



Prognostic role of tissue eosinophilia in *Cryptosporidium parvum*-induced colorectal cancer

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Abstract

Cryptosporidium parvum has been increasingly implicated as a potential contributor to colorectal cancer (CRC), particularly in immunocompromised hosts. This study aimed to evaluate the prognostic role of tissue eosinophilia in *C. parvum*-induced CRC. Fifty female, immunosuppressed white Albino CDI strain mice were divided into 5 groups: 10 mice each. GI was the negative control, GII-GV were infected with *C. parvum* oocysts in the following pattern: GII and GIII were positive control groups. GVI and GV were infected with 100 and 1000 *C. parvum* oocysts per week for 90 days, respectively. Parasitological examination of mice feces, histopathological examination of liver, lung, spleen, ileocaecal sections, and assessment of peripheral blood eosinophilia were performed. No statistically significant difference ($P > 0.05$) between the test and the corresponding control groups at the 10th and 60th dpi regarding the number of oocysts in the feces of the study mice, whereas a highly statistically significant difference ($P < 0.01$) between the test and the corresponding control groups at the 30th and 90th dpi. The infection triggered a strong systemic immune response characterized by significant eosinophilia. However, the outcome was organ-specific: The liver successfully contained and resolved the infection. The lung and spleen failed to resolve, developing chronic and even worsening pathology. Ileocaecal sections showed progress from marked inflammatory infiltrates to focal surface ulceration, then low-grade dysplasia. The highest leucocytic and eosinophilic responses were observed in GV, followed by GIV, indicating a clear dose-dependent inflammatory reaction. Tissue eosinophilia should be considered as an important marker for immunopathology of cryptosporidiosis.

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Introduction

One of the main causes of cryptosporidiosis, a diarrheal illness mainly spread by the fecal-oral route by unclean food or water, or direct contact with infected individuals, is the zoonotic apicomplexan protozoan *Cryptosporidium parvum*. When hardy *C. parvum* oocysts are consumed, they infiltrate the intestinal epithelial cells, causing mucosal destruction, higher intestinal permeability, inflammation, and copious

watery diarrhea. In immunocompetent people, this infection usually resolves on its own, but in immunocompromised patients and young children, it can become chronic and even fatal. Cattle are frequently crucial reservoirs that increase the danger of human infection from *C. parvum*, which causes a sizable amount of human cryptosporidiosis worldwide and exhibits zoonotic transmission. According to the latest studies, animal exposures and poor water quality are important epidemiological determinants, and infections alter

the composition of the microbiota and the function of the gut barrier, worsening the severity of disease (1–3).

The most common sign of a human *C. parvum* infection is acute gastrointestinal sickness, with watery diarrhea being the most prevalent symptom. Abdominal discomfort, nausea, headache, exhaustion, and vomiting are often described in conjunction with diarrhea in human case studies, suggesting a wide range of gastrointestinal clinical symptoms linked to cryptosporidiosis. Immunocompetent hosts usually experience non-bloody, self-limiting diarrhea after an incubation period of several days. However, symptoms like fatigue and ongoing gastrointestinal discomfort have been reported long after the acute phase, and some people experience prolonged bouts of nausea and abdominal pain. In the small intestine, where the parasite replicates and interferes with regular absorptive processes, these symptom patterns show direct mucosal involvement and inflammation (4).

The *C. parvum* infection in immunocompromised people can cause severe, persistent diarrhea, dehydration, loss of weight, and extended digestive problems, all of which frequently call for medical attention. Diarrhea in hospitalized *Cryptosporidium*-infected patients can persist for up to two weeks, according to a recent research study. It is strongly linked to dehydration and dietary loss, especially in younger individuals, suggesting that populations at risk have a higher clinical incidence of disease. Although less frequent, extra-intestinal symptoms, including fever and other systemic symptoms, as well as possible complications from prolonged diarrheal illness, can happen, especially in hosts with weakened immune systems. These results emphasize the significance of species-specific evaluation and therapy in patients presenting with chronic diarrheal disease and show how the clinical presentation of cryptosporidiosis varies depending on host variables (5).

