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IN VIVO STUDY ON THE ROLE OF INTERFERON- γ AND INTERLEUKIN-4 IN CONTROL OF *CRYPTOSPORIDIUM* INFECTION

By

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Abstract

Cryptosporidium species are protozoan parasites that cause severe diarrhea in immunocompromised individuals and children, especially in developing countries. This study investigated the roles of IFN-γ and IL-4 in controlling *Cryptosporidium* infections in neonatal mice. Mice were grouped into four groups: normal control, infected control, infected/anti-IFN-γ mAb treated, and infected/anti-IL-4 mAb treated groups. Fecal assessments for *C. parvum* oocysts, cytokines detection, histopathological evaluations, and gene expression investigations for IL-4 and IFN-γ were conducted.

The results showed a 22% mortality rate was among the infected anti-IFN-γ mAb-treated mice (11%) and anti-IL-4 mAb-treated ones (6%). Infected mice showed significantly higher IFN-γ levels than controls. The anti-IFN-γ & anti-IL-4 treated ones raised serum cytokines with blocked biological activity by compensatory responses. The increase in IFN-γ secretion in infected mice attempted to overcome infection correlated with oocyst counts, histopathological, and gene expression changes. But, a negative correlation was between oocyst shedding and IFN-γ levels in serum as well as intestinal expression implying the IFN-γ role in infection control. Consequently, neutralization with anti-IL-4 mAb increased oocyst shedding early in cryptosporidiosis, clarifying the IL 4 role in early infection control.

Keywords: *Cryptosporidium parvum*, controlling, IFN-γ, IL-4, RT-qPCR

Introduction

Cryptosporidium species are protozoan parasites that infect the epithelial cells of the gastrointestinal tract of vertebrates (Gerace *et al*, 2019). Infection is initiated by ingestion of oocysts, which release sporozoites in the small intestine that then invade intestinal epithelial cell (IEC), where they reside in a parasitophorous vacuole at the apical tip of the cell (Guerin and Striepen, 2020). The parasites undergo three asexual cycles of replication and infection before differentiation into male and female parasites. The release of males allows fertilization of intracellular females and production of new oocysts that are shed in the feces, or excyst within the host and thereby maintaining infection (English *et al,* 2022).

In humans, cryptosporidiosis is usually a self-limiting infection in immunocompetent individuals and is a major cause of severe, life-threatening diarrhea in immunocompromised individuals and children, particularly in developing countries (Khalil *et al,* 2018). Outbreaks of human cryptosporidios- is are not only reported in developing count-ries but also in industrialized countries (Sh- irley *et al,* 2012), mainly attributable to its low infective dose, presence of a wide ran- ge of animal reservoirs, and ability to withstand chlorination (Adeyemo *et al,* 2019).

Cryptosporidiosis infection is acquired by oral route ingestion (feces, foodborne, waterborne…etc.) or by oocyst inhalation (Gaafar, 2007). Incubation period (pre-patent) depends on various factors (host susceptibility, strain virulence, or infection route, but may be from 5-28 days (Højlyng *et al,* 1987).

Cryptosporidiosis was identified as one of the chief causes of diarrhoeal diseases in man and animals and even birds in developing and developed countries (Sulaiman *et al,* 2004). In Egypt, cryptosporidiosis together with giardiasis pollutes the water source (El-Shazly *et al,* 2007) and *Crptosporidium* species is one of the main virulent agents of di-

arrhea, especially in the childhood, with varied prevalence (Youssef *et al,* 2008).

Cryptosporidiosis is usually an acute, self-limiting gastrointestinal disease, characterised by watery diarrhea, abdominal cramps, vomiting, low-grade fever, and appetite loss. Symptoms can last for up to a month, during apparent recovery and may relapse in about one third of cases (Kotloff *et al,* 2013).

In immune response to *C. parvum* infection, CD4+ T cells play an important protective role. In contrast, there appears to be only a limited or no requirement for CD8+ T cells and antibody in control of infection (Ungar *et al,* 1991). Interferon-gamma (IFN-γ) activity is associated with a partially protective innate immunity against parasite with the adaptive immune response eliminated infection (Hayward *et al,* 2000). Thus, cytokines including IFN-γ, TNF-α, interleukin-2 (IL-2), IL-4 and IL-6 play a vital role in immune regulation capable of hindering the disease development due to their rapid recruitment after *C. parvum* invasion and infection (Han *et al,* 2011). While the importance of pro-inflammatory cytokines such as IFN-γ in regulating cryptosporidiosis is well documented, their dysregulated activity also, contribute to some of the characteristic disease features, including increased permeability in the intestines (Roche *et al,* 2000).

