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Assessment of vaccination with gamma radiation-attenuated infective *Toxocara canis* eggs on murine toxocariasis

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Abstract. *Toxocara canis* is a major parasite that infects many animals with high risk of human infections. This study aims at assessing the immunization with gamma radiation-attenuated infective stage on rats challenged with non-irradiated dose. Level of vaccine protection was evaluated in liver and lung_regarding parasitological, histopathological, biochemical and molecular parameters. Fifty rats were enrolled in three groups: group A (10 rats) as normal control; group B (20 rats) subdivided into subgroup B1 (infected control) and subgroup B2 infected then challenged after 14 days with the same dose of infection (challenged infected control); and group C (20 rats) subdivided into subgroup C1 vaccinated with a dose of 800 gray (Gy) gamma-radiated infective eggs (vaccine control) and subgroup C2 vaccinated then challenged on 14th day with same number of infective eggs (vaccinated-challenged). Tissues were stained with Haematoxylin and Eosin (H&E) for histopathological studies. Biochemical studies through detection of nitric oxide (NO) and Caspase-3 were conducted. Extent of DNA damage by Comet assay was assessed. Vaccinated-challenged subgroup revealed a marked reduction in larvae in tissues with mild associated histological changes. In addition there was accompanied reduction of NO, Casepase-3 level and DNA damage compared to the control infected group. It could be concluded that vaccination of rats with a dose of 800Gy gamma radiation-attenuated infective stage improves immune response to challenge infection and drastically reduces the morbidity currently seen.

INTRODUCTION

Human toxocariasis is considered one of parasitic diseases that have high risk of human infection due to its broad distribution and various routes of transmission. Larval migration in the body may be asymptomatic or may cause various clinical syndromes including visceral larva migrans (VLM), ocular larva migrans (OLM) and covert toxocariasis (Kleine *et al.*, 2017; Ma *et al.*, 2018).

During migration of *Toxocara* larvae (L2) inside the host tissues, nitric oxide (NO) which is a highly reactive and unstable free radical is produced by host cells or activated by the parasite. Its production facilitates larval migration and represents parasite adaptation mechanism so deleterious effects are attributed to the host due to its release (Durante *et al.*, 2007; Muro & Arellano, 2010).

As a result of infection, caspases which are proteolytic enzymes are involved in cell death. Some are apoptotic as caspase-2, -3, -7, -8, -9 and -10, whereas others are involved in inflammation as caspase-1, -4, -5, -11 and -12 (Shalini *et al.*, 2015). Activation of these caspases causes digestion of cytoplasmic proteins and degradation of chromosomal DNA (Matsuura & Kurokawa, 2016; Team *et al.*, 2017).

Comet assay was adopted in 2016 by the Organization for Economic Co-operation and Development as a guideline for *in vivo*

testing of DNA strand breaks, repair screening and study of apoptosis in different cell models of animals. Its versatility was demonstrated by previous studies, which hold promise that it is appropriate for the next 30 years (Muller, 2019). Viable cells showed a large head with a minute tail and apoptotic one represented as comet-like with a small head and a large tail while dead cells showed large nuclear remnants (Archana *et al.*, 2012).

Vaccination by live-attenuated, killed microbe or components of its remains is one of the best ways to protect against infectious diseases. Live-attenuated vaccine tricks the body into thinking an infection has occurred preparing the host's immune system for further attacks. This supports the hypothesis that activating multiple innate receptors is better than activating only one (Lombard *et al.*, 2007).

Using ionizing radiation in the production of attenuated vaccines against various parasitic infestations has been taken into consideration. It was reported that gamma radiation when applied to cells can basically induce DNA damage by direct effects resulting from their interaction or indirect effects resulting from free radicals that are generated (Seo, 2015; Vasou *et al.*, 2017).

The aim of the present work is to assess the effect of vaccination of rats with infective *Toxocara canis* eggs attenuated with 800 Gy gamma radiations and challenged with normal infection regarding assessment parasitological, histopathological, biochemical and molecular changes on liver and lung tissues.

MATERIAL AND METHODS

Experimental animals

The study was conducted on fifty clean laboratory inbred male albino rats (Rattus norvegicus) that have been pointed out as usual paratenic host of *T. canis* (Chieffi *et al.*, 1981). They were weighing 100-160 gm and received a diet of standard rodent pellets produced by Cairo Company for Oil and Soap. They were housed in cages (10 rats in each) with water and food available *ad libitum*, maintained under controlled conditions of lighting and temperature and were euthanized using anesthesia.

