



## HISTOPATHOLOGICAL CHANGES OF *HELICOBACTER PYLORI* IN MICE

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### Abstract

Fifteen BALB/c mice were divided into three equal groups (5 animals each). The first group gave an infective dose of *H. pylori*  $1.2 \times 10^9$  cell/ml/os, the second group at dose  $9 \times 10^8$  cell/ml/os and the third group gave phosphate buffer saline-PBS 1 ml/os as a control group. The results showed the animals of the first group suffer from loss of mucosal architecture with evidence of mucosal metaplasia, irregular appearance of gastric glands together with mucosal irregularity of epithelial cells, vacuolization involving both parietal and chief cells accompanied with destructive findings in submucosal connective tissue with epithelial damage characterized by mucosal necrosis with evidence of nuclear pyknosis in the stomach. Liver shows moderate disruption of tissue with disorganization of hepatic cords associated with sinusoidal dilation. Focal necrosis was noticed mainly in the portal region with mononuclear cell infiltration and slight ductal dilation. Spleen showed moderate to severe lymphoid depletion of white pulp with severe atrophy of lymphoid follicles. Kidney showed severe tubular epithelial necrosis with sloughed epithelial cells, hyaline eosinophilic casts mainly seen in tubules with perivascular mononuclear cell aggregation. In the second group, the stomach showed irregular shortening of gastric glands, blood vessel congestion, surface epithelial sloughing of gastric columnar cells with mild submucosal cellular infiltration with ulceration. Liver showed severe vacuolation of hepatocytes, mild sinusoid dilation, degenerative changes in parenchyma with intense perivascular mononuclear cell aggregation concentrated in the central portion of the hepatic lobe. Spleen showed moderate to severe vascular congestion and dilation of splenic vessels with destructive changes in red pulp tissue with lymphoid depletion of white pulp tissue and severe atrophy of lymphoid follicles. Kidney showed cystic tubular distention, predominant mild interstitial mononuclear cell infiltration, intense epithelial swelling of major tubules with focal mononuclear cell aggregation. In the third group, no obvious histological changes were seen in the stomach, liver, spleen and kidney.

**Keywords:** *Helicobacter pylori*, gastritis, peptic ulceration, stomach.

### Introduction

The genus *Helicobacter* contains more than 35 species and *H. pylori* is the most important in terms of human health (Mladenova-Hristova *et al.*, 2017). *H. pylori* is a small, curved, highly motile bacterium (Jones *et al.*, 1984; Marshall *et al.*, 1989; Goddard and Logan, 2003), it has two to six flagella (Brown, 2000), Gram negative bacterium that colonizes the gastric epithelium, is the major cause of gastric carcinogenesis, other gastric diseases such as chronic gastritis, gastro duodenal ulcers, gastric mucosa associated lymphoid tissue lymphoma (McColl, 2010; Lee and Kim, 2015) and it is the etiologic agent of gastritis and peptic ulceration and may infect the gastric mucosa of over half of the world's population (Warren and Marshall, 1983; Graham, 1989; Osaki *et al.*, 1998). The route of transmission of *H. pylori* has not been clearly proved, one of the theories is via raw uncooked milk from animals (Mladenova-Hristova *et al.*, 2017), in developed countries the most likely route is oral-oral, probably via regurgitation and vomits, which occurs mainly in childhood, while in developing countries the fecal-oral route is feasible (Megraud and Broutet, 2000). The immunocompromised hosts are particularly susceptible (Mladenova-Hristova *et al.*, 2017). A number of animals have been suspected of harboring the organism in their stomach and therefore to be involved in the transmission (Megraud and Broutet, 2000). The existence of these diseases is higher in developing countries compared to developed countries (Dorer *et al.*, 2009). *H. pylori* increases with increasing age and higher in males than females and in adults than young (El-Gohary *et al.*, 2015). The prevalence of infection in young people from developed countries is already low ~15-25% (Goddard and Logan, 2003), that varies with age, socioeconomic status (Whitaker *et al.*, 1993;

Brown, 2000; Logan and Walker, 2001; Goddard and Logan, 2003), race and geographic area (Brown, 2000), and it is believed that once a subject is infected, the bacterium persists for life (Pounder and Ng, 1995). Also, host and environmental factors such as hygiene practices and diet may play a role in the acquisition of infection and the expression of clinical disease (Fallone, 1999). Infection is generally asymptomatic with the majority of infections not developing into clinical disease (Blaser, 1995). Vaccination has been demonstrated in animal models (Goddard and Logan, 2003).

