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INFLUENCE OF DEXAMETHASONE AND SEX ON SUSCEPTIBILITY OF MICE TO PRIMARY INFECTION WITH *TOXOPLASMA GONDII*

By

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Abstract

Toxoplasma gondii is an intracellular protozoan parasite of health hazards, particularly to immunocompromised individuals. Sexes difference in immune responses to infections are well-documented. This study evaluated the effects of sex on latent toxoplasmosis progression and treatment efficacy in immunocompetent and immunocompromised mice. A total of 100 Swiss Albino mice (50 males & 50 females) were infected with *T. gondii* (ME49 strain). Mice were distributed into groups based on sex and immune status, receiving treatment with Spiramycin (SP) and Metronidazole (MTZ) or remaining untreated. Assessments included mortality rate, cyst counting in brain, histopathological studies of liver and brain, and molecular analysis of parasitic DNA in brain.

The results showed that treatment significantly reduced brain cysts in immunocompetent males (24.68%) and females (23.2%), but in immunocompromised males reduction was (15.62%) and (11.72%) in females. Histopathological examination showed higher inflammatory responses in females than in males. Molecular analysis showed that infected females showed significantly higher parasitic DNA concentrations than males.

Keywords: *Toxoplasma gondii,* Spiramycin, Metronidazole, Sexes, Dexamethasone mice

Introduction

Toxoplasma gondii is an intracellular protozoan zoonotic parasite of worldwide zoological and geographical distribution particulary among weak immune hosts (Morsy *et al,* 2025). Epidemiologically from 30% to 50% of the global population may be infected or toxoplasmosis seropositive (Galeh *et al,* 2020). In immunocompetent individuals, infections usually are asymptomatic (Durieux *et al,* 2022), but in immunocompromised patients with acquired immunodeficiency syndrome or undergoing immunosuppressive therapies, toxoplasmosis caused severe or even sometimes fatal and systemic manifestations (Ammar *et al,* 2024).

Toxoplasmosis has a unique complex life cycle included asexual reproduction in intermediate hosts and sexual one in definitive hosts mainly felines (Flegr *et al,* 2022). This parasite adaptation adds to widespread environmental oocysts transmission in contaminated food and water (Al-Malki, 2021), as well as congenital transmission (Xiao *et al,* 2013). Pyrimethamine and sulfonamides are among the current therapy choices, but with more or less different success degrees, especially in immunocompromised patients (Konstantinovic *et al,* 2019).

The influence of host variables on infection outcomes significant depend on host sex (Lyons *et al,* 2024) and his/her immunity (Alonaizan *et al,* 2021). Host's sex and immunity changes both cytokine patterns and antibody production to treatment efficacy and toxoplasmosis severity (Hegazy *et al,* 2019).

Dexamethasone (DEX) is widely used in the clinic for treatment of inflammatory and autoimmune diseases. However, long-term use of DEX is often easy to lead to acute toxoplasmosis in patients, and the potential molecular mechanism is still not very clear (Zhang *et al,* 2017).

In host-parasite coevolution, a parasite is selected to increase its infectivity, while a selected host resisted the parasitic infection (Woolhouse *et al,* 2002). Aavani and Rice (2022) in USA reported that the host immune system was likely to ultimately evolved and adapted to parasitic infection, when the

sexual selection was a part of this process.

This study aimed to evaluate the effects of host sex on the latent toxoplasmosis progression and treatment efficacy in the immunocompetent and immunocompromised mice.

Materials and Methods

This case-control study was done from Jly, 2024 to October, 2024 at Department of Parasitology, Faculty of Medicine, Benha University and the National Research Center's Zoonotic Diseases Department in Cairo.

Parasites: The *Toxoplasma gondii* cysts of ME49 strain was kindly provided by the Zoonotic Diseases Department, National Research Centre, isolated from brain of eight weeks infected mice. Brain was homogenized with 1ml of phosphate-buffered saline suspension was diluted and adjusted to $1x10^2$cysts/ml (El-Sayed and Aly, 2014).

Animals: One hundred clean laboratory-bred Swiss Albino mice (aged six to eight weeks and weighed 20-25gm) with equal numbers of males and females. They were kept in a housed under a 12-h light cycle at a controlled temperature of 22–25°C and were offered drinking water and regular mouse feed following the Helsinki declaration for experimental animal care. Each experimented with mouse (except negative control) was orally infected by 0.1ml of 10 *T. gondii* cysts in brain suspension.

