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IJPBS |Volume 3| Issue 3 |JUL-SEP|2013|220-227

Research Article
Biological Sciences

HISTOPATHOLOGICAL CHANGES IN MICE INFECTED WITH *CRYPTOSPORIDIUM* SPP

Harith S. Al-Warid [1*], Ihsan M. AL-Saqur [2], Souhaila H. Mahmood [1]

[1]*Biology Department- College of Science-University of Baghdad- Baghdad-IRAQ*

[2]*Biological Researches Unit for Tropical Disease- College of Science-University of Baghdad-Baghdad-IRAQ*

***Corresponding Author Email:** harithalward@yahoo.com, harithalward@scbaghdad.edu.iq

ABSTRACT

Experimental infection was established in immunosupreesed and non-immunosupressed BALB/c mice using 100 oocyst /0.2 ml of two different Cryptosporidium spp. isolates : fecal origin and water origin, the histopathological changes were noticed in these groups of mice, results indicated that these two isolates were able to induce experimental infection in both immunosuppressed and non-immunosuppressed mice as well as the histopathological changes were varied from mild in non- immunosupressed to severe in immunosupressed mice when it's compared with the control group, the most important histopathological changes were inflammatory infiltration and blunting of the villus, the villi were also observed to be shorter and wider than normal, finally the villus height / cryptic depth ratio was less in immunosuppressive mice than the ratio in the control group of mice.

KEY WORDS

Cryptosporidium spp., Histopathology, BALB/c mice

INTRODUCTION

Cryptosporidiosis is a zoonotic and anthroponotic disease which caused by protozoan parasites genus *Cryptosporidium*, it has a worldwide distribution and in the most surveys it is considered to be among the four major pathogens causing diarrheal diseases in children [1]. Humans can acquire *Cryptosporidium* infections through several transmission routes, such as direct contact with infected persons or animals, and ingestion of contaminated food and water [2]. On the other hand several sensitive rodent models have been developed, standardized, and optimized over the last two decades, the majority of investigators, presumably for reasons of availability and costs, continue to use either the neonatal mouse or the immunosuppressed adult mouse, which have major limitations. Although cryptosporidiosis occurs in most vertebrates, human cryptosporidiosis is quite distinct from that seen in other mammals. The infection in humans induces serious symptoms of gastrointestinal illness lasting several days in all age groups. Whereas infections cause acute watery diarrhea only in neonatal ruminants and piglets, no symptoms are observed in most other infected animals of any age [3]. Because of these variations, different animal models are required for different types of scientific studies such as therapeutic [4,5], immunization [6] and histopathological studies [7,8].

Materials and Methods

Laboratory animals

BALB/C mice were obtained from "The National Centre for Drug Control and Research /Baghdad-Iraq " and were bred in "The Animal House / Biology Dept./ University of Baghdad" for reproduction in clean cages with feeding bottle for water supply and dry sawdust with cotton.

Three mice were put together in one cage, the room temperature was between (22°C-28°C), animals were provided with food that consists of dry vegetation, parsley, celery and powdered milk.

Preparing of Cryptospridium infected dose

Four high positive human fecal samples and two high positive water samples were used for preparing the infected dose after concentration with Sheathers's sugar solution [9]. The oocyst from the different two

origins were counted with haemocytometre, the final concentration for each fecal and water samples were 500 oocyst per (1 ml) phosphate buffer saline.

EXPERIMENTAL DESIGN

Forty male mice aged (8-10) weeks were separated into two groups each group consists of (20) mice the, first group was given 1.5 mg dexamethasone subcutaneously twice a week for eight weeks to develop immunodeficiency (immunosupressed mice) the second group was not given any immuosuppressed drugs (Non-Immunosupressed mice), before giving dexamethasone treatment, all mice were checked to be free of infection.

Each group then separated into two sub-groups each sub-group consisting (10) animals and were orally administrated using a special catheter as follows:

Immunosupressed group 1: were administrated with 100 fecal origin oocyst/ 0.2 ml.

Immunosupressed group 2: were administrated with 100 water origin oocyst/ 0.2 ml.

Non-Immunosupressed group 3: were administrated with 100 fecal origin oocyst/ 0.2 ml.

Non-Immunosupressed group 4: were administrated with 100 water origin oocyst/ 0.2 ml.