Due to the importance of the bacterium *H. pylori* in humans and its effect as an association with cancer of the stomach, this study was conducted to use mice as a model for experimental infection and estimate the histological changes in the vital organs.

### Materials and Methods

#### Bacterial isolate

The isolate of *Helicobacter pylori* was adequate from the Department of Microbiology, College of Veterinary Medicine, University of Baghdad. Cultured on selective media (Columbia blood base agar) and confirmed by the biochemical tests (Haffman *et al.*, 1979).

#### BALB/c mice

Fifteen BALB/c mice were divided into equal three groups (5 animals each): The first group gave an infective dose of *H. pylori*  $1.2 \times 10^9$  cell/ml, the second group at dose  $9 \times 10^8$  cell/ml (McFarland, 1907) and the third group gave phosphate buffer saline 1 ml as a control group. All animals were dosed per os.

## Histological examination

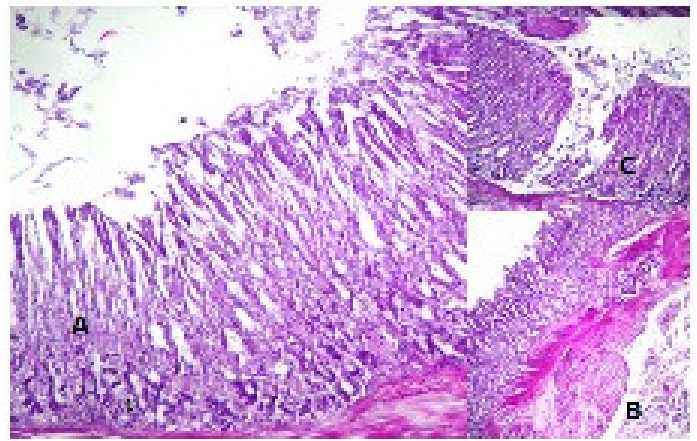
The histological examination was done after 5 days by sacrificed all animals for obtained the vital organs samples (Stomach, liver, spleen and kidneys) and preparation the tissues sections were done according to **Luna and Lee (1968)** as follows : the organs were fixed in formalin solution (10%) ,after that all samples were putted in concentrations (70, 80, 90 and 100%) of ethyl alcohol ascending and descending, then a paraffin blocks prepared and sectioning as a tissue films 4 $\mu$  thickening by histokinase micrometer, fixed on slides and stained by hematoxylin and eosin stain.

## Results and Discussion

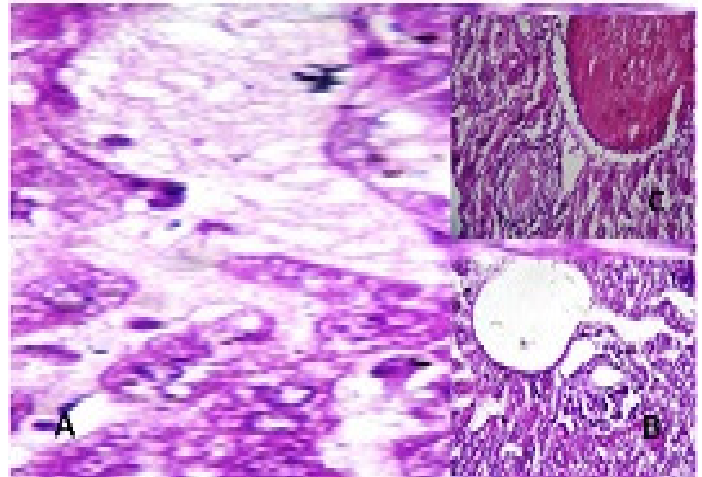
In the first group that infected by  $1.2 \times 10^9$  CFU/ml was showed that stomach revealed lost of mucosal architecture with evidence of mucosal metaplasia irregular appearance gastric glands together with mucosal irregularity epithelial, vacuolization involving both partial and chief cells seen in other section accompanied with destructive finding in submucosal connective tissues with mucosal metaplasia accompanied with destructive finding in submucosal connective tissues and epithelial damage characterized by mucosal necrosis with evidence of nuclear pyknosis (Fig. 1). Liver showed moderate disruption of tissue with disorganization of hepatic cords associated with sinusoidal dilation focal necrosis noticed mainly in portal region with mononuclear cells infiltration and slight ductal dilation with apoptosis of hepatocytes mainly noticed near central vein with ductal epithelial hyperplasia accompanied with mild inflammatory response (Fig. 2). Spleen showed moderate to severe lymphoid depletion of white pulp with severe atrophy of lymphoid follicle (Fig. 3). Kidney showed severe tubular epithelial necrosis with sloughed epithelial, hyaline eosinophilic cast mainly seen in tubules and edema between dilated tubules accompanied by severe vascular lesions with perivascular mononuclear cells aggregation (Fig. 4).