Drugs: Three drugs were prepared with the ultra-pure water and given via a 22G feeding needle after an overnight fast. These were 1- Spiramycin (SP) purchased as tablets of 3 MIU (Pharaonia Pharmaceuticals, Alexandria), and administered as 200mg/kg/day for 14 successive days (Grujić *et al,* 2005). 2- Metronidazole (MTZ) was purchased as 500 mg tablets (Amriya for Pharmaceutical Industries), and administered orally as 250mg/kg/day for 14 successive days, half an hour before SP to allow tissue absorption and diffusion (Chew *et al,* 2012). 3- Dexamethasone or DEX (Dexazone, Kahira Pharmaceuticals and Chemical Industries Co, Cairo) was administered at a dose of 0.25mg/kg/day for seven days before to induce and to maintain immune suppressed immunocompromised mice and continued to experimental end (Rehg *et al,* 1988).

Experimental design: Mice (100) were separated into males (50) and females (50) in each of five groups. G1: immunocompetent neither infected nor treated (negative control), G2: immunocompetent, infected, not treated (positive control), G3: immunocompetent, infected and treated with SP & MTZ, G4: immunocompromised, infected, not treated (immunocompromised positive control, and G5: immunocompromised, infected, and treated with SP &MTZ. Treatment was given for forty-day post-infection for 14 successive days, and observed for two days post treatment; survived ones were gently sacrificed by neck dislocation (Djurković-Djaković *et al,* 2002).

Sex effect on toxoplasmosis dexamethasone mice: Mice mortality rate in each group was estimated (Allam *et al,* 2020).

$$MR = \frac{\text{Dead mice no. at scarification}}{\text{Total number of mice used}} \times 100$$

Brain cysts count: One-half of each mouse brain was harvested, washed with sterile saline solution, weighed, and homogenized with 1ml of sterile saline for 5 min. Then, 25 ml of brain homogenate was spread on a clean glass slide (four slides for each sample), air dried, fixed in 100% ethanol for 15min, air dried again and stained with Giemsa 10% stain for 1hr, rinsed with water, dried at 60°C, mounted on xylene and covered with a suitable coverslip. Cysts were microscopically for each brain as cysts/mouse brain= number in 100µl x10x2, and mean number was considered for each group. Reduction% = (mean cysts number in control-mean cysts number in treated mice) x 100/mean cysts number in controls (Chew *et al,* 2012).

Histopathological changes were examined in cerebral and hepatic tissue sections with hematoxylin and eosin (H&E) stained for toxoplasmosis changes Brain and liver tissues of all mice were fixed in 10% buffe red formalin and processed for paraffin em-

bedding and sectioning (4-5µm), mounted onto slides and examined.

Molecular assessment of DNA extraction: About 20-25mg of brain tissue was rinsed three times in sterile phosphate-buffered saline (PBS, pH 7.4). DNA extraction was done using the MagMAX™ CORE nucleic acid purification kit (Cat. No. A32700, Thermo-Fisher Scientific Inc., USA with DNA eluted in 50µl of elution buffer), following the manufacturer's instructions,

Real-Time PCR: Quantitative analysis utilized ViPrime PLUS Taq qPCR Green Master Mix I (SYBR® Green Dye, Cat QLMM12, Vivantis Co., Malaysia). The process used 2 µl of extracted DNA, 10 µl of 2X PCR master mix, and 0.1µl of forward and reverse primers targeting the P29 gene, for a total volume of 20 µl. The qPCR was conducted using a thermal cycler (qTOWER³G, Analytikjena, Germany) as the next cycling conditions: initial denaturation at 95°C for 5 minutes, thereafter 40 cycles of 95°C for 30 seconds and 60°C for 1 minute. Melting curve analysis confirmed the specificity of the PCR products.

Primer information: P29 Q-f: CAGCATG GATAAGGCATCTG & P29 Q-r: GTTGCTCCTCTGTTAGTTCC.