Control group: Ten mice were added as a control group.

Histopathlogical changes

All animals belonging to the previous group were dissected after 15 days of experimental infection with *Cryptosporidium* spp. The method of [10] was followed in the preparing of histological section.

Small intestine of each animal was cut into small piece (1 cm) and kept in formalin saline, then in 70%, 90%, and 95 % alcohol, respectively for 30 minutes and then in 100% alcohol for 60 minutes, then submerged in paraffin wax at 55-60^{o}C. Paraffin was cut and stabilized on slides by the use of Mayer's albumin and then stained with hamatoxline-eosin stain as following:

Slides were put in xylene to dissolve the paraffin wax, then at 35%, 70%, 90 % and 95% alcohol respectively about 1 minute for each concentration.

Slides were stained with hematoxline for 2 minutes then washed with water and 35% alcohol, then stained with eosin for 1.30 minutes and put in a series of alcohols.

Clearing was done by xylene. Slides were covered with cover slide, which was fixed with Canada balsam.

RESULT AND DISCUSSION

Mice were used in this study, to evaluate the histopathological changes induced by two *Cryptosporidium* spp isolates (fecal origin and water origin), the mouse model represents a competitive alternative for many reasons: less expensive and labor-intensive than using cattle, can be used in any laboratory with basic microbiological containment and housing facilities for mice. [11].

Dexamethasone was used in the present animal design to immunosuppressed two groups of mice because it was considered as a good immunosuppressive agent in several studies dealing with *Cryptosporidium* spp infection especially in rat and mice model (12,13) , hair of immunosuppressed animals had deteriorated and bleeding of skin was observed after 18 day of immunosuppression in the present study (Figure 1).Possibly due to papillary edema and pseudotumor cerebri developed in some animals [12].

Infection is initiated when the host ingests oocysts [14], so oral inoculation were used in this study, investigators showed that the infection with *Cryptosporidium* spp may initiate by using 2-10 oocyst [15], but this number of oocyst may not show typical symptoms of cryptosporidiosis, Al-Gelany (1998) [16] established experimental infection using 50 oocyste, others used 10^3,10^4 and 10^5 oocyst [7,17] depend on the species of parasites and the experimental host, so the determination of oocyte dose is important to guarantee the best infection with *Cryptosporidium* spp. In the present study 100 oocysts were in the acceptable range of oocyst to create an experimental infection.

Histopathological changes were observed in all groups in this experimental design except the control group, inflammatory infiltration and blunting of the villus were seen in the intestine of infected immunosuppressed group 1 and 2 of mice, Figure (1 and 2), when it's compared with the control group which were no any histopathopalogical changes.

More pronounced inflammatory changes such as disruption of the epithelial barrier and more extensive infiltration of the lamina propria with inflammatory cells in both group 1 and group 2 of immunosuppressed infected mice (Figure 3,4 and 5), when it compared with a Non immunosuppressed group which had a moderate infiltration (Figure 6), and with a control group which had no any histopathological changes (Figure 7).

The villi were also observed to be blunt, shorter, and wider than normal and are sometimes fused to other villi, whereas crypts are elongated and hyperplastic in infected immunosuppressed mice and also the villus height / cryptic depth ratio was less in immunosuppressive mice than the ratio in control group of animals (Figure 8, 9 and 10).

These histopatological changes which varied between mild in Non-immunosuppressed mice, to severe in immunosuppressed mice may reflect the extent of infection and severity of illness [3, 18]. These changes may correlate with the number of infecting organisms [19]. The characteristics of histopatological changes in the present study were similar to those in other experimental animals such as kids and guinea pigs [20, 21].

Results indicated that BALB/c mice are a good model to study the progressive of *Cryptosporidium* spp infection in intestine tissue. The two different origin isolates were able to induce experimental infection in immunosuppressed and non-immunosuppressed BALB/c mice, and histopathological changes indicated strongly the level of infection which were more severe in immunosuppressed mice and low or moderate in non immunosuppressed mice.

