Study the pathogenicity of Providencia Vermicola in mice

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Abstract---P. vermicola is an opportunistic pathogen of human and animals. To estimate the pathogeneses of P. vermicola in mice infected orally and I/P with 1x10^8 CFU/ml). The histopathological changes at 24 hrs showed infiltration of inflammatory cells and apoptosis in hepatocytes in the liver, infiltration of inflammatory cells around a congested blood vessel and interstitial tissue of kidney; lung revealed congestion of blood vessels, infiltration of inflammatory cells and alveolar emphysema; intestine appeared infiltration of mononuclear cells in mucosa layer with hyperplasia of goblet cells. In mice infected I/P, the liver showed infiltration of inflammatory cells in a necrotic area with granulomatous lesion formation, the spleen showed depletion of white pulp. The kidney revealed dilatation of bowman’s space with vacuolation of glomeruli. The intestine appeared infiltration of inflammatory cells in mucosa and submucosa layers with hyperplasia of goblet cells, also, the lung showed infiltration of inflammatory cells in interalveolar septa and alveolar emphysema. At 72 hrs, one week and two weeks, necrotic area containing macrophage, neutrophils, and lymphocytes with fibrin in the liver, the kidney appeared dilatation of bowman’s space, vacuolation of glomerular tuft and degeneration of renal tubules, granular exudates of pneumonia and presence of bacteria and macrophage in the interstitial tissue of the lung, intestine shows sloughing and necrosis of intestinal mucosa and separated of muscular layer and hyperplasia of goblet cells and infiltration of inflammatory cells. In I/P, lung appeared filled of alveolus with granular exudate of pneumonia, presence of bacteria and macrophage in the interstitial tissue.

Keywords---Providencia, P. vermicola, histopathology.
**Introduction**

The genus *Providencia* are gram-negative opportunistic pathogens that have been isolated from a wide variety of environments and many living beings ranging from humans, insects, sea turtles and shark mouths (*Foti et al.*, 2009; *Nascimento et al.*, 2010). *Providencia* species are found in multiple animal pools including flies, cats, birds, dogs, cattle, sheep, penguins and guinea pigs and are resident oral flora in reptiles like pythons, vipers and boas (*Juneja and Lazzaro*, 2009; *Khunthongpan et al.*, 2013). In 2006, *P. vermicola* spp. was described for the first time in infective juveniles of the entomopathogenic nematode by (*Somvanshi et al.*, 2006), then in diseased freshwater fish, an acute watery diarrheal patient, and from human fecal samples (*Rajpara et al.*, 2015, *Ramkumar et al.*, 2014; *Duan et al.*, 2020). Besides, *P. vermicola* were detected from Canine feces (*Whittaker et al.*, 2016), and from milk (*Al-Gburi*, 2020). The first report of isolated *P. vermicola* was from a diarrheal cow (*Abees*, 2021). This study aimed to study the pathogenicity of *P. vermicola* in mice.

**Materials and Methods**

Bacterial isolate and Prepare the bacterial inoculum: *P. vermicola* obtained from diarrheal cow (MW301153) from previous study (*Abees*, 2021), the bacteria was cultured on Brain heart infusion broth and incubated at 37°C for 24 hours. Then the culture was washed three times with PBS (pH=7.2), then bacterial suspension containing 1x10^8 CFU/ml was prepared using (0.5) McFarland tube.

Experimental infected mice: Forty white mice were taken from (Iraqi Center For Cancer Research), weight about 20-30 gm., they were housed and adapted. Mice were divided randomly into four groups, 1st group contain (12 Mice) inoculated intraperitoneally (I/P) 0.5 ml (contain 1x10^8 CFU/ml), 2nd group contains (eight mice) were inoculated I/P 0.5 ml of normal salin as control. While the 3rd group (12 Mice) were inoculated orally one ml with the same dose. The 4th group (eight mice) were inoculated one ml of normal salin the same number of animals that were injected control I/P; then, the mice were observed for any clinical signs and mortality. After that three mice from the 1st and 3rd group and four from the 2nd and 4th group were ansthesia at 24 hours, 72 hours, seven and 14 days. Pathological and histopathological changes of the infected and control mice were recorded.

