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Clinical and pathological study of rabbits experimentally infected with *E. coli* O157: H7 isolated from human

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Abstract
This study was conducted to investigate the clinico-pathological changes in rabbits after infected experimentally with *E. coli* O157: H7 isolated from human. This study was carried out on sixty domestic rabbits of both sexes in two experiments, in the first experiment, 20 rabbits were used to estimate the infective dose of *E. coli* O157: H7 and the remaining (40) rabbit were infected with the estimated infective dose of *E. coli* O157:H7**.** The results showed that the infective dose (ID) of *E. coli*O157: H7was (5×10^{-8}C.F.U/ml) which appeared the clinical signs of *E. coli* without mortality in this group. Post inoculation, all rabbits were examined clinically, and postmortem changes (macro and microscopic) were also examined. With isolation of the organism from different organs at 24,48,72,96, 120, 144 and 168 hours post infection. The body temperature, heart and respiratory rates were elevated accompanied with diarrhea, depression and loss of appetite. The lesion included the internal viscera of rabbits which show enlargement and congestion. The intestine revealed proliferation in the payer patch and infiltration of mononuclear cell. Other organ like brain, spleen, Lung, liver and kidney revealed congestion of blood vessels and infiltration of mononuclear cell.

Keywords: *E. coli* O157: H7, pathological study, human, rabbits

Introduction
Escherichia coli important zoonotic agents transmitted from cow to human especially veterinarian and persons who lived with these animals in the same place (Dolejska *et al.* 2011) [7]. *E. coli* are regarded as the normal bowel flora that is commonly found in the lower intestine of warm-blooded organisms including humans and other animals and can be pathogenic both within and outside of the GI tract (Singleton, 1999; Harvey *et al*., 2013) [30, 12]. Katani *et al.* (2015) [15] and Bonardi *et al.* (2015) [3] reported that the Shiga toxin-producing *Escherichia coli* O157:H7 as a cause of food borne infections and ruminants were regarded as the natural reservoir for these toxins producing in *E. coli* (STEC) especially serogroups O157.Enterohemorrhagic*Escherichia coli* (EHEC) is the most important cause of the recent outbreaks of diarrhea, hemolytic-uremic syndrome (HUS) as well as hemorrhagic colitis worldwide (Kwon and Cho, 2015) [18].
E. coli O157:H7 serotype is worldwide zoonotic and major foodborne pathogens responsible for the majority of severe cases of human enterohemorrhagic *Escherichia coli* (EHEC) disease (Dulo, 2014; Lime *et al*., 2010) [8, 21]. A study of Garcia *et al.* (2002) [9] on naturally infected white rabbit by EHEC was performed to demonstrate the pathogenicity, they found that presence of erosive and necrotizing enterocolitis with adherent bacterial rods, tubular necrosis proliferative, glomerulonephritis and fibrin thrombi within small vessels and capillaries on histopathological slides, also they found that there were increasing in creatinine and BUN levels which indication for kidney damage. The aim of this study was to investigate the clinico-pathological changes in the Rabbits inoculated with a slime producing *Escherichia coli* O157:H7.

Materials and methods
Animals
A total number of sixty domestic rabbits of both sexes with an age range (8–12) months old and weighing between (1500 and 1900)gm, were obtained from local markets, during the experimental period, They were housed in clean metal cages at room temperature about (22 ± 3, at an experimental animal house in college of Veterinary medicine/ Diyala University, the rabbits were fed commercial pellet, green food (alpha alpha) and water was supplied.

They had free access to water and food and were exposed to artificial light for (12 hours) per day and Animals were adapted for 10 days before starting the experiment, then used in two experiments:

First experiment (I): twenty rabbit were used in this experiment to estimate the infective dose (ID) of the *E. coli* (O157:H7).

Second experiment (II): forty rabbit were used in this experiment to infect the rabbit with the estimated infective dose of *E. coli* (O157:H7).

Bacteria

The *E. coli* O157:H7 which was isolated from child with severe diarrhea and the diagnosis was confirmed according to (Chow *et al.*, 2006) [4].