The Th2 antibody-mediated response is characterized by production of IL-4, IL-5, IL-10, and IL-13, and it is recognized that Th2 activity may exacerbate intracellular infections (Wakeham *et al,* 2000). However, with *C. parvum* infection, there is evidence that Th2 cytokines may play a protective role. In mice, reproduction of parasites increased in adult BALB/c mice after treatment with anti-IL-5-neutralizing antibodies (Enriquez, 1993).

In humans with HIV infection, increased expression of IL-4 mRNA was associated with recovery from *C. parvum* infection after antiretroviral therapy (Okhuysen *et al,* 2001). In a study of adult C57BL/6 mice, IL-4–deficient (IL-4−/−) mice had increased levels of excretion of oocysts, but only during infection recovery and Th1 & Th2 pathways gave significant protective immune response against cryptosporidiosis different infections (Aguirre *et al*, 1998).

This work aimed to evaluate the role of IFN-γ, and IL-4 in controlling *Cryptosporidium parvum* infection in neonatal mice and to highlight the host cell and immune responses in the early and late stages in cryptosporidiosis infection control.

Material and Methods

The present study was carried out at Theodor Bilharz Research Institute (TBRI), Giza, during the period from June 2024 to March 2025. Specific pathogen-free 100 Swiss albino mice, aged 6-8 weeks and weighing 22-25g, were obtained from the Schistosome Biological Supply Program (SBSP) at Theodor Bilharz Research Institute (TBRI).

Mice were maintained in a controlled conditioned room at 25°C on sterile water ad libitum and a balanced dry 14% protein food for one week before the experimental study.

Ethical approval: The protocol was revised and approved by the Ethics Committee, Faculty of Medicine, Al-Azhar University after Helsinki Declaration (WMA, 2024).

Mice immunosuppression: They were given orally by an esophageal tube dexamethasone sodium phosphate as 0.25mg/kg/day in distilled water (Dexazone, Kahira Pharmaceuticals & Chemical Industries Co., Cairo). Dexazone was given daily for 2 weeks prior to oral *Cryptosporidium parvum* oocysts infection and contained weekly to the experimental end (Rehquel *et al,* 1998).

Parasite and infection: *C. parvum* oocysts were obtained from the outpatient clinic at TBRI and administered orally in approximatelly 10,000oocysts/ mouse.

Neutralization of IL-4 & IFN-γ cytokines: Anti-IL-4 and IFN-γ mAb (Elabscience Bionovation Inc., USA Cat.# E-MSEL-M0008 & E-EL-M0048) were given intraperitoneally a week before infection (10mg anti-IL-4 & 2mg anti-IFN-g) three times during the first week and then twice weekly to the exp-

erimental end. An equivalent dose of the isotype-matched rat IgG2a mAb (BD/Pharmingen, San Diego, CA. Cat. #70-4321) was given intraperitoneally at same dose and time as challenge control.

Study design: Mice were divided into four main groups: GI: Ten neither infected nor treated mice as negative control. GII: 30 infected mice and subdivided into 3 subgroups of 10 mice each. GIIa: Mice sacrificed 4 days post-infection (PI), GIIb Mice sacrificed 7 days PI & GIIc: Mice sacrificed 14 days PI. GIII: 30 infected mice given anti- IFN-γ mAb and subdivided into 3 subgroups of 10 mice each. GIIIa: Mice sacrificed 4 days PI, GIIIb: Mice sacrificed 7 days PI, & GIIIc: Mice sacrificed 14 days PI. GIV: 30 infected mice given anti-IL-4 mAb and subdivided into 3 subgroups of 10 mice each. GIVa: Mice sacrificed 4 days PI, GIVb: Mice sacrificed 7 days PL. & GIVc: Mice sacrificed 14 days PI.

