Immunohistochemical Detection of Cryptosporidium-Induced Intestinal Tissue Alterations in Dexamethasone Treated & Untreated Mice

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Abstract

Cryptosporidium parvum (C. parvum) is a coccidian protozoan that causes cryptosporidiosis, a parasitic disease of the mammalian intestinal tract. C. parvum is considered one of the most important waterborne pathogens among the most relevant parasitic enteric agents in man and animals. It is resistant to all practical levels of chlorination and it is an obligate intracellular pathogen. It has been the cause of multiple diarrhea outbreaks in developed and developing countries. The present work was carried out to evaluate the pathological, immuno-histochemical and molecular changes in the ileocecal region induced by chronic irritation with different inoculum sizes of cryptosporidium (50,500 oocysts) in immunocompetent and immunosuppressed mice. The mice were euthanized at different dates starting from 14, 21, 36, 45, 57 till day 64 to study these transformations. Histopathological examination of the ileocecal region revealed neoplastic changes in the form of dysplasia, polypoid structures, architectural distortion, glandular crowding, marked cellular atypia, exophytic adenomatous polypi, intramuscular adenocarcinoma and marked nuclear anaplasia. Immunohistochemical results showed positive K-ras expression. The present study proved that Cryptosporidium species is one of the infectious agents that may induce intestinal neoplasia, which is highly affected by repeated exposure and elevated parasite loads.

Keywords: Cryptosporidium, Mice, Intestinal tissue alterations, Histopathology, Immunohistochemistry.

Introduction

Cryptosporidium parvum (C. parvum) is a coccidian protozoan causes cryptosporidiosis, a parasitic disease of the mammalian intestinal tract. C. parvum is considered to be the most important waterborne pathogen in developing countries and due to the zoonotic character of some of its species is among the most relevant parasitic enteric agents in human and veterinary medicine. It is resistant to all practical levels of chlorination and it is an obligate intracellular pathogen [1]. It has been the cause of multiple diarrhea outbreaks in developed and developing countries [2]. Cryptosporidium spp. infection is a leading cause of diarrhea in Egypt. Cryptosporidium spp. prevalence among individuals with diarrhea visiting inpatient and outpatient clinics in Egypt ranges from 0-49% [3]. The most common protozoan infection in immunosuppressed (IS) Egyptian patients has been found to be C. parvum (60.2%) [4]. A study was done to identify the genotypes of the C. parvum isolates from clinical samples from diarrheic children in Egypt using polymerase chain reaction (PCR) amplification and restriction fragment length polymorphism (RFLP), the results showed that 12% of the specimens were positive for Cryptosporidium spp. revealing that 82% had C. parvum, 12% had C. hominis, and (6%) had mixed infections [5].
Moreover, the zoonotic potential for cryptosporidiosis has been proven in Egyptian farmers and their farm animals infected with *C. parvum* [6]. Different species of *Cryptosporidium* have been reported among human patients, including *C. parvum*, *C. hominis* and *C. bovis* [7]. In animals, *Cryptosporidium spp.* infection implies both an economic loss and a significant source for zoonotic infection. *C. parvum* causes severe and life-threatening diarrhea in immunocompromised hosts.

Although this protozoan is far more common in immunocompromised patients, it is also known to induce diarrhea in immunocompetent (IC) persons where infection is generally self-limiting [8]. Infection is caused by ingestion of sporulated oocysts transmitted by the feco-oral route or indirect via contaminated water supply, food, or environment [9]. Invasion of the apical tip of ileal enterocytes by sporozoites and merozoites causes pathology seen in the disease. *C. parvum* has been correlated with digestive carcinogenesis.