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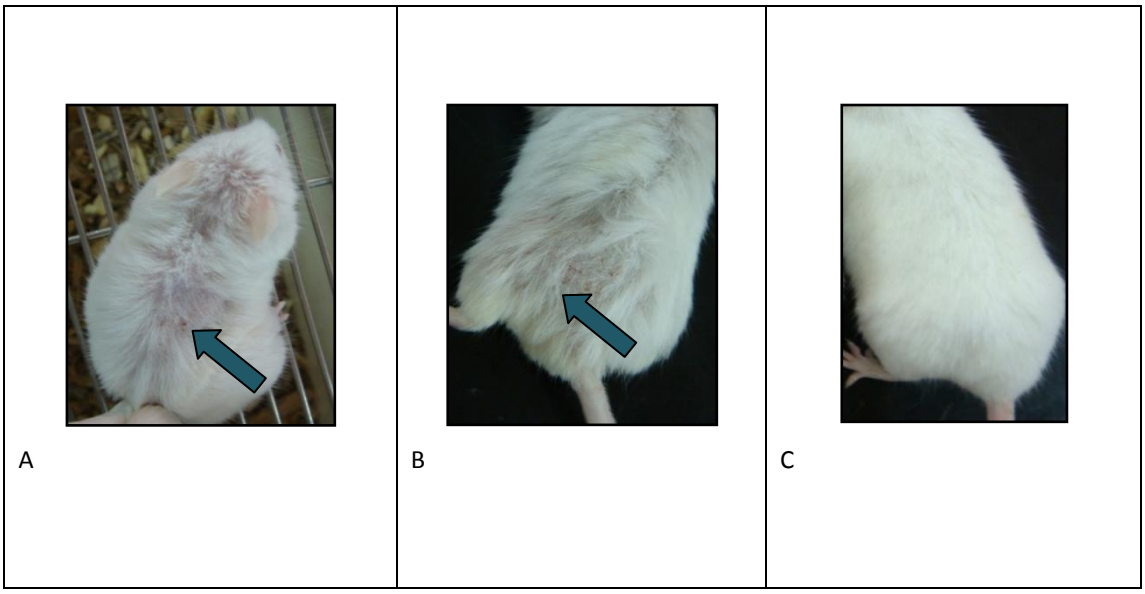

Figure 1. Hair of mice belong to different experimental groups after 18 days of immunosuppression
A: Hair loss in Immunosuppressed group 1
B: Hair loss in Immunosuppressed group 2
C: No hair loss in Non-immunosuppressed mice

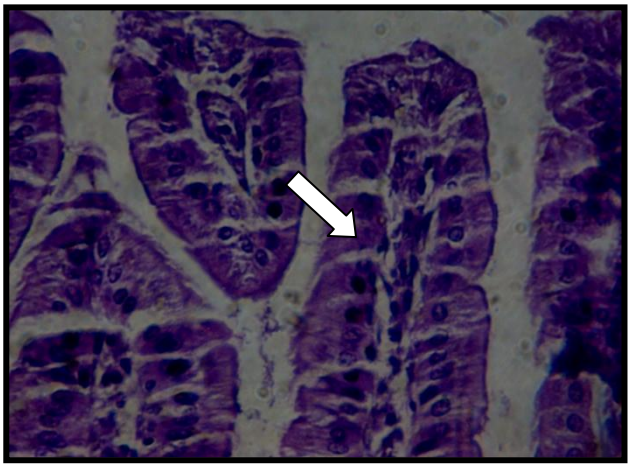

Figure 2 .Disruption of the epithelial barrier in immunosuppressed mice group 2 after 15 day of infection, Haematoxline- Eosin 1000 X

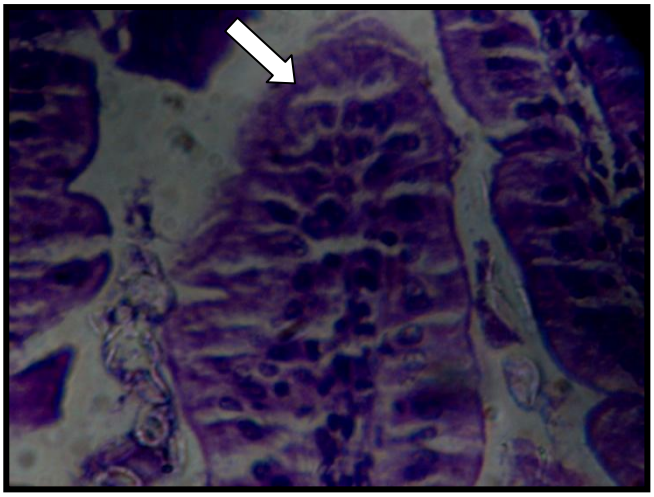

Figure 3 .Disruption of the epithelial barrier in immunosuppressed mice group 1 after 15 day of infection, Haematoxline- Eosin 1000 X