After complete reaction, cycle threshold (CT) values were recorded as parasitic load among the experimental with mice. Standard ranges were calculated by a real-time quantitative PCR assay, DNA of *T. gondii* was extracted from tachyzoites under the same conditions, with a series of dilutions ranged from $1x10^5$-10ng/µl were done. Then, PCR was performed under the same conditions to establish the standard calibration curve.

Statistical analysis: Data were analyzed by using SPSS software package version 20. Data was represented using mean ± standard deviation (SD). Student t-test and analysis of variance (ANOVA) were used to detect the significance among groups. *P*-values less than 0.05 showed significant differences.

Ethical approval: The study protocol was accepted by the Ethical Committee of Faculty of Medicine, Benha University with the Code number: RC662023.

Results

At the experimental end, mortality rates in immunocompetent infected untreated mice were 20% in females and 10% in males, while in immunocompromised infected untreated ones were 40% in females and 20% in males. No mice died in the other groups.

Treated immunocompetent and immunocompromised mice showed significantly lower cysts than in positive control; due to SP & MTZ treatment, with reduction of (24.68%) in immunocompetent males and (15.62%) in immunocompromised males, with significant increase in cysts in immunocompromised mice compared to immunocompetent ones.

Treated immunocompetent female showed significant reductions in cysts (23.2%) than in immunocompromised ones (11.72%) post treatment.

Cyst counts across analysis showed significant sex-based differences in positive immunocompetent control. In females immunocompetent positive control showed higher significant cyst counts than males. But, the positive immunocompromised control didn't show significant differences in cyst counts. Immunocompromised treated females showed higher significant cysts than in males.

Details were given in tables (1, 2, 3 & 4), and figures (1, 2, 3, 4, 5, 6, 7, 8, 9, 10 & 11).

Table 1: *Toxoplasma* brain cyst counts in male mice.

Males	Mean ± SD	Reduction	P value
G1: Negative control	0.00±0.00		P3-2 <0.001**
G2: Immunocompetent non-treated	1575.5±55.05		P5-4 <0.014*
G3: Immunocompetent treated with SP+MTZ	1186.6±86.5[a]	24.68%	
G4: Immunocompromised non-treated	1757.0±129.96[ab]		
G5: Immunocompromised treated with SP+MTZ	1482.4±73.32[abc]	15.62%	

[a] Significant versus G2, [b]Significant versus G3; [c] Significant versus G4; **:Significant (P<0.001); *Significant (P<0.05); SP: Spiramycin; MTZ: Metronidazole.

able 2: *Toxoplasma* brain cyst counts in female mice.

Females	Mean ± SD	Reduction	P value
G 1: Negative control	0.00±0.00		
G2: Immunocompetent non-treated	1733.7±88.12		P3-2 <0.001**
G3: Immunocompetent treated with SP+MTZ	1331.5±109.72[a]	23.2%	
G4: Immunocompromised non-treated	1821.5±85.18[ab]		P5-4 <0.001**
G5: Immunocompromised treated with SP+MTZ	1608.0±83.0[abc]	11.72%	

Table 3: Comparison of *Toxoplasma* brain cyst counts between male and female mice.

groups	M\ales	Females	Total
G 1: Negative control	0.00±0.00	0.00±0.00	
G2: Immunocompetent non-treated	1575.5±55.05	1733.7±88.12	<0.001**
G3: Immunocompetent treated with SP +MTZ	1186.6±86.5	1331.5±109.72	0.004**
G4: Immunocompromised non-treated	1757.0±129.96	1821.5±85.18	0.21
G5: Immunocompromised treated with SP +MTZ	1482.4±73.32	1608.0±83.01	0.002**

SD: Standard deviation; **: Significant (P<0.001); SP: Spiramycin; MTZ: Metronidazole.