An epidemiologic study in Poland reported a frequency of 18% of cryptosporidiosis in patients with colorectal cancer [10]. However, in this report it was unclear whether *C. parvum* behaved as a carcinogenic factor or simply as an opportunistic agent whose development was enhanced by host immunosuppression. More consistent with a potential tumorigenic role of this parasite, it was recently proved that IOWA and TUM1 strains of *C. parvum* of animal origin induced digestive neoplasia in a rodent model [11]. This study aimed to detect of *Cryptosporidium parvum* induced intestinal tissue alterations in dexamethasone treated & un-treated Albino mice

**Material and Methods**

**Animal Source**

Laboratory bred female, white Albino mice of CDI strain, about 4-6 weeks old, weighing 20-25gram, were obtained from the European countryside. Animal experiments were performed in the biological unit of Theodor Bilharz Research Institute (TBRI), in a well-ventilated plastic cage with clean wood-chip bedding in conditioned rooms (27±2°C) and away from direct sunlight, ensuring good sanitary condition. All applied experiments on animals were carried out according to the internationally valid guidelines after the approval of the institutional ethical committee of TBRI.

**Experimental Design**

One hundred and fifty laboratory bred white albino female mice were used in this study. Mice were divided into two main groups according to their immune status (whether treated with Dex or not): Group A (immunocompetent) was divided into the following subgroups according to the infecting dose and number of exposure to *C. parvum*, each consisted of 15 mice: GA 1: Negative control (Control non-infected), GA 2: repeatedly infected with 50 oocysts/mouse/ week for nine weeks, GA 3: repeatedly infected with 500 oocysts/mouse / week for nine weeks, GA 4 a: Positive control (infected with 500 oocysts/mouse once) and GA 4 b: Positive control (infected with 500 oocysts/mouse once).

Group B (immunosuppressed group) was divided into the following subgroups according to the infecting dose and number of exposure to *C. parvum*, each consisted of 15 mice: GB 1: Negative control (Control non-infected), GB 2: repeatedly infected with 50 oocysts/mouse/ week for nine weeks, GB 3: repeatedly infected with 500 oocysts/mouse / week for nine weeks, GB 4 a: Positive control (infected with 50 oocysts/mouse once) and GB4 b: Positive control (infected with 500 oocysts/mouse once).

Immunosuppression: Mice were administered with 0.25 μg/g/day of dexamethasone sodium phosphate (Dexazone) orally via esophageal tube. Dex administration started daily for two weeks prior to oral inoculation with *Cryptosporidium* oocysts and was maintained weekly during the whole experiment [12].

Animal infection: Mice were orally infected with *C. parvum* oocysts. The infection dose was calculated as some groups received 50 oocysts and others received 500 oocysts. The dose was repeated every week for nine weeks to cause chronic irritation and infection in groups A2, A3, B2 and B3. *C. parvum* was dissolved in 200μL of PBS and was given to each mouse using esophageal tube. Animal scarification: Scarification was done at different durations starting from day 14, 21, 36, 45, 57 till day 64 post first infection and euthanasia was performed by decapitation.
The ileocecal region was removed and subjected to histopathological examination. Parasitological examination: One week after infection of the mice, stool samples were collected and subjected to parasitological examination after staining by MZN stain according to [13] to detect and count C. parvum oocysts per oil immersion lens (x100) and to ensure that mice have been infected. Stool samples were collected every week throughout the experiment to assess the effect of weekly chronic irritation.

Histopathological criteria: ileocecal region was removed from each animal, fixed in 10% buffered formalin solution, embedded in paraffin wax blocks that were sectioned then stained in the pathology lab of TBRI, staining was done using Hematoxylin and Eosin (H&E) to assess the pathological changes and explore any abnormal pattern of proliferation, dysplasia, cellular atypia, or any neoplastic lesions. Immunohistochemical technique: tissue sections from colonic lesions were immunohistochemically stained by K-ras, this was performed with immunohistochemical technique (Hsu et al., 1981), using rabbit monoclonal anti human K-ras (Cat# 54-0017, CA 94080) (Oncogene, San Francisco, USA) beside a detection kit DAKO LSAB® System- HRP (DAB, DAKO, Denmark).