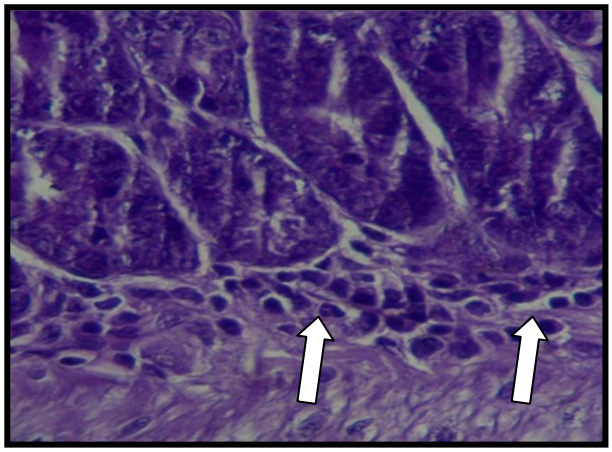

Figure 4 : Infiltration of lamina propria in immunosuppressed mice group 2 after 15 day of infection, Haematoxline- Eosin 1000 X

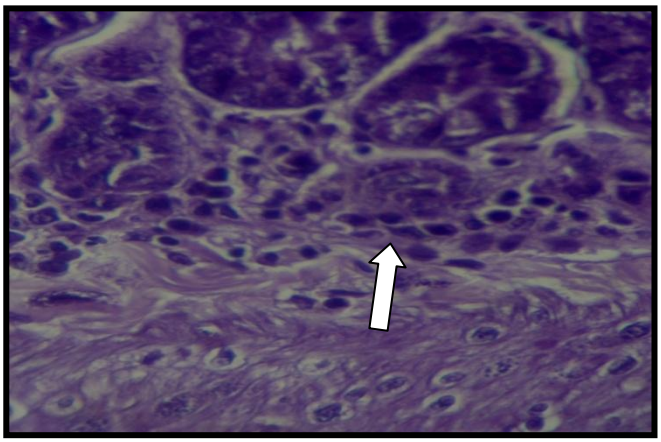

Figure 5 .Infiltration of lamina propria in immunosuppressed mice group 1 after 15 day of infection, Haematoxline- Eosin 1000 X

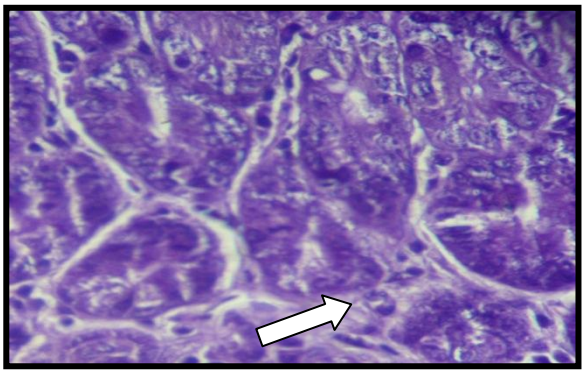

Figure 6. Moderate Infiltration of lamina propria in non immunosuppressed mice group 3 after 15 day of infection, Haematoxline- Eosin 1000 X

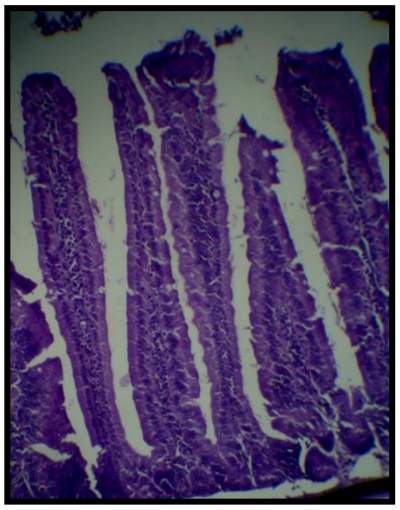

Figure 7. No disruption, no fusion of villus and no filtration in lamina properia in control mice, Haematoxline- Eosin 400 X

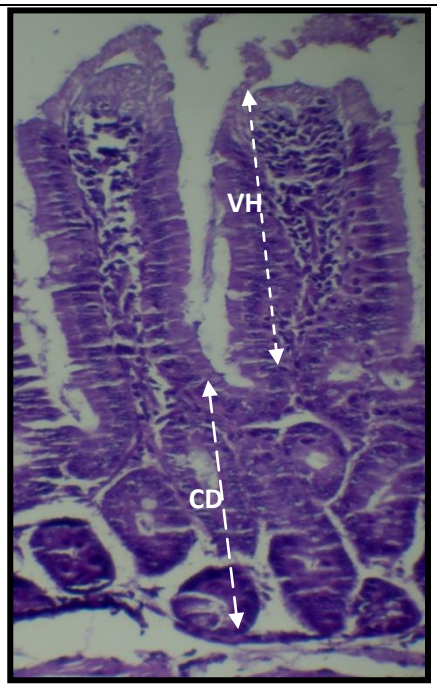

Figure 8 -A- . short and wide villi of immunosuppressed mice group2 after 15 day of infection, Haematoxline- Eosin 400 X
VH: Villus Height
CD:Crypt Depth

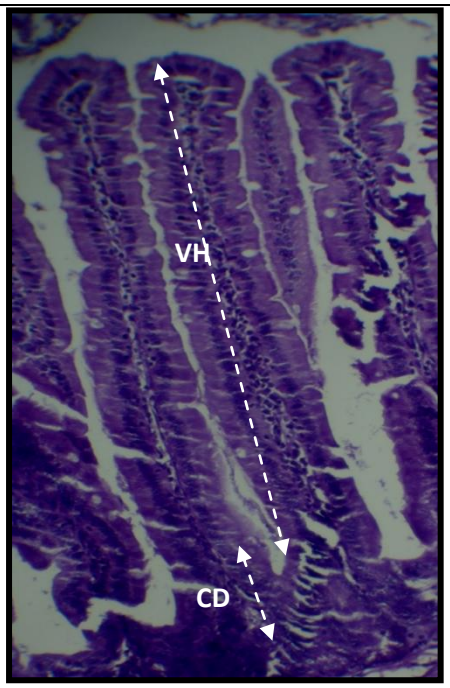

Figure 8 -B- .normal villi of Control, Haematoxline-Eosin 400 X
VH: Villus Height
CD:Crypt Depth

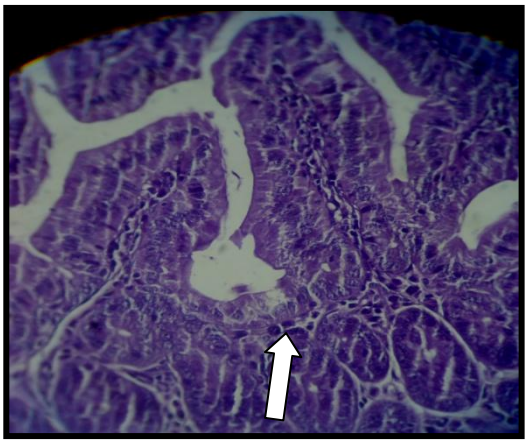

Figure 9: Fusion of villus in immunosuppressed mice group 1 after 15 day of infection, Haematoxline- Eosin 400 X

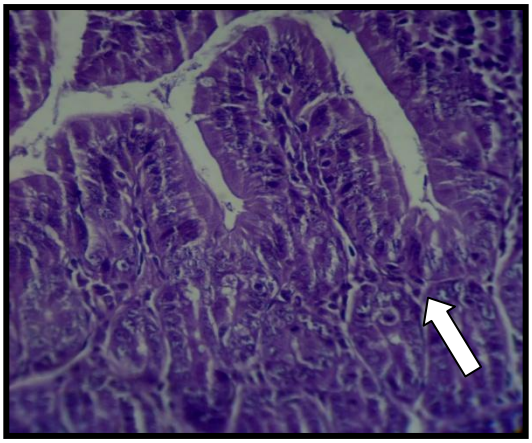

Figure 10: Fusion of villus in immunosuppressed mice group 2 after 15 day of infection, Haematoxline- Eosin 400 X

***Corresponding Author:**
E-Mail:harithalward@yahoo.com,
harithalward@scbaghdad.edu.iq