Parasitological examination: All experimental with and negative control mice were examined to insure the *C. parvum* infection. Morning fecal samples were individually collected in labeled clean, wide-mouthed covered containers. The feces were homogenized and stained in cold Kinyoun's acid-fast stain and microscopically examined for oocysts. Other fecal samples were collected for the mean oocyst number per each group. A milligram of fecal pellet was fixed in 10% formalin and centrifugation for 10 minutes at 1000×g. A 100µl aliquot of the fecal sample was dried, dyed, and microscopically (x100) examined to calculate oocysts/1ml sample, and the average of three counts was multiplied by 10 as oocysts/gm (Garcia, 2016).

Mice scarification for blood samples: Mice were euthanized under isoflurane inhalation and decapitated; blood samples were collected into labeled centrifuged tubes for 5 minutes to separate sera, which by ELISA detected IFN-γ and IL-4 cytokines.

Histopathological examination: Small intestine tissues were fixed in 10% formalin, processed for 5µm paraffin sections, stained in H & E and Masson Trichrome stains (Drury and Wallington, 1980).

Immunological assessment: Sera were tested by ELISA for IL-4 & IFN-γ & OD was measured at 450nm (Engvall and Perlmann, 1971).

Molecular evaluation of IL-4 & IFN-γ gene expressions in tissues: Total RNA was extracted from intestinal tissues by a commercial RNA isolation kit according to the manufacturer's instructions. The cDNA was amplified to PCR by using specific primers for IFN-γ gene, IL-4 gene, and bactin gene (Table). Quantitative RT-PCR was performed using SYBR Green master mix, according to the initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15s, annealing and extension at 60°C for 1min. extended at 60°C for 30s. Gene expression levels were quantified using Step-One Software (Biosystems, Foster City, California, USA), and calculated by $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Table: Primer sequences used in qRT-PCR

Gene	Forward (5'-3')	Reverse (5'-3')
IL-4	5'-GTCTCTGCGACCTAGAAGTGGA-3'	5'-CGGAGGGAATAGAGGTGAAAGA-3'
IFN-γ	5'-AGCAGAGGAGAAAGCATCTATGATGC-3'	5'-GGTTTAGGCCCCAGAGTTTTTCTCC-3'
b-actin	5'-TGGAATCCTGTGGCATCCATGAAAC-3'	5′–TAAAACGCAGCTCAGTAACAGTCCG–3′

Statistical analysis: Data were collected, computerized and analyzed by using IBM-SPSS 24.0 (IBM-SPSS Inc., Chicago, IL, USA). Descriptive statistics: Means, standard deviations, medians, range, frequency, and percentages were calculated. Chi-square /Fisher's exact tests compared significant differences among groups. ANOVA (F-test) and Kruskal-Wallis test (K-test) were used to assess significance between quantitative variables, followed by a post Hoc test. A significant p-value was considered when it was <0.05.

Results

Mortality rate in mice was 22% (22/100) at the experimental end. Mortality rate was

significantly high ($p<0.05$) among infected anti-INF-γ mAb treated mice (11%) followed by infected anti-IL-4 mAb treated (6%), and infected control (5%) compared to compared to infected control ($P= 0.146$ & $P=0.238$, respectively). INF-γ at 4, 7, & 14 days PI showed significantly ($p<0.001$) lower INF-γ level in negative control mice (168.3±9.4pg/ml) than in all infected ones ($P<0.01$), but INF-γ cytokine levels showed a significant decrease in infected anti- IFN-γ mAb treated and anti-IL-4 mAb mice compared to infected mice ($P<0.001$).

There was a significant ($p<0.001$) elevation of IL-4 in infected control at 4, 7, & 14 days PI as compared to negative control (22.9±3.9pg/ml), but IL-4 cytokine levels showed a significant decrease in infected/ anti-IFN-γ mAb treated and infected/anti-IL-4 mAb treated mice compared to infected control mice ($P<0.001$).

Neutralization of INF-γ and IL-4 increased sera INF-γ & IL-4 levels by compensatory mechanisms. On neutralization biological activities were blocked, but protein was in sera and more cytokines compensated for blocked activity led to increase in INF-γ and IL-4levels.

In infected control mice, there was a posi negative control (0%), but without significant differences between GII, GIII and GIV Anti-IFN-γ mAb and anti-IL-4 mAb treated mice passed insignificantly high oocysts as tive expression of IFN-y. IFN-γ gene expression showed significant down-regulation in infected anti-INF-γ mAb treated mice, but up-regulation in infected anti-IL-4 mAb treated than in infected control ones ($P<0.001$), without IFN-γ expression in negative control mice. Also, in infected control mice, there was a positive IL-4 gene expression with significant down-regulation in infected/ anti-INF-γ mAb treated mice and infected/ anti-IL-4 mAb treated than in infected control ones ($P<0.001$). Low IL-4 mRNA expression was in negative control mice.