Statistical Analysis

Data were analyzed using SPSS© Statistics version 24 (SPSS© Corp., Armonk, NY, USA). Skewed numerical data were presented as median and interquartile range and between-group differences were compared using the Mann-Whitney test (for 2-group comparison) or the Kruskal-Wallis test (for multiple-group comparisons). The Jonckheere-Terpstra test was used to compare numerical data across ranked groups. Post hoc comparisons were done using The Conover test if needed. Categorical variables were presented as number and percentage. Fisher’s exact test was used to compare nominal data and the chi-squared test for trend to compare ordinal data. Inter-method agreement was tested using the Cohen kappa (κ) coefficient P-values less than 0.05 were considered as statistically significant. Ethical consideration: This study was approved by Scientific Research Ethics Committee of TBRI.

Results

Results of Parasitological Criteria

Cryptosporidium Oocysts Shedding

Oocysts of Cryptosporidium spp. were visualized as red spots 4-6µm and with refractile round thick capsules on blue background when examined by light microscope with ×1000 objective. Some of the four sporozoites may be visible in the Cryptosporidium spp. oocysts (Kaushik et al., 2008). Micrometry was used to confirm the size of Cryptosporidium oocysts which is 4-6µm as shown in Figures (1) and (2).

The mean shedding of oocysts in different groups was 306 oocysts, with maximum shedding of 2250 oocysts as shown in Table (1). The number of shed oocysts in the different studied groups showed statistically significant difference between the different groups, with the highest shedding in group B3 (IS inoculated by 500 oocysts) as shown in Table (2) and Figure (3). There was a statistically significant correlation between shedded oocysts and number of inoculated oocysts indicating that the more the dose of inoculation, the more the shedding of oocysts (Figure 4).

![Fig. 1: Cryptosporidium oocysts (red spots), stained by MZN](image-url)
Fig. 2: Micrometer to measuring oocysts, they measured 4-6 um in diameter.

Table 1: Fecal oocyst shedding in studied mice

<table>
<thead>
<tr>
<th>Variable</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal oocyst shedding (oocysts/mg feces)</td>
<td>0</td>
<td>2250</td>
<td>90</td>
<td>473</td>
<td>0</td>
<td>90</td>
<td>485</td>
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Table 2: Comparison of fecal oocyst shedding in studied groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Median</th>
<th>IQR</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0</td>
<td>0-0</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>A2</td>
<td>68</td>
<td>32-114</td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>552</td>
<td>401-1205</td>
<td></td>
</tr>
<tr>
<td>A4a</td>
<td>5</td>
<td>2-9</td>
<td></td>
</tr>
<tr>
<td>A4b</td>
<td>27</td>
<td>20-32</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>0</td>
<td>0-0</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>173</td>
<td>103-243</td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td>768</td>
<td>575-1090</td>
<td></td>
</tr>
<tr>
<td>B4a</td>
<td>15</td>
<td>5-22</td>
<td></td>
</tr>
<tr>
<td>B4b</td>
<td>31</td>
<td>8-115</td>
<td></td>
</tr>
</tbody>
</table>

*Kruskal-Wallis test

Fig. 3: Box plot showing fecal oocyst shedding in groups. Markers represent individual observations. Box represents range from 1st quartile to 3rd quartile. Line inside box represents median (2nd quartile). Error bars represent minimum and maximum values.
Fig. 4: Bar chart showing dose inoculated versus shedded oocysts number, with a significant correlation between shedded oocysts and number of inoculated oocysts indicating that more dose caused more oocysts shedding, with an r value of +0.417, and a p value of 0.005.

Evaluation of Unintended Death of Mice

On comparing mean days of death of mice, there was a statistically significant difference among the different studied groups, with the least survival in group B3 (IS, inoculated regularly by 500 oocysts) with mean survival of 34.3 days and the maximal survival was in group A1 and B1 (negative control) with mean survival of 64 days as shown in Table (3) and Figure (5).