In the second group that infected  $9 \times 10^8$  CFU/ml , stomach showed irregular shortening of gastric glands, blood vessel congestion, surface epithelial sloughing of gastric columnar cells with mild submucosal cellular infiltration, ulceration , irregular shortening of gastric glands with widening of some gastric pits (Fig. 5). Liver showed severe vacuolation of hepatocytes, mild of sinusoid dilation, degenerative changes in parenchyma with intense perivascular mononuclear cells aggregation concentrated in central portion of hepatic lobe (Fig. 6). Spleen showed moderate to severe vascular congestion and dilation of splenic vessels with destructive changes in red pulp tissue with lymphoid depletion of white pulp tissue and severe atrophy of lymphoid follicle (Fig. 7). Kidney showed cystic tubular distention predominant, mild interstitial mononuclear cells infiltration, intense epithelial swelling of major tubules with focal mononuclear cells aggregation (Fig. 8).

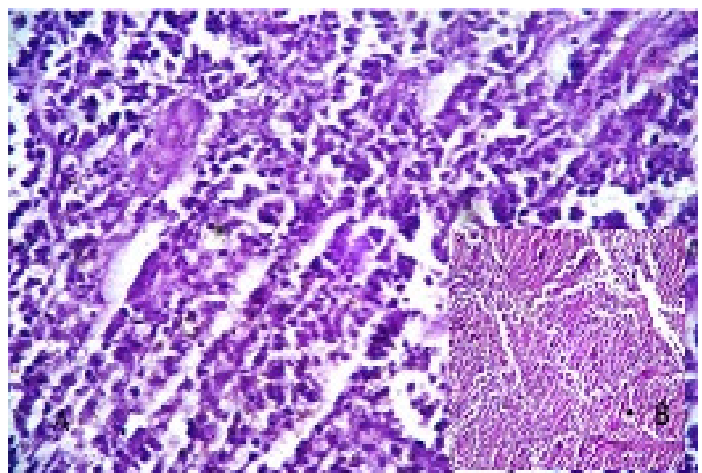
In the third group (control group) which given phosphate buffer saline (PBS) /ml was showed in Stomach, liver, spleen and kidney typical details of normal structures (Fig. 9, 10, 11, 12).



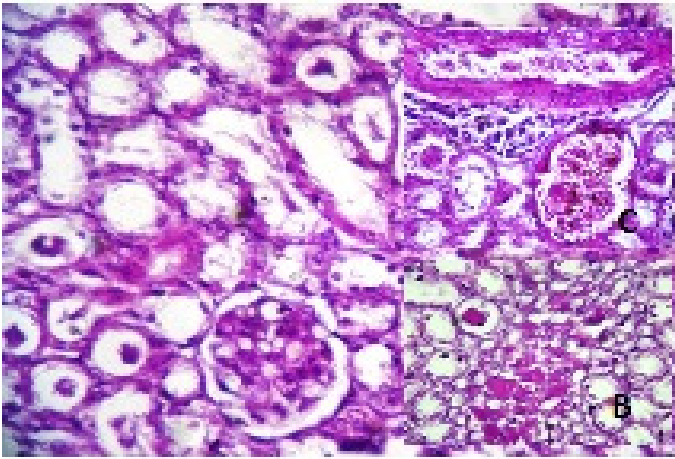
**Fig. 1** : Stomach section in BALB/c mice infected by  $1.2 \times 10^9$  CFU/ml reveal lost of mucosal architecture with evidence of mucosal metaplasia (A), irregular appearance gastric glands together with mucosal irregularity epithelial, vacuolization involving both partial and chief cells seen in other section accompanied with destructive finding in submucosal connective tissues with mucosal metaplasia accompanied with destructive finding in submucosal connective tissues and epithelial damage characterized by mucosal necrosis with evidence of nuclear pyknosis (Fig. 1).