Table 4: Immunosuppression and sex effect on *T. gondii* DNA concentration in brain of males &females

	Males (groups)		Females (groups)		Total
	Mean ±SD	P value	Mean ±SD	P value (groups)	P value
G1	0.00±0.00		0.00±0.00		-
G2	505.20±32.46	<0.001**[a]	664.20±41.97	<0.001**[a]	<0.001**
G3	278.80±41.71	0.103 [c]	331.40±31.86	<0.001** [c]	0.005**
G4	706.70±27.98	<0.001** [b]	849.60±55.97	<0.001** [b]	<0.001**
G5	324.40±34.83	<0.001** [d]	457.80±38.38	<0.001** [d]	<0.001**

**Highly significant, [a] P- value between G2 & G33, [b] P- value between G2 & G4, [c] P- value between G3& G5, [d] P- value between G4 & G5

The histopathological pictures of liver and brain of experimental groups showed significant differences in toxoplasmosis, and treatmint. Normal histological structures were liver and brain of females and males negative controls but, changes were cleared in females and males positive control. Positive female control showed the liver with infiltration of by high number of mononuclear inflammatory cells portal area with blood vessels congestion and brain hemorrhage at cerebral cortex. Male ones showed liver congestion of central vein with mononuclear inflammatory cells infiltration and congestion of brain blood capillary.

Treated males and females mice showed mild inflammatory responses. Females showed focal peri-central vein infiltration and diffuse gliosis in brain, but males showed central vein congestion, and diffuse cerebral gliosis. Immunocompromised females positive control showed severe pathological changes in the form of extensive inflammatory infiltration in liver, bradyzoite cysts and brain gliosis, but males showed focal inflammatory infiltration between hepatocytes, and cerebral gliosis. Treated immunocompromised mice showed liver with marked portal fibrosis and inflammatory cell infiltration, and brain hemorrhage.

Molecular assessment: Known standards *Toxoplasma* DNA dilution assessed the limit detection of modified real-time PCR to create a calibration curve ranged from $1x10^5$-10ng/µl of *Toxoplasma* DNA. As SYBR Green, a fluorescent dye was used as a detective method, real-time PCR included a melting analysis to differentiate between specific and non-specific products, with melting temperature (Tm) of positive controls and positive samples was 86°C.

Immunocompetent infected female mice exhibited a significantly higher parasitic load than males ($p< 0.001$). Treatment with SP & MTZ in immunocompetent infected mice reduced in parasite load compared to G2 in both sexes ($p<0.001$). However, immunocompetent infected mice had lower parasitic load than (G5) in female mice ($p< 0.001$), while the reduction in males was not significant ($p=0.103$). Also, immunocompetent infected female mice showed more parasitic load than male mice ($p=0.005$).

In the present study, immunocompromised infected mice showed significantly higher parasitoc load than in immunocompetent in-

fected mice ($p< 0.001$), with a weak immune system enhanced parasite persistence.

Treatment with SP & MTZ in immunocompromised infected mice significantly reduced parasite load in both sexes as compared to immunocompromised infected mice ($p< 0.001$), females showed the higher counts.

Discussion

T. gondii is one of the main opportunistic pathogens of immunocompromised people (Wang *et al,* 2017). However, the understanding of mechanisms of sex-dependent differences in course of parasitic, bacterial, and/or viral infectious diseases might be relevant for the infected host management (Lipoldová and Demant, 2021).

In the present study, male and female infected mice showed different parasitological, histopathological, and molecular findings, as the females' mortality rate was higher than in males, indicated that they were more susceptible to toxoplasmosis. This agreed with Roberts *et al.* (1995); Liesenfeld *et al.* (2001), and Alonaizan *et al.* (2021), they suggested a stronger immune response in female mice compared with male mice enhanced their ability to control parasite replication, but increased their morbidity and mortality.

In the present study, the immunocompetent male showed (24.68%) reduction in cyst counts, but immunocompromised one showed (15.62%) reduction. The treated immunocompetent female showed significant reductions (23.2%) than in immunocompromised one (11.72%). This agreed with Roberts *et al.* (1995), who found that female mice were more susceptible to acute infection by higher mortality rates, than male mice, and female mice survived chronic infections with more brain cysts in than males. Also, this agreed with Martynowicz *et al.* (2019), who reported that both male and female mice had lower cyst counts after specific treatments, but females showed the huge cysts' number. Besi des, Alonaizan *et al.* (2021) reported that female mice were more sensitive to acute infection shown by increased mortality rates and weight loss compared to males. Again, this agreed with Troublefield *et al.* (2023), they reported that female mice were more susceptible to tachyzoites with higher cyst counts than males.