Table 3: Mean day of death among different studied groups

<table>
<thead>
<tr>
<th>Group number</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>p-value</th>
</tr>
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<tr>
<td>A1</td>
<td>64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>38.7</td>
<td>16.9</td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>38.9</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>A4a</td>
<td>44.6</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>A4b</td>
<td>44.6</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>35.8</td>
<td>18.5</td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td>34.3</td>
<td>20.1</td>
<td></td>
</tr>
<tr>
<td>B4a</td>
<td>44.6</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>B4b</td>
<td>44.6</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

*P-value calculated using Kurskall Wallia test

Fig. 5: Graphic presentation of mean day of death among studied groups
Results of Histopathological Criteria

Histopathological examination of the ileocecal region of mice in different groups showed several degrees of pathological and neoplastic lesions among different groups. Several degrees of inflammatory changes were seen in the groups infected with the parasites, both immunocompetent and immunosuppressed. Neoplastic lesions ranging from low grade dysplasia, polypoid structures, nuclear pleomorphism, hyperchromasia and exophytic adenomatous polypi appeared in groups A2, A3, B2, and B3 with different degrees of severity according to the group as shown in Figures (6-13).

Figure 6: Section of small intestine from a mouse that was euthanized at 36 days post-infection in group (B4) (infected control group) showing Cryptosporidium oocysts (tiny purple stained structures) in the intestinal lumen and on the mucosal brush border (red arrows) with shortened broad villi (H&E stain x 1000 "oil immersion").

Figure 7: Exophytic adenomatous polyp from a mouse that was euthanized at 57 days post-infection; showed an increasing architectural distortion and glandular crowding (H&E stain x 100).

Figure 8: High power view of the adenomatous polyp that showed an increasing architectural distortion, glandular crowding, marked cellular atypia with large nuclei showing prominent nucleoli (H&E stain x 400).
Figure 9: Ileocecal region from a mouse that was euthanized at 64 days post-infection revealed intramucosal adenocarcinoma with marked architectural atypia and marked glandular crowding (star) (H&E stain x 200).

Figure 10: High power view of the previous section revealed intra-mucosal adenocarcinoma with marked nuclear anaplasia showing large nuclei with prominent nucleoli (black circles) and increased mitotic activity (black arrows) (H&E stain x 400).

Figure 11: Intramucosal adenocarcinoma showing marked cytological anaplasia with large nuclei and prominent nucleoli. Frequent mitoses are also detected (black circles) (H&E stain x 1000 "oil immersion").
Results of Immunohistochemical Criteria

Immunohistochemistry was done to explore the metabolic alterations that could be involved in the neoplastic process using antibodies against K-ras. Evaluation was done according to the intensity of K-ras staining. In the current work, all negative control cases were negative for K-ras immune-expression. On the other hand, IHC assay of the positive cases for K-ras expression showed statistically significant difference between different studied groups with the highest K-ras expression in group B3 as shown in Figures (14, 15).
Discussion

The current work was designed to explore the potential role of *C. parvum* in inducing neoplastic changes in the digestive epithelium of IC and IS mice, using different parameters including; parasitological (oocyst shedding and death rate evaluation) and histopathological methods. Regarding the infecting dose of *Cryptosporidium* oocysts, mice were challenged with low dose of (50 oocysts) aiming to study the effect of low dose of *Cryptosporidium* oocysts (a low dose which could be found in environmental samples as in tap water and vegetables) compared to high dose (500 oocysts) to assess the role of different doses in induction of neoplastic changes.

In the same context, Benamrouz et al. [14] inoculated the mice with different doses of oocysts to study the effect of low and high dose, mice were challenged with low inoculum (1 and 10 oocysts) and a high inoculum (100 and $10^5$ oocysts). On the other hand, Abdou et al. [15] and Cetrad et al. [16], inoculated their mice with only one infective high dose of $10^5$oocysts per mouse.