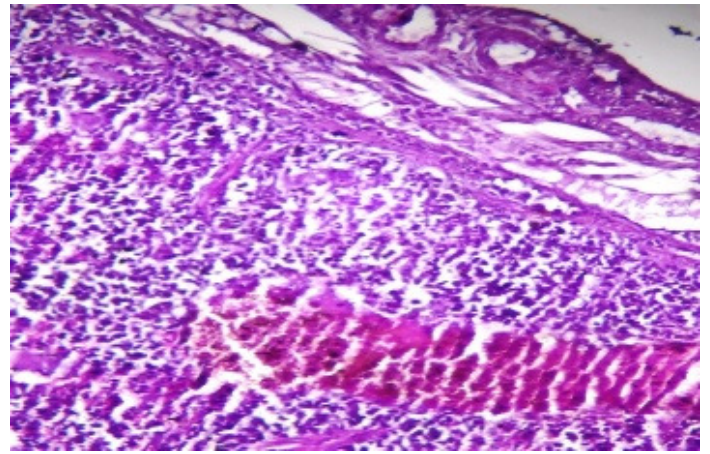
**Fig. 2** : Liver section in BALB/c mice infected by  $1.2 \times 10^9$  CFU/ml show moderate disruption of tissue with disorganization of hepatic cords associated with sinusoidal dilation(A) , focal necrosis may notice mainly in portal region with mononuclear cells infiltration and slight ductal dilation(B) , and great portal vein dilation and congestion with ductal epithelial hyperplasia accompanied with mild inflammatory response (C) (X40).



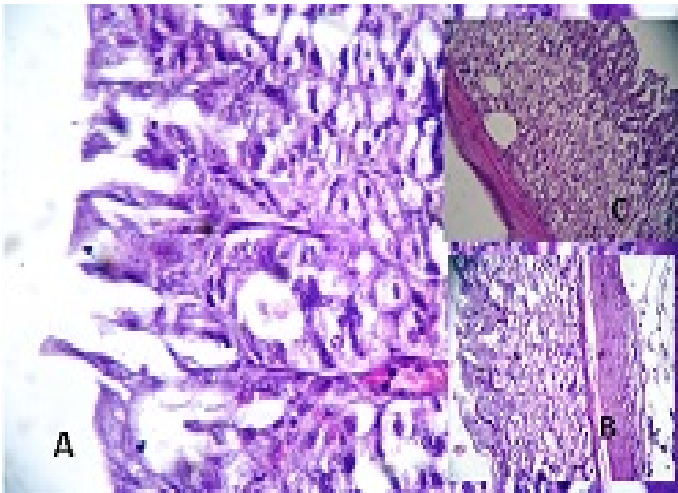
**Fig. 3** : Spleen section in BALB/c mice infected by  $1.2 \times 10^9$  CFU/ml show severe atrophy of lymphoid follicle (A), and moderate to severe lymphoid depletion of white pulp tissue(B) (X10).



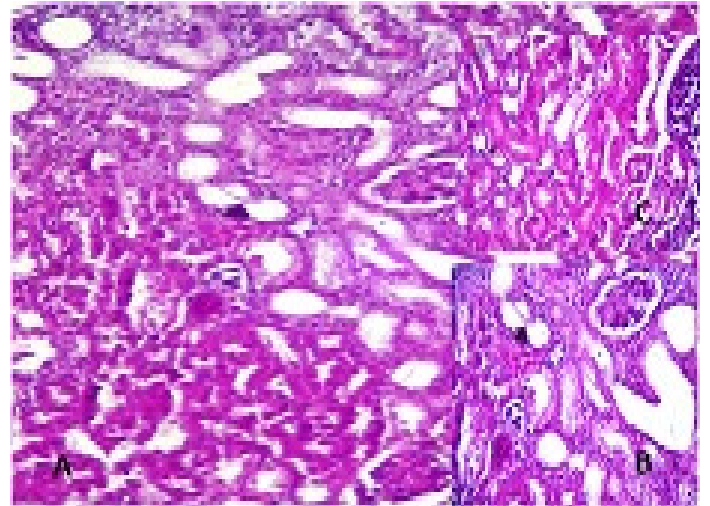
**Fig. 4 :** Kidney section in BALB/c mice infected by  $1.2 \times 10^9$  CFU/ml show severe tubular epithelial necrosis with sloughed epithelial (A), hyaline eosinophilic cast mainly seen in tubules (B) with severe vascular lesions with perivascular mononuclear cells aggregation (C) (X40)