Ethical Approval Consent

All procedures were performed according to standard guidelines for researches on experimental animals after permission from the Research Ethics committee of the National Center for Radiation Research and Technology, the Atomic Energy Authority, Cairo, Egypt (REC-NCRRT) (Permissin code 8A/18).

Parasite

Egyptian isolate *Toxocara canis* eggs were freshly collected from adult female worms obtained from small intestine of puppies inbred in animal house of parasitology department of Collage of Veterinary Medicine, Cairo University. Eggs were incubated 0.5% of formalin solution at 28°C for 4 weeks till embryonated then were kept at +4°C until being used (Galvin, 1964).

Radiation source

Irradiation of *Toxocara* eggs was conducted at the National Center for Radiation Research and technology (NCRRT), the Atomic Energy Authority, Cairo, Egypt. They were exposed to 800 Gy gamma-radiation rays using Indian Cobalt-60 gamma chamber 4000 A Irradiator at a dose rate of 2.5 Krad / h at the time of irradiation.

Infection of rats

Enrolled rats were divided into three groups, Group A (10 rats) as normal control, group B (20 rats) subdivided into subgroup B1 orally infected with embryonated eggs using stomach tube syringes under mild anesthesia (2500 infective eggs / rat) as infected control subgroup, and Subgroup B2 challenged with the same infective dose 14th day after infection (challenged infected control). Group C (20 rats) was subdivided into subgroup C1 vaccinated with 800 Gy gamma-irradiated infective eggs (2500 eggs) as vaccine control and subgroup C2 vaccinated with 800 Gy gamma-irradiated infective

eggs (2500 eggs) then challenged with the same infective dose 14th day after vaccination as vaccinated-challenged (Table 1).

Animals of different groups were sacrificed by decapitation after anesthesia on the 14th day post infection (B1 and C1) and post challenge (B2 and C2). Pieces from liver and lung tissues were dissected from all groups for parasitological study and histopathological changes detection. Biochemical determination of nitric oxide (NO) production and Caspase-3 were assessed. A molecular study by comet assay to assess the quantitative and qualitative extent of DNA damage in the cells of the examined tissues was also performed.

Counting larvae in liver or lung tissue

One third of the liver and lung tissues from each rat were individually taken to count the number of larvae/g tissue. Each piece (liver or lung) was weighed and placed in 2ml of digestive fluid which consisted of 9 gram pepsin, 7 ml hydrochloric acid and one liter water. They were incubated at 37°C for 24 h until the tissues were digested. The digest was well shaken and three samples were counted under the microscope by putting 25 µl of each on a slide. The average number was calculated. The rest of digest was poured in graduated cylinder and measured and total number of larvae was calculated as follows, according to Chung *et al.* (2004) and Fan *et al.* (2004).

Total number of larvae = mean larvae count X volume of the digested sample.

$$\text{Number of larvae/g tissue} = \frac{\text{Total number of larvae}}{\text{Weight of tissue sample}}$$

Histopathology

Another third of the liver and lung tissues was washed in normal saline. Specimens were fixed in 10% phosphate buffered formalin (pH 7.4) for at least 24 h. They were embedded in paraffin wax, sectioned at thickness of 4 micron. Slides were stained with haematoxylin and Eosin for histopathological examination (Nilsson, 1999).

The remaining third of the liver and lung tissues from each sacrificed rat were homogenized in Phosphate-buffered saline (PBS), pH 7.4 and centrifuged at 10000 x g for 20 minutes. Supernatant was ultra-centrifuged at 100, 000 x g for 15 minutes then kept to be used for biochemical study and Comet assay.

Nitric oxide

Filtration of supernatant solution through 0.45 micron filter, then ultra filtration using a 10 or 30 Dalton (Da) molecular weight cut-off filters were conducted. 200 µL of water or Assay Buffer were added to two wells to serve as blank for the microtiter plate. Then

Table 1. Represents the experimental groups

Groups	Item
Group A (n=10)	Normal control.
Group B (n=20)	
B1(n=10)	Infected with 2500 infective eggs per rat as infected control.
B2(n=10)	Infected with 2500 infective eggs then challenged with the same dose 14th day after infection as challenged infected control.
Group C (n=20)	
C1(n=10)	Vaccinated with 800 Gy gamma-irradiated infective eggs (2500 eggs) as vaccine control.
C2(n=10)	Vaccinated with 800 Gy gamma-irradiated infective eggs and challenged after 14 days with the same dose of infection (2500 eggs) as vaccinated-challenged.