As for the frequency of infection exposure, some studied groups were exposed to repeated weekly infection by *Cryptosporidium* oocysts to induce chronic irritation. To our knowledge, chronic irritation in our study was the first to be done with *Cryptosporidium spp*. The role of chronic irritation was done to ensure the maintenance of infection all through the experiment in both IC and IS mice and to assess the role of chronic irritation in induction of neoplastic changes.

Chronic irritation was applied by Watanapa [17] who chronically irritated the mice by infecting them with *Opisthorchis viverrini* to assess its role in development of cholangiocarcinoma.

Also, Tian et al. [18] chronically irritated mice to assess the pathogenesis of chronic schistosomiasis in hepatic fibrosis and carcinogenesis. Scarification days in our experiment were chosen to detect neoplastic changes in the ileocecal region histopathologically.

Mice were euthanized at different durations starting from day 14, 21, 36, 45, 57 till day 64 post infection. The choice of different dates for scarification of mice was done according to observations recorded by previous studies. Cetrad et al. [19] observed an apparent increase of the mitosis number in cells from ileocecal sections between days 20 and 35 post infection and continued to increase till day 46 post infection. A strong positive significant correlation was found between shedded oocysts and the day of stool examination indicating that the longer the duration of infection and inoculation, the more the shedding of oocysts.

This went in accordance with Benamrouz et al. [14] who showed that the day post infection significantly influence the number of oocysts shedded so that as the days increase, oocyst shedding increases. IC chronically irritated mice (Groups A2, A3) showed sustained levels of oocyst shedding throughout and at the end of our experiment, mice challenged with low and high inoculums
developed chronic infection and shedded oocysts without stationary or decline phase until the end of the experiment.

Regarding the IC positive control mice (Group A4) that were inoculated once at the beginning of the experiment (chronic infection) showed decline phase till they reached complete cessation at the end of our study. This coincided with Miller et al. [20] and Certad et al. [11] who found that oocyst shedding declined till it stopped completely at the end of the experiment. Benamrouz et al. [14] revealed that oocyst shedding in IC mice showed decline phase of shedding with almost complete cessation of oocyst production at the end of the experiment.

Lacroix et al. [21] established that the immune system and defense mechanisms were able to fight the infection and reject the parasite rapidly in the IC host. Chronically irritated mice in the present work were inoculated weekly by Cryptosporidium oocysts, which helped maintain the infection without decline or stationary phase till the end of the experiment while in positive control groups, infection was induced only once at the beginning of the study which lead to decline phase till the end of the experiment. Pertaining to IS chronically irritated mice and positive control infected once mice; they showed high levels of oocyst shedding throughout and at the end of the experiment.

These agreed with McDonald et al. [22] who found that IS mice showed high and sustained levels of oocysts shedding. Moreover, Ahmet et al. [23] proved that IS mice were chronically infected with Cryptosporidium spp. and continued shedding oocysts all through the experiment without decline or stationary phase. Abdou et al. [15] found that IS mice showed high levels of oocyst shedding till the end of the experiment. In the current work, evaluation of the intensity of oocysts shedding showed variability between the different groups, this variability was influenced by some factors as the immune status and the inoculum size.

With regards to the effect of the immune status on the intensity of oocyst shedding in the current work, the current study had both IC and IS mice. The intensity of oocyst shedding was higher in IS mice than in IC mice throughout the duration of the experiment, however, it was not statistically significant.

Similar results were reported by Ahmet et al. [23], they found that immunosuppression increases the intensity of oocysts shedding. Chai et al. [24] proved that immunosuppression has a direct correlation with the intensity of oocysts shedding. This went well with Abdou et al. [15] who found that the intensity of oocyst shedding was significantly higher in IS mice than in IC ones throughout the duration of the experiment. But, Certad et al. [11] found that the mean number of excreted oocysts tended to decrease in Dex-treated mice receiving the highest challenge inoculum.