A fascinating connection between *C. parvum* infection and colorectal cancer (CRC) has been suggested by a new study. The idea that the parasite might be a risk factor for tumor formation is supported by epidemiological studies that show noticeably greater frequencies of *C. parvum* infection in people with CRC than in healthy controls. For instance, genetic subtyping verified the parasite's presence in tumor tissue, suggesting a possible zoonotic role in carcinogenesis. A case-control study conducted in China revealed that *C. parvum* was present in 17.24% of CRC patients, compared to significantly reduced rates in non-cancer controls (6). Furthermore, the total frequency of *C. parvum* in gastrointestinal malignancies was considerably higher in these investigations, indicating that sustained colonization and contact with host tissues may be a contributing factor to epithelial metamorphosis. The possibility that *C. parvum* influences colorectal carcinogenesis is further supported by scientific findings from in vitro investigations and experimental studies. Chronic infection seems to interfere

with normal host cell signaling, which includes stimulating oncogenic pathways such as PI3K, NF- κ B, and Wnt. These pathways can enhance cell life and hinder apoptosis, which are indicators of the occurrence of cancer (7–9). It has been demonstrated that chronic *C. parvum* infection in experimental mice causes tumor development in the ileo-cecal region, along with an immunosuppressive and inflammatory condition that promotes the development of neoplasms (10). These results show how persistent parasite infection may contribute to conditions favorable to CRC, especially in immunocompromised hosts, even if *C. parvum* is not yet formally recognized as a tumor-causing agent (7).

This elucidates the urgent need to examine the prognostic role of tissue eosinophilia in *C. parvum*-induced CRC.

Materials and methods

Ethical approval

This study was performed following the ethical guidelines and approved by the research ethical committee at Theodor Bilharz Research Institute (TBRI-REC) with approval number PT (905).

Experimental animals

Fifty female, white Albino CDI strain mice (laboratory-bred, 4–6 weeks old, weighing 20–25 g each) were obtained from the Biological Unit of the Theodor Bilharz Research Institute (TBRI). The animals were kept in a well-ventilated plastic cage with clean wood-chip bedding. The animal cage was kept in conditioned rooms (27 $\pm$ 2 °C) and away from direct sunlight. The internationally valid guidelines were applied during the animal experiments. The approval of the institutional ethical committee was obtained before the start of the experiment.

Induction of immunosuppression

The experimental mice were chemically immunosuppressed by the administration of dexamethasone sodium phosphate (Dexazone) at a dosage of 0.25 mg/g/day orally via an esophageal tube for two weeks before the induction of infection; then, they continued to receive the same dose of dexamethasone throughout experiments (11).

Induction of infection with *C. Parvum* oocysts

C. parvum oocysts were concentrated from the feces of infected calves. *Cryptosporidium* species was confirmed genetically as *C. parvum* (12). Oocysts were isolated and purified from stool samples, preserved in a 2.5% solution of potassium dichromate, and kept at 4 °C until required. To determine how much fluid volume was in the inoculum per mouse, the infective inoculum was prepared, and the number of infective oocysts in the concentrated stock inoculum was counted. Mice were infected orally with the oocysts via oral-gastric gavage (13).

Animal groups

Immunosuppressed mice were divided into five groups: 10 mice each. GI: Non-infected (Negative control). GII: Infected with 100 oocysts (positive control). GIII: Infected with 1000 oocysts (positive control). GIV: Infected with 100 oocysts per week for 90 days. GV: Infected with 1000 oocysts per week for 90 days.

Parasitological examination

On days 10 and 30 after infection (dpi) for acute infection and on days 60 and 90 dpi for chronic infection, fecal pellets were taken from the infected mice. The Modified Zehil Neelson acid-fast stain was used to parasitologically analyze the fecal pellets and determine the quantity of *Cryptosporidium* oocysts present (14). Samples of feces were taken from every infected mouse. For every mouse group, the average number of oocysts was determined. After being weighed, one milligram of the fecal pellet was kept in one milliliter of 10% formalin. Centrifugation was used to concentrate the fecal suspension for 10 minutes at 500× g. 100 µL of dried and stained fecal sediment was used to measure the number of oocysts in 1 mL of fecal sample, which was then seen via an oil immersion lens (×100). The average of three counts was estimated and multiplied by ten to find the number of oocysts in one milliliter of fecal pellet (15). The number of oocysts was estimated per gram of stool (16).

Histopathological examination

Mice were sacrificed by light isoflurane inhalation anesthesia (Forane®, Baxter, Deerfield, MA, USA) (17). Blocks of paraffin wax were used to embed the removed liver, lung, spleen, and ileocecal segments after they had been fixed in a 10% buffered formalin solution. Following sectioning, Hematoxylin and Eosin (H&E) staining was performed in accordance with Drury and Wallington's instructions (18).

Peripheral blood eosinophilia examination

Blood samples (0.5 mL) were collected in vacutainer tubes with ethylene diamine tetraacetic acid tripotassium (K3EDTA) and used for evaluation of the leukogram (Total leukocytic counts and eosinophilic count). Blood smear is prepared on a slide and stained with Hematoxylin and Eosin (H&E) stain. Eosinophils are identified by their characteristic bilobed nucleus and large, red/orange-red cytoplasmic granules. A differential count (counting the percentage of eosinophils among a total count of white blood cells) is performed under a microscope, and this percentage is then multiplied by the total white blood cell count to estimate the absolute eosinophil count (19).

Statistical Analysis

Data were analyzed using One-way ANOVA. *P*-value was considered significant when <0.05.

Results

Parasitological results

At the acute stage of infection, there was no statistically significant difference ($P>0.05$) between the test and the corresponding control groups at the 10th dpi. A high statistically significant difference ($P<0.001$) was reported at the 30th dpi. At the chronic stage of infection, there was no statistically significant difference ($P>0.05$) between the test and the corresponding control groups at the 60th dpi. However, a high statistically significant difference ($P<0.001$) was reported at the 90th dpi. GV showed greatest mean±SD of *C. parvum* oocyst at both acute and chronic stages (95±11.2 and 130±9.7, respectively) (Table 1).

Table 1: *C. parvum* oocysts count (Mean±SD) in different groups during the acute and chronic stages

Groups	Acute stage		Chronic stage	
	10 th dpi	30 th dpi	60 th dpi	90 th dpi
GII	84.1±7.2	10.6±2.8	116.8±8.4	20.7±4.2
GIII	83.8±7.5	24.2±4.6	117.3±7.9	44.1±5.8
GIV	84.6±6.9	91.5±7.1	116.1±8.1	123.2±9.4
GV	87.6±7.8	95±11.2	120±8.7	130±9.7
P-value	0.94 (ns)	<0.001**	0.87 (ns)	<0.001**

One-Way ANOVA test for Mean±SD, *P*-value >0.05 is insignificant; **P*-value <0.05 is significant; ***P*-value <0.01 is highly significant.

Histopathological results

Histopathological examination of liver tissues

During the acute stage of infection

The liver tissue exhibited significant acute inflammation consistent with a robust host response. There were multifocal to coalescing areas of moderate to severe inflammatory infiltrates, primarily located within the portal triads (portal hepatitis). The infiltrate was characteristically dominated by a prominent number of eosinophils, identifiable by their bright eosinophilic cytoplasmic granules. Alongside eosinophils, lymphocytes, plasma cells, and some neutrophils were present. Mild hepatocellular degeneration was observed, including scattered individual hepatocyte necrosis (pyknotic nuclei) and occasional ballooning degeneration. The hepatic cord architecture was generally maintained but disrupted in focal areas of intense inflammation (Figure 1a,b and Table 2).

During the chronic stage of infection

The inflammatory response appeared to be evolving at 60th dpi. The dense eosinophilic infiltrates seen at 30th dpi have diminished in severity and are now graded as moderate, while some granulomas appeared. The inflammation

remained portal but may show early extension into the lobules. A shift in the cellular composition was noted, with lymphocytes and plasma cells becoming more predominant relative to eosinophils. Evidence of early tissue repair and chronicity may be present, such as slight portal fibrosis and biliary hyperplasia. Focal areas of hepatocellular degeneration persisted but were less widespread than in the acute phase (Figure 1c,d and Table 2).

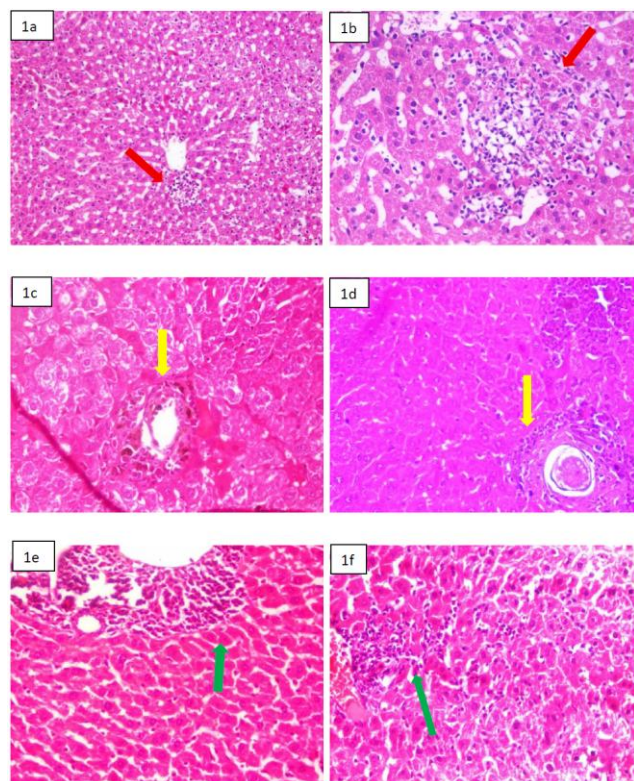


Figure 1: Liver sections at 30th, 60th, and 90th dpi. a and b: sections at 30th dpi showing significant acute inflammation, prominent eosinophilia, with mild hepatocellular degeneration as pointed by red arrow. c and d: sections at 60th dpi showing diminished inflammatory response, some granulomas, with evidence of early tissue repair as pointed by yellow arrow. e and f: sections at 90th dpi showing signs of resolution as pointed by green arrow.

The liver architecture at 90th dpi showed signs of resolution or chronic, low-grade damage. Inflammation was generally mild and more sparsely distributed. Eosinophils were rare or absent, with the inflammatory foci now consisting almost exclusively of lymphocytes and plasma cells. In a scenario of incomplete resolution, mild portal fibrosis may be a prominent feature, representing scarring from the previous inflammatory injury. The parenchyma appeared largely regenerated with minimal ongoing

degeneration. The overall picture was one of a resolved acute insult or established chronic, non-destructive inflammation (Figure 1e,f, and Table 2).

Histopathological examination of lung tissues During the acute stage of infection

The lungs displayed a marked allergic-type pneumonitis at 30th dpi. There was a mild inflammatory reaction around airways (peribronchial) and blood vessels (perivascular). Eosinophils were few and may also be observed within the alveolar spaces. The alveolar interstitium was mildly thickened due to edema and cellular infiltration. Some perivascular edema might be evident. Despite the inflammation, the overall alveolar architecture remained intact (Figure 2a,b and Table 2).

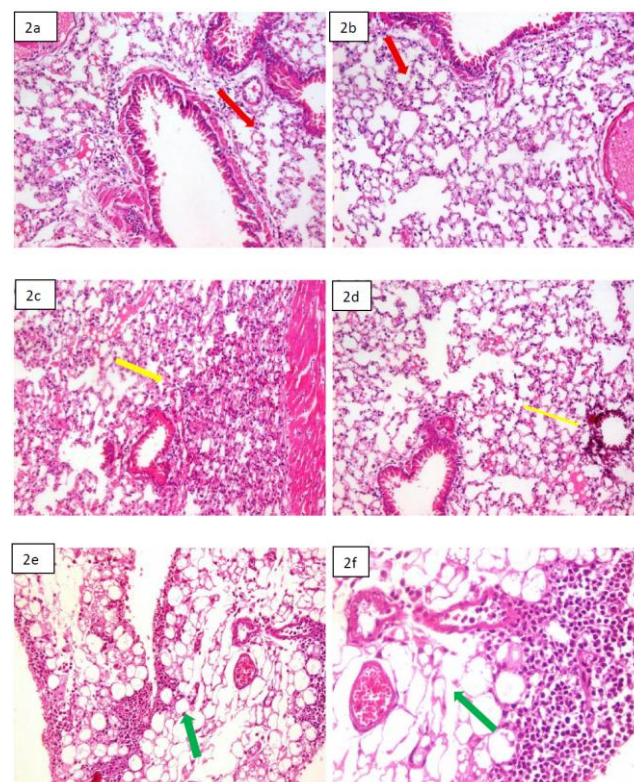


Figure 2: Lung sections at 30th, 60th, and 90th dpi. a and b: sections at 30th dpi showing signs of allergic pneumonitis as pointed by red arrow. c and d: sections at 60th dpi showing increased peribronchial and perivascular inflammation and the interstitial thickening as pointed by yellow arrow. e and f: sections at 90th dpi showing evident remodeling as pointed by green arrow.

Table 2: Histopathologic examination of liver, lung, spleen, and ileocaecal tissues

Examination	Time point	Inflammation (0-3)	Degeneration (0-3)	Eosinophils (0-3)	Key observations and notes
Liver tissue	30 th dpi	3	1	2	Severe portal hepatitis. Infiltrate dominated by eosinophils. Mild focal hepatocyte necrosis.
	60 th dpi	2	1	1	Moderate, evolving inflammation. Granuloma formation. Shift to lymphocytic predominance. Early portal fibrosis.
	90 th dpi	1	0	1	Mild, scattered lymphocytic foci. Eosinophils are rare. Minimal degeneration, architecture largely restored.
Lung tissue	30 th dpi	1	0	1	Mild allergic-type pneumonitis (peribronchial/perivascular). Few eosinophils present.
	60 th dpi	1	0	1	Slight increase in interstitial thickening. Mixed cellular infiltrate (lymphocytes/macrophages).
	90 th dpi	3	0	2	Severe, patchy lymphocytic inflammation. Eosinophils absent. Evidence of chronic tissue remodeling.
Splenic tissue	30 th dpi	2	0	1	Immune activation: white pulp hyperplasia. Noticeable eosinophil infiltrates in red pulp/PALS.
	60 th dpi	3	0	2	Increased and more focal eosinophil infiltrates. Prominent red pulp congestion. Ongoing immune response.
	90 th dpi	3	0	3	Distorted architecture. Severe congestion, extramedullary hematopoiesis, and high eosinophilia. Hyperactive state.

During the chronic stage of infection

At 60th dpi, the intensity of the peribronchial and perivascular inflammation had increased slightly. The pronounced eosinophilic component was reduced, with a more mixed cellular infiltrate (lymphocytes, macrophages) now visible. The interstitial thickening increased. In some cases, this stage may show the beginning of structural remodeling, such as mild smooth muscle hyperplasia in the bronchiolar walls, indicative of a chronic irritant (Figure 2c,d and Table 2).

At 90th dpi, the lung tissue was largely remodeled. Inflammation is evident and predominantly lymphocytic, localized to occasional perivascular areas. Eosinophils were typically not found. The alveolar septa appeared normal away from the patchy inflammation, and the airways were mostly clear, possibly with residual, insignificant lymphocytic aggregates (Figure 2e,f and Table 2).

Histopathological examination of splenic tissue

During the acute stage of infection:

The spleen showed features of significant immune activation. The white pulp (lymphoid follicles) was hyperplastic and expanded, indicating a strong antigen-specific immune response. Within both the red pulp and the periarteriolar lymphoid sheaths (PALS) of the white pulp,

there were noticeable infiltrates of eosinophils. This picture reflected the spleen's role in both generating and housing an immune response against the parasite (Figure 3a,b and Table 2).

During the chronic stage of infection

At the 60th dpi, the splenic changes were less pronounced. The white pulp hyperplasia has slightly regressed. The infiltrates of eosinophils in the red pulp and PALS are increased and more focal (mild to moderate). Congestion in the red pulp was still prominent. The spleen's architecture reflected ongoing immune response (Figure 3c,d, Table 2).

At the 90th dpi, the spleen's architecture was distorted. The white pulp may appear normal or only slightly prominent. Eosinophils are increased within the splenic tissue. The red pulp showed significant congestion and extramedullary hematopoiesis. The spleen appeared hyperactive (Figure 3e,f and Table 2).

Histopathological examination of ileocaecal tissues

During the acute stage of infection

Intestinal section at 30th dpi exhibited ulceration of the lining intestinal epithelium with marked mononuclear inflammatory infiltrate and lymphoid follicle hyperplasia (Figure 4a and Table 2).

During the chronic stage of infection

Intestinal section at 60th dpi exhibited focal surface ulceration of the mucosal brush border (Figure 4b and Table 2). At 90th dpi, a polypoid adenoma was noted with focal depletion of the goblet cells and low-grade dysplasia (Figure 4c and Table 2).

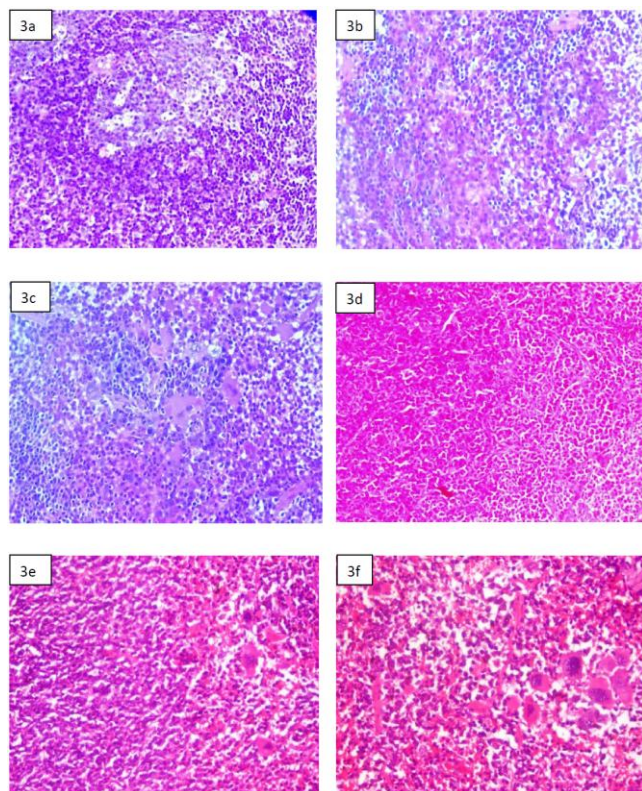


Figure 3: Lung sections at 30th, 60th, and 90th dpi. a and b: sections at 30th dpi showing evident immune activation. c and d: sections at 60th dpi showing the ongoing immune response. e and f: sections at 90th dpi showing hyperactive splenic features.

Peripheral blood eosinophilia

WBC and eosinophil counts showed consistent differences between groups across the three sampling times, 30th, 60th, and 90th dpi. The highest leukocytic and eosinophilic responses were observed in GV, followed by GIV (infected groups with 1000 and 100 oocysts/ week for 90 days, respectively), indicating a clear dose-dependent inflammatory reaction. The lowest values throughout the study were recorded in GII (the positive control infected with 100 oocysts). These data were obtained from K3EDTA blood samples, using total leukocyte counts combined with H&E-stained blood smears, where eosinophils were identified morphologically and their absolute counts calculated from differential percentages (Table 3).

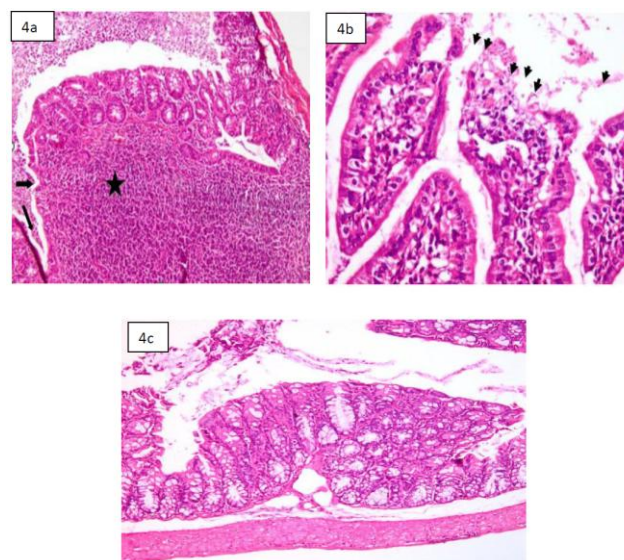


Figure 4: Ileocaecal sections at 30th, 60th, and 90th dpi. a: sections at 30th dpi showing ulceration of the lining intestinal epithelium (black arrow) with marked mononuclear inflammatory infiltrate and lymphoid follicle hyperplasia (black star) (H&E stain x 100). b: sections at 60th dpi showing *Cryptosporidium* oocysts (tiny purple-stained structures) in the intestinal lumen and on the mucosal brush border (black arrow heads) with focal surface ulceration (H&E stain x 400). c: sections at 90th dpi showing a polypoid-adenoma section with focal depletion of the goblet cells and low-grade dysplasia (H&E stain x 100).

Table 3: Total WBCs and eosinophil count of different study groups at 30th, 60th, and 90th dpi

Time	WBCs Eosinophils ($\times 10^3$ /ml)	GII	GIII	GIV	GV
30 th dpi	WBCs	4.3	5.7	6.65	8.1
	Eosinophils	0.16	0.22	0.537	0.69
60 th dpi	WBCs	4.2	5.8	6.76	8.8
	Eosinophils	0.17	0.26	0.61	0.75
90 th dpi	WBCs	4.31	5.5	6.70	9.1
	Eosinophils	0.18	0.29	0.69	0.84

GII and GIII: infected with 100 and 1000 oocysts, respectively. GIV and GV: infected with 100 and 1000 oocysts per week for 90 days, respectively.

Discussion

The histopathological analysis reveals a complex and compartmentalized immune response to *C. parvum* infection over time, with a surprising and significant failure to resolve in the lung and spleen. In the

present study, the histopathologic progression observed in the livers of *C. parvum*-infected mice from intense, eosinophil-rich acute inflammation at 30 dpi, through a transitional granulomatous phase at 60 dpi, to predominantly lymphocytic, low-grade chronic foci by 90 dpi reflects a coordinated host response that successfully controls infection and drives tissue repair. The recruitment of innate effector cells, such as eosinophils, which can localize to damage sites and aid in pathogen containment as well as the control of later immune responses, is a common characteristic of acute hepatic inflammation. Recent research emphasizes the importance of eosinophils in early host defense and in determining the resolution course of inflammation by showing that they take part in acute liver injury responses and can affect downstream immune signaling and wound healing processes (20,21). An established model in hepatic responses to persistent pathogens, organized granuloma formation is an intermediate histologic pattern that occurs as the response progresses. In this pattern, immune cells, such as macrophages and lymphocytes, encapsulate residual antigenic stimuli to limit tissue damage and facilitate parasite clearance. The eventual dominance of mononuclear lymphocytic infiltrates, accompanied by a significant decrease in eosinophils and degenerative changes by 90 dpi, is consistent with immune regulation and inflammation resolution mechanisms, wherein adaptive immune processes facilitate the restoration of tissue homeostasis and acute pro-inflammatory signals subside. According to current models of controlled inflammation resolution in the liver, this gradual transition from acute, granulomatous to mild chronic histopathologic patterns shows efficient local infection management and the start of repair pathways (22). In the current experiment, the pulmonary histopathologic trajectory deviates markedly from the classic resolution seen in competent hosts, instead demonstrating persistent and evolving chronic inflammation. Mild allergic pneumonitis at 30 dpi probably represents an early Th2-specific response with eosinophil mobilization and type 2 cytokine release, which is in line with processes that use IL-4, IL-5, and IL-13 signaling to generate eosinophilic pulmonary inflammation in allergy models (23,24). However, rather than resolving, this initial response fails to abate and transitions into severe, patchy lymphocytic inflammation by 90 dpi with an absence of eosinophils, suggesting a shift away from a Th2-type (eosinophil-associated) phenotype toward a non-Th2 chronic inflammatory milieu. In pulmonary immunobiology, such sustained lymphocytic predominance is frequently associated with Th1 or mixed immune activation, where IFN- γ -producing T cells and associated proinflammatory pathways replace type 2 effector mechanisms in driving tissue pathology (25,26). The loss of eosinophils at later stages cells that are hallmarks of Th2 responses supports the interpretation that the ongoing lung injury is not maintained

by classic type 2 immunity but instead reflects persistent Th1-like or alternative chronic inflammation, which is linked to sustained tissue damage and ineffective resolution in other chronic lung disease contexts (27). This pattern underscores that in the absence of effective immunoregulation, immunosuppression may permit *C. parvum* to drive a dysregulated chronic pulmonary response with pathological significance, marked by unresolved lymphocytic inflammation and a shift in immune phenotype. In this study, the splenic pathology reveals escalating rather than resolving immune activity, with the organ transitioning from early activation at 30 dpi to marked focal eosinophilia at 60 dpi and culminating at 90 dpi in a profoundly distorted, hypercellular spleen dominated by eosinophils and extramedullary hematopoiesis (EMH). Instead of a complete resolution, this progressive pattern points to uncontrolled hematopoietic responses and ongoing systemic immune involvement. When bone marrow output is inadequate or distorted by systemic signals, the spleen may resume a fetal-like hematopoietic process to support the synthesis of myeloid and erythroid lineages. Under normal circumstances, the spleen is a vital lymphoid organ and a secondary site of blood detoxification and immunological surveillance. The proliferation of hematopoietic progenitors in the spleen and related splenomegaly have been linked to prolonged EMH in murine models of inflammatory and infectious stimuli, particularly when immunosuppression and ongoing infection are present alongside chronic inflammation and persistent antigenic stimulation (28). The observed increase in eosinophilia points to aberrant eosinophil-lineage cell migration or retention in splenic tissue, which has been linked in other contexts to improper leukocyte transport or extended type-2 immune signals (29). When combined with a strong EMH, these results show that the spleen in this paradigm continues to be maladaptively active, generating effector immune cells and progenitors even after hepatic damage has started to heal. Models of persistent immunological stimulation, in which extramedullary hematopoietic zones compensate for altered marrow function and contribute to continued pathogenesis, are consistent with such chronic systemic hematopoietic and inflammatory stimulation (30). The progressive increase in total leukocyte and eosinophil counts observed across all infected groups highlights a dose- and exposure-dependent systemic immune activation in mice infected with *C. parvum*. Animals receiving repeated oocyst exposure (GIV and GV) exhibited consistently higher WBC and eosinophil counts at all time points compared with single-dose groups (GII and GIII), indicating that chronic antigenic stimulation sustains leukopoiesis and eosinophil mobilization rather than permitting immune contraction. One well-known symptom of parasite infections is eosinophilia, which is a reflection of persistent type-2-associated immunological activation, especially in cases of immunological dysfunction or

persistent infection. Through IL-5-mediated pathways, ongoing parasite exposure has been shown to sustain eosinophil generation and survival. This is frequently accompanied by splenic hematopoietic activation and systemic leukocytosis. Furthermore, the idea that *C. parvum* might cause persistent immunological stimulation when clearance is insufficient a phenomenon made worse by immunosuppression is supported by the slow increase in eosinophil counts from 30 to 90 dpi, particularly in groups that have been infected repeatedly. In chronic parasite and inflammatory models, such extended leukocyte and eosinophil growth has been associated with EMH and immune organ overactivity, which is consistent with the severe splenic pathology later in this work. All of these hematologic results support the idea that recurrent or highly prevalent *C. parvum* infections cause systemic immunological activation to remain rather than resolve, which leads to long-term immunopathology (29,31–33).

Conclusion

The infection triggers a strong systemic immune response characterized by significant eosinophilia. However, the outcome is organ-specific: The liver successfully contains and resolves the infection. The lung and spleen fail to resolve, developing chronic and even worsening pathology. The spleen, in particular, shows signs of being a major site of ongoing immunopathology, potentially acting as a reservoir for the immune response that drives the chronic inflammation seen in the lungs. This discordance suggests that the control of *Cryptosporidium* infection is not uniform across all tissues, and the long-term health implications may be dominated by chronic inflammatory damage in the respiratory and systemic immune systems, rather than the initial hepatic involvement. Ileocecal sections showed focal surface ulceration and low-grade dysplasia at the chronic stage of cryptosporidiosis. The dose-dependent inflammatory reaction and eosinophilia that were recorded in different stages of this study could present the eosinophilia level as an important prognostic marker for cryptosporidiosis-induced CRC.

Authors' contribution:

H.F. A conducted the investigation, performed the formal analysis, conceptualized the study, supervised the present work, and wrote the manuscript. A.M. El-A conceptualized the methodology and drafted the manuscript Rabab Fouad performed the histopathological examinations. H.F. A. conducted the laboratory analyses. A.M. El-A. wrote the manuscript and revised it. The manuscript was read and approved by all named authors. We confirm that the order of authors listed has been approved by all authors.

Compliance with ethical standards

The authors confirm that all the research ethical standards were applied in this study.

Conflict of interest and Funding

None

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الدور التنبؤي لفرط الحمضات النسيجي في التغيرات المرضية الناتجة عن عدوى خفية الأبواغ

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الخلاصة

يساهم *Cryptosporidium parvum* وبشكل متزايد كمحتمل في سرطان القولون والمستقيم، وخاصة في المضيفين الذين يعانون من نقص المناعة. هدفت هذه الدراسة إلى تقييم الدور التوقعي لفرط الحمضات في الأنسجة في سرطان القولون والمستقيم التي يسببها *C. parvum*. قسمت خمسون من إناث الفئران البيضاء المثبطة للمناعة إلى ٥ مجموعات؛ ١٠ فئران لكل منها. المجموعة الأولى كانت السيطرة السلبية، المجموعات الأربع الباقيات أصيب ببويضات *C. parvum* على النحو الآتي؛ المجموعة الثانية والثالثة اعتبرت مجموعة سيطرة إيجابية. المجموعة الرابعة والخامسة تم إصابتها بـ ١٠٠ و ١٠٠٠ من بويضات *C. parvum* في الأسبوع ولمدة ٩٠ يوماً، على التوالي. تم إجراء فحص للكشف عن الطفيليات في براز الفئران، وفحص الأنسجة المرضية للكبد والرئة والطحال والأقسام اللفائفية، وتقييم فرط الحمضات في الدم المحيطي. لم يكن هنالك فرق معنوي بعدد البويضات في براز فئران الدراسة عند اليوم ١٠ واليوم ٦٠ بعد الإصابة، في حين كان الفرق معنوي وعالي ($P < 0.01$) بين عند اليوم ٣٠ و ٩٠ بعد الإصابة. تسببت العدوى في استجابة مناعية جهازية قوية تتميز بفرط الحمضات بشكل كبير. ومع ذلك، كانت النتيجة حسب الأعضاء: إذ نجح الكبد في احتواء العدوى والتخلص منها. أما الرئة والطحال فقد فشلا التخلص من الإصابة أو تطورها إلى الإصابات المزمنة وحتى المتفائمة. أظهرت المقاطع اللفائفية تقدماً من الارتشاح الالتهابي الملحوظ إلى تقرح السطح البؤري، ثم خلل التنسج منخفض الدرجة. وقد لوحظت أعلى الاستجابات للكريات البيض والحمضات في المجموعة الخامسة، تليها المجموعة الرابعة، مما يدل على رد فعل التهابي واضح يعتمد على الجرعة. يمكن اعتبار فرط الحمضات في الأنسجة علامة مهمة في علم الأمراض المناعي للإصابة بـ *Cryptosporidium*.