40 µL of tissue sample homogenates, 10 µL of freshly prepared NADPH solution and 10 µL of Nitrate Reductase solution were added to each sample well so that final volume was adjusted to 60 µL. The plates were incubated at room temperature for 60 minutes then 10 µL of the Co-Factor Preparation solution and 10 µL for the lactate dehydrogenase solution were added to each well, followed by incubation at room temperature for 20 minutes. 50 µL of Griess Reagent R1 and 50 µL of Griess Reagent R2 were added to each well. After ten minutes, at room temperature, color development was observed. The plate blank wells were used to zero the plate reader and the absorbance was read at 540 nm (Bories & Bories, 1995).

Caspase-3 (CASP3)

Determination of Caspase-3 level was performed using Enzyme Linked Immuno-Sorbent Assay (ELISA) sandwich kit for *in vitro* quantitative measurement of CASP3 in tissue homogenates (Cloud- Clone Corp. SEA626Mu) according to Engvall and Perlmann (Engvall & Perlmann, 1971).

Single Cell Gel (Comet) Assay

Volumes of 6 µl of Liver and lung tissues homogenate were suspended in 100 µl of 0.5% low-melting agarose (LMA) (Sigma-Aldrich, St Louis, MA, USA) then placed onto microscope slides and coated with 300 µl of 0.6% of normal melting point agarose. Slides were solidified on ice for 10 minutes, covered with 0.5% low melting point (LMP) agarose, and then they were immersed for one hour in an ice-cold lysis solution consisting of 100 mM Na2EDTA, 2.5 M NaCl, 10 mMTris-HCl, and 1% sodium sarcosinate, which was adjusted to pH10, using 1% Triton X-100 and 10% dimethyl sulfoxide (DMSO) that were added immediately prior to use. Slides were removed from lysing solution before electrophoresis and placed for 20 minutes in a horizontal electrophoresis unit (near the anode) that was filled with an alkaline buffer to allow unwinding of DNA and to express alkali labile damage. After Unwinding, electrophoresis was done in freshly prepared alkaline solution for 20 minutes at 25 V (300 mA). Structures such as comets were observed where the intensity of the tail relative to head reflected the number of DNA damage. All steps were implemented under dimmed light to prevent additional damage of DNA. Images of 100 randomly selected cells were analyzed using Leitz Orthoplan Epifluorescence microscope (magnification 250x) which was connected to a camera to computer-based image analysis system (Bories & Bories, 1995).

Statistical analysis

Results were expressed as Mean and standard error (mean±SE). The statistical analysis of differences was carried out using t-test, and multifactor analysis of variance (ANOVA) was used to check the significant differences, where $P<0.05$ was considered significant. All analyses were performed using the SPSS 10.0 version software packages.

RESULTS

Parasitological results

Larvae counts/ gm liver and lung tissues are shown in Table 2 and Figure 1. Vaccine control (C1) and vaccinated-challenged subgroup (C2) showed a high significant reduction in comparison to infected control and challenged infected control subgroups B1 and B2 respectively ($P <0.001$).

Table 2. *Toxocara canis* larvae counts in liver and lung tissues

Group	Liver mean ± SE	Lung mean ± SE
Group B		
B1	924 ± 25.3	524 ± 18.4
B2	519 ± 22.1^{a*}	278 ± 3.7^{a*}
Group C		
C1	356 ± 6.3$^{a**, b*}$	190 ± 4.9$^{a**, b*}$
C2	145 ± 5.7$^{a**, b**}$	20 ± 3.0$^{a**, b**}$

Data are expressed as mean ± SE.
a: Significant change from B1.
b: Significant change from B2.
*: Significant ($P<0.01$).
**: High significant ($P<0.001$).

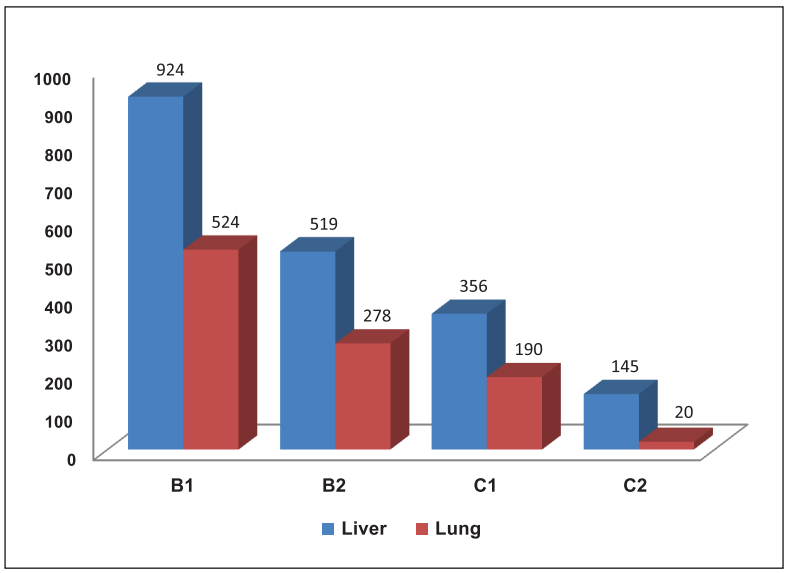

Figure 1. The individual larva counts for the animals in each group.