Sequestration of the parasites out of the intestine and into other sites could also explain a diminished detection of Cryptosporidium oocysts in the feces [25].

Concerning the effect of the inoculum size on the intensity of oocyst shedding in the current work, there was a statistically significant correlation between shedded oocysts and number of inoculated oocysts indicating that the more the dose of inoculation, the more the shedding of oocysts so, oocyst excretion increased according to inoculum size ranging between 50 and 500 and was substantially higher in Dex-treated mice than in the Dex untreated ones.

This coincided with Certad et al. [16] who also stated that the higher the dose of the inoculated oocysts, the more the shedding, he found that oocyst excretion increased according to inoculum size and was substantially higher in Dex-treated mice than in the untreated ones. This varied from Miller et al. [20] who found higher oocysts shedding with lower inoculation doses and explained it by the fact that oocyst inoculated doses raise the level of infectivity but not necessarily the shedding of parasites and the pathological outcome.

On comparing fecal oocyst shedding between the different studied groups, it showed statistically significant correlation between the shedded oocysts and number of inoculated oocysts indicating that the more the dose of inoculation, the more the shedding of oocysts, with the highest shedding in GB3 (IS inoculated by 500 oocysts) and mean oocysts shedding of 306 oocysts.

This is compatible with Benamrouz et al. [14] who showed that the inoculum size
significantly influences the oocysts shedding in a directly proportional correlation. In the present study, the unintended death of mice showed a significant difference among the different studied groups regarding unintended death of mice, with the least survival found among GB3 (IS, inoculated regularly by 500 oocysts – 34 days) and the maximal survival found equally in groups A1 and B1 (negative controls 64 days).

This work was the first to evaluate the relation between the number of inoculated oocysts and the unintended death of mice. In the present study, the ileocecal regions were observed with microscopically examination. C. parvum develops usually in enterocytes of small intestine and colon of mice, gastric and duodenal locations are reported less often [25].

In the present work, C. parvum development site was assessed through histopathological examination, it was found that C. parvum parasites developed predominantly in the ileocecal region and in the colon of both IC and IS mice. Abdou et al. [15] found that the terminal part of the ileum was found to be the site with the heaviest burden of infection in both IC and IS mice; Gookin et al. [26] also found that the exact location of C. parvum is in the lamina propria of the terminal part of the ileum. Certad et al.[11] found that C. parvum localization and histopathological changes were mainly localized to the ileocecal region. It has been suggested that conditions in the ileum were favorable, including biochemical conditions and the presence of specific receptors that contribute to the development of C. parvum [27].

In the present study, non-infected healthy mice (GA1 & GB1) showed normal villous architecture with normal brush border and normal glandular architecture in the negative control groups, while histopathological examination of the ileocecal region of the infected groups who were infected with Cryptosporidium oocysts either once or weekly revealed profound effect on the structure of the intestinal mucosa in the form of villous shortening and atrophy, decrease in the ratio of villous height to crypt length, goblet cell depletion, mucosal ulceration and infiltration of lamina propria with inflammatory cells mainly lymphocytes and eosinophils with diffuse loss of brush border micro villous surface area.

These findings comport with Waters and Harp [28] who revealed variable histopathologic changes ranging from partial to complete villous atrophy and inflammatory infiltrate attributed to Cryptosporidium infection. Similar histopathological findings were reported by Gaafar [29] and Al-Mathal and Alsalem [30] who reported villous shortening and villous atrophy with ulcerations associated with Cryptosporidium spp. infection. Cryptic hyperplasia was described by McDonald et al. [22].

Heine et al. [31] found inflammation of the crypts in the large intestine, Enemark et al. [32] observed stunting and fusion of vili, replacement of enterocytes by immature cells and eosinophilia of lamina propria, small and large intestine mucosa severely damaged with villous contraction and little or absent epithelial layer. Another report described an association between Cryptosporidium sp. and aural-pharyngeal polyps, these polyps were pedunculated masses composed of glandular cystic structures lined by hyperplastic cuboidal to columnar epithelium, containing numerous parasites along the apical surface of the epithelial cells [33].