Histopathological examination Approximately one cm of the specimen was taken from the lung, liver, spleen, intestine, kidney, and sometimes urinary bladder. These specimens were fixed in formalin solution 10% for three days; then, the specimens were washed with tap water, and then the processing was done according to (*Luna*. 1968).

**Results**

In general, in the control group of the animals, there is no development of clinical signs, gross or histological changes. Grossly, the prominent features of *P. vermicola* infection different tissues revealed acute inflammatory reaction at 24,
3 hours and 7 days extended to day 14 post-infection. Infected mice presence clinical signs on mice including fever, anorexia after five hours. At 24 hours characterized by grossly, in oral infection mice and intraperitoneally showed watery ingesta inside the intestinal lumen and colon, splenomegaly, and hepatomegaly (Figure1). Also, at 3 hours and 7 days extended to 14 day grossly mice injected orally post mortem finding of the presence of petechial hemorrhage on the liver with watery ingesta inside the intestinal lumen and colon with splenomegaly. Grossly lesions in intraperitoneally injected mice were the same in oral infection (Figure-2).

**Histopathological changes**

**At 24 and 72 hours post infection**

At 24 hours, group infected orally for 24 hours shows infiltration of inflammatory cells (neutrophils) and apoptosis in hepatocytes in the liver, Kidney showed infiltration of inflammatory cells around a congested blood vessel and in interstitial tissue; the depletion of white pulp in the spleen, lung revealed congestion of blood vessels, infiltration of inflammatory cells and alveolar emphysema; while in intestine appeared infiltration of mononuclear cells in mucosa layer with hyperplasia of goblet cells. Intraperitoneally, the liver showed infiltration of inflammatory cells in a necrotic area with granulomatous lesion formation. The kidney revealed dilatation of Bowman’s space with vacuolation of glomeruli (Figure-9), the spleen showed depletion of white pulp. Also the lung showed infiltration of inflammatory cells in interalveolar septa and alveolar emphysema. The intestine appeared infiltration of inflammatory cells in mucosa and submucosa layers with hyperplasia of goblet cells.

At 72 hours, Group infected orally for 72 hours with *P. vermicol* revealed infiltration of inflammatory cells in necrotic area with granulomatous lesion around blood vessels in the liver, dilatation of Bowman’s space with vacuolation of glomeruli of the kidney, in spleen revealed depletion of the white pulp, intestine showed hyperplasia of goblet cells and infiltration of inflammatory cells, the granular exudates of pneumonia, also aggregation of bacterial colonies, macrophage with neutrophil infiltration in the lung. Intraperitoneally, kidney showed dilated of Bowman’s space, Liver showed infiltration of inflammatory cells in interstitial tissue, lung appeared infiltration of macrophage and around bronchioles, spleen showed mild depletion of white pulp, and infiltration of inflammatory cells in mucosa and submucosa around acini and hyperplasia of goblet cells of the intestine as in (Figure 1 a,b, c,d and e).
Figure 2a: Section in spleen of group infected orally for 24 hours P. vermicola shows depletion of white pulp (H and EX400).

Figure 2b: Section in lung of group infected orally for 24 hours shows congestion of blood vessels, infiltration of inflammatory cells (red arrow) and alveolar emphysema (black arrow) (H and EX400).

Figure 2c: Section in kidney of group infected orally for 24 hours shows infiltration of inflammatory cells around congested blood vessel and in interstitial tissue (H and EX400).
Figure 2d: Histopathological section in liver of group infected orally for 72 hours shows infiltration of inflammatory cells in necrotic area with granulomatous lesion around blood vessels (H and EX400).

Figure 2e: Histopathological section in intestine of group infected orally for 72 hours shows hyperplasia of goblet cells (red arrow) and infiltration of inflammatory cells (blue arrow) (H and EX400).

At 1 and 2 weeks

At one week, section of group infected orally shows aggregation of mononuclear cells as granulomatous lesion around blood vessels with infiltration in sinusoids in the liver; the lung showed congestion of blood vessels and infiltration of macrophage. The spleen revealed mild depletion of white pulp, dilated of bowman’s space and vacuolation of glomerular tufts of kidney, intestine appeared mild infiltration of inflammatory cells in mucosa and submucosa layers. Intraperitoneally the lung showed the granular exudates in bronchiole contain mononuclear cells, spleen showed prevascular hyperplasia, mild infiltration of inflammatory cells in submucosa in the intestine, kidney showed dilated of bowman’s space, and liver shows apoptosis and kupffer cells with mononuclear cells infiltration in sinusoids.

At two week, Histopathological section of group infected orally for 2 weeks shows necrotic area contain macrophage, neutrophils, and lymphocytes with fibrin in the liver, spleen revealed near lesion in white pulp, the lung showed granular exudates of pneumonia. The presence of bacteria and macrophage in the
interstitial tissue, some sections alveolar emphysema, the kidney appeared dilatation of bowman’s space, vacuolation of glomerular tuft and degeneration of renal tubules, and intestine shows slouphing and necrosis of intestinal mucosa and separated of muscular layer. Intraperitoneally spleen showed depletion of the white pulp as in, liver appeared aggregation of inflammatory cells around blood vessels as a granulomatous lesion, lung revealed interstitial tissue, mild dilatation of bowman’s space of kidney, and intestine showed mild infiltration of inflammatory cells in submucosa with hyperplasia of acinar cells (Figure 2 a,b, c, and d).

Figure 2a : Histopathological section in liver of group infected orally for two weeks with *P.vermicola* shows necrotic area contain macrophage, neutrophile and lymphocytes with fibrin (H and EX400).

Figure 2b: Histopathological section in kidney of group infected orally for one week shows dilated of bowman’s space(bleck arrow) and vacuulation of glomerular tufts(red arrow) and degeneration of renal tubules (blue arrow) (H and EX400).
Figure 2c: Histopathological section in lung of group infected intraperitoneally for two weeks shows alveolus is filled with granular exudate of pneumonia (red arrow), presence of bacteria (blue arrow) and macrophage in interstitial tissue (green arrow) (H and EX400).

Figure 2d: Histopathological section in intestine of group infected orally for two weeks shows sloughing and necrosis of intestinal mucosa (red arrow) and separated of muscular layer (blue arrow) (H and E X 100).

Discussion

The current results explained the serious pathogenic effects of *P. vermicola* post oral and intraperitoneal infection in mice at different times. There are no studies on the pathogenesis of *providencia* spp especially *P. vermicola* in experimental animals, the current dose was depending on other studies, Ramkumar et al., (Ramkumar et al., 2014) found the LD50 of *P. vermicola* in fish for intramuscular infection was $1.98 \times 10^6$ - $1.01 \times 10^7$ /animal, Al-Janabi (Al-Janabi, 2012) detected the LD50 of *P. rettgeri* in mice was $5.62 \times 10^7$ cell /mouse injected intraperitoneal, so in this study used higher dose ($1 \times 10^8$ CFU/ ml) to infected mice, and this dose was used in studies of the histological changes of *P. rettgeri* in mice by (Jassim, 2015; Ibrahim. 2016).
P. vermicola as gram-negative belong to Enterobacteriaceae have LPS and outer-membrane proteins and during infection, this LPS activates macrophages to produce IL-1 particularly IL-1β and TNF, in addition to toxic oxygen radicals, leading to many effects in the host such as fever, hypertension, sickness behavior and lethal shock (Rietschelet al., 1994; Schiffelholz and Lancel, 2001; Ulmer et al., 2002). Also, the current study revealed that P. vermicola caused diarrhea that attributed to invasive factor and suggesting that bacterium consider as an invasive enteric pathogen, Similar to other Providencia spp such as P. stuartii, P. rettgeri, and P. alcalifaciens, which are implicated as a cause of diarrheal diseases (Guth and Perrella, 1996; Albert et al., 1998). The results accordance with a study, when mice infected intraperitoneally with P. rettgeri and LPS of P. rettgeri caused death in mice after 24 - 48 hours and the clinical signs were temperature arising, anorexia and diarrhea, and the internal organs were congested of the liver and abnormal of color and size of spleen (Al-Janabi, 2012).

The present results explained the severe pathogenic effects of P. vermicola post oral and intraperitoneal infection in mice which persistence up to two weeks, the lesions characterized by the appearance of apoptosis, infiltration of inflammatory cells in the necrotic area, an increase in the number of macrophage cells in the necrotic areas, which led to cell aggregation and granulomatous formation around blood vessels with infiltration in sinusoids in the liver. Fibrinous granular exudates of pneumonia, aggregation of bacterial colonies, macrophage with neutrophil infiltration and emphysema in lung and, in spleen revealed depletion of white pulp, intestine showed hyperplasia of goblet cells and infiltration of inflammatory cells, infiltration of inflammatory cells around a congested blood vessel and in the interstitial tissue, dilatation of Bowman’s space with vacuolation of glomeruli, degeneration of renal tubules. These may be attributed to strong virulence factors such as LPS, urease, siderophores, and adherence factor, especially LPS which affect liver enzymes also, the liver histopathological changes may be attributed to the transfer of bacteria from the bile sac to the liver, and liver sinusoids damage as a result of superoxide free radicals released by LPS (Yokoyama et al., 1998; Ishikawa et al., 1999).

P. rettgeri found effects on an elevation of liver enzyme activity and caused cholecystitis and hepatitis in cats (Newton and Fry 2018). These reasons explain the changes that occurred in the liver in the current study. Present results following Al-Janabi (Al-Janabi, 2012), who injected mice with LPS of P. rettgeri and live bacteria intraperitoneally, caused death after 24-48 hours post-infection and observed the histopathological changes of congestion and edema, hemorrhage effects on the spleen, liver, and lung, and this is in line with Ibrahim, (Ibrahim, 2016) when investigated acute inflammatory reaction extended from 24, 48 hours, and four days to fourteen-day in mice infected with P. rettgeri, and the histopathological changes were mild to moderate acute inflammatory cells reaction, edema, fibrinous exudate, necrosis and focal aggregation of mononuclear cells.

The results indicated that P. vermicola caused pneumonia, macrophage and neutrophil infiltration, and emphysema, and the presence of bacterial colonies. This similar to others, fatal hemorrhagic pneumonia has been reported caused by Providencia spp (P. alcalifaciens) in piglets and isolated P. alcalifaciens from the
A study reported that *P. rettgeri* was found associating with granulomatous pneumonia and hepatitis in the monitor lizard (*Kycko et al.*, 2013). Furthermore, it has been shown that septicemic India Rhesus macaque infected naturally by *P. stuartii*, and the histological changes in lungs were edema, fibrin, numerous degenerate neutrophils and erythrocytes, and fewer macrophages, lymphocytes and plasma cells, emphysematous alveoli and exudate and presence of bacteria and chronic hepatitis (*Liu et al.*, 2016). Another study indicated that *P. vermicola* infected fish experimentally, which showed hyperplasia, shrinking lamellar fusion, epithelial necrosis and desquamation, and the hepatic parenchymatous increased cytoplasmatic vacuolation, blood sinusoids, and central nucleus in the liver (*Ramkumar et al.*, 2014).

The kidney showed infiltration of inflammatory cells around congested blood vessels and in the interstitial tissue, dilatation of bowman’s space with vacuolation of glomeruli, degeneration of renal tubules, uropathogenicity may be related to identifiable factors associated with virulence such as urease. Bacterial urease represents a potential virulence factor that is produced by nearly half of the gram negative bacteria present at 10^5 CFU/ml of urine and may therefore represent a common pathogenic factor (*Jones and Mobley*, 1987; *Griffith et al.*, 2011). This factor partially reported by others, who determined the milder than *P. rettgeri* when found severe histopathological changes (*Al-Janabi*, 2012; *Jassim*, 2015; *Ibrahim*, 2016). This may be attributed to the species, strain, or dose of bacteria.

The current study demonstrated the ability of *P. vermicola* to translocate from the intestinal lumen to extra intestinal when infected orally, Vieira *et al* found that *P. alcalifaciensis* as the Enterobacteriaceae member translocation from the gastrointestinal tract to extra intestinal sites and its ability to resist human serum bactericidal activity. (*Vieira et al.*, 2003). The histological changes in intestine infiltration of inflammatory cells in mucosa and submucosa layers, hyperplasia of goblet cells, accordance to study, there is partial necrosis of the intestinal villi with complete necrosis of surface epithelium and partial necrosis of colonic crypts in the large intestine in mice infected with *P. rettgeri* (*Al-Janabi*, 2012). *P. vermicola* was caused acute septicemia and death in a rabbit; there were multifocal petechial hemorrhages on the cecal serosa. Histologically, the infiltrated of heterophils mixed with a small amount of fibrin in the cecum and colon, bacteria were present within the fibrin and isolated from lung, liver, spleen and kidney (*Peddireddi et al.*, 2010).

**References**