Histopathological results

Figure 1 reveals that Group "A" (normal control) showed a normal lobular pattern of hepatocytes. *Toxocara canis* larva with proliferating, regenerating, loss of the normal pattern of regular liver cell plate was detected in Subgroups "B1" (infected control). Inflammatory cellular infiltration of lymphocytes, plasmocytes, and a small number of eosinophils could be seen in subgroup "B2" (challenged infected control). Vaccine control subgroup "C1" demonstrated a mild inflammatory cellular infiltration in localized foci while vaccinated challenged subgroup "C2" (vaccinated-challenged) showed nearly no histopathological changes (Figure 2).

Regarding histopathological changes in lung tissue (Figure 3), the study revealed normal lung tissues in group "A". Severe inflammatory reactions with numerous nodular leukocyte infiltrations in both infected control subgroup "B1" and challenged infected control "B2" were detected. Vaccine control subgroup "C1" showed a mild focal interstitial pneumonia while vaccinated-challenged subgroup "C2" demonstrated minimal histopathological changes in lung tissues.

Biochemical results

Nitric oxide level

Information presented in Figure 4 shows that mean±S.E. of NO in normal control group A was 3.59 ± 0.20 and 1.68 ± 0.07 in liver and lung tissues respectively. In subgroups B1 (infected control) and B2 (challenged infected control), it was 6.42 ± 0.22 and 7.014 ± 0.12 in liver tissues. In lung tissues, it was 4.32 ± 0.17 and 5.36 ± 0.18 respectively with significant increase ($p<0.05$). Regarding subgroups C1 (vaccine control) and C2 (vaccinated-challenged), mean±S.E. of NO was 5.80 ± 0.13 and 5.02 ± 0.10 in liver and 4.02 ± 0.08 and 4.64 ± 0.09 in lung respectively with significant changes from control normal group (A) and both control infected (B1) and challenged control infected (B2) subgroups ($P<0.05$).

Caspase-3 level

Figure 5 shows that mean±S.E. of Caspase-3 in normal control group A in rats liver was 27.6 ± 0.58 and in lung was 41.5 ± 0.59. While in infected control subgroup (B1) and challenged infected control (B2), it was 202.4 ± 1.1 and 294.0 ± 1.68 respectively in liver and 194.4 ± 0.95, and 254.6 ± 1.19 in