Undoubtedly, early *T. gondii* immunity activated type 1 inflammatory response with TNF-α & IFN-γ (Jones *et al,* 2006). *T. gondii* resistance is primarily mediated by IFN-γ that initiated intracellular processes to kill and prevent parasite multiplication (Suzuki *et al,* 2011). Also, Xiao *et al.* (2013) added that males have greater antibody to *Toxoplasma* matrix antigen than females. Hegazy *et al.* (2019) reported that serum IL-12 levels were considerably greater in male mice with latent toxoplasmosis than in females. But, Alonaizan *et al.* (2021) reported significantly higher of IFN-γ &TNF-α concentrations in female mice than in male. Consequently, differential response based on sex is a biological feature influenced how immune system functions, and that sex hormones affect innate immune system cells such as NK cells, macrophages, eosinophils, and mast cells as well as dendritic cells (Sciarra *et al,* 2023).

In the present study, positive immunosuppressed control mice of both sexes showed significant increase in brain cysts compared to positive immunocompetent control ones, and that immunocompetent mice showed significant reductions in cysts post-treatment, but immunocompromised ones showed less efficacy. This agreed with Dropulic and Lederman (2016), who reported that immunosuppression severely, impaired host's ability to infections, with less treatment efficacy. Also, this agreed with Zhang *et al.* (2017), they found that dexamethasone promoted proliferation of *T. gondii* tachyzoites, and Giles *et al.* (2018) added that dexamethasone suppressed the T cell proliferation and differentiation.

The present study showed a considerable reduction in cyst counts in immunocompetent and immunocompromised mice after SP & MTZ treatment. This agreed with Chew *et al.* (2012); Montazeri *et al.* (2017) and Hegazy *et al.* (2019), they reported that the effic-

acy of SP & MTZ lowered *Toxoplasma* load, mainly in immunocompetent host as combined MTZ improved brain in SP absorption, led to a significant brain cysts reduction compared to either drug alone.

In the present study, infected female mice showed greater *Toxoplasma* DNA concentrations than male in treated or untreated mice. This agreed with Alday and Doggett (2017), they reported that development of a drug to reduce toxoplasmosis impact was a challenging task. *Toxoplasma* has a complex life cycle of different stages with different metabolic requirements, and potential emergence drug resistance poses a potential problem (Rifaat and Morsy, 1965).

In the present study, the histopathological pictures of liver and brain of immunocompetent and immunocompromised female and male *T. gondii* infected mice showed that infection and treatment differently affected sexes and immunological status. This agreed with Hegazy *et al.* (2019), they reported that positive male and female control mice showed significant *T. gondii* pathological changes mainly in female mice brain cysts than males with higher cyst counts and more severe brain inflammation

In the present study, treated male and female immunocompetent mice had residual liver inflammation, tissue distress, localized infiltration and congestion as well as brain gliosis. This agreed with Fuentes-Castro *et al.* (2017), who reported that some drugs efficacy were achieved, but without total recovery. Also, it agreed with Omar *et al.* (2021), who reported that therapies lowered the infection severity and improve survival rates, but neither completely eradicated *Toxoplasma* nor restored normal tissue structure.

In the present study, liver in immunocompromised mice showed marked necrotic alterations, and inflammatory cell infiltration due to host' toxoplasmosis susceptibility, as well as bleeding and fibrosis were cleared. This agreed with Lewis *et al.* (2015), they reported that toxoplasmosis treated immunocompromised mice; the outcome data depended on sex of host and immunological state.

Conclusion

The sex variations in latent *T. gondii* infected mice showed susceptibility and treatment effectiveness; particularly males caused greater resistance to infection and improved treatment response. Immunocompromised female and male mice experienced the worse treatment outcomes and continued to have significant cyst loads post treatment.

Recommendation

Host sex and immune status must be considered in treating chronic toxoplasmosis.

*Authors' contributions***:** All authors equally contributed in the field and practical studies as well as in writing, reviewing the manuscript and approved its publication.

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Explanation of figures

Fig. 1: Female negative control (H & E stained). A: Liver showed normal histologic structure. B: Brain showed normal histologic structure.

Fig. 2: Male negative control. A: Liver showed normal histological structure. B: Brain showed normal histological structure.

Fig. 3: Female positive control. A: Liver showed high number of mononuclear inflammatory cells (arrow) in portal area and portal blood vessels congestion (star). B): Brain showed hemorrhage in cortex (star).

