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Systemic Histopathological and Immunological Changes in the Liver of Immunosuppressed Mice Infected with *Acanthamoeba polyphaga* Isolated from Contaminated Contact Lenses

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ABSTRACT

Disseminated Acanthamoebiasis poses a grave threat that can potentially lead to fatal outcomes in immunosuppressed individuals. This study investigates the immunological and histopathological effects in immunosuppressed animals after a systemic infection by *A. polyphaga* strain isolated from contaminated contact lenses. Fifty male Swiss albino mice were divided into three groups: immunosuppressed control (CS), immunosuppressed *A. polyphaga* infected (AS), and negative controls (C). The CS and AS groups were sacrificed at 2, 7, and 15 days post-infection. The results showed that the AS mice displayed a range of clinical signs including emaciation, changes in coat color, ulcerations, enlargement of the testicular region, circular moving, bilateral eye lesions, diminished mobility, and alterations in posture. There was a highly significant increase in the cytokines IL-1β, TNF-α, and IL-10 levels, especially at the onset of infection in AS groups. The infected liver tissues demonstrated an

inflammatory response, characterized by an intercellular distribution of parasite stages, multiple focal areas of inflammatory cell infiltration, diffuse vacuolar degeneration with sporadic hepatocyte necrosis, dilatation of blood sinusoids, and congestion in the blood vessels. Additionally, significant changes were observed in liver enzyme levels. These findings indicated the potential impact of systemic infection by *A. polyphaga* in immunosuppressed hosts, highlighting the need for awareness and intervention strategies to mitigate such life-threatening infections. Further research is crucial to better understand the disseminated Acanthamoebiasis by analyzing changes in gene and protein expressions in liver tissues.

INTRODUCTION

Acanthamoeba spp are ubiquitous and opportunistic parasites, that possess the capability to overcome natural barriers, leading to diseases in both humans and animals [1]. They are widely distributed in diverse environmental niches such as lakes, pools, air, and soil. Acanthamoeba spp exist in two distinct stages: the motile trophozoite stage, which ranging from 12 to 35 μm in diameter and is characterized by metabolic activity, a conspicuous central nucleus, and food vacuoles; and the resistant cyst stage, which varies from 5 to 20 μm in diameter, exhibits metabolic dormancy, and encased within a protective bilaminated cellulose wall [2].

Acanthamoeba sp represents a threat, particularly in immunocompromised individuals, such as those following organ transplantation, chemotherapy, or those patients infected with HIV-[3]. It has a penchant for infecting immune-privileged organs like the eye and brain, which have limited regenerative capacity causing conditions such as Acanthamoeba keratitis (AK) and granulomatous amebic encephalitis (GAE) [4]. Post-mortem investigations have revealed that animals may gain infection through inhalation of cysts/trophozoites or exposure of skin lesions to contaminated environmental elements, subsequently leading to hematogenous dissemination [5]. Disseminated Acanthamoebiasis (DA) occurs in multi-organs, including the liver, kidneys, and lungs with multi-symptom which can rapidly result in host death [6-10]. Disseminated infections are typically associated with an average time from 3 months to 6 years [6].

Scientific literature indicates the virulence associated with intravenous inoculation of *Acanthamoeba* spp. [13]. Intraperitoneal multiplication of amoebae accentuates systemic tissue invasion [14], with infection severity influenced by environmental dissemination, *Acanthamoeba* spp. virulence, and host immunity [15]. Some strains exhibit organ-specific tropism, while others induce lesions in diverse organs [16]. Experimental models have been developed to demonstrate the pathogenicity of environmental isolates, highlighting the adaptability and survival of *Acanthamoeba* spp. in mammalian hosts [17, 18, 19, 20]. De Jonckheere [21] demonstrated the different sensitivity of experimental models to the inoculation routes and this influence of pathogenicity.

The liver is frequently exposed to numerous systemic infectious pathogens which can directly or indirectly cause liver pathology [48]. One of these pathogens is *Acanthamoeba* sp., which found in patients with compromised immunity, such as organ transplantation recipients and individuals receiving corticosteroids [11, 12]. Our previous study demonstrated a high prevalence of *A. polyphaga* and *A. castellanii* in both new and used cosmetic contact lenses and their disinfectant solutions [22]. This study aims to examine histopathological changes in the liver of immunosuppressed mice systematically infected with an *A. polyphaga* strain. Furthermore, it aims to examine the immune response dynamics alongside liver function enzymes.

MATERIALS AND METHODS

2.1. Ethics approved*:*

All procedure of the experiment was approved by the Ethical committee of the Faculty of Science, Assiut University, Assiut, Egypt (FSREC) with a protocol number (No. 01-2024-0001, Assiut, Egypt) in accordance with Egyptian laws and University guidelines for the ethical treatment of experimental animals. The study was conducted in accordance with the ARRIVE (Animals in Research: Reporting In Vivo Experiments) criteria [23]

2.2. Isolation and Cultivation of *A. polyphaga* strain

The *A. polyphaga* strain was previously isolated from contaminated contact lenses and identified morphologically and molecularly as belonging to the T4 genotype (GenBank

OL336326.1) in the Parasitology Laboratory, Zoology Department, Faculty of Science, Assiut University, Egypt [22].

2.3. Animals

A total of 50 male Albino mice (age 8-10 weeks, weight 25-30 g) were obtained from the Theodor Bilharz Research Institute, Cairo, Egypt, and maintained under standard conditions. They were housed in our animal house on a 12 /12 h light/dark cycle, with constant temperature and humidity, and had *ad libitum* access to standardized granulated food and water. The animals were acclimatized 15 days before the experiment.

2.4. Experiment design

The animals were divided into three groups: negative control mice (C, n=5), immunosuppressed control mice (CS, n= 15), and immunosuppressed *A. polyphaga* infected mice (AS, n= 30). Animals in the CS and AS groups were immunosuppressed by intraperitoneally administering 0.25 mg/g of methylprednisolone sodium succinate (MPS, Solu-Medrol, Pfizer, Puurs, Belgium, Middle East) dissolved in 0.1 ml of normal saline before each injection for 5 days before *A. polyphaga* infection [25].

Infected mice (AS) were intravenously injected with 80 µL of *A. polyphaga* suspension containing 5×10^4 amoebae (> 90 % trophozoites) in the tail vein after sterilizing it [13]. Negative control mice (C) received an equal volume of sterile saline. *Acanthamoeba polyphaga* was initially grown on 1.5% non-nutrient agar (NNA) with Pages' amoeba saline (PAS), covered with a thin layer of heat-killed *Escherichia coli* (ATCC 25922) [24]. The PAS mixture is composed of 15 g agar, 0.12g NaCl, 0.004g $MgSO_4.7H_2O$, 0.004g $CaCl_2.2H_2O$, 0.142g Na_2HPO_4, and 0.136g KH_2PO_4 dissolved in 1000 ml distilled water. The mixture was autoclaved and poured into Petri dishes to solidify [49]. Amoebae were maintained and grown on (NNA) plates seeded with deactivated *Escherachia coli,* incubated at 30°C, and examined daily for 7 days for trophozoites and 14 days for cysts. Active trophozoites were collected gently after 72-96 hours of the culture, washed in phosphate- buffer saline (PBS) three times, and centrifuged at 500 ×g for 10 min/each. The obtained pellet was suspended in PBS, and

a hemocytometer devise was used to count the cells, adjusted to a final concentration of 5×10^4 (> 95 % trophozoites) for experimental infection [14].

2.5. Clinical signs

The animals were observed along 15 days and any clinical signs recorded. To show the main changes that occurred in the body of the mice, 5 mice from each group were sacrificed at each period, while the remaining mice continued in the experiment until day 15. Sacrifice was performed by cervical decapitation in the CS and AS groups after 2, 7, and 15 dpi. The C group was sacrificed at the beginning of the experiment.

2.6. Re-isolation test

After sacrifice, liver tissue fragments (5*5 mm) were cut into small segments, minced, and inoculated onto a non-nutrient agar plate (1.5%) seeded with *E. coli*. The plates were examined for 15 days under an inverted microscope to detect trophozoites and/or cysts [24].

2.7. Cytokine analysis

50 µL of serum samples were used to determine the cytokines IL-1β (Cusabio, Cat. No. CSB-E08054m), TNF-α (Cusabio, Cat. No. CSB-E04741m), and IL-10 (Cusabio, Cat. No. CSB-E04594m) according to the respective kits using the sandwich enzyme immunoassay technique (ELISA). Concentrations were expressed in pg/ml and determined using a microplate reader (Stat Fax 2001, USA) at an optical density of 450 nm.

2.8. Histopathology

Liver tissues from all groups were collected and immediately fixed in formal alcohol for 24-48 h. The fixed tissues were further processed in 70% ethanol for three days to remove any fixative remnants. Then, the specimens were dehydrated in an ascending series of ethanol (80%, 95%, 100% (I), 100% (II). Dehydrated specimens were cleared in methyl benzoate for three days, infiltrated with paraffin at 56 ℃, and subsequently embedded in paraplast (Sigma Aldrich, USA). Sections were cut at 5 µm using a Reichert-Leica RM2125 Microtome (Germany), stained with hematoxylin and eosin, and photography with an OPTICA microscope (Italy) supported with a colored camera [26].

2.9. Biochemical analysis

Using a homogenizer, 0.3 g of liver tissue was homogenized in 1 ml (0.1 M) phosphate buffer (pH 7.4). The homogenates were centrifuged and the supernatant cytosols were frozen at −20 °C for the biochemical evaluations. Tissue hemolysates were used to measure the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), determined colorimetrically following the instructions of representative commercial kits (Spectrum Diagnosis, Egypt).

2.10. Data analysis

Statistical analyses were performed using SPSS software (version 20). Data were tested using one-way analysis of variance (ANOVA), followed by Duncan as post- hoc test. Independent Samples t- test were used to compare between the infected and its control in the same period. Normality of the data was evaluated using the Shapiro Wilk test, and homogeneity of variances was confirmed. The data are expressed as mean ± standard deviation. *P* values less than or equal to 0.05 were considered statistically significant.

RESULTS

3.1. Clinical signs

Immunosuppressed *A. polyphaga* infected mice displayed a spectrum of clinical signs, including emaciation, changes in coat color appeared rough, ulceration in various body regions, and enlargement of the testicular area were observed from 2 dpi to 7dpi (see Table 1 and Fig. 1). Additionally, neurological manifestations such as circular moving, bilateral eye lesions characterized by ocular discharge, sluggish or diminished mobility, and alterations in posture were reported after 7 day post-infection period, accompanied by a mortality rate of 26.66%. At the same times, changes in the body weight between different groups were not statistically significant at $P<0.05$ (Table 2). A significant decrease in body weight was observed only after 15 dpi in the AS group compared to the negative control (C) ($P<0.05$).

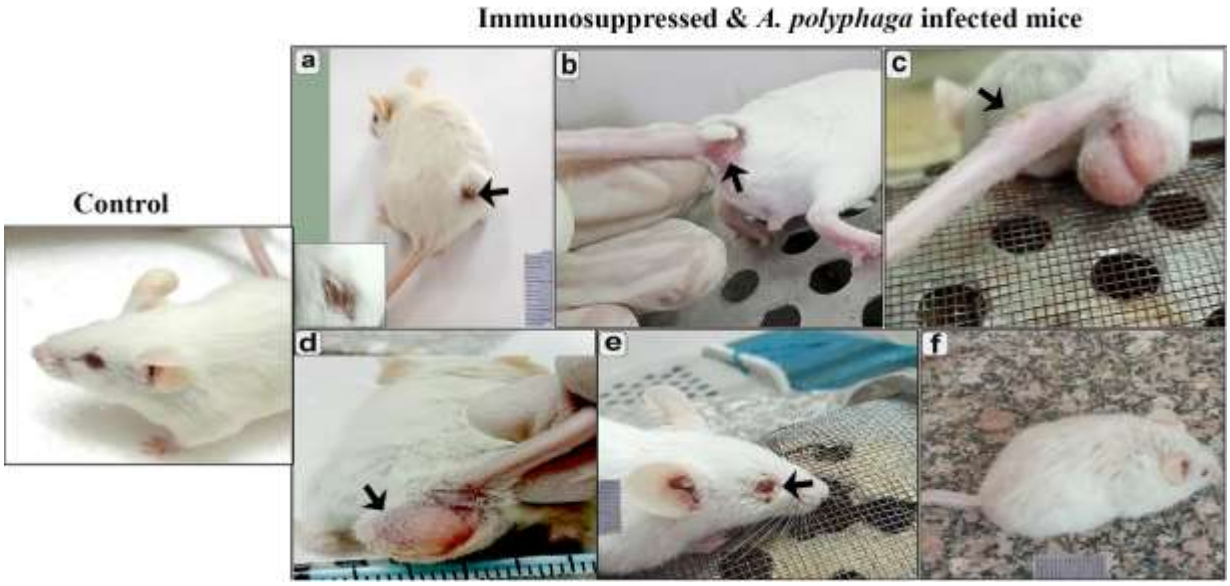

Figure (1): Clinical signs in immunosuppressed intravenously *A. polyphaga* infected mice including (a) skin ulceration in the back of mice; (b) perianal ulceration with hair loss; (c) skin ulceration in the tail; (d) enlargement in the testicular region; (e) ocular discharge; and (f) sluggish movement with emaciation with appeared rough. All of this compare with the normal mice (negative control (C)).

Table (1): The clinical signs were observed in different groups during the experiment along with 15 dpi.

Group / Signs	C	CS	AS
Emaciation	-	-	3 (10%)
Coat color change	-	-	2 (6.6%)
Ulceration in different areas (tail and skin)	-	-	9 (30%)
Testis enlargement	-	-	2 (6.6%)
Lachrymation	-	-	1 (3.3%)
Circular moving	-	-	1(3.3%)
Sluggish movement	-	-	2 (6.6%)
Mortality rate	-	-	8 (26.66%)

C: control negative, CS: immunosuppressed control animals, and AS: immunosuppressed *A. polyphaga* infected animals.

Table (2): The changes in body weight of controls and infected groups.

Group / Body weight	C	2 days		7 days		15 days		ANOVA (P value)
Mean ± SD	32.5 ± 0.5^{bc}	CS	AS	CS	AS	CS	AS	
		34.7± 0.3 ^{c}	30± 0 $^{ab **}$	31 ± 1 ^{abc}	30.5 ± 2.5^{abc}	30.3 ± 4.5 ^{abc}	27.7 ± 3.01^{a}	0.059

C: control negative, CS: immunosuppressed control animals, and AS: immunosuppressed *A. polyphaga* infected animals.

^{abc} letter is a significant difference between groups. Data expressed as mean ± SD. ***** showing the significant difference between the infected and its immunosuppressed control group at ($P < 0.05$).

3.2. Re-isolation test

The re-isolation test of amoebae from liver tissue samples yielded negative results.

3.3. Cytokine analysis (ELISA)

Serum IL-1β level by showed a highly statistically significant difference between the different groups (P= 0.000). Highly significant increase was observed in IL-1β in infected animals at 2 dpi (t= 10.56, P=0.000) and 7 dpi (t= 4.47, P=0.01) compared to the immunosuppressed control (CS). However, a significant decrease was observed in infected animals at 15 dpi compared to the CS (t= 7.28 and P=0.002) (Fig. 2a). Similarly, TNF-α levels showed marked statistically significant difference between different groups (P= 0.000). A significant increase in infected animals in TNF-α at 2 dpi compared to the CS (t= 7.161 and P=0.002) with a decrease at 15 dpi (t=4.943 and P=0.01, Fig. 2b).

IL-10 levels also showed a significant difference in its level between the groups (P= 0.000). The infected animals showed an increase in the level of IL-10 at 2 dpi (t= 5.41 and P=0.006) and 7 dpi (t= 4.29 and P=0.013) compared to the CS, then restored its normal level at 15 dpi (Fig. 2c).

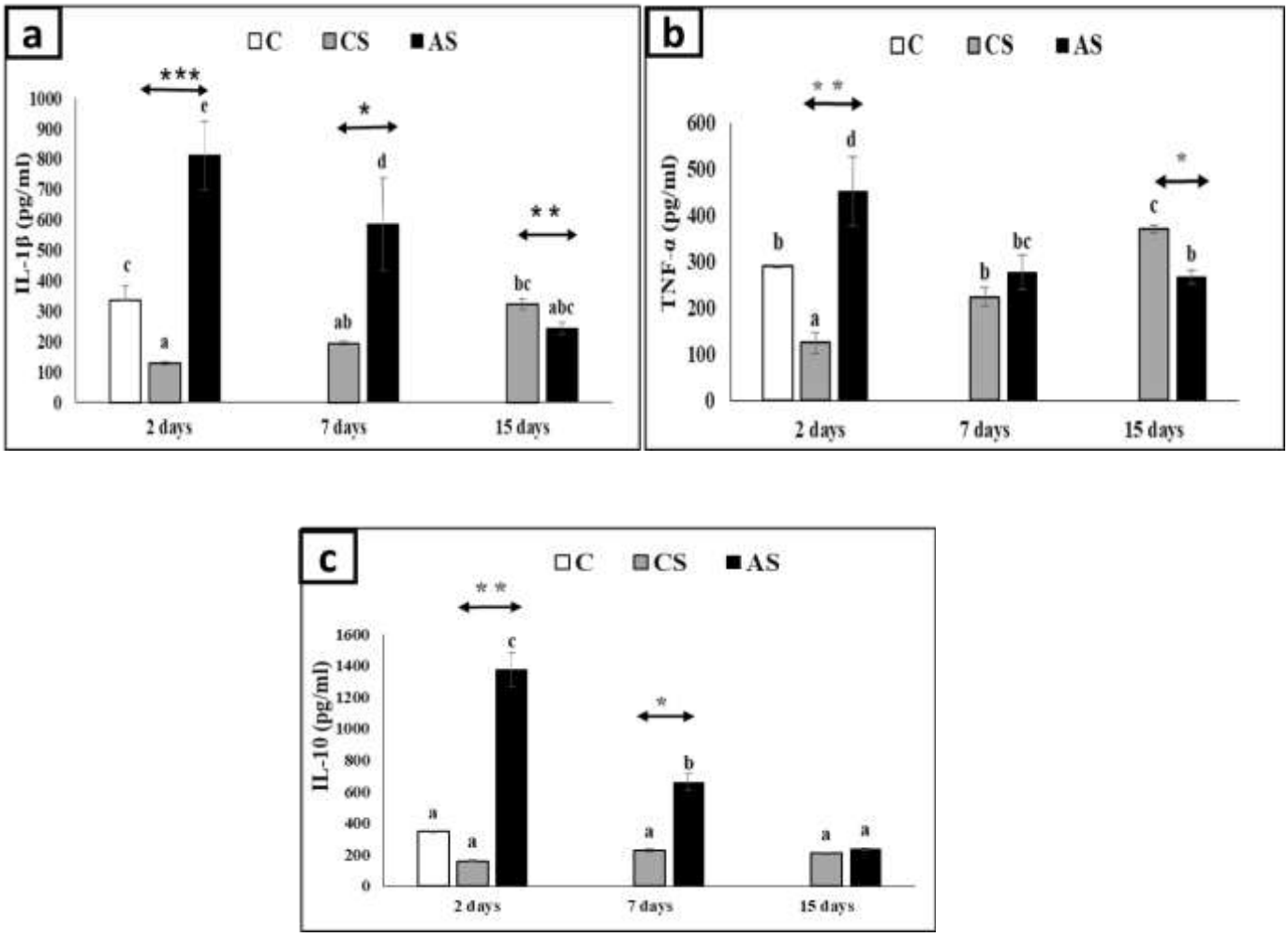

Figure (2): Histogram illustrating that: (a) IL-1β, (b) TNF-α, and (c) IL-10 level in serum of different groups at days 2, 7, and 15 post infection. Data are represented as Mean ± SD. ^{abc} different letters indicate significant difference at P< 0.05 (One-way ANOVA followed by Duncan post-test). (*) indicate a significant difference at $P \leq 0.05$ by (T- test), (**) at $P \leq 0.001$, and (***) a highly significance at $P \leq 0.0001$. C: negative control, CS: immunosuppressed control, and AS: immunosuppressed & *A. polyphaga* group.

3.4. Histopathology

Liver sections from negative control (C) and immunosuppressed control groups (CS) appeared normal, showing cords of hepatocytes arranged in lobules around the central vein and Kupffer cells located inside the sinusoids Figs. 3, 4, and 5 (a & b). The immunosuppressed infected animals showed multiple focal areas of inflammatory cell infiltration, the presence of parasite stages, mild dilatation of blood sinusoids, and necrotic changes in some hepatocytes at the early stage of infection Figs. 3 and 4 (c & d). Moreover, an accumulation of eosinophilic homogenous material in the central vein, possibly indicating a white thrombus, was noted (Fig. 3 c). Although some hepatocytes showed necrosis, the histological arrangement of hepatic cell cords are still present. Perivascular inflammatory cellular reactions were observed in the infected animals at 7 dpi (Fig. 4c), diffused vacuolar degeneration was observed Fig. 4 (c& d). In other cases, inflammatory cells replaced necrotic hepatocytes (Fig. 4 d).

By day 15 pi, extensive inflammatory cell infiltration of macrophage and leukocytes (eosinophils and neutrophils) was observed in the portal tract area and liver parenchyma, accompanied by congestion in the portal vein (Fig. 5c). Additionally, dilatation in blood sinusoids, vacuolar degeneration, and necrosis in some hepatocytes was noted, disrupting the lobular architecture, with *A. polyphaga* stages distributed throughout the blood sinusoids and portal area, (Fig. 5 ; Table 3). Histopathological changes in infected liver tissues were associated with myriads of intralesional basophilic cysts, roughly oval or rectangular in shaped have a bilaminated cyst wall. Moreover, the stages exhibited a round, eccentric nuclei with a conspicuous karyosome and granular or vacuolar cytoplasm were morphologically suggestive of amoebic trophozoites surrounded by a clear halo.

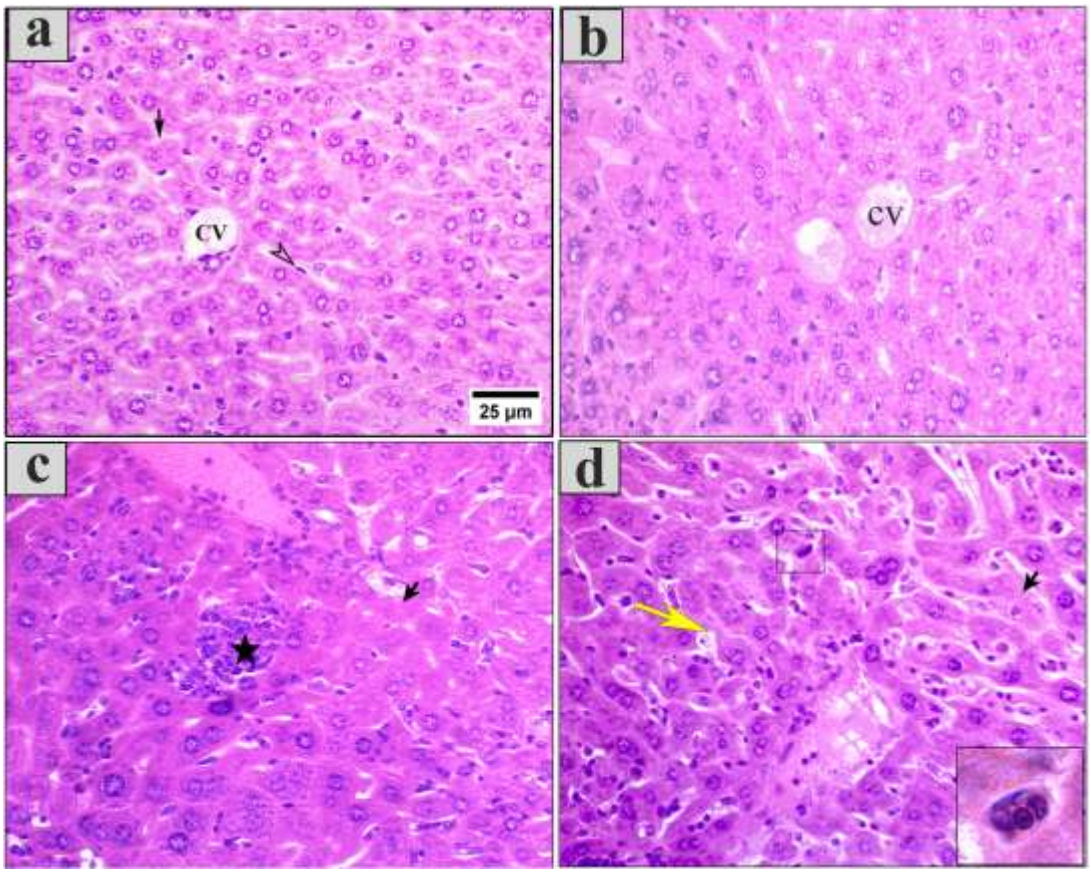

Figure (3): Showing histopathological section of mice liver at 2 dpi, stained by H & E (400×). (a) Control negative (C) showing normal hepatocytes (arrow) and kupffer cells in blood sinusoids (head arrow). (b) Immunosuppressed control (CS). (c&d) immunosuppressed *A. polyphaga* infected group (AS) showing focal inflammatory cell infiltration (star), mild dilatation in blood sinusoids (yellow arrow), necrosis in hepatocytes (arrow), central vein with thrombus, and trophozoite (magnified). CV: central vein.

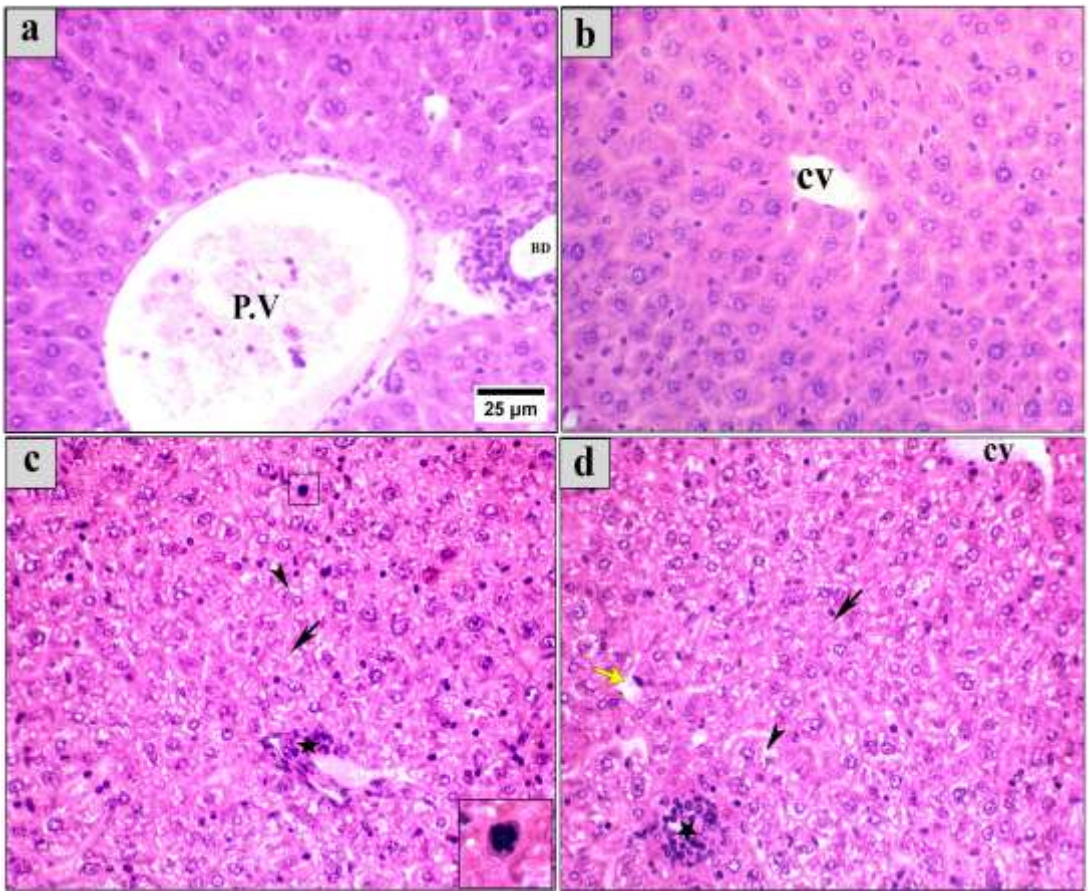

Figure (4): Showing histopathological section of mice liver at 7 dpi, stained by H & E (400×). (a) Control negative (C) showing normal hepatocytes around portal vein (P.V) and bile duct (BD). (b) Immunosuppressed control (CS). (c&d) immunosuppressed *A. polyphaga* infected group (AS) showing inflammatory cell reaction (star), parasite cyst (magnified), necrosis in hepatocytes (arrow), vacuolar degeneration (head arrow), and slight dilatation in blood sinusoids (yellow arrows). CV: central vein.

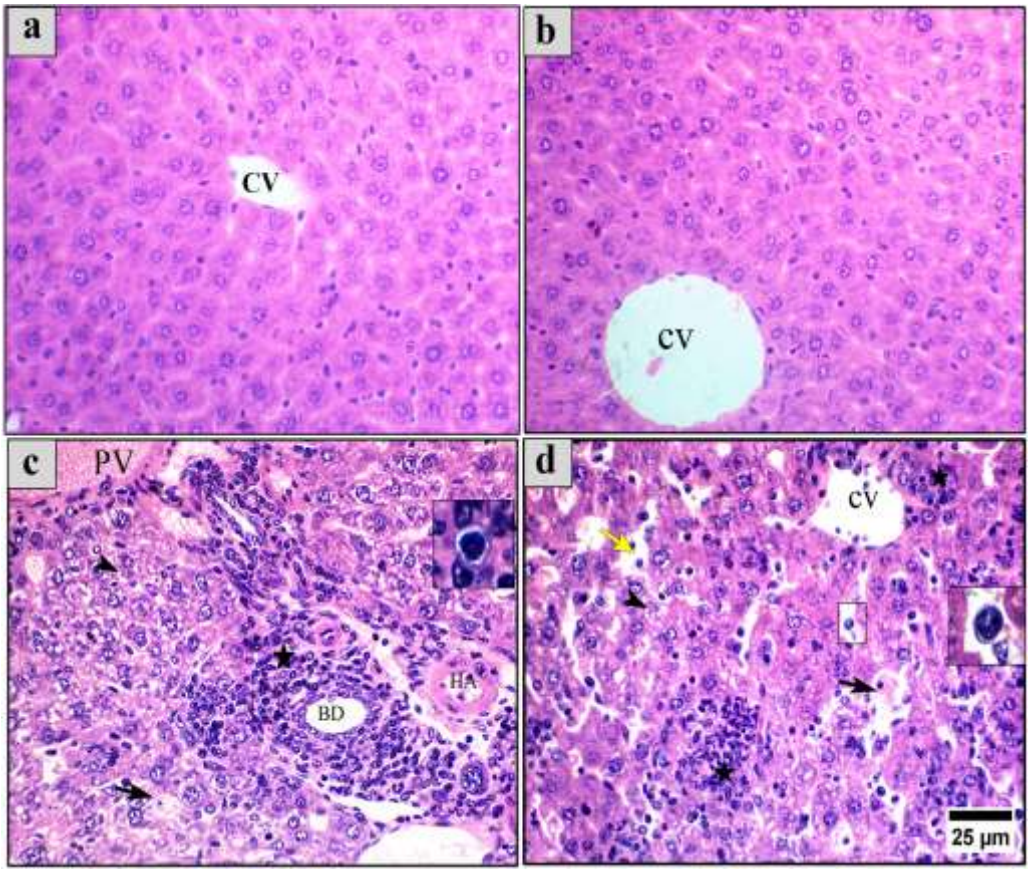

Figure (5): Showing histopathological section of mice liver at 15 dpi, stained by H & E (400×). (a) Control negative (C) showing normal cords of hepatocytes around the central vein. (b) Immunosuppressed control (CS). (c & d) immunosuppressed *A. polyphaga* infected group (AS) showing extensive inflammatory cell infiltration (star), congestion in portal vein, necrosis in hepatocytes (black arrow), diffused vacuolar degeneration (head arrow), dilatation in blood sinusoids (yellow arrow), and parasite cysts (magnified). CV, central vein. PV, portal vein. BD, bile duct.

Table 3: Histopathological score of the changes in liver tissues in different groups**.**

Group / Parameters	C	CS (2,7,and 15 days)	AS 2 dpi	AS 7 dpi	AS 15 dpi
Focal inflammatory reaction	-	-	++	++	+++
Dilatation of blood sinusoids	-	-	++	++	+++
Vacuolar degeneration	-	-	-	+	++
Eosinophilic reaction	-	-	-	-	++
Necrotic changes or necrosis	-	-	-	+	+++
Thrombosis	+	+	++	++	++
Portal tract lesions	-	-	+	++	++
Parasite distribution	-	-	++	++	+++

Scores: (-) absent; (+) mild; (++) moderate; and (+++) severe.

3.5. Biochemical analysis

For ALT and AST levels, a statistically significant difference was observed between the different groups (P = 0.001 and P = 0.003, respectively). In the AS group, a significant decrease was noted at 7 dpi compared to the CS group (t = 5.75, P = 0.005, and t = 5.63, P = 0.005), while a significant increase was observed at 15 dpi compared to the CS group (t = 5.32, P= 0.006, and t = 3.57, P= 0.023) (Fig. 6a & 6b).

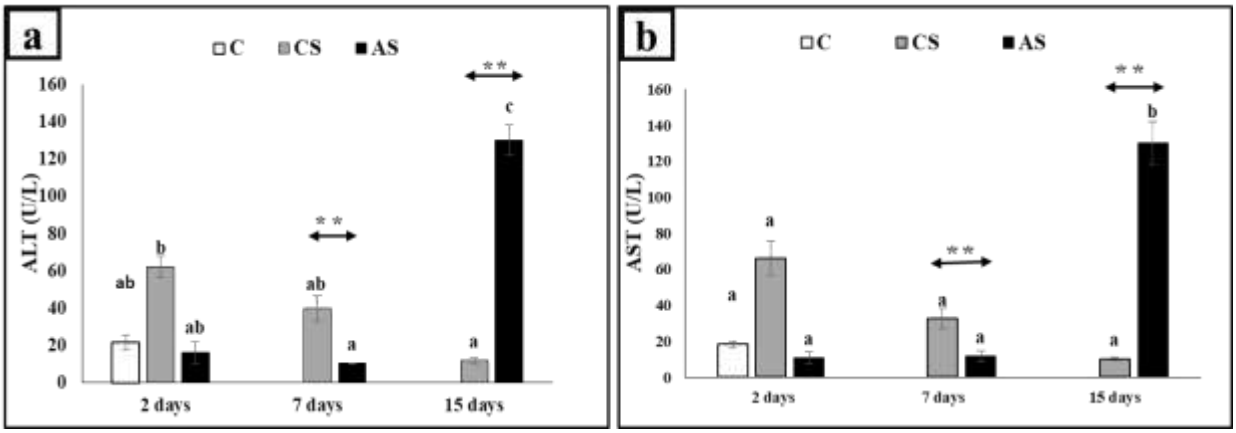

Figure (6): Histogram illustrating that: (a) ALT and (b) AST level in different groups at days 2, 7, and 15 post infection. Data are represented as Mean ± SD. ^{abc} different letters indicate significant difference at P< 0.05 (One-way ANOVA followed by Duncan post-test). (*) indicate a significant difference at $P \leq 0.05$ by (T- test), (**) at $P \leq 0.001$, and (***) a highly significance at $P \leq 0.0001$. C: negative control group. CS: immunosuppressed control group. AS: immunosuppressed & *A. polyphaga* group.

DISCUSSION

The *Acanthamoeba* strain (*A. polyphaga*) belongs to genotype T4, is associated with GAE cases in immunocompromised patients and amoebic keratitis (AK). This study determined the pathogenicity of *A. polyphaga* isolated from contact lenses through intravenous inoculation route. Virulent strains of *Acanthamoeba* sp. have been shown to induce mortality within 4-7 days in mice administered intranasally [13]. This study recorded a mortality rate of 26.66% in immunosuppressed *A. polyphaga* infected animals over the 15 dpi, consistent with findings by Clarke &Niederkorn [50] where, *A. polyphaga* induced approximately 30% mortality rates. In contrast, *A. castellanii* strains in immunosuppressed mice reported 50% mortality rates; however, another study, *A. castellanii* didn't cause any mortality, while *A. culbertsoni* caused 50% mortality [28, 41]. The variability in outcome may stem from factors such as host age and immune status of the host, route of inoculation, the dose of infection, and the genetic diversity of strain [28]. our previous study confirmed the in vitro pathogenicity of the isolate based on physiological conditions such as temperature and osmotic tolerance [27].

Górnik and Kuźna-Grygiel [29] showed the clinical signs appeared between the 3rd and the 14th days post-infection in strains inoculated intranasally. Similarly, this

study showed clinical signs during the first 15 dpi such as emaciation, changes in coat color, enlargement of the testicular region, circular moving, bilateral ocular discharge, and diminished mobility. These findings align with those of Kot *et al*. [16] who reported similar physiological and behavioral observations in intranasally infected mice by *Acanthamoeba* spp. In addition, the results indicated cutaneous lesions or ulcerations which may arise from the localization of *A. polyphaga* in the skin after parasitemia. Circular marching, a common sign of central nervous system involvement, especially in the cerebellum, along with ocular discharge, may occur due to parasite migration via the optic nerve from the brain to the eyes [1]. Furthermore, Chandra *et al*. [30] demonstrated that eye infection may ensue in hosts with disseminated acanthamoebiasis. The skin, brain, and eye lesions will be shown in further studies. This study reported negative re-isolation of *A. polyphaga* from the infected liver tissues. Additionally, Górnik and Kuźna-Grygiel [29] reported negative re-isolation of some *Acanthamoeba* types from different organs. They suggested that this might be due to *Acanthamoeba* sp. being considered secondary infection, which confirmed the organ specificity of the parasite [16].

The immune response against *Acanthamoeba* sp. has not been well characterized, as there are IgG antibodies to the ameba in the serum of (90 to 100%) of healthy individuals, so they are mostly asymptomatic [31]. While, in immunosuppressed individuals, the relationships between the host-parasite shifts to acute widespread disseminated parasitic infections affecting many organs, ultimately leading to the death of the host, as immunosuppressive drugs widespread the invasion of infection causing acanthamoebiasis [6, 32]. Studies have shown protection from infection with free-living amoebas may involve both innate and acquired immunity [33]. Innate immunity is mediated by the migration of phagocytic cells to the site of infection, macrophages play a significant role than neutrophils in killing *Acanthamoeba*, as activated macrophages produce TNF-α and IL-1α or IL-1β cytokines [34]. In this study levels of IL-1β and TNF-α in immunosuppressed control mice (CS) decreased, as methylprednisolone, a synthetic glucocorticoid made a reducing for inflammation and impaired the immune response which facilitates infection by *Acanthamoeba* sp. [35]. At the same time, the levels of pro-inflammatory cytokines

IL-1β and TNF-α in intravenously *A. polyphaga* infected animals was significantly up-regulated, especially at 2 dpi, followed by a gradual decrease at 7 & 15 dpi. Marciano-Cabral & Toney [34] demonstrated that activated macrophages secreting cytokines are not amoebicidal for *Acanthamoeba* sp. when used alone or in combination in vitro. However, Stewart *et al*. [36] reported that rat macrophages resemble murine macrophages, which undergo chemotaxis to eliminate amoebic trophozoites in vitro.

The timing and dynamics of cytokine responses can inform therapeutic strategies. For instance, targeting early cytokine responses may help mitigate acute inflammatory symptoms, while later interventions could focus on managing chronic inflammation and preventing tissue damage. Cytokines such as IL-1β and TNF-α are typically among the first released in response to infection, and their early peak levels are crucial for initiating the inflammatory response, and essential for controlling the initial stages of infection. This early response is characterized by the activation of immune cells and the recruitment of additional inflammatory mediators to the site of infection [53]. In our study, the clinical signs observed from 2 dpi to 7 dpi, such as emaciation, rough coat color, ulceration, and testicular enlargement, align with the early peak of pro-inflammatory cytokines, indicating an acute inflammatory response driven by the actions of IL-1β and TNF-α [54]. Following this initial peak, levels of IL-1β and TNF-α typically decrease as the immune response transitions from an acute phase to a more regulated state. This decline may correspond with the onset of neurological manifestations observed after 7 dpi, such as circular movement and diminished mobility, suggesting a shift in the immune response dynamics as the infection progresses [55].

IL-10 is an inhibitory cytokine secreted by macrophages and lymphocytes, crucial for maintaining a balance between the inflammatory process and immunopathological responses [37]. Cano *et al*. [33] found that pathogenic *Acanthamoeba* strains induce IL-10, while nonpathogenic strains predominantly elicit a pro-inflammatory response. In this study, IL-10 level significantly increased at 2 & 7 dpi, then occured down-regulation at 15 dpi. This align with findings by Łanocha-Arendarczyk *et al*. [25] Who reported downregulation of IL-10 level in

the immunosuppressed *Acanthamoeba* spp. infected mice at 16 dpi. Nevertheless, pathogenic *Acanthamoeba* strains transform into intact cysts to evade the inflammatory responses and deter macrophage or neutrophil attraction [38]. Additionally, IL-10 and proinflammatory Th17 cytokine expression are often elevated in human tears with severe Acanthamoeba keratitis compared to mild cases [39]. The mechanisms underlying IL-10 immune events remain unclear, numerous studies suggest that the intracellular *Leishmania* spp., *Plasmodium* spp., and *Trypanosoma cruzi* trigger similar responses [40]. The elevation in IL-10 level likely results from its regulatory function to suppress excessive immune responses, as seen in pro-inflammatory cytokines levels (IL-1β, TNF-α), which can protect the pathogen and promote persistence by suppressing the effective immune responses [37]. This phenomenon is observed in other protozoan infections such as *Toxoplasma gondii* and *Plasmodium* sp [51, 52]. The subsequent down-regulation of IL-10 at later times may indicate a shift in the immune response, potentially indicating either the resolution of the infection or the onset of immune exhaustion. As the infection progresses, the immune system's ability to produce IL-10 may diminish, influenced by factors such as persistent antigen exposure or the exhaustion of immune resources.

Histopathological changes caused by different *Acanthamoeba* spp. stains remains a subject of debate. This study clarified that systemic infection by *Acanthamoeba polyphaga* supports occurring inflammatory response during the early stages of infection and this is contrary to [41] , which reported an absence of inflammatory reaction in the early stages of infection. The liver, being a highly vascular organ, serves as a target for various diseases, toxins, and foreign pathogens. It is also a constant source of inflammation, housing different cell types such as endothelial, hepatic stellate, and Kupffer cells that aid in infection eradication and prevent propagation throughout the body [42]. In this study, the livers of immunosuppressed *A. polyphaga* infected animals exhibited notable histopathological lesions in the early stage, including focal inflammatory cell infiltration and congestion in central veins resulting in mild dilatation in sinusoids. At 7 &15 dpi, perivascular inflammatory cell reaction indicated the vascular route of infection, with immune cells gathering around the parasite. Additionally, vacuolar degeneration, necrosis, and inflammatory cells

replaced necrotic hepatocytes, indicating a direct reaction of the parasite on the hepatic tissue in the portal area and liver parenchyma. *A. polyphaga* stages were distributed around the portal area and in blood sinusoids. These findings align with those of Górnik & Kuźna-Grygiel [29] and Omaña-Molina *et al*. [41] who reported widespread necrotic alterations, inflammatory infiltrates, polymorphism of hepatocyte nuclei, and petechia in liver and as well as trophozoites attached to hepatocytes in the space of Disse and the hepatic sinusoidal endothelium. The liver sections of immunosuppressed control animals appeared with normal structure as methylprednisolone used in various liver diseases such as autoimmune hepatitis, where it has been shown to enhance survival and prevent rejection post-liver transplantation [43].

Changes in liver enzymes AST and ALT are crucial indicators of structural liver damage and dysfunction. In this study, analysis of liver enzymes revealed a reduction in ALT and AST levels at 2 and 7 dpi and high significant elevation in 15 dpi in immunosuppressed infected animals compared to the controls in liver tissues. This could stem from alterations in energy metabolism involving amino acid propagation [44]. Conversely, a sharp increase in the levels of ALT and AST was observed at 15 dpi in the immunosuppressed infected animals compared to the immunosuppressed control group. This could be attributed to methylprednisolone, which enhances survival and is used in various liver diseases [43]. Additionally, Yusuf *et al*. [45] demonstrated increased ALT activity in experimental trypanosomiasis, resulting from tissue destruction and the host defense mechanism against parasites. Furthermore, immunosuppressed infected mice exhibited a non-significant increase in the level of AST compared to immunosuppressed uninfected mice [16]. Contrary to our results, patients with disseminated infection with *Acanthamoeba* spp. exhibited normal urea, creatinine, and liver function enzyme activities [46, 47].

Acanthamoeba spp. are classified into different genotypes (T1- T23) based on 18S rDNA [56] .T4 is the most frequently isolated from nature and includes many pathogenic strains that associated with diseases [58]. T4 contains several species, including *A. castellanii, A. polyphaga, A. lugdenensis, A. mauritaniensis, A. triangularis, A. rhysodes, A. royreba, A. divionensis, A. paradivionenesis,* and others.

[57]. Comparing *A. polyphaga* with other pathogenic *Acanthamoeba* strains (like *A. castellanii* or *A. culbertsoni*) in immunosuppression mice revealed that some strains might exhibit higher virulence in immunosuppressed hosts [58]. The different tissue tropism of *A. polyphaga* infection from other strains may also play a role. Additionally, the host immune response might vary depending on the infecting *Acanthamoeba* strain, influencing disease progression.

The results showed the severity of *A. polyphaga* on the liver of immunosuppressed animals at immunological, histopathological, and physiological parameters. The course of infection may stem from factors such as the host's immune status, route of inoculation, the dose of infection, and the genetic diversity of the strain [28]. In conclusion, the current findings could contribute to a deeper comprehension of the systemic pathogenesis of *Acanthamoeba polyphaga* strains isolated from contact lenses in the liver of immunosuppressed animals, caused by disseminated infection. These findings highlighted the early phase of infection and exerted its significant impact on host histopathological, immunological, and physiological parameters. In the context of organ transplantation, careful consideration should be given to donors presenting with unexplained cutaneous lesions or meningoencephalitis. Additional research focusing on protein analysis and gene expression in infected hosts is warranted to gain further insights into disseminated infection.

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