). The samples were cultured aerobically on enrichment and selective media, then the isolates were identified by Vitek 2 and they were confirmed by latex agglutination test. Eight isolates were diagnosed and identified as *Escherichia coli O157:H7*. The pathogenicity of the stool recovered isolates were study to recognize the alterations in some organs of mice after experimentally infected with this pathogen. Twelve mice provided by animal house of AL-RAZI center in Baghdad, divided into two groups each group consist of 6 mice. The first group injected with 0.2 ml of de-ionized water and left as control group, whereas the second group infected orally with 0.2 ml of $1.8x10^6$cfu/ml. The animals of two groups sacrificed 24-48 hours post infection. The histopathological examination of intestine for infected mice showed infilteration of inflammatory cells, focal lining epithelial stratification, with multiple layer basal lamina degeneration, then distention of villi appeared with increase inflammatory cells infiltration. The liver showed accumulation of lymphocyte, hemorrhage in central vein with sinuses expansion and, hepatocyte cells showed, degeneration, increase nuclear size and increase hyperchromasia with irregular chromatin distribution. The histopathological examinations of the control group were naïve.
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**Keywords:** *Escherichia coli O157:H7*, Bloody diarrhea, Histopathological study, Experimentally infection.

## Introduction

The *Escherichia coli* serotype *O157:H7* is a rare variety of *E. coli* that produces large quantities of one or more related, potent toxins that cause severe damage to the lining of the intestine, [1]. The bacteria do not invade mucosal cells as readily as *Shigella, Yersinia* or *Aeromonas*, which are armed with fimbrae, [2; 3]. It is an emerging pathogen that causes acute human gastroenteritis and hemorrhagic colitis [4] and a major causative agent of severe UTI in children [5]. *Enterohemorrhagic Escherichia coli* as a subgroup of Shiga-toxin (Stx)-producing *E. coli* (STEC) are characterized by certain serotypes that are frequently occurring in outbreaks and are associated with severe clinical illnesses such as hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS), [6].

There are several virulence factors that contribute to *E. coli* pathogenicity, such as pilli, enterotoxins (LT, ST), shiga-like toxins, endotoxin (lipopolysaccharide), hemolysin, aerobactin, cytonecrotizing factor, intimin, and biofilm formation [7:8]. Endotoxin lipopolysaccharide (LPS) and Vero toxin (VT) are released and absorbed cross the gut epithelium. LPS, VT and maybe other virulence factors lead to an increase in pro inflammatory cytokines from host cells and subsequent release of chemokines from inflammatory cells. The capability for VT in the intestine micro vascular endothelial cells is exacerbated by this pro inflammatory response, [9]. LPS and VT activate the

endothelial injuries and thrombocytes, activation of the coagulation cascade and inhibition of fibrinolysis lead to formation of thrombi that lead to block capillaries and small arterioles. The intestinal vascular damage leads to ischaemia and necrosis that together with the inflammatory response may be cause the bloody diarrhea, [10]. LPS and VT can also enter the blood stream where bound to thrombocytes (LPS) and polymorph nuclear leukocytes (VT), [11; 10].

Histopathological examination of tissue biopsies for the identification of infectious organisms is a very important diagnostic tool specifically, in clinical medicine; histo-pathology has contributed to advances in our understanding of disease leading directly to new and more effective treatment. Some new infection involving histopathology in their discovery, have also led to fresh diagnostic challenges. Histopathology has become increasingly common in recent decades, [12].

## Material and Methods

### Isolation and Identification

A total of 200 bloody diarrhea samples were collected from children of both sexes aged of 3 - 10 years. They were all suffered from bloody diarrhea, in the period extended from beginning of September to end of December 2016 in Al-Eskan pediatrics hospital and children safe hospital.

Full loop of each sample was cultured aerobically on enrichment and selective media, as modified tryptic soy broth (mTSB) supplemented with Vancomycin (4 mg/L) according to [13]. Then full loop was streaked onto CT-SMAC to seek sorbitol non-fermenting bacteria (colorless colonies) after that streaked onto Hicrome media and Eosin methylen blue (EMB) as differential Media. The culture incubated at 37°C for 24 h., [13] The isolates were identified by Vitek 2 and they were confirmed by latex agglutination test depend on, [3].

### Histopathological study

### Animals grouping:

Twelve mice of 22-25 grambody weight with age of 9-15 weeks were provided by animal house of Al-RAZI center in Baghdad, housed in previously sterilized and cleaned plastic cages. Mice were transferred to animal house of biotechnology research center/ Al-Nahrain University and stay for seven days before administration started. The animals divided into two groups of mice each group consist of 6 mice.

1- The first group used as control group was administrated with 0.2 ml of de-ionized water.
2- The second group infected orally with 0.2 ml of $1.8\times10^6$ cfu/ml.

The animals placed under observation and the symptoms daily recorded, then animals of two groups sacrifice after 24-48 hours.

### Histological examination

The intestine removed from control and infected mice and cut in long pieces, then fixed in 10% neutral buffered formalin. Further routine processing for hematoxylin2 and eosin staining was performed in the biotechnology research center at the Al-Nahrain University according to reference, [14].

## Results and Discussion

### Results:

### Isolation and Identification

Results revealed that 120 out of 200 stool sample were positive to *E. coli*. Only 8 isolates were diagnosed as *E. coli O157:H7* that appeared on SMAC as small, circular and colorless colonies with smoky center and (1-2) mm in diameter. This bacteria is identified by classical microbiological diagnostic procedures based on its inability to ferment sorbitol [15], which aids in the initial recognition of suspicious colonies isolated from bloody stools.

Culture on Hicrome media that appeared dark purple to magenta colored moiety colonies. Then, the isolates were identified by Vitek 2 and they were confirmed by latex agglutination test as mentioned by [16].

### Pathogencity investigation of *E coli O157: H7* isolates by experimental Infection of Mice (*invivo* study):

The symptoms appeared in all mice with morbidity (100 %) after 24-48 hrs. post infection. The clinical sings represented by bloody diarrhea, raised fur, shivering labored

breathing, Fig.(1). This finding also described by [17] and [18]. No signs of disease or mortality were observed in animals of control group that administrated with normal saline.

***Fig.(1) Mice of infected group with clinical signs as bloody diarrhea, raised fur during 24-48 hrs. post infection.***

The intestines of infected mice were examined macroscopically after two days post infection, it appeared congested and sticky in comparison with intestine of control group, Figs.(2) and (3). The liver macroscopically appeared congested after 2 days post infection in comparison with control.

***Fig.(2) Small intestines of mice appear congested were examined macroscopically after two days post infection.***

***Fig.(3) Small intestine of mice was appeared normal when examined macroscopically after administered with de-ionized water .***

**Histopathological Examination**

The pathogenicity of the stool recovered isolates were appeared in the infected animals as different lesions in different organs especially intestine and liver that attributed to virulence factors of this isolates which leads to the development of these histological alterations.

**Histological Examination of Intestine**

The macroscopic and microscopic examination of intestinal section of control group animals showed normal intestinal architecture, Fig.(4).

Histopathological examination of Intestine of experimentally infected mice showed that intestine was affected with variable lesions with infilteration of inflammatory cells, focal lining epithelial stratification and basal lamina degeneration, Fig.(5) at one day post infection. This agreement with [19] who found symptoms appeared on all mice after one day post infection with *E coli O157:H7* that administered orally and changes appeared in the intestine when they examined histopathology. On the other hand [20] recorded sticky, considerably empty, flimsy, of miceintestine when macroscopic examination after 1 day post infected with *E coli O157:H7*.

At 2th day post infection, intestine of infected mice showed distention of villi with increase inflammatory cell infiltration, Fig.(6) multiple areas show stratification with mixed inflammatory cell (lymphocyte, plasma and eosinophil cell). Stratification of lining epithelial cell with destruction of basal lamina, reactive epithelial atypia secondary to inflammation with multiple layer, Fig.(7).

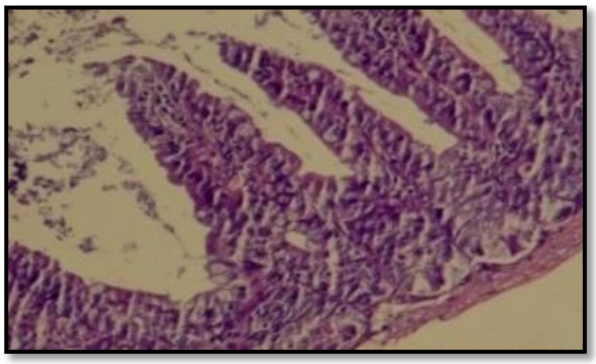

***Fig.(4) Histopathological section of intestine of control group mice shows normal histology of intestine (H&E stain 10X).***

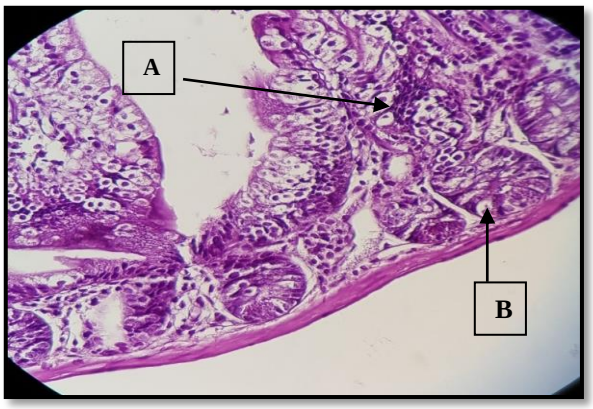


***Fig.(5) Histopathological section in the intestine of infected mice at one day post infection shows focal lining epithelial stratification (A), basal lamina degeneration (B) (H&E stain 40X).***

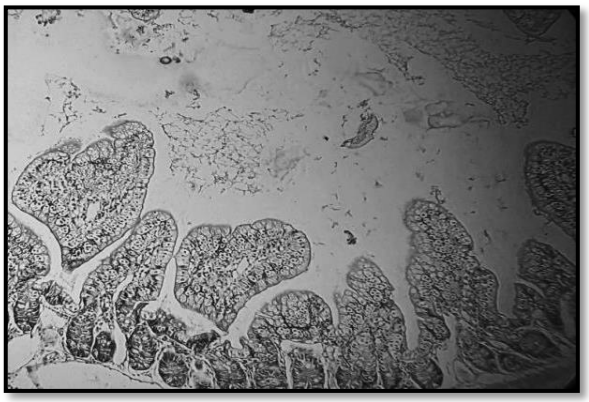

***Fig.(6) Histopathological section in the intestine of infected mice at day 2nd post infection showdistention of villi (H&E stain 40X).***

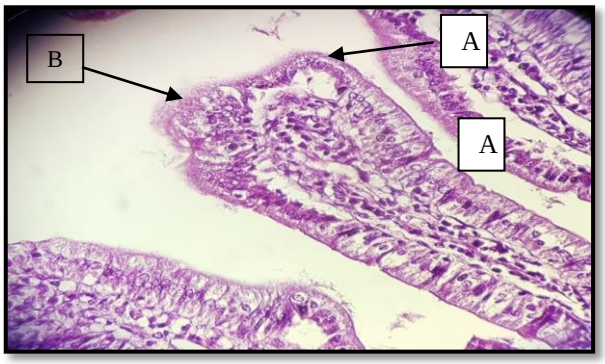


***Fig.(7) Histopathological section in the intestine of infected mice at day 2nd post infection shwos reactive epithelial atypia secondary to inflammation (A) with multiple layer (B) (H&E stain 40X).***

**Histological Examination of Liver**

Microscopic examination of liver section of mice in control groups presented in Fig.(8).

At second day post infection, liver sections showed accumulation of lymphocyte, hemorrhage in central vein with sinuses and, Hepatocyte cells appeared, degenerative cells with sinuses expansion, Figs.(9) and (10). increase nuclear size and increase hyperchromasia with irregular chromatin distribution), with abnormal shape of some nuclei and some have prominent nucleoli. Increase N/C ratio with area of necrosis, Fig.(11). The congestion and hemorrhage noted in this study accounted for activation of endothelial cells by inflammatory mediators.

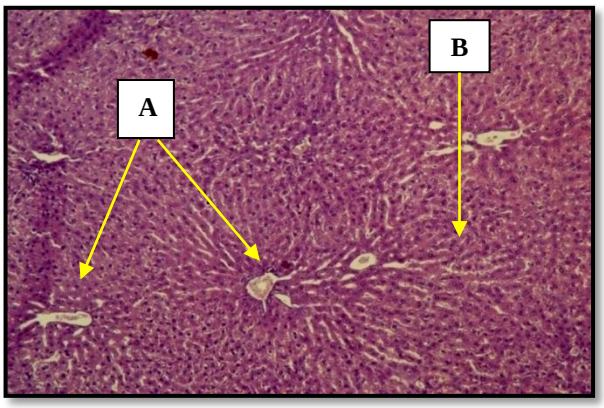


***Fig.(8) Histopathological section in liver of control group mice, shows normal liver histology, (A) Normal centrilobular area, (B) Normal hepatocyte (H&E 10X).***

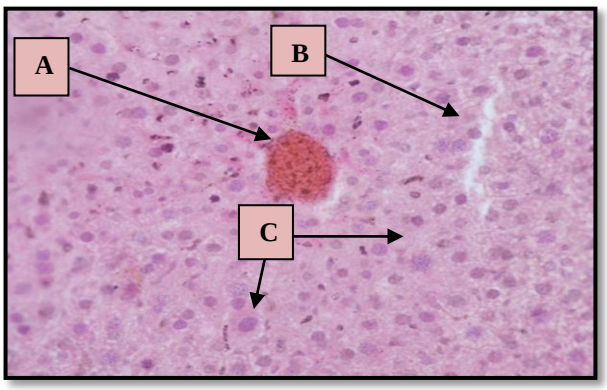


***Fig.(9) Histopathological section in liver of infected mice at second day post infection shows hemorrhage in central vein(A) with sinuses (B) and Hepatocyte cells (C) (H&E stain 40X).***

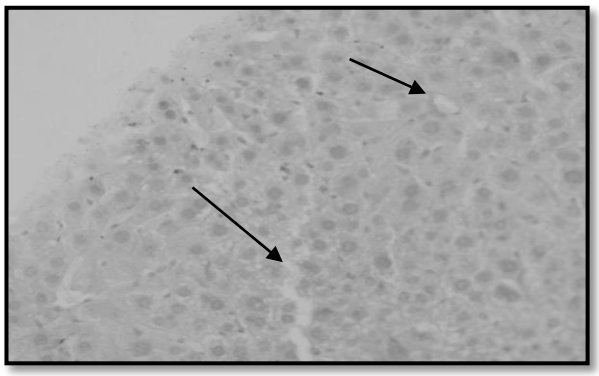

***Fig.(10) Histopathological section in liver of infected mice at second day post infection shows degeneration cells and sinuses expansion (H&E stain 40X).***

***Fig. (11) Histopathological section in liver of infected mice at second day post infection shows area of necrosis (H&Estain 40X).***

**Histological Examination of kidney**

Normal looking tubules and glumeruli, Fig.(12).

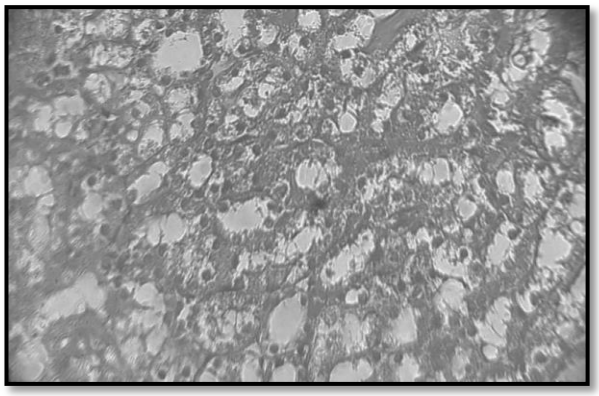

***Fig.(12) Histopathological section in kidney of mice shows Normal looking tubules and glumeruli figure (H&Estain 40X).***

In this study, intestinal sections shwoed lymphocyte aggregation below the mucosa and distention of villi with increase inflammatory cell infiltration. multiple areas shows stratification with mixed inflammatory cell (lymphocyte, plasma and eosinophil cell) and destruction of basal lamina. Most of these finding are described by other researchers as [21] and [22].

The intestinal histopathological changes observed in this study could in part be explained by the effect of endotoxin (lipopolysaccharides), the destruction of epithelial layer of mucosal glands and infiltration of inflammatory cells observed in this study also mentioned by [23] who inject lipopolysaccharides of E coli O157:H7 intra peritoneally into mice.

Histopathological changes of liver observed in this study were similar to that mention by [24] who found congestion in liver of mice that infected by E coli O157:H7 that isolated from rectal swab of pet dogs and cats in west Bengal.

[25] showed necrosis in liver of pigeons after infected by E coli O157:H7 in India. Hepatic paranchymal necrosis has been reported a consequence of tissue anoxia resulting from vasoconstriction or by extracellular bacterial toxin [26].

## Conclusions

Current study revealed distinguish percentage of E. coli O157: H7 isolation recovered from bloody diarrhea and urinary tract infections in children. The results of experimental infection in mice (in vivo) indicated the pathogenicity of E. coli O157:H7 isolate through sever lesions scattered in intestine and liver.

## References

[1] Kaistha, S.D., & Sinha, R. “Homology modeling of phosphoryl thymidine kinase of enterohemorrhagic Escherichia coli O157: H7”, 3(6): 240-24, (2009).

[2] Nataro, J. P. and Kaper, J. B. “Diarrheagenic Escherichia coli”. Clin. Microbiol. Rev. 11:142-201 (1998).

[3] Al-Ayash M A. “Detection of E. coli O157:H7 Bacteria from Raw Cow's Milk and Soft Arab Chesses by Using Molecular and Conventional Methods”. M.Sc. thesis;

Genetic Engineering and Biotechnology: University of Baghdad, (2016).

[4] Rabinovitz, B.C; Gerhardt, E; Tironi Farinati, C; Abdala, A; Galarza, R; Vilte, D.A; Ibarra, C; Cataldi, A; Mercado, E.C. "Vaccination of pregnant cows with EspA, EspB, Y-intimin, and Shiga toxin 2 proteins from Escherichia coli O157:H7 induces high levels of specific colostral antibodies that are transferred to newborn calves". J Dairy Sci. 95:3318-3326, (2012).

[5] Navidinia, M; Karimi, A; Rahbar, M; Fallah, F; Ahsani, R.R; Malekan, M.A, Jahromi, M.H and GholineJad, Z. "Study Prevalence of Verotoxigenic E.coli Isolated from Urinary Tract Infections (UTIs) in an Iranian Children Hospital". Open Microbiol J. 6: 1-4, (2012).

[6] Levine, M. M. "Escherichia coli that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and entero adherent". J. Infect. Di, 55: 377-389, (1987).

[7] Blanco, M.; Blanco, J.E.; Blanco, J.; Gonzalez, E.A.; Mora, A.; Prado, C.; Fernandez, L.; Rio, M.; Ramos, J. and Alonso, M.P. Prevalence and characteristics of Escherichia coli serotype O157:H7and other verotoxin-producing E. coli in healthy cattle. Epidemiol Infect 117:251-257, (1996).

[8] Wong, C.S; Mooney, J.C; Brandt, J.R; Staples, A.O; Jelacic, S; Boster, D.R; Watkins, S.L; Tarr, P.I. Risk factors for the hemolytic uremic syndrome in children infected with Escherichia coli O157:H7: a multivariable analysis. Clin Infect Dis. 2012 Jul; 55(1):33-41, (2012).

[9] Palermo, M.S.; Exeni, R.A. and Fernandez, G.C. Hemolytic uremic syndrome: pathogenesis and update of interventions. Expert Rev Anti Infect Ther 7: 697-707, (2009).

[10] Baqer A A (2013). A comparative study of methods to detect E.coliO157:H7 isolated from food and patients in Baghdad. M.Sc thesis; Genetic Engineering and Biotechnology: University of Baghdad, (2013).

[11] Brigotti, M.; Carnicelli, D.; Ravanelli, E.; Barbieri, S.; Ricci, F.; Bontadini, A.; Tozzi, A.E.; Scavia, G.; Caprioli, A. and Tazzari, P.L. Interactions between Shiga toxinsand human polymorphonuclear leukocytes. J Leukoc Biol 84: 1019-1027, (2008).

[12] James CE. More than meets the eye: the enhancing face of histopathology; Histopathology 70, 4-9. DOI: 10.1111/his. 13047, (2017).

[13] Sanderson, MW; Gay, JM; Hancock, DD; Gay, CC; Fox, LK and Besser, T.E. Sensitivity of bacteriologic culture for detection of Escherichia coli O157:H7inbovine feces. J. Clin. Microbiol., 33: 2616-2619, (1995).

[14] Muslim Dhaher Musa. Molecular Characterization and Pathological study of Aeromonas species Isolated from Animal feces and Water in Thi-Qar Province, (2017).

[15] Lee and choi. Isolation and characteristics of sorbitol-fermenting Escherichia coli O157 strains from cattle: Research Gate; DOI: 10.1016, (2006).

[16] Al-Jobori M B, Althwani A N and Najeeb L M. Investigation of E coli O157:H7 strain and study the effect of some plant abstract and nanoparticles on pathogencity. Ph.D thesis, (2017).

[17] Ko, W.C., Chiang, S.-R., Yan, J.-J., & Chuang, Y.C. Comparative pathogenicity of bacteraemic isolates of Aeromonas hydrophila and Klebsiella pneumonia. Clin Microbiol Infect, 11:553–558, (2005).

[18] Musa M. D. Molecular Characterization and Pathological study of Aeromonas species Isolated from Animal feces and Water in Thi-Qar Province, (2017).

[19] Jennifer L Roxas, Athanasia Koutsouris, Amy Bellmeyer, Samuel Tesfay, Sandhya Royan, Kanakeshwari Falzari, Antoneicka Harris, Hao Cheng, Ki Jong Rhee and Gail Hecht. Enterohemorrhagic E. coli alters murine intestinal epithelial tight junction protein expression and barrier function in a Shiga toxin independent manner 90, 1152-1168, (2010).

[20] Romina J. Fernandez-Brando, Elizabeth Miliwebsky, Marı´a Pilar Mejı´as, Ariela Baschkier, Cecilia A. Panek, Marı´a Jimena Abrey-Recalde, Gabriel Cabrera, Marı´a Victoria Ramos, Marta Rivas and Marina S. Palermo. Shiga toxin-producing Escherichia coli O157:H7 shows an

increased pathogenicity in mice after the passage through the gastrointestinal tract of the same host Journal of Medical Microbiology, 61, 852-859, (2012).

[21] Mansour, A.M.A., Zaki, H.M., Hassan, N.A., & El Nashar, N.A.M.. Phenotyping, virulence characteristics of Aeromonas species and effects of essential plant oils as antimicrobial agents against pathogenic isolates from different sources. American Journal of Infectious Diseases, 10(1), 21-35, (2014).

[22] Longa-Briceno, A.; Pena-Contreras, Z.; Dávila-Vera, D.; Mendoza-Briceño, R.V., & Palacios-Pru, E., Experimental toxicity of Aeromonas spp. in mouse small intestine: Ultrastructural aspect. Nterciencia, 33(6):457-460, (2008).

[23] Milan Chromek, Ida Arvidsson, Diana Karpman. The Antimicrobial Peptide Cathelicidin Protects Mice from Escherichia coli O157:H7-Mediated Disease, (2012).

[24] Das S., C. Guha, U. Biswas, A. Chatterjee, P. S. Jana, S.K. Biswas, L. Sharma and A. Singha. Isolation, identification, pathotyping and antibiogram of Echerichia coli from rectal swab of pet dogs and cats. Animal and Medical Research vol.2, isso-p26-33, (2012).

[25] Pankaj Dutta, Manoj Kumar Borah, Ratul Sarmah, Rakhi Gangil. Isolation, histopathology and antibiogram of Escherichia coli from pigeons (Columba livia), DOI: 10.5455/vetworld.2013.91-94, (2013).

[26] Brenden, R. A., & Huizingat, H. W.. Pathophysiology of experimental Aeromonas hydrophila infection in mice. Journal of Medical Microbiology, 21:311-317, (1986).