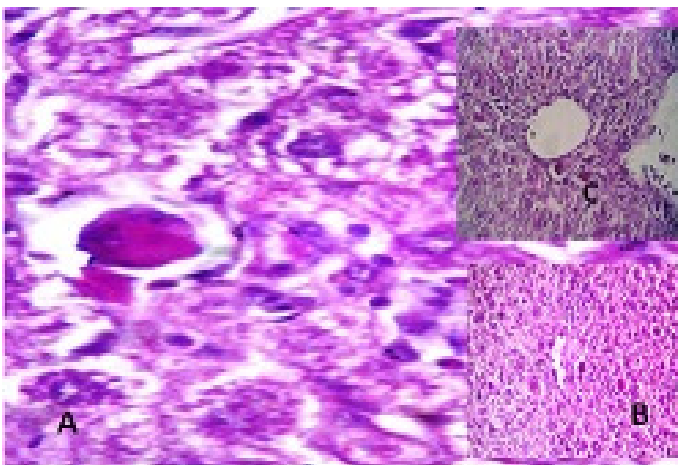
**Fig. 7 :** Spleen section of BALB/c mice infected by  $9 \times 10^8$  CFU/ml show moderate to severe vascular congestion and dilation of splenic vessels with moderate mononuclear cells infiltration mainly in capsular region associated with destructive changes in red pulp tissue (X10).



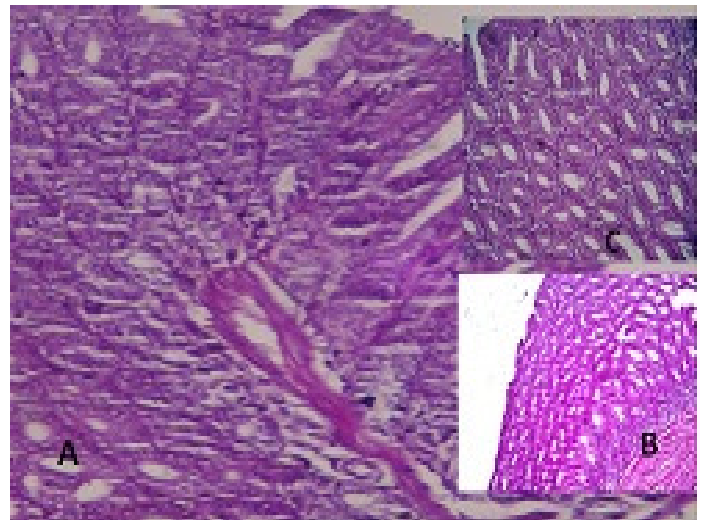
**Fig. 5 :** Stomach section of BALB/c mice infected by  $9 \times 10^8$  CFU/ml show irregular shortening of gastric glands with blood vessel congestion (A), focal epithelial sloughing with irregular shortening of gastric glands (B), and widening of some gastric pits (X10,40).



**Fig. 8 :** Kidney section of BALB/c mice infected by  $9 \times 10^8$  CFU/ml show cystic tubular distention predominant in renal tissue with mild interstitial mononuclear cells infiltration (A), cystic tubular distention predominant in renal tissue with mild interstitial mononuclear cells infiltration (B), and intense epithelial swelling of major tubules with focal mononuclear cells aggregation (C) (X10,40)