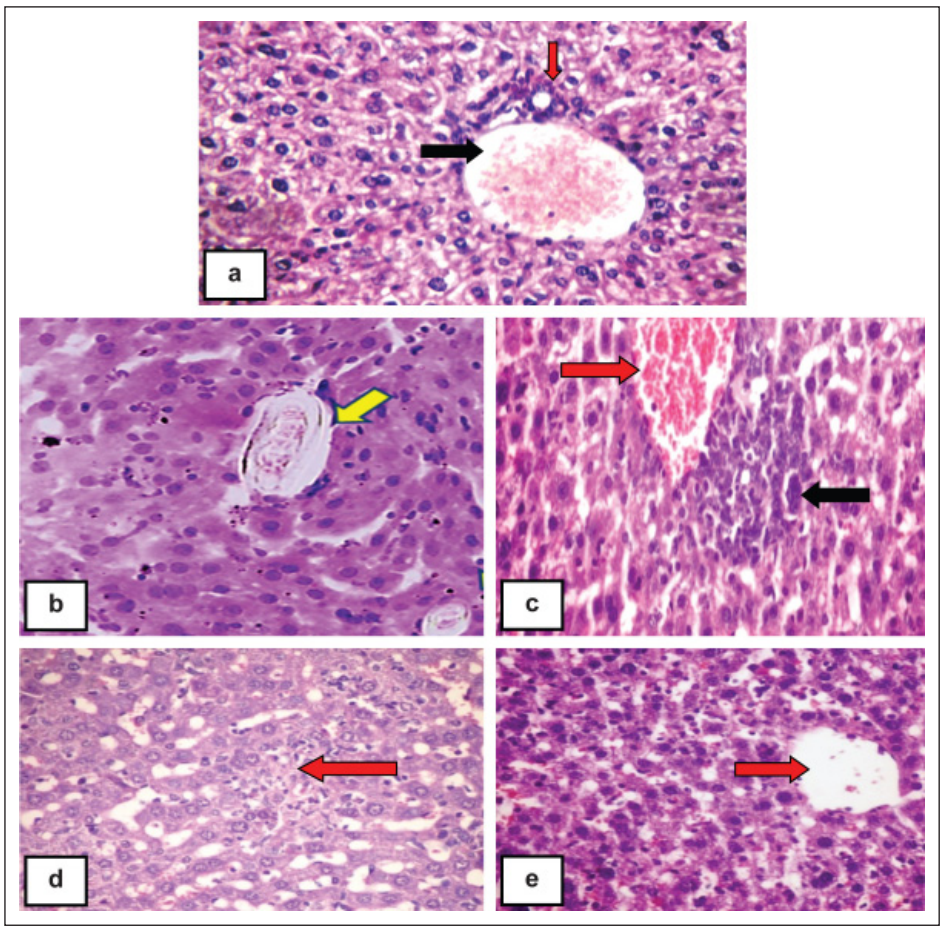

Figure 2. Liver sections of group A showing normal hepatocytes with average portal vein (black arrow) and pile duct (red arrow) (a). Section of subgroup B1 showing *Toxocara canis* larva (yellow arrow) associated with mixed cell infiltrate (b). Section of subgroup B2 showing marked portal infiltration with inflammatory cells (black arrow) and congested vessel (red arrow) (c). Liver section of subgroup C1 showing focal hepatic necrosis with inflammatory cellular infiltrates (red arrow) (d). Liver section of subgroup C2 showing nearly mild histopathological changes with mild dilation of central vein (red arrow) and few inflammatory cells (e) (H&E X400).

lung tissues of with a significant increase compared to those of the normal control group A ($P< 0.05$). Subgroups C1 (vaccine control) and C2 (vaccinated-challenged) showed a significant reduction ($p<0.05$) compared to the infected control (B1) and challenged infected control (B2) subgroups as it was 186.4 ± 1.65 and 190.8 ± 1.46 in liver and 186.7 ± 1.58 and 197.8 ± 0.86 in lung respectively.

DNA Comet assay

Evaluating results of comet by image analysis are evident in Figures 6 and 7 and illustrated in Tables 3 and 4 for both liver and lung cells. The changes of tail DNA%, tail moment and tail length displayed a marked increase in the infected control subgroup B1 and the challenged infected control subgroup B2 compared to the normal control group A. While, the subgroups C1 (vaccine control) and subgroup C2 (vaccinated-challenged) showed a significant decrease in tail DNA%, tail moment and tail length compared to that B1 & B2 especially in subgroup C2 in both liver and lung cells. In damaged cells, breaks appear as fluorescent tails extending from the core toward the anode. The tail length