Preparation of *E. coli* O157:H7 infective dose

To prepare the bacterial suspension of *E. coli* (O157:H7), the bacteria inoculated in 10 ml of brain heart infusion broth at 37 °C for 18 hours, then centrifuged in cold centrifuge at 8000 rpm for 15 minutes, the sediment washed three times with phosphate buffer saline (PBS) (pH=7.2).The viable plate count of the bacteria in each diluent was made according to (Miles and Misra, 1938) [24] and selected the diluent which represents The infective dose (ID) and was estimated by choosing the group of rabbits which showed clinical signs with no mortality after appearance of clinical signs, two rabbits were killed for necropsy finding and isolate *E. coli O157:H7* from internal organs.

Post inoculation, all the rabbits were clinically examined daily for appearances of

clinical signs including temperature, pulse and respiratory rates and other signs.

Histopathlogical examination

Specimens were taken from internal organs including: kidney, intestine, liver, spleen, lung and brain at (24hrs, 48hrs, 72hrs, 96hrs, 120hrs, 144hrs and 168hrs) from infected and control groups, one centimeter cubes from different organ were taken and fixed in 10% buffered formalin, dehydrated in ascending concentrations of ethanol and cleared inxylene followed by embedding in paraffin. Sections (5µm) were prepared from eacht issue block and stained with Hematoxylin-Eosin stain (H&E) for histological examination. (Luna and Lee, 1968) [22].

Ethics Approval

This study was approved by the ethical and research committee of Veterinary

Medicine College/University of Diyala.

Results and Discussion

Clinical and bacteriological finding

All rabbits in the experiment were clinically healthy before inoculation, and no abnormal clinical findings were present. Post inoculation (after 24 hr.)the rabbits in the infected group showed emaciation, weight loss and lethargy compared with the pre inoculation weight, and lack of movement around the cage when manually stimulated by toe or ear touch or pinch, also showed cloudy or milky urine and frequent urination, dyspnea, and diarrhea occurred only in 6 infected rabbits after 2 day post infection. And then, were shown high increase in body temperature (40.15± 0.17°C). Pulse rate (170.67±1.23beat/min) and respiration rate (70.0 ± 0.73breath/min).In comparison with other studies in different animals, many researchers noted different clinical signs, Dean-Nystrom *et al*. (2008) [6] recorded the clinical signs of *E. coli* O157:H7 in experimentally Inoculated Weaned calves, some calves showed watery diarrhea at days 3 and 4 (the feces of one calf contained blood at day 4).The inoculated mice with *E. coli* O157 exhibited the signs of anorexia, diarrhea, increased in the respiration rate, severe dehydrated and recumbent till death in 1-3 days post infection (Yousif *et al*., 2013) [36]. Also, the current results showed that most clinical signs were resolved after 5 days post infection. This result is in agreement with Besser *et al*. (1999) [2] who found that infection with *E. coli* O157:H7 usually resolved after one week.

Re-isolation of the bacteria from the internal organs

The bacterial spreading and disappearance in different organs for 7 days from starting the experiment. In intestine and liver *E. coli* O157:H7was re-isolated from the intestine after 24,48,72,96,120, 144, and 168 hours post infection from most rabbits which infected orally with infective dose. The bacterial re-isolationin Spleen of infected group recorded only in these times 96,120 and 144hours post infection. Inheart blood recorded only after 72 and 96 hours post infection, in Kidneyafter 72h,,120h 144h and 168 hours post infection. While in lung the bacteria was re-isolated only in two rabbits at 96 and 144 hours post infection.Inbrainthe *E. coli* O157:H7 reported only in two rabbits at 96,120 hours post infection during the first week.

Re-Isolation of *E. coli* O157:H7 bacteria from the large intestine of the rabbits at 24hrs post infection may indicate that these bacterial isolates were highly virulent and had the ability to destroy the natural defense barrier of the intestinal host and they colonized in the intestinal epithelial cells, these facts agree with the observation of Large *et al*. (2005) [19] who demonstrated that the main features of *E. coli* O157 infection are resistance the gastric acidity and important normal defense of gastrointestinal tract, therefore this pathogen can survive in acid food including apple juice and salami which considered one important source of infection for human (Tilden *et al*., 1996) [33].

E. coli O157:H7 can survive and colonize the intestine and disseminate to main target organs especially the kidney and other organs like liver post secret the toxins during these periods through overcoming the cellular and humoral innate immune system. This observation is in agreement with Lathem *et al*. (2002) [20] who found that *E. coli* produced StcE that play an important role in regulating classical complement pathway via destroying serpinC1 esterase which are encoded on PO17 plasmid.

Histopathological studies

1-macroscopicaly:-The infected group showed diarrhea and there is emaciation figure (1). The infected rabbits which killed at 24 hours show slight significant gross lesion but all rabbits at 48, 72, 96,120,144 and 168 hours showed intestinal alterations of marked catarrhal entero colitis, The intestine were flaccid, thin-walled and filled by clear to yellow watery contents with variable amounts of mucus. Internal viscera of rabbits showed enlargement and congestion figure (2). Kidney enlargement, swollen and congestedas manifestation of toxemia in the infected rabbits figure (3). Liver enlargement, fragile and extend fibrosis on surface of liver figure (4).Heart and lung of infected rabbits were congested figure (5). Intestine were engorged figure (6).

Fig 1: infected rabbits show diarrhea and emaciation.

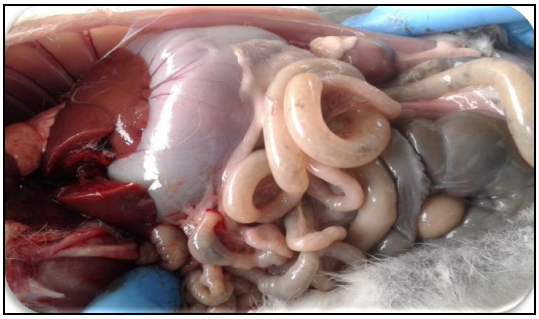

Fig 2: Macrscopical section of internalorganin rabbits show enlargement and congestion in the viscera.

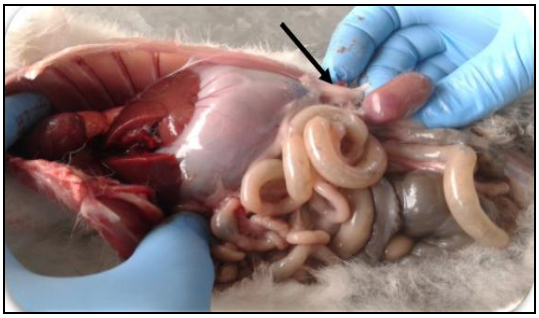

Fig 3: Macroscopicalsection of kidney show enlargement in the infected rabbits

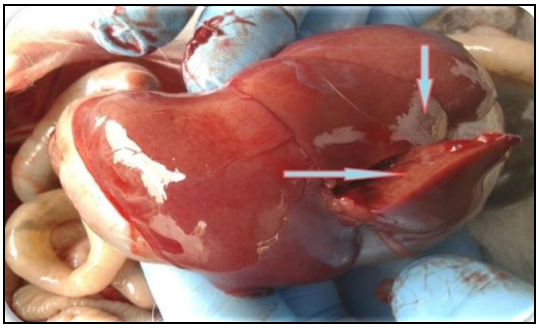

Fig 4: Macrscopical section of liver appear enlargement and fragile and extend fibrosis on its surface.

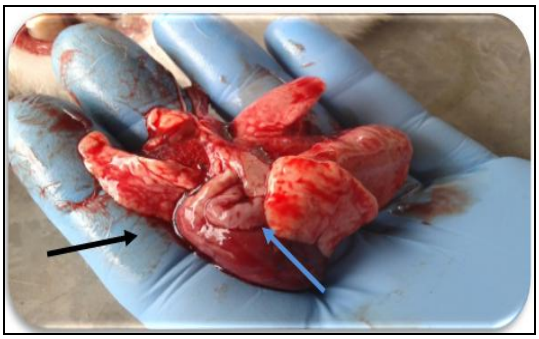

Fig 5: Macrscopical section of heart and lung of infected rabbits show congestion of lung.

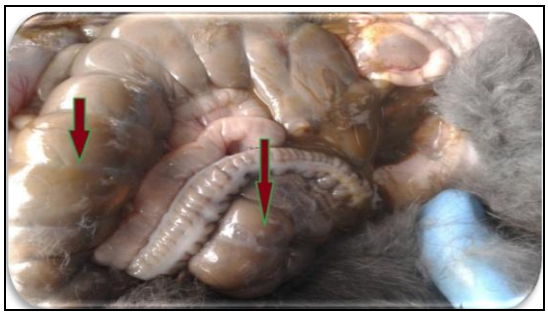

Fig 6: Macrscopical section of intestine there is engorged and there is diarrhea.

Microscopically:-The intestine of the control group showed normal payer patch without any pathological lesion (figure 7).but in the infected animals the intestine revealed proliferation and sloughing in (epithelial lining) in the payer patch and infiltration of mononuclear cell. (figure 8). in brain there is hyperemia and congestion of blood vessels and infiltration of mononuclear cell (figure 9).

In the spleen showed proliferation in the white bulb and red bulb and there is amyloid degeneration (there is infiltration of homogenous protein Material) (figure 10).The Lung showed emphysema and thickening between interstitial septa of the wall &infiltration of mononuclear cell and congestion of blood vessels (figure 11). In liver there is coagulative necrosis and loss of cellularity details due to karryo riches and karryolysis of nuclei's of the hepatocytes (figure 12).in the kidney there is cloudy swelling and congestion of blood vessels and infiltration of mononuclear cell (mesengeal cell) (figure 13). In the heart mild infiltration of inflammatory cell like mononuclear cell in striated muscle (figure 14).

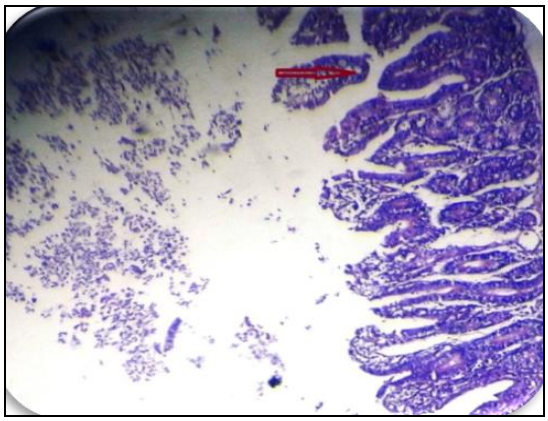

Fig 7: The intestine of control group showed normal payer patch without any pathological lesion.(H&E stain 20x).

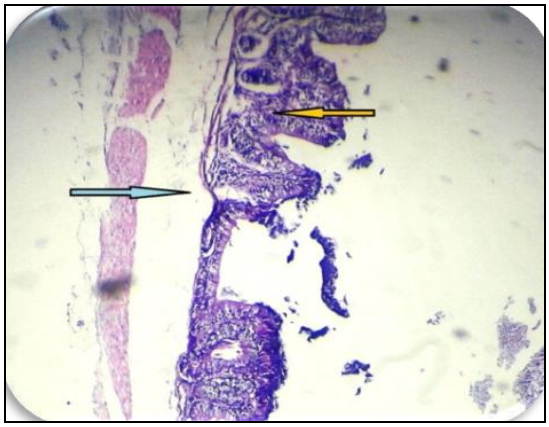

Fig 8: Intestine there is sloughing in(epithelial lining) the payers patch and infiltration of mononuclear cell (H&E stain 20 x).

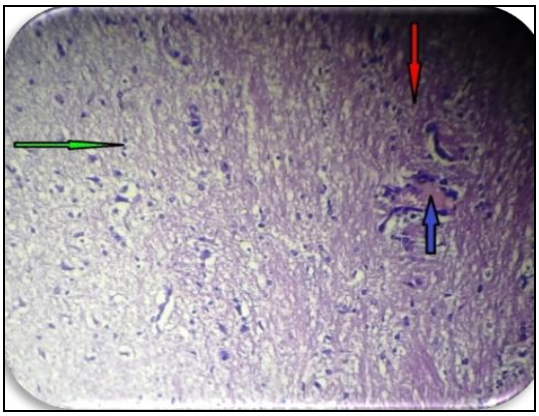

Fig 9: Brain showed hyperemia and congestion of blood vessels and infiltration of mononuclear cell (H&E stain 20 x).

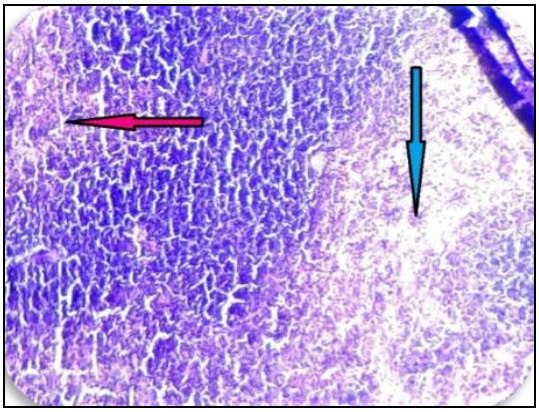

Fig 10: The Spleen show proliferation in the white bulb and red bulb and there is amyloid degeneration (there is infiltration of homogenous protein Material) (H&E stain 20 x).

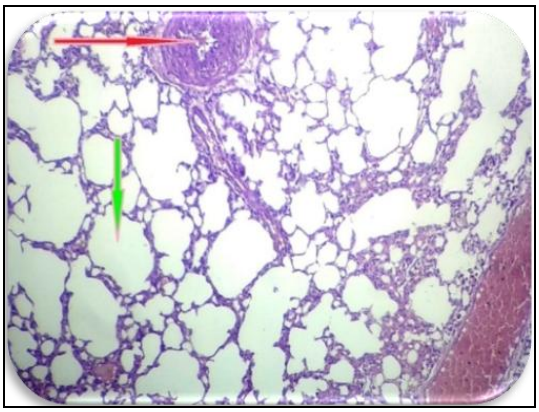

Fig 11: The Lung revealed emphysema and congestion of blood vessels (H&E stain 20 x).

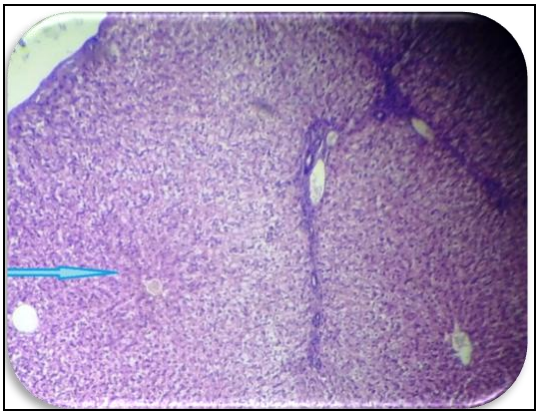

Fig 12: liver showcoagulative necrosis and loss of cellularity details (H&E stain 20 x).

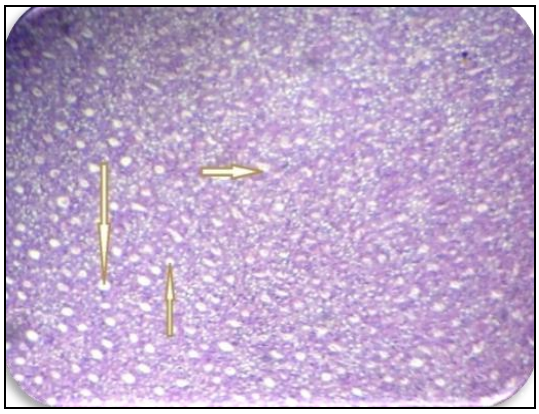

Fig 13: Kidney revealed cloudy swelling and congestion of blood vessels and infiltration of mononuclear cell (mesengeal cell) (H&E stain 20 x).

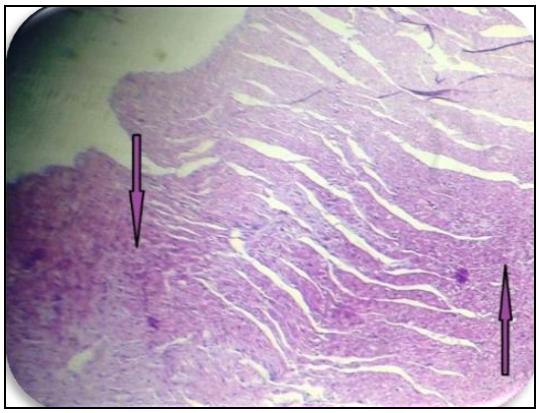

Fig 14: Heart with mild infiltration of inflammatory cell like mononuclear cell in striated muscle (H&E stain 20 x).

Discussion

In current study the infected group shows diarrhea and there is emaciation its agree with (Joseph, (1993); Christian, R. and chris, W. 2002) [14, 5] they revealed that the septic shock can manifested by sever congestion, edema, hemorrhage, and neutrophils infiltrates in the internal organs of mice. Also reported the Septic shock and septicemia occurred as a result of lipopolysaccharides which is amajor constituent of gram negative bacteria (EHEC in this study) which lead to diarrhea and enteritis in mice (Joseph, E. P. 1993; Christian, R. and chris, W. 2002) [14, 5]. Our study was compatible with the study made by Suzana D. *et al.* (2005) [31]; Mundy *et al.* (2003) [25] they found the enteritis accompanied by goblet cells proliferation, diarrhea, mucinsecretion, kidney and liver enlargement and fragile and extend fibrosis to the surface of liver. The our result agree with Beery *et al.* (1984) [1]; Keenan *et al.* (1986) [16]; Padhye *et al.* (1987) [26]. all those authors reported The changes in kidneys, liver, spleen, lymph nodes, heart and lung of infected rabbits by *E. coli O157:H7*, intestine were engorged with diarrhea. the study of Iron side *et al.*(1970) [13]; Kessner *et al.*(1962) [17] show that liver was firm and fragile and there is extend fibrous on the surface of liverdue to *E. coli* infection. Tesh *et al.* (1994) [32] who found that prion flammatory cytokines play roles in overproduction of Stxs Gb3 receptors on vascular endothelial cells. Guessous *et al.* (2005) [11] didn't show any pathological lesionin the intestine microscopically of control group, but in the infected animals the intestine show proliferation in the payer patch and infiltration mononuclear cell, there is sloughing in (epithelial lining) the payers patch and infiltration of mononuclear cell. Zhou *et al.* (2003) [37] who reported that A/E pathogens induced cellular damage characterized by cellular necrosis,

disruption of the epithelium and occasionally bleeding, in addition may be due to occlusion of vascular sinusoids by thrombus and inflammatory cells. In the brain there infiltration of mononuclear cell (glia cell) and there is congestion of blood vessels, this condition is called a double population which are seen in other bacteria such as *Listeria*. *monocytogenes and Salmonella*. Gentry-Week *et al.*,(1999) [10]: Riley *et al*. (1983) [28]; Riley, (1987) [29] those show proliferation in the spleen (include white bulb and red bulb) and there is amyloid degeneration (there is infiltration of homogenous protein Material),focal necrosis in the spleen, also congestion, edema and accumulation of fibrin in the liver, in lung there is emphysema and congestion of blood vessels and infiltration of mononuclear cell (alveolar macrophages) and thickening in the interstiasal septa. In Liver there is coagulative necrosis and loss of cellularity details due to karryolyesis of nuclei's of the hepatocytes these agree with (Wadolkowski *et al.,* 1990) [34]. Xu and Qi. (1988) [35] reported that Systemic syndromes such as HUS result from the action of toxin of bacteria, The kidney undergo hydropic degeneration and infiltration of mononuclear cell (mesengealcell) and there is congestion of blood vessel and also the kidney show cloudy swelling and congestion of blood vessels. The our result also agrees with Tesh *et al.*, (1993) [23] found that SLT-II- causes acute renal tubular necrosis in the mice infected with *E. coli* O157:H7 in addition to hypercellularitey of sub epithelial layers, these lesions may be due to direct effects of bacterial toxin. In the heart mild infiltration of inflammatory cell mononuclear cell in striated muscle (figure 13) and also pathological changes in the cardiac muscles. In heart there is infiltration of mononuclear cell and congestion of blood vessels (figure 14) these results agreement with Parillo *et al.* (1993) [27] who noticed that the consequence of septic shock are myocardial dysfunction, hepatic failure, acute renal failure and dissemination of intravascular thrombus, our evidence supported by previous data recorded by Luster (1998) [23] who investigated that the APC mediated inflammatory response is the result of activation of both pro and anti-inflammatory signaling pathway in host cells**.**

Conclusion

1. *E. coli* O157:H7 can survive and colonized in the intestinal epithelial cells.
2. Re-Isolation of *E. coli* O157:H7 bacteria from the large intestine of the rabbits at 24hrs post infection may indicate that these bacterial isolates were highly virulent.
3. *E. coli* O157:H7 disseminate to main target organs especially the kidney and other organs like liver post secret the toxins and overcome the innate immune system.
4. *E. coli* O157:H7 is able to cause systemic infection with severe diarrhea when infect the rabbits.

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