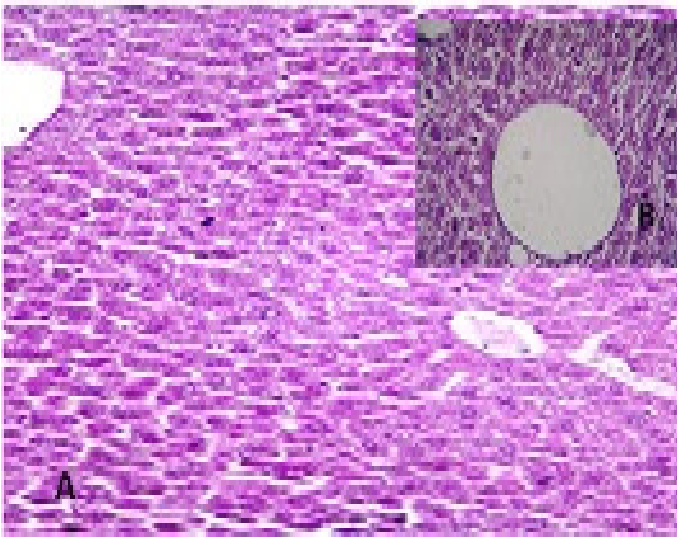


**Fig. 6 :** Liver section of BALB/c mice infected by  $9 \times 10^8$  CFU/ml show severe vacuolation of hepatocytes with individual appearance of Councilman bodies (A), mild sinusoid dilation with focal mononuclear cell infiltration (B) and perivascular mononuclear cells aggregation concentrated in central portion of hepatic lobe (C) (X10,40).

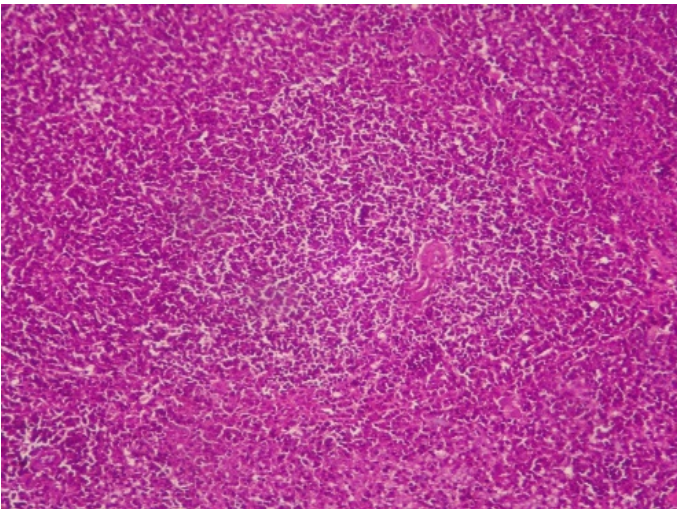


**Fig. 9 :** Stomach section of BALB/c mice ingested 1 ml of phosphate buffer saline-PBS show typical details of normal structure limit (A, B, C) (X10)

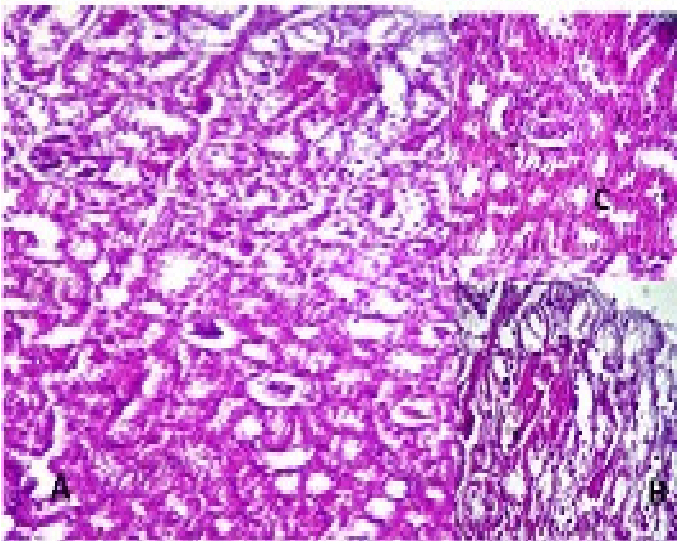




**Fig. 10 :** Liver section of BALB/c mice ingested 1 ml of phosphate buffer saline-PBS show normal structural details with no obvious changes (A,B) (X10)



**Fig. 11 :** Spleen section of BALB/c mice ingested 1 ml of phosphate buffer saline -PBS show normal structural details (X10)



**Fig. 12 :** Kidney section of BALB/c mice ingested 1 ml of phosphate buffer -PBS show no structural changes with normal renal tissues (A,B,C) (X10, 40)

The gastric epithelium has typically been regarded as a physical barrier, plays a key role in the inflammatory and immune responses induced by *H. pylori*. The epithelium is

the only cell phenotype in the gastric mucosa that is indirect contact with the pathogen. This feature places the epithelium in a strategic situation to interact with this bacterium and with the immune elements (Suarez *et al.*, 2006).

Up to 14% with *H. pylori* will not have natural infection, but will have bacterium elsewhere in the stomach, especially if there is gastric atrophy, intestinal metaplasia or bile reflux (Goddard and Logan, 2003), while persistent infection in gastric mucosa has been reported worldwide (Osaki *et al.*, 1998) and in about 15-20% of subjects long-term infection can lead to peptic ulcer or gastric cancer (Goddard and Logan, 2003). *H. pylori* produce a number of virulence factors, including vacuolating cytotoxin (vac A) that may have different disease association (Atherton, 1997). Pan gastritis, with an inflamed corpus is associated with the loss of acid secretion, which leads to atrophic gastritis and a cancer (Blaser, 1992; Baron and logan, 1994; Goddard and Logan, 2003). The interaction between specific microbial virulence factors and host genetic factors (pro-inflammatory genotypes are the main determinants of the pattern and severity of gastritis (Goddard and Logan, 2003), and establishment of chronic infection maybe influence by host genetic factors (Moriai and Hirahara, 1999). and differences in susceptibility to particular strains (Dubois *et al.*, 1996; Brown, 2000). It is thought to be indigenous to the human population and is well adapted to existing in the human stomach for the life time of its host (Blaser, 1997). On the same hand, animal models have provided an invaluable resource with which to study *H. pylori* pathogenesis and carcinogenesis (Peek Jr, 2008). Animal models of *H. pylori* infection have been developed, but their clinical validity has yet to be established (Goddard and Logan, 2003). It is colonize in the animal and human gut (Mladenova-Hristova *et al.*, 2017). *H. pylori* maybe recognized on H and E stained section alone and atrophy or intestinal metaplasia assessed (Goddard and Logan, 2003). Infection with *H. pylori* can result in chronic gastritis, a cellular infiltrate of immunocompetent lymphocytes and of IgA, IgG and IgM secreting plasma cells in the gastric mucosa (Spipponen, 1997). Adherence plays an important role in the induction of pathologic squeal and *H. pylori* adhesions have been studied in animal models (Peek Jr, 2008). Also, infection with *H. pylori* results in a typical sequence of events, ultimately resulting in the development of gastric diseases. Colonization of gastric mucosa by *H. pylori* first results in the induction of an inflammatory response, predominantly of the Th1 type. The initial acute gastritis is followed by active chronic gastritis, which last for life. This inflammatory response is characterized by an influx of neutrophils, mononuclear cells, and T- helper1 (Th1) cells. Th1 response results in epithelial cell damage rather than in the removal of *H. pylori*, thus causes a lifelong proinflammatory response coupled to cellular and initiate the histological cascade. The continuous production of reactive oxygen species that results from the ongoing inflammation an give rise to DNA damage, thus inducing the multiple mutations thought to be required for initiation of cancer cascade (Kusters *et al.*, 2006) It can use antigenic variation to regulate its interaction with host cells (Peek Jr, 2008) and induce the recruitment of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells into the gastric mucosa (Lundgren *et al.*, 2003) with reactive CD8<sup>+</sup> T cells can be efficiently stimulated by *H. pylori* antigen-pulsed B cell and DCs, and that most of the CD8<sup>+</sup> T cells in the infected gastric mucosa are memory T cells (Azem *et al.*, 2006) and a novel mechanism of immune

avoidance used by *H. pylori*, which involves the induction of coinhibitory molecule expression on gastric epithelial cells by the bacterium (Suarez *et al.*, 2006), all that maybe explain the different results of histopathological changes and the inflammatory cells infiltration in the present study, which synchronizing with dose of infection, but the infected mice don't developed cancer that referred in the previous studies, most mouse strains do not develop cancer, but only mild inflammation (Peek Jr, 2008) and that persistently elevated gastrin levels synergize with infection to augment the progression to gastric cancer (Peek Jr, 2008), and It is well documented that the epithelium has the ability to produce cytokines that trigger the recruitment of inflammatory cells into the gastric lamina propria (Beswick *et al.*, 2005). IL8 recruits immunological components into the gastric mucosa from the periphery, particularly polymorphonuclear cells, which contribute to epithelial damage (Yoshikawa and Naito, 2000) and macrophages contribute to epithelial damage by producing nitric oxide in response to bacterial urease (Gobert *et al.*, 2002). In conclusion, this study was reflect the possibility of using mice as a model for studying the pathogenesis and pathological changes of *H. pylori*, that synchronize with the dose of infection.

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