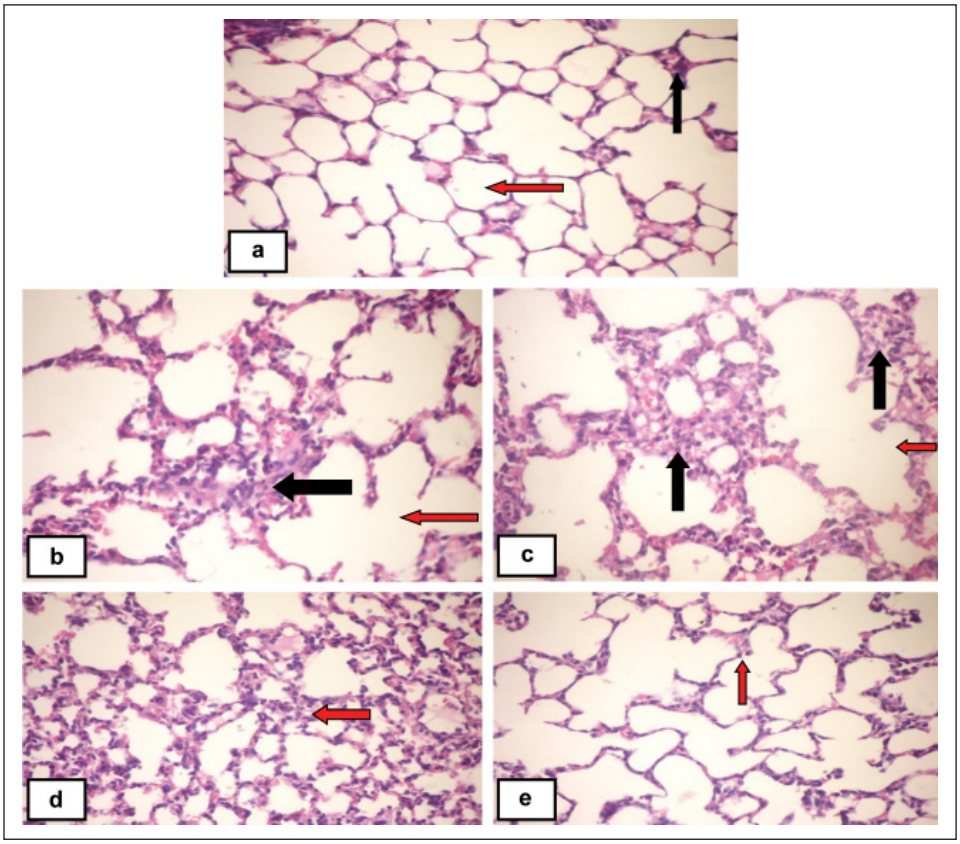

Figure 3. Lung sections of normal group (A) showing average alveoli (red arrow) and blood vessel (black arrow) (a). Section of lung tissues of subgroup B1 showing numerous nodules resulting from inflammatory cellular infiltration (black arrow) and dilated alveoli (red arrow) (b). Section of lung tissues of subgroup B2 showing multiple focal interstitial pneumonia (black arrow) with markedly dilated alveoli (red arrow) (c). Lung section of subgroup C1 showing numerous cellular infiltration (red arrow) (d). Lung section of subgroup C2 showing nearly normal lung with mild cellular infiltrates (red arrow) (e) (H&E X400).

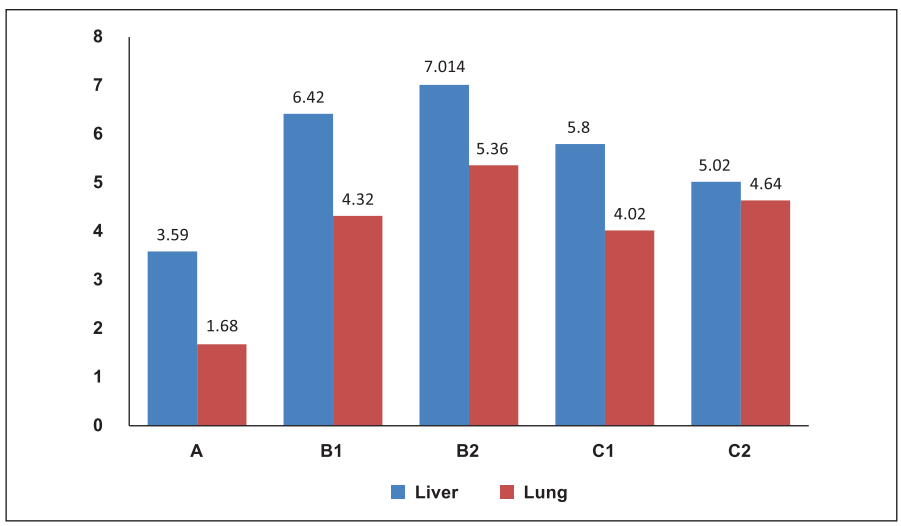

Figure 4. Nitric oxide level (µmol/L) in liver and lung tissues of experimental groups.

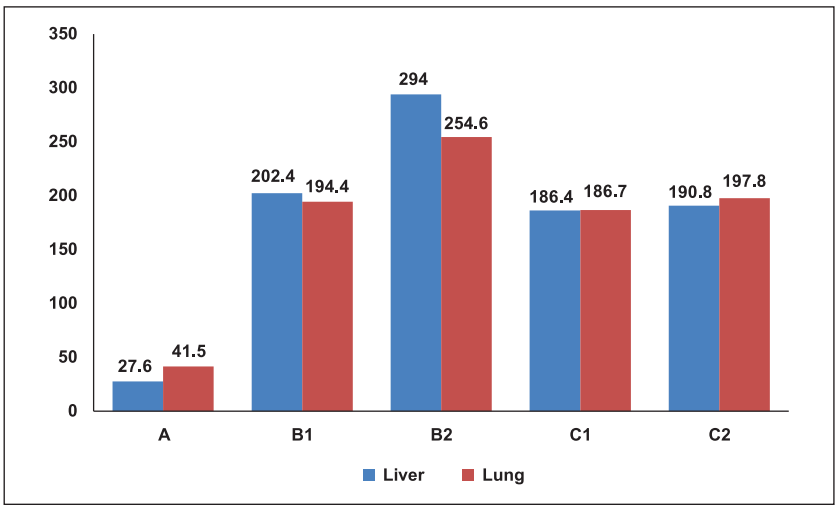

Figure 5. Caspase 3 level (Pg/L) in liver and lung tissue of experimental groups.