A negative correlation between oocyst shedding and serum IFN-gamma levels infected mice showed the role of IFN-γ in controlling infection. As serum IFN-γ levels increase caused oocyst shedding decrease. A positive correlation between oocyst shedding and serum IL-4 levels in infected mice showed the role IL-4 may in exacerbating infection as serum IL-4 levels increase with increase in oocyst shedding.

Details were given in table (1) and in figures (1, 2, 3, 4, 5, 6, & 7)

Table 1: Correlation between Severity (Oocyst Stool Excretion) and Biomarkers' level

Parameters	Oocyte Stool Excretion		
	Infected Control	Infected anti-INF-γ mAb treated	Infected anti-IL-4 mAb treated
Serum INF-γ level	0.678 ($p<0.001$)	0.819 ($p<0.001$)	0.903 ($p<0.001$)
Serum IL-4 level	0.569 ($p=0.002$)	0.808 ($p<0.001$)	0.870 ($p<0.001$)
Gene INF-γ expression	0.832 ($p<0.001$)	0.837 ($p<0.001$)	0.868 ($p<0.001$)
Gene IL-4 expression	0.601 ($p=0.001$)	0.856 ($p<0.001$)	0.895 ($p<0.001$)

*Pearson correlation coefficient, **Spearman Ranked correlation coefficient

Discussion

No doubt, the IL-4 may play a role in exacerbating cryptosporidiosis by increasing oocyst shedding (Robertson *et al*, 2020). In the present study, the increase of cyst shedding early in- cryptosporidiosis with anti-IL-4 mAb neutralization showed that IL-4 plays a role in the early fection control of acute cryptosporidiosis. *C. parvum* is an opportunistic widely prevalent parasite among both healthy and immunocompromised individuals (Urrea-Quezada *et al*, 2022). Symptoms can be resolve naturally, but *C. parvum* can be life-threatening to the imunocompromised patients (Checkley *et al*, 2015).

In the present study, Swiss albino mice proved to be model for *C. parvum* infection by response to immunosuppression and prolonged infectivity. However, two weeks of post Dexa treatment, they showed signs of reduced immunity, such as hair loss, abscess, subcutaneous edema, and ulcers formation, which signs markedly increased in the late stage of the infection. This agreed with Chang *et al.* (2022), who reported that mice showed poor spirit, disordered hair, reduced

skin elasticity, lost appetite, and activity.

In the present study, a mortality rate (22%) was detected all mice treated with anti-INF-γ mAb at the experimental end, with a mortality rate (11%), followed by (6%) among mice treated with anti-IL-4 mAb and then infected control mice (5%), but normal control was (0%), but without significant differences among all infected treated mice ($p>0.05$). The present data disagreed with Chang *et al.* (2022), who reported 16.7% mortality rate, which may due to different *C. parvum* strains, or neutralization with anti-IFN-γ mAb and anti- IL-4 mAb or even immunosuppression factors or secondary infections.

In the present study, mice were orally infected with *C. parvum* oocysts at a dose of about 10,000oocysts/mouse, and the role of IFN-γ, and IL-4 detection in early stages control of *Cryptosporidium* infection was 4, 7, & 14 days post infection. This agreed with McDonald *et al.* (2004), and with Cetrad *et al.* (2010), they both infected mice with high dose of 10^5 oocysts/mouse and scarification days were on the same times.

In the present study, oocyst shedding began on 2^{nd} day post infection in immunosuppressed mice. This agreed with Abdelmksoud *et al.* (2018), who didn't report viable parasites 14 day post infection in the positive control mice. But, this disagreed with Abdou *et al.* (2013), and Atia *et al.* (2018), who reported oocyst shedding up to 21 or 30 days post infection.

In the present study, infected mice treated with anti-IFN-γ mAb, and anti-IL-4 mAb showed insignificantly higher oocysts shedding as compared to infected control mice ($p=0.146$ & $p=0.238$, respectively), indicated that anti-IL-4 mAb neutralization increased early cyst shedding, implicating IL-4 in early infection control. This showed a negative correlation between oocyst shedding and serum IFN-γ levels, highlighting IFN-γ's role in infection control.

In the present study, the innate and adaptive immunity contribute to resistance against cryptosporidiosis. This agreed with Clark (1999), who reported that the exact nature of *Cryptosporidium* immune response in humans was not clear. Nevertheless, Abouel-Nour *et al.* (2015) reported that experimentally the immunologic mediated elimination of *C. parvum* requires CD4+ T cells and IFN-γ. But, the innate immune responses also have the significant protective role in both man and animals.

The present study showed that serum INF-γ was significantly lower in negative control mice compared to infected ones ($p<0.001$). Also, INF-γ levels significantly decreased in mice treated with anti-IFN-y and anti-IL-4 mAbs compared to the positive control ones ($p<0.001$). This increase in INF-γ in infected mice showed the infection control immune response on oocyst numbers and histopathological changes. This in agreed with Codices *et al.* (2013) and Abdelmksoud et al. (2018), who found that IFN-γ was increased in infected mice about eight times higher after 7 & 28 days post infection as compared to control mice. Barakat *et al.* (2009); Gullicksrud *et al* (2022) showed that in immunodeficient mice IFN-γ plays a crucial role in limiting *C. parvum* infection by macrophage activation, upregulation of MHC class II, nitric oxide synthesis induction, NK cell activation, specific cytotoxic responses, and B cells switching to IgG2a. Perez-Cordon *et al/* (2014) reported that administration of IFN-γ neutralizing antibodies to mice with SCID exacerbated *C. parvum* infection and significantly reduced its survival

In this present study, CD4+ T lymphocytes caused IL-4 deficiency due to treatment with anti–IL-4–neutralizing antibodies, on susceptibility to *C. parvum* infection, with significant ($p<0.001$) elevation of IL-4 in positive control at 4, 7, & 14 days post infection as compared to normal control, but levels of IL-4 cytokine showed significant decrease in infected/anti- IFN-γ mAb treated and infected/anti-IL-4 mAb treated mice compared to positive control mice ($P<0.001$). This agreed with McDonald *et al.* (2000),

who reported that neutralization of IL-4 by mAb treatment enhances early oocyst excretion in neonatal mice clarified the role of IL-excretion in the adult C57BL/6 mice with anti-IFN-γ mAb treatment due to IL-4 role in preventing a prolonged *C. parvum* infection (Aguirre *et al,* 1998). Also, role of IL-4 in human cryptosporidiosis, peripheral mononuclear cells showed significant expression of both IL-4 & IFN-γ (Gomez Morales *et al,* 1996). But, contradictive hypotheses suggested that IL-4 is either: (i) involved in the late infection stages, (ii) promotes DCs activation, (iii) facilitates protective Th1 reactions, (iv) plays a role in immune memory or (vi) has suppressive or no role in immunity against cryptosporidiosis (Mc-Donald *et al,* 2013).

In the present study, there were histopathological changes in the lower ilium and ileocecal regions changes associated with *C. parvum*. *Cryptosporidium* oocysts, tiny purple-stained structures measured 4-6µm, scattered along the mucosal brush border and in the small intestinal lumen. *Cryptosporidium parvum* typically develops in enterocytes of the small intestine and colon, with the terminal ileum being the most affected site both immunocompetent and immunosuppressed mice, attributed to favorable biochemical conditions in this ileum part for the parasite, and to presence of specific receptors (Abdou *et al,* 2013). Also, the present examination of ileal sections in infected control showed significant mucosal pathological changes as compared to the negative control mice, such as the crypt hyperplasia, villous atrophy, and infiltration by inflammatory cells caused by pathogen-secreted toxins that directly damage epithelial cells. This agreed with Atia *et al.* (2018), who reported similar pathological changes in infected and negative control.

In the present study, the anti-INF-γ treated infected mice at 4 days showed mostly preserved villous and goblet cell pattern with few scattered crypto-oocysts, at 7 days showed that the distorted villous pattern was with many scattered crypto-oocysts, and at 4 in early *Cryptosporidium* infection control. Also, depletion of IL-4 by mAb treatment didn't cause early enhanced oocysts 14 days showed mostly expanded villi with distorted villous to crypt ratio and moderate infiltration with inflammatory cells. But, in anti-IL-4 treated mice at 4 days showed distorted villous patterns expanded by inflammatory cells with minimal scattered villous patterns with mild expansion by inflammatory cells, and at 14 days showed preserved villous patterns without abnormal expansion by inflammatory cells and regenerating goblet cells. Also, the expression of IL-4 was stable during infection process and without significant difference as compared to negative control mice at 1, 3, & 7 dpi ($P > 0.05$). This agreed with Ungar *et al*. (1991); Soufy *et al.* (2017); Chang *et al.* (2022), and Abdel-maksoud *et al,* 2023), reported that blocked IFN-γ by anti-mAb treatment caused a severe early *C. parvum* infection in adult C57BL/6 mice.

Conclusion

In *Cryptosporidium* infection, a negative correlation exists between oocyst shedding and IFN-γ expression in intestinal tissue, indicating that higher IFN-γ levels may help control shedding. Conversely, there is a positive correlation between oocyst shedding and IL-4 expression, suggesting that increased IL-4 levels promote shedding. This suggests that IL-4 produced in the intestinal tissue may play a role in promoting oocyst shedding and exacerbating *Cryptosporidium* infection. This showed neutralization with anti-IL-4 mAb increased oocyst shedding in early infection and that IL-4 plays a role in controlling early acute cryptosporidiosis.

INF-γ plays a key role of immunity to cryptosporidiosis as mice with neutralized IFN-γ didn't control infection but, may have future potential immunotherapeutic applications. No doubt, IL-4 production has an important role in the early control cryptosporidiosis as in mice cytokine lack increased their susceptibility to infection. So, cryptosporidi-

osis has a significant impact on the immunocompromised patients and children health in developing countries.

Extensive study on pathogenesis and host immune response in cryptosporidiosis patients is ongoing and will be published in due time.

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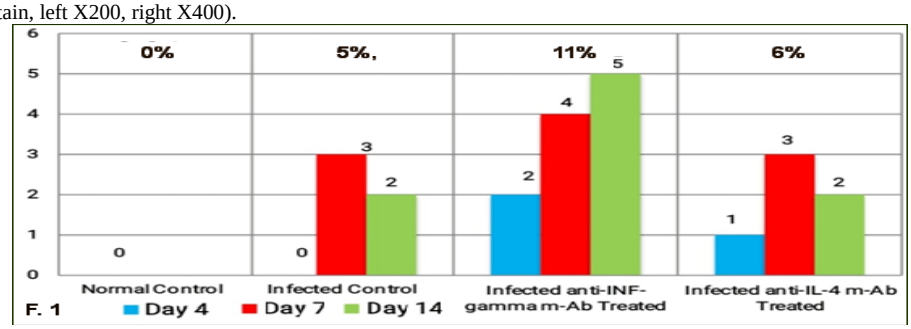
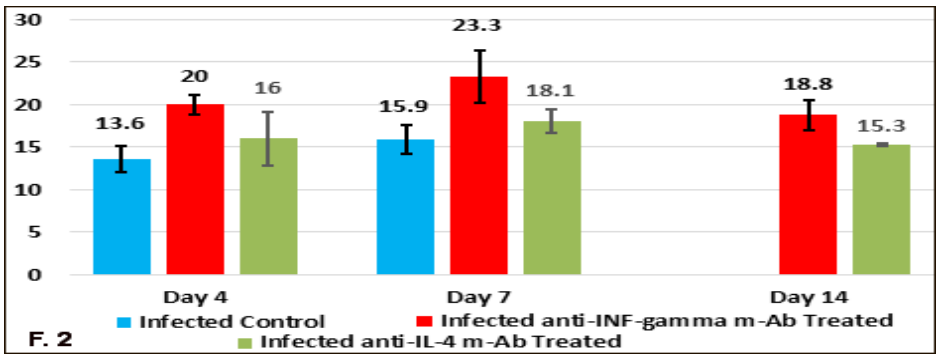
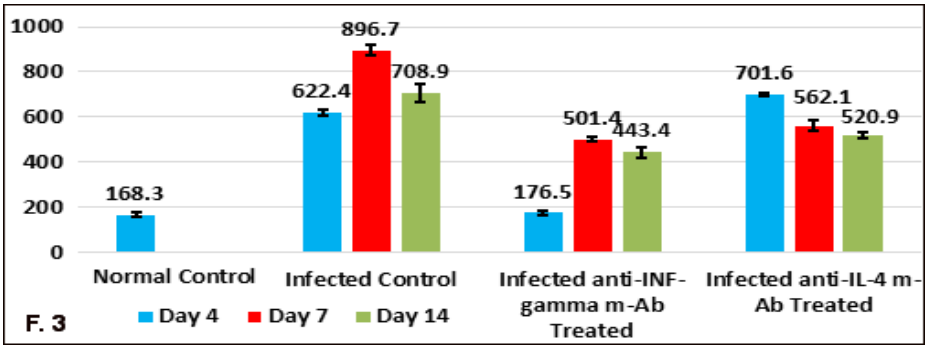
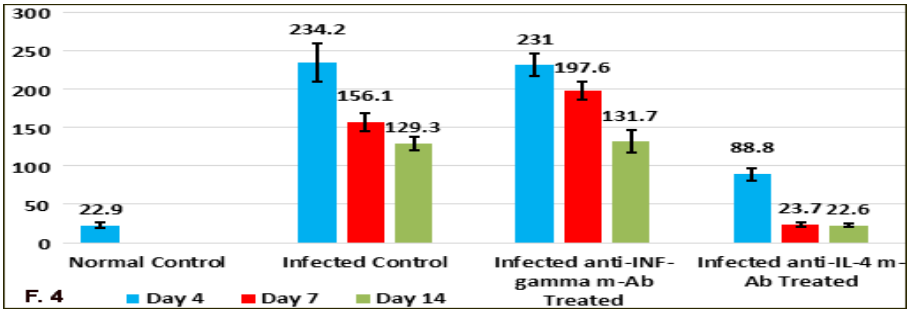
Explanation of figures

Fig. 1: Difference between groups regarding mortality rates over experimental time.
Fig. 2: Difference in stool oocyt shedding count between groups.
Fig. 3: Difference in INF-y Level between groups.
Fig. 4: Difference in IL-4 Level between groups.
Fig. 5: Difference in INF-y Gene Expression Level between groups.
Fig. 6: Difference in IL-4 Gene Expression Level between groups
Fig.7: Histopayhological sections in mice intestine (A) Normal control showed mostly regular villi, crypts were regularly arranged with a preserved villous to crypt ratio. (B) 4 days infected control showed mostly distorted villous pattern with many scattered crypto-oocysts (yellow arrows) and moderate expansion by inflammatory cells. (C) 7 days infected control showing mostly distorted villous patterns with many scattered intracellular crypto-parasite (yellow arrow) and high expansion by inflammatory cells. (D) 14 days infected control showed mostly preserved villous patterns, decreased inflammatory cells with few goblet cells. (E) 4 days anti-INF-γ treated infected showing mostly preserved villous and goblet cell pattern (black arrows) with few scattered crypto-oocysts (yellow arrows). (F) 7 days anti-INF-γ treated infected showed mostly distorted villous pattern with many scattered crypto-oocysts (yellow arrow). (G) 14 days anti-INF-γ treated infected showed mostly expanded villi with moderate infiltration with inflammatory cells (red arrows). (H) 4 days anti-IL-4 treated infected showing mostly distorted villous pattern expanded by inflammatory cells (red arrows) with minimal scattered crypto-oocysts (yellow arrows). (J) 7 days anti-IL-4 treated infected showed mildly distorted villous pattern with mild expansion by inflammatory cells (red arrows). (K) 14 days anti-IL-4 treated infected showed preserved villous pattern with no abnormal expansion by inflammatory cells and regenerating goblet cells (black arrows) (H & Estain, left X200, right X400).





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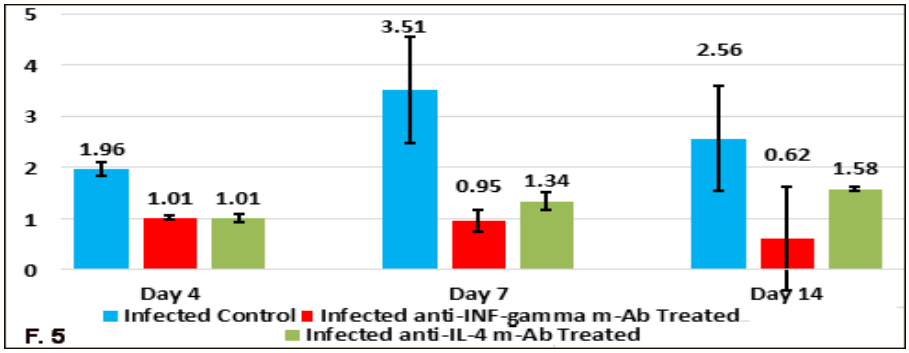
200
150
100
50
0
22.9
234.2
156.1
129.3
231
197.6
131.7
88.8
23.7
22.6
Normal Control
Infected Control
Infected anti-INF-gamma m-Ab Treated
Infected anti-IL-4 m-Ab Treated
Day 4
Day 7
Day 14
F. 4


5
4
3
2
1
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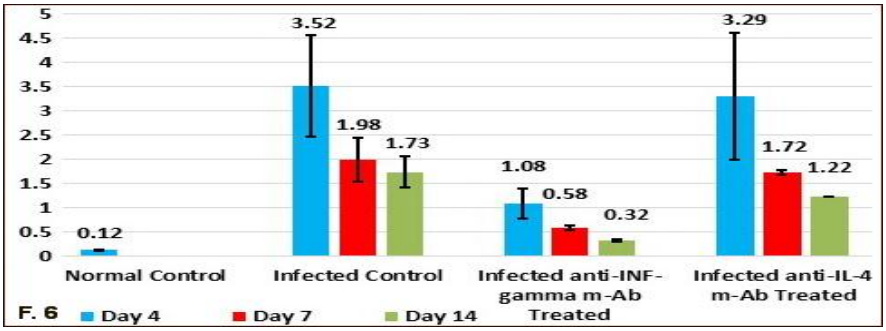
1.96
1.01
1.01
3.51
0.95
1.34
2.56
0.62
1.58
Day 4
Day 7
Day 14
Infected Control
Infected anti-INF-gamma m-Ab Treated
Infected anti-IL-4 m-Ab Treated
F. 5


5
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4
3.5
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2.5
2
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1
0.5
0
0.12
3.52
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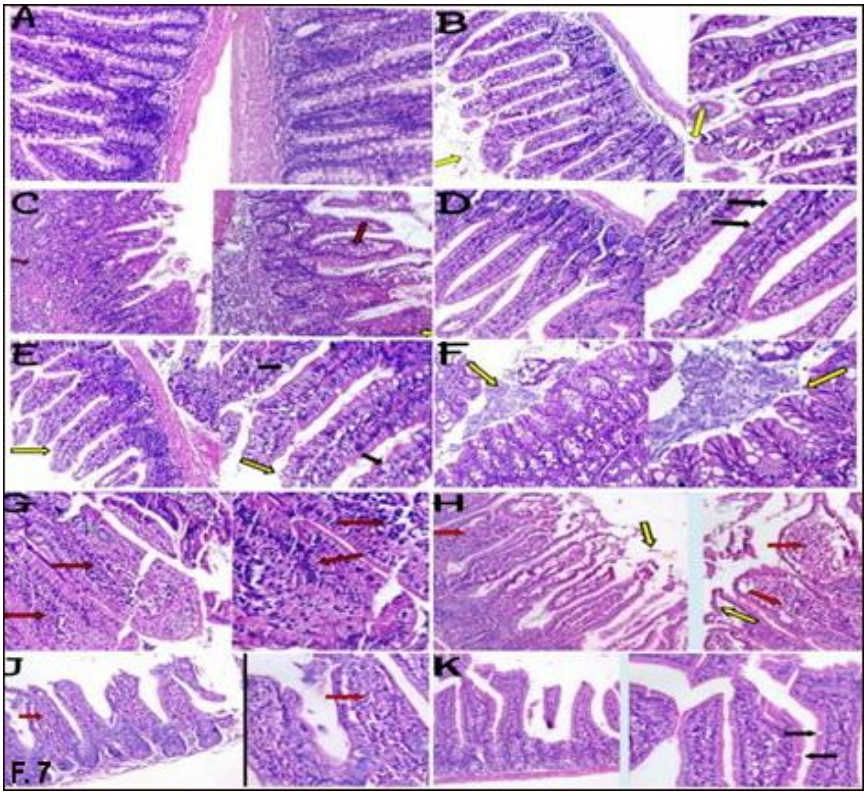
1.73
1.08
0.58
0.32
3.29
1.72
1.22
Normal Control
Infected Control
Infected anti-INF-gamma m-Ab Treated
Infected anti-IL-4 m-Ab Treated
Day 4
Day 7
Day 14
F. 6


A
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F. 7