Fig. 4: Male positive control. A: Liver showed congestion of central vein (star) with focal infiltration by mononuclear inflammatory cells (arrow) between hepatocytes. B: Brain showed focal gliosis (arrow) and blood capillary congestion (star).

Fig. 5: Immunocompetent females infected and MTZ & SP treated. A: Liver showed mononuclear inflammatory cells infiltration around central vein (blue arrow), vacuolar degeneration in some hepatocytes (red arrow) and some sinusoid engorged with blood (black arrow). B: Brain showed diffuse gliosis in cortex (arrow).

Fig. 6: Immunocompetent infected males and MTZ & SP treated. A: Liver showed congestion of central vein (star) with focal infiltration by mononuclear inflammatory cells (black arrow) and engorgement of some hepatic sinusoids with blood (red arrow). B: Brain showed diffuse gliosis in cerebral cortex (arrow).

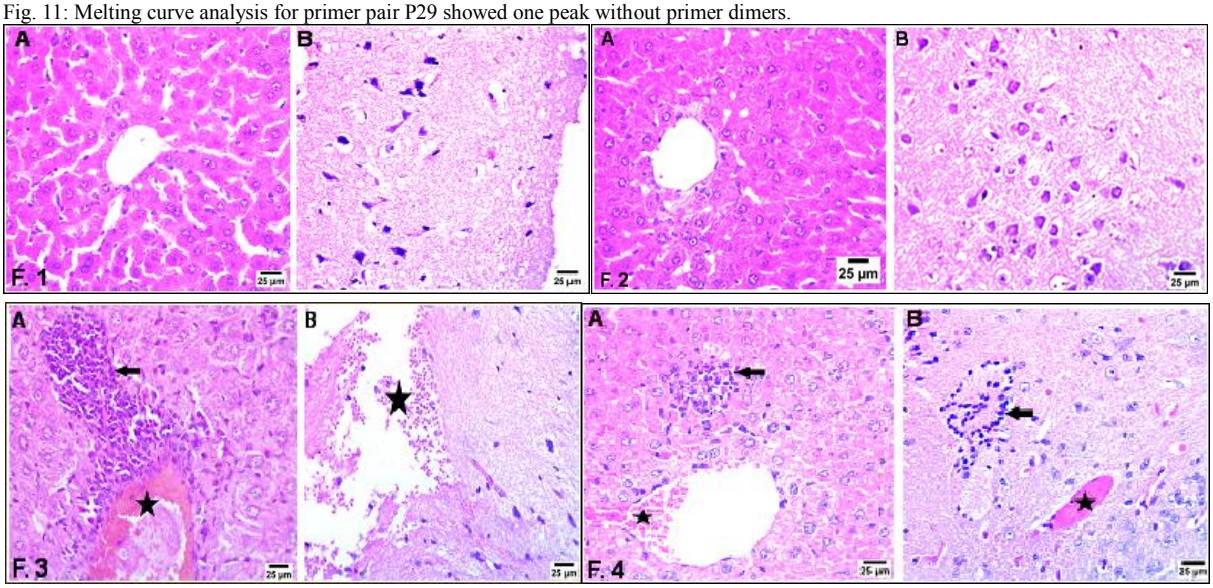
Fig. 7: Immunocompromised positive female control. A: Liver showed portal infiltration with mononuclear inflammatory cells (red arrow) with detection of bradyzoites (black arrow). B: Brain showed multiple cysts contained bradyzoites (arrow) in cerebral cortex. C: Necrotic neurons with acidophilic cytoplasm and acidophilic nuclei (black arrow) with gliosis (blue arrow).

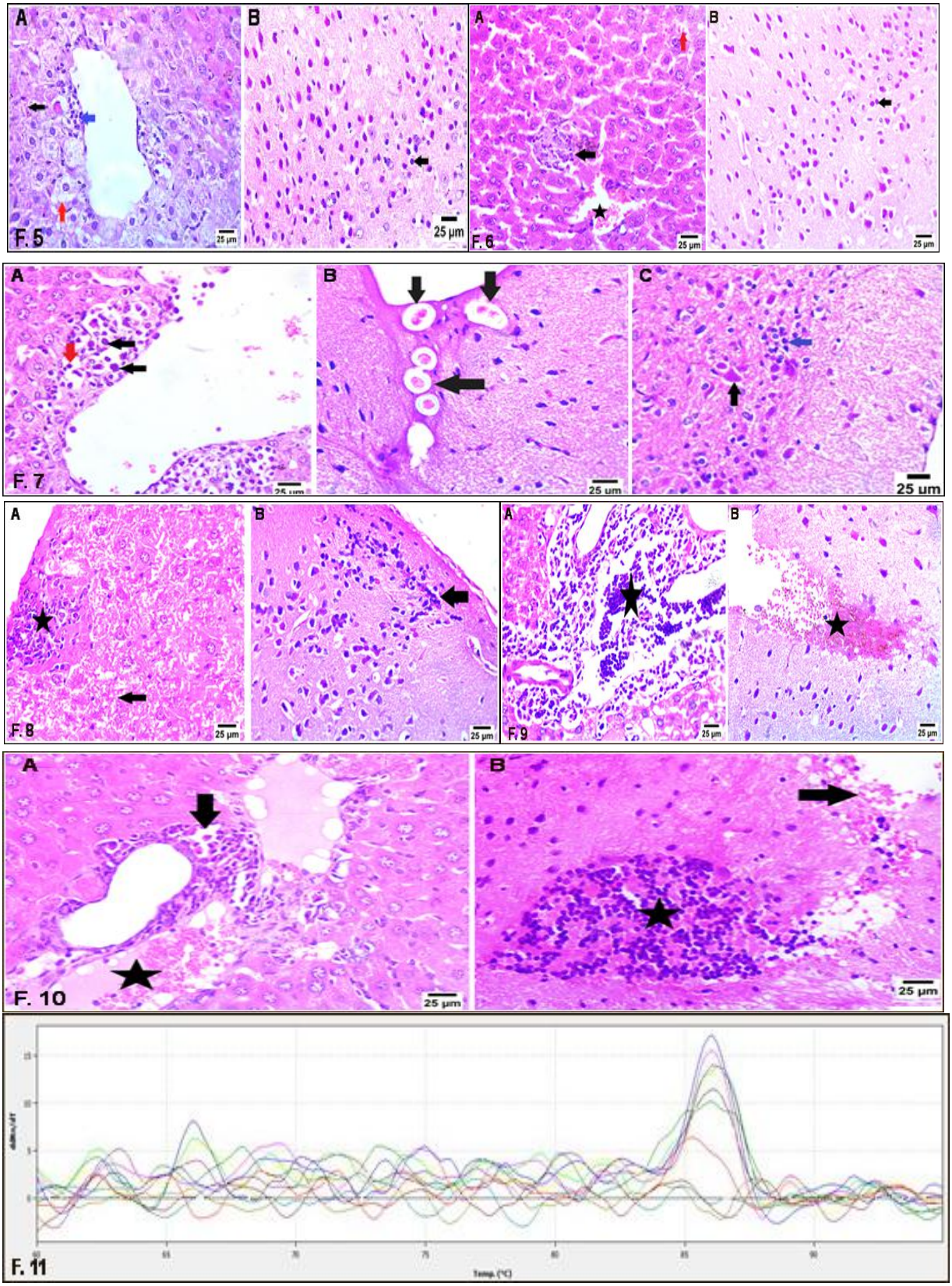
Fig. 8: Immunocompromised positive male control. A: Liver showed focal infiltration by mononuclear inflammatory cells between hepatocytes (star) and some hepatocytes showed necrobiotic changes (arrow). B: Brain showed diffuse gliosis in cortex (arrow).

Fig. 9: Immunocompromised infected treated with (MTZ & SP) females. A: Liver tissue showed portal fibrosis with infiltration by high mononuclear inflammatory cells (star). B: Brain tissue showed hemorrhage in cerebral cortex (star).

Fig. 10: Immunocompromised infected treated (MTZ & SP) males. A: Liver tissue showed portal blood vessels congestion (star) and with infiltrated by mononuclear inflammatory cells (arrow). B: Brain tissue showed focal gliosis (star) with hemorrhage (arrow).

Fig. 11: Melting curve analysis for primer pair P29 showed one peak without primer dimers.

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