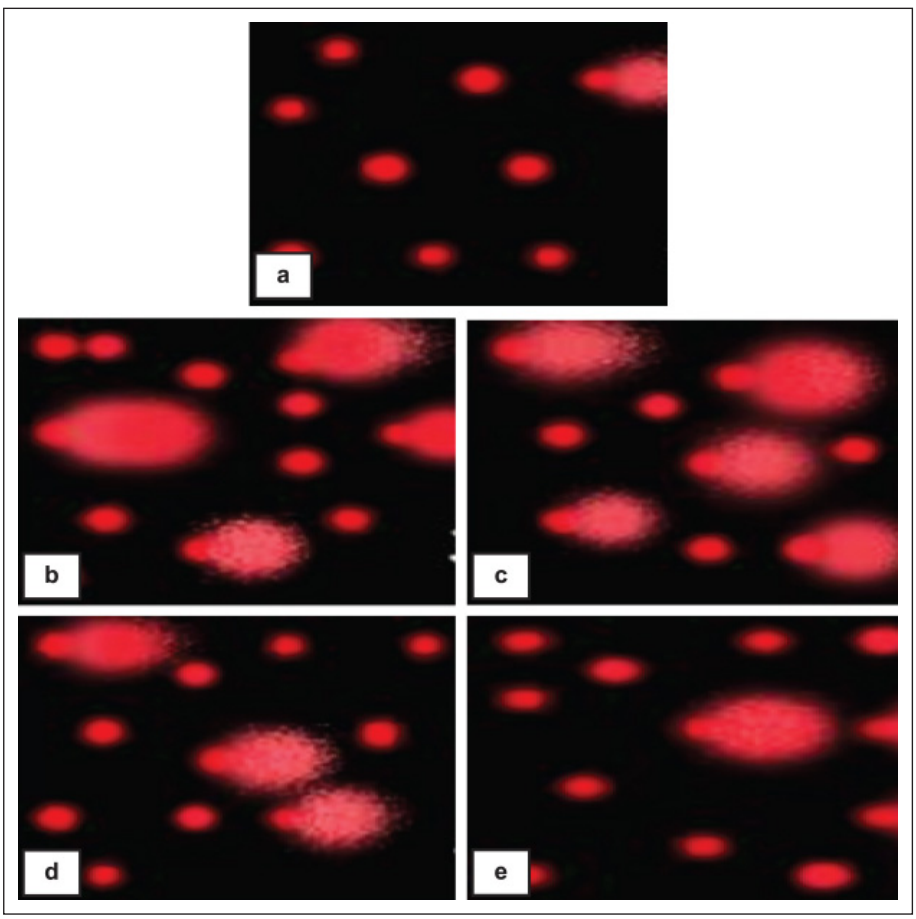

Figure 6. Nearly intact nuclear DNA in rat liver cells of normal control group A (a). Infected control subgroup B1 and challenged infected control subgroup B2 show strongly damaged DNA expressed by tail moment in liver cells (b, c). Vaccine control subgroup C1 showing DNA damage expressed by tail moment (d). Less DNA damaged spot expressed by tail moment in rat liver cells of vaccinated-challenged subgroup C2 (e) (x250).

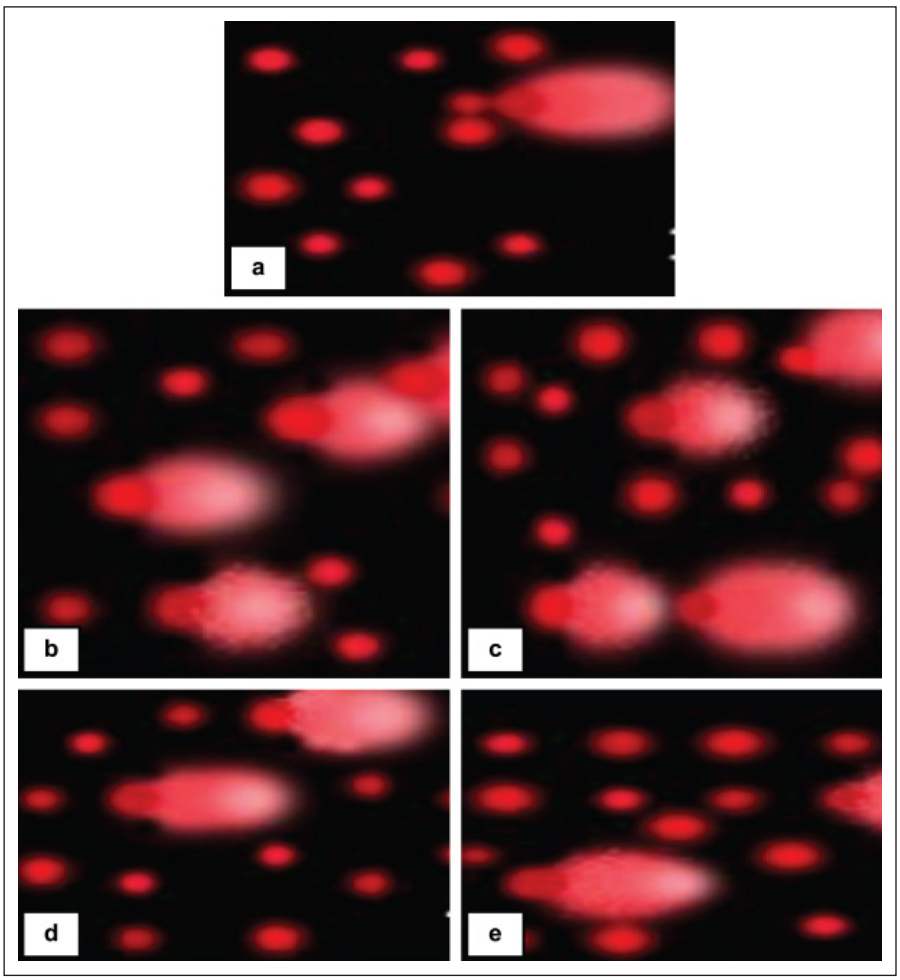

Figure 7. Intact nuclear DNA in normal rats of group A lung cells (a). Infected control subgroup B1 and challenged infected control subgroup B2 show strongly DNA damaged expressed by tail moment in rat lung cells (b, c). Vaccinated subgroups (C1 & C2) showing DNA damage expressed by tail moment in cell with less damaged spot (d, e) (x250).

Table 3. Tail moment, tail length, tailed and untailed % DNA in liver

Groups	Tailed %	Untailed %	Tail Length (µm) mean ± SE	Tail moment mean ± SE
Group A	4	96	1.63 ± 0.15	2.65 ± 0.15
Subgroup B1	14	86	3.79 ± 0.05[a]	16.1 ± 0.44[a]
Subgroup B2	16	84	4.08 ± 0.05[a,b]	19.4 ± 0.24[a,b]
Subgroup C1	12	88	5.07 ± 0.09[a,b,c]	24.0 ± 0.32[a,b,c]
Subgroup C2	11	89	4.81 ± 0.16[a,b,c]	21.32 ± 0.33[a,b,c]

Data are expressed as mean ± SE. P<0.05.
a: significant change from normal control group A.
b: significant change from infected control subgroup B1.
c: significant change from challenged infected control subgroup B2.
Tail moment = Tail DNA% X tail length. Tail DNA% = percent of DNA in the comet tail.
Tail length = length of the comet tail measured from right border of head area to end of tail (µm = pixels).

Table 4. Tail moment, length, tailed and untailed % of DNA in lung

Groups	Tailed %	Untailed %	Tail Length (µm) mean ± SE	Tail moment mean ± SE
Group A	2	98	1.34 ± 0.139	1.8 ± 0.12
Subgroup B1	18	82	3.01 ± 0.046[a]	18.3 ± 0.38[a]
Subgroup B2	20	80	3.67 ± 0.09[a,b]	22.6 ± 0.29[a,b]
Subgroup C1	10	90	4.13 ± 0.07[a,b,c]	16.3 ± 0.31[a,b,c]
Subgroup C2	8	92	3.56 ± 0.06[a,b,c]	14.2 ± 0.16[a,b,c]

Data are expressed as mean ± SE. $P<0.05$.
a: significant change from normal control group A.
b: significant change from infected control subgroup B1.
c: significant change from challenged infected control subgroup B2.
Tail moment: Tail DNA% X tail length. Tail DNA%: percent of DNA in the comet tail.
Tail length: length of the comet tail measured from right border of head area to end of tail (µm = pixels).

reflects the amount of DNA breakage in the cell. Therefore, the migