



## Histopathological and Histochemical Study of Intestinal Cryptosporidiosis in Mice

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### Abstract:

This study was conducted to investigate the histopathological and histochemical alteration of intestinal mice infected with *C. parvum* isolated from calf feces. The result elucidate that histopathological changes represented by hypertrophy and hyperplasia of epithelium villi in mucosa and submucosal gland, presence of different developmental stages of parasite attached to brush border of epithelium and in crypt in addition to proliferation of mononuclear inflammatory cell and blunts of villi have been observed as compared with control group. Histochemically, histological sections of infected mice revealed strong positive reaction with AB pH 1.0. In addition to positive reaction with PAS – AB pH 2.5 as compared with control group.

**Conclusion:** This study investigated that infection with *C. parvum* cause changes in mucosubstances secreted from goblet cells of mucosal villi and submucosal glands.

**Key words:** Cryptosporidiosis, Intestinal pathology, Histochemistry, *Cryptosporidium parvum*.

### دراسة مرضية نسجية وكيميائية لداء البويغات المعوي في الفئران

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### الخلاصة:

صممت هذه الدراسة لمعرفة التغيرات المرضية النسجية وكيميائية النسيج لامعاء الفئران المصابة ببطفيلي البويغات الخبيثة المعزولة من براز العجول. اظهرت النتائج تغيرات مرضية نسجية تمثلت بضخامة وفرط تنسج ظاهرة الزغابات في المخاطية والغدد تحت المخاطية ووجود مراحل متعددة من تطور الطفيلي تلتصق بالحافة الفرشائية للظهارة وعند الخباء، فضلاً عن تكاثر الخلايا الالتهابية وحيدة النواة مقارنة مع مجموعة السيطرة. كيميائية النسيج، اظهرت المقاطع النسجية للفئران المصابة بالمخجمة تفاعل موجب شديد مع تقنية الاليشيان الزرقاء عند الدالة الحامضية 1.0 فضلاً عن التفاعل الموجب الشديد مع تقنية فوق حامض البرويديك تبعثها تقنية الاليشيان الزرقاء عند الدالة الحامضية 2.5 مقارنة مع مجموعة السيطرة.

**الاستنتاج:** بينت هذه الدراسة بان الخمج باليويغيات الخبيثة سببت تغيرات في المواد المخاطية المفرزة من الخلايا الكاسية للزغابات المخاطية وتحت المخاطية.

**الكلمات المفتاحية:** داء الأبواغ الخبيثة، أمراض الأمعاء، كيمياء النسيج، كريبتوسبورديوم بارفم.

### Introduction:

*Cryptosporidium parvum* is now recognized as primary enteric pathogen in animals (1, 2). The parasite is in Phylum Apicomplexa and part of the group of parasites commonly referred to as Coocidia which included cryptosporidium, Eimeria, Isospora and Cyclospora (3). The parasite can cause diarrhea in calves (as well as other mammals) (4). This extracytoplasmic organism invades enterocytes in the distal small intestine and large intestine. The infection is acquiring through the ingestion of sporulated oocyst (5). Each oocyst contains four sporozoites, after passage through the stomach the sporozoites emerge from the oocyst and attach to intestinal epithelial cells, instead they induce an extension and fusion of microvilli resulting in the parasite becoming surrounded by double membrane of host origin (6), cryptosporidia now called arophozoit, likely derives nutrients from the host, all via this junction, called the feeds organelle. Cryptosporidial infection can cause disruption of enterocytes which increased intercellular permeability and inflammation in the submucosal layer (7). So many changes could be including chemical composition. Gastric and intestinal mucins have been investigated biochemically and histochemically and the changes in their composition have been reported in disease (8). Mucous are composed mainly of layer glycoproteins called mucin and inorganic salt, glycoproteins serve as antioxidative properties in the gastrointestinal tract since structural changes in mucous can lead to some gastrointestinal disease (9). The studies on the changes of mucosubstances in the gastrointestinal tract (histochemically) in pathological problems have great importance. Study of histochemical changes give us our

knowledge of mucins secretion in infected area to asses it's variation in diseases.

Aim of this study was conducted to throw more light on histopathological and histochemical changes which might occur due to the presence of cryptosporidia in intestine of the host.

### Material and Methods:

**Mice:** 20 mice of species Balb/C were obtained from the laboratory house of veterinary college, Mosul University. They were reared in clean cages in the laboratory; they were used after they were determined to be free of intestinal parasites by microscopic examination of fecal samples.

***Cryptosporidium parvum*:** were obtained from calf fecal samples. Isolated according to the methods of Thompson *et al.*, (10).

**Oocyst:** were inoculated into ten mice at 3 weeks old via gastric intubation of a dose  $1 \times 10^4$  oocyst per mice preparation of dose rate according to Freire – Santos *et al.*, (11) while control group inoculation by normal saline.

**Experimental Design:** On three days post infection (PI), oocyst was detected in fecal samples, and then mice were sacrificed on three day PI to observe any gross pathological lesions. After anesthetized and dissected 1 cm segment of the duodenum, carefully oriented on a filter paper and fixed in 10% neutral buffer formalin. After routine processing and staining with haematoxyline – eosin (H&E), a 4-6  $\mu$ m thick section were examined.

**Histochemical techniques:** The histochemical techniques listed below, with appropriated control, were under taken according to Pears (12) and Culling *et al.*, (13):

1- Carbohydrates.

Periodic – Acid Schiff's reagent (PAS)

Alcian blue (AB) 8GX (pH 2.5).

Alcian blue (AB) 8GX (pH 1).

**Chemical conversion:**

1- Acetylation (acetic anhydride/Pyridine)  
1-5 hours.

2- Saponification (1% KOH in 70% methyl  
alcohol for 20 minutes).

3- Methylation 60C° for 4 hours.

4- Acidic hydrolysis (H<sub>2</sub>SO<sub>4</sub>)

**Results:**

The histopathological changes occurred in the intestine infected with *C. parvum* showed lesions characterized by hyperplasia and hypertrophy of epithelial cells lining villi in addition to blunting and shortening of some villi and elongation of others. Different stages of parasites (oocyst, trophozoites) was noticed at brush border and in epithelium and at villi and submucosal glands (Figures 1-4).

Histochemically: In control group, the goblet cells in intestinal villi and submucosal glands secrete a, PAS- positive material which contain neutral and sialomucins revealed by the acetylation – saponification.

PAS sequence and by the alcianophilia, it means that intestinal villi and submucosal gland show PAS reactivity which is abolished by acetylation – PAS treatment and loss colored, whole often acetylation –

saponification – PAS, the colors become magenta (purple) (figures 5 and 6). Goblet cell in villi and submucosal gland are blue in color (strong positive) with AB pH 2.5 give negative reaction after methylation – AB and restored after methylation, saponification – AB pH=2.5 (strong positive) blue in color. Reaction with AB pH=1 give strong positive reaction acidic hydrolysis – AB at pH = 2.5 give moderate positive reaction (blue in color), (table 1).

Sections from the intestinal mice of infected groups revealed moderate positive reaction with PAS and AB pH2.5 and pH 1. Indicated the presence of sulphated and acid glycoconjugated (Figures 7 - 9). Positive reaction was observed in goblet cells of villi and epithelium of gland in lamina propria of infected one. The intensity of AB at pH 2.5 and pH 1.0 PAS – positive reaction particularly in apical part of villi. On the other hand, negative reaction was observed with acetylation – PAS and methylation – AB pH 2.5 then, the reaction was return after acetylation – saponification – PAS, methylation – saponification – AB pH 2.5. PAS positive material was less densely stained in the infected groups than in the control. Table 2 show the reactivity mucin was slightly more densely stained by AB at pH 2.5 (Figure 10).

**Table 1:** Histochemical reaction of normal intestinal mice.

staining techniques	intestinal villi	submucous gland	interpretation
<b>A</b>			
Periodic Acid Schiff's (PAS)	+++M	+++M	neutral mucopolysaccharide
Acetylation p PAS	-	-	
Acetylation - Saponification - PAS	+++M	+++M	
<b>B</b>			
Alcian Blue (AB) pH 2.5	++B	++B	acidic sulphated mucopolysaccharide
AB pH 1.0	+++	+++	
AB pH 2.5 - PAS	++RB	+RB	
AB pH 1.0 - PAS	++B	++B	
<b>C</b>			
Methylation – AB pH 2.5	-	-	
Methylation – AB pH 1.0	-	-	
Methylation – Saponification AB pH 2.5	++B	++B	acidic mucopolysaccharide (sialomucin)
Acidic hydrolysis (H <sub>2</sub> SO <sub>4</sub> ) AB pH 2.5	++B	++B	
Acidic hydrolysis (H <sub>2</sub> SO <sub>4</sub> ) AB pH 1.0	-	-	

+++ Strong; ++ Moderate; + Mild; - No reaction.

M: magenta.

B: blue.

RB: red blue.

**Table 2:** Histochemical reaction of intestinal mice intestine infected with *C. parvum*.

staining techniques	intestinal villi	submucous gland	interpretation
<b>A</b>			
Periodic Acid Shifts (PAS)	++M	+M	neutral mucopolysaccharide
Acetylation – PAS	-	-	
Acetylation - Saponification - PAS	++M	+M	
<b>B</b>			
Alcian Blue (AB) pH 2.5	+++M	+++M	acidic sulphated mucopolysaccharide
AB pH 1.0	+++B	+++B	
AB pH 2.5 - PAS	+	+	
AB pH 1.0 - PAS	++RB	++RB	
<b>C</b>			
Methylation – AB pH 2.5	+RB	+RB	
Methylation – AB pH 1.0	-	-	
Methylation – Saponification AB pH 2.5	++B	++B	acidic mucopolysaccharide (sialomucin)
Acidic hydrolysis (H <sub>2</sub> SO <sub>4</sub> ) AB pH 2.5	++B	++B	
Acidic hydrolysis (H <sub>2</sub> SO <sub>4</sub> ) AB pH 1.0	± B	± B	

+++ Strong; ++ Moderate; + Mild; ± Reaction or non-reaction; - No reaction.

M: magenta.

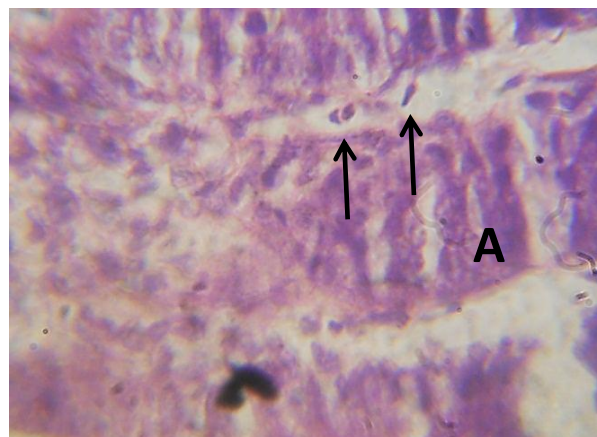
B: blue.

RB: red blue.

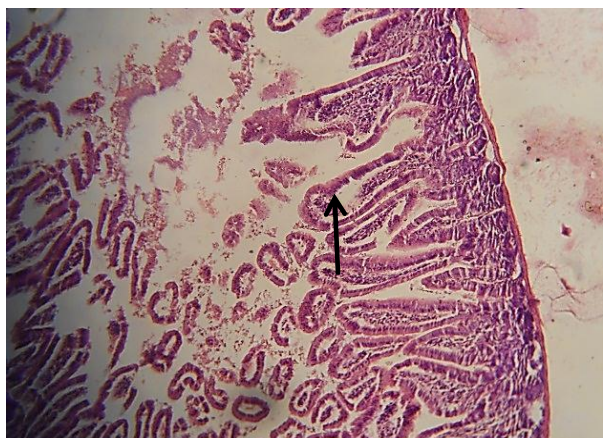




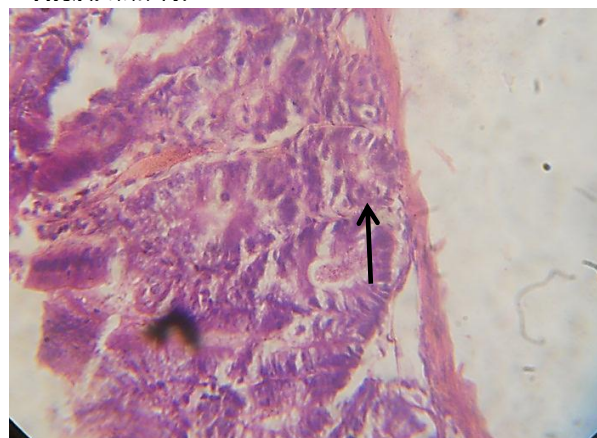
**Figure 1:** Histological sections of intestinal mice infected with *C. parvum* showed presence of cyst in epithelium and at brush borders (head arrow)(arrow). H&E, 335X.



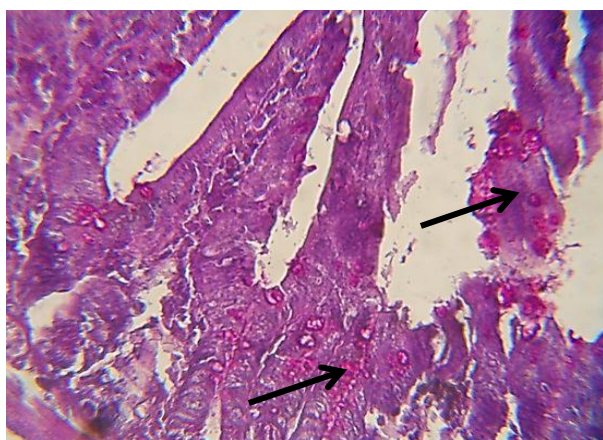
**Figure 2:** Histological sections of intestinal mice infected with *C. parvum* revealed hyperplasia of epithelium mucosa (A), presence of parasite at brush border (arrows), H&E, 335 X.



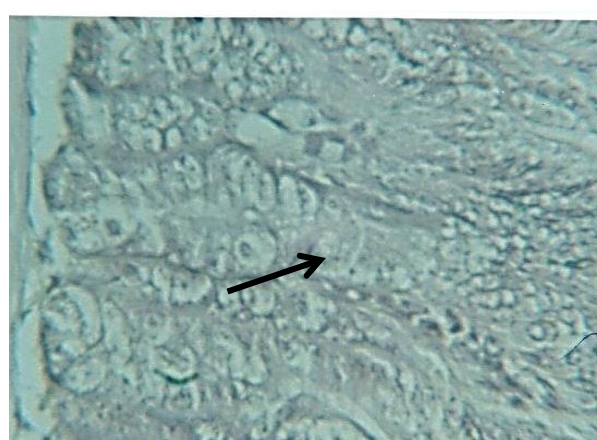
**Figure 3:** Histological sections of intestinal mice infected with *C. parvum* showed hyperplasia of epithelium villi (arrow), H&E, 145 X.



**Figure 4:** Histological sections of intestinal mice infected with *C. parvum* showed stages of parasite in epithelium of submucosal gland (arrows), H&E, 265 X.

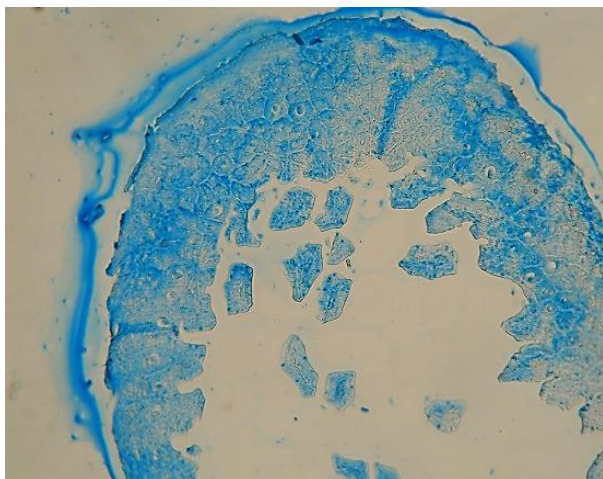


**Figure 5:** Histological sections normal intestinal mice showed positive reaction with PAS stain (arrow), 265 X.

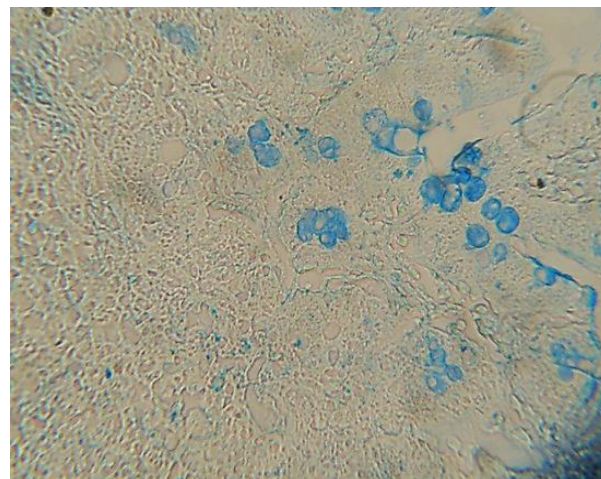


**Figure 6 :** Histological sections of intestinal mice infected with *C. parvum* showed negative reaction with PAS stain after Acetylation (arrow). 335X.

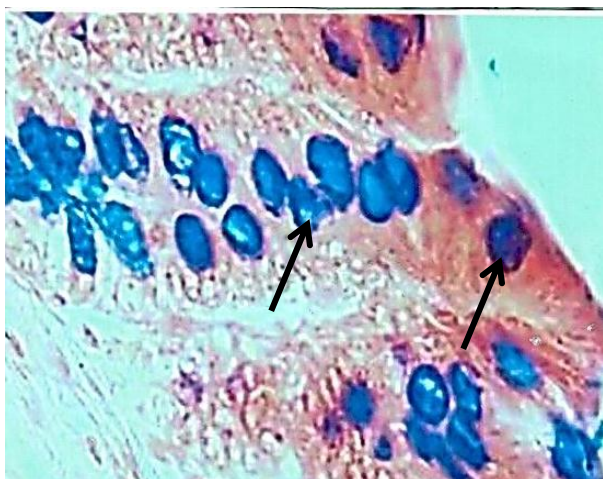




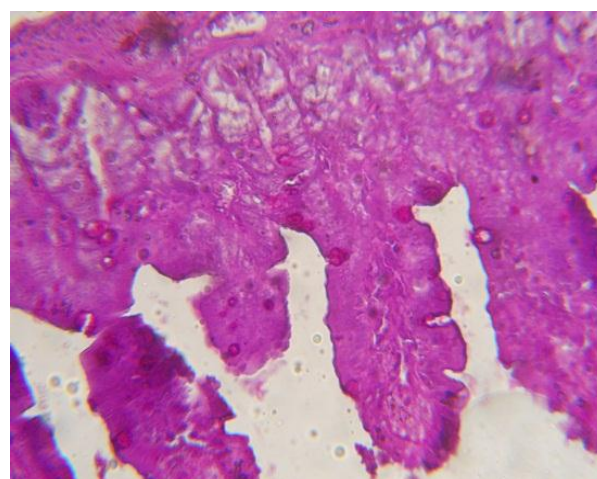
**Figure 7:** Histological sections of normal intestinal mice showed strong positive reaction with AB pH 1.0, 145X.



**Figure 8:** Histological sections of mice infected with *C. parvum*, Showed positive reactions with AB pH 1.0, 265 X.



**Figure 9:** Histological sections of intestinal mice infected with *C. parvum* showed strong positive reaction with AB pH 2.5 (arrow), 335 X.



**Figure 10:** Histological sections of intestinal mice infected with *C. parvum*, showed positive reaction with PAS after Acetylation and Saponification, 265 X.

### Discussion:

This study revealed the histopathological and histochemical alteration of small intestine infected with *C. parvum* experimentally, the epithelial lining villi in infected group revealed hyperplasia and hyperatrophy in addition to presence of many developmental stages of parasite. Our result was in agreement with (6). Histochemical investigation of mucosubstance that secreted from goblet cell of villi was studies in intestinal infection with *C. parvum* as compared with control non – infected group. The epithelial

villi and mucosal gland revealed moderate positive reaction with PAS for neutral mucosubstance this result indicate for that infected with *C. parvum* cause increase mucus secretion, it means increase the production of glycoproteins of mucosubstances that is using from parasite to facility the penetrating mechanism process into the cell, so, reduction acid mucosubstance in goblet cell have been reported in some disease (14) the normal functionality and biochemistry of the mucous barrier appear to be lost in disease

the colon rectal mucosa (15). This result denotes an increase in secretion of mucopolysaccharide facilitating of the parasite to adhesion and penetration of the intestinal epithelium of villi as suggested by (16) who mentioned that parasite might secrete mucin – degrading enzyme, enabling the penetration of protective mucus gels that overlife the mucosal surface of their potential hosts. Furthermore, they might generate binding lignads on the membrane – bound mucins of host cells by using specific glycosidases, it is possible that host mucins and mucine – like molecules prevent the establishment of parasites or facilitate parasite expulsion.

Carbohydrate both on the outer surface of cellular and secreted macromolecules, mediate many events in cell and cell – matrix interaction leading to the development and functions of organism, many diseases are characterized by changes in carbohydrate, so they are highly considered in therapeutic investigation (17). Alteration in mucins glycoproteins in gastrointestinal disease play an important role in the pathogenesis of the disease (18, 19). On the other hand, mucosubstance have been reported as one of the intestinal protective tools against *C. parvum* due to their contact of IgA and IgM, so, because that some parasite may be *C. parvum* have ability to secrete enzyme cause disruption in the secretion of host mucosubstance to facilitate interaction with host cell of intestine or might be utilized throughout its metabolic activity and adhesion lignads for these mucinous and mucin – like molecules in relation to interaction (20).

Furthermore, the result revealed strong positive reaction with AB in infected groups it's indicated for presence of high secretion of sulfamucin and sialomucine which act as a protection against pathogenic infection. Particularly in cryptosporidiosis, in attachment phase, it may be utilized the secretion of goblet cell due to changes the chemical composition during colonization of parasite on mucosal

surface large number of cryptosporidia adherent to villus function (21, 22).

Therefore, their findings have necessity for further study for research on the correlation of mucosubstance with parasites particularly intracellular, also detected the favorable drugs effects on those infections. The detection of histochemical changes in composition of mucinus in the gastrointestinal disease has received a great attention (23, 24, 25).

### Conclusion:

In this study histochemical analysis revealed that those infections with *C. parvum* cause slight changes in intensity of mucosubstance (neutral and acidic mucopolysaccharide). However, further investigations are needed in order to understand the molecular bases of *C. parvum* on mucosubstance of gastrointestinal tract.

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