The present results showed that as the inoculated Cryptosporidium dose increased, the pathological lesions were more severe in the form of villous atrophy, ulcerations and severe inflammatory infiltrate, and profound effect on the structure of the intestinal mucosa, this agreed with Certad et al. [11] who found that histopathological evidence of dysplasia in any organ was always associated with the presence of C. parvum and that an elevated parasite load was correlated with the severity of ileocecal pathological changes.

Histopathological changes didn’t show a difference between IC and IS mice. This varied from Certad et al. [19] who suggested that combination of C. parvum with Dex administration is involved in the generation of significant histological changes.

This can be explained as we inoculated the mice weekly causing chronic irritation to both IC and are mice. Apropos our histopathological neoplastic changes, histopathological examination of the ileocecal region of the negative and positive control groups didn’t show any neoplastic changes, neither parasites nor neoplastic changes.
were detected, while histopathological examination of the ileocecal region of the chronically irritated infected groups revealed neoplastic changes that exhibited a markedly abnormal pattern of proliferation, similar to that observed in advanced neoplasms.

The neoplastic changes ranged from mild dysplasia, polyloid structures, architectural distortion, glandular crowding and marked cellular atypia (that started to appear at 36-45 days post infection), to exophytic adenomatous polypi and intramuscular adenocarcinoma and marked nuclear anaplasia (that appeared at 57-64 days post infection). The severity of lesions increased steadily according to the duration and the number of the inoculated oocysts post-infection.

These neoplastic findings agreed with Certad et al. [16] who observed the development of intramucosal carcinoma with a suspicion of submucosal invasion in mice and the development of intraepithelial adenocarcinoma in C. parvum infected SCID mice, and with Benamrouz et al. [14] who observed neoplastic lesions in all Dex-treated SCID mice infected by C. parvum, whatever the inoculum.

The present findings were in divergence with Abdou et al. [15] who found that dysplastic changes were mainly of low-grade category, with only few cases showing high-grade dysplasia, no frank carcinoma was detected in his work. The current work might be the first to report neoplastic lesions in IC mice, this was explained as the mice were chronically irritated (weekly) by the inoculated oocysts, which caused maintenance of the infection all through the experiment and subsequent irritation of the intestine leading to dysplastic and neoplastic changes.

In the current work, all negative control cases were negative for K-ras immunohistochemistry and this coincided with Andersen et al. [34] who found that colonic control cases with normal mucosa were negative for K-ras immunoreactivity and Abdou et al. [13] who used cyclin D-1 as a dysplastic marker for colorectal carcinoma whose upregulation is an early event in intestinal carcinogenesis, it did not stain normal areas as well.

On the other hand, IHC assay of the positive cases for K-ras expression showed statistically significant difference between different studied groups with the highest K-ras expression in group B3 (IS inoculated by 500 oocysts). This went in agreement with Bartkova et al. [35] who detected positive immuno-histochemical staining in SCID mice infected with Cryptosporidium oocysts. Our results oppose with the results obtained by Benamrouz et al. who did not find any difference in the labeling of K-ras in the ileocecal epithelia, from both negative control and Cryptosporidium-infected mice, normal membrane staining of K-ras was observed.

Conclusion

The present study proved the high infectious power of Cryptosporidium oocysts associated with both immunosuppressed and immunocompetent mice after repeated infection. It also proved its ability to develop histopathological changes in the ileocecal region even with low dose of oocysts as well as its ability to induce neoplastic changes after chronic irritation. Cryptosporidium species is one of the infectious agents that may induce intestinal neoplasia, which is highly affected by repeated exposure and elevated parasite loads. Cryptosporidium species cancerogenic role was confirmed [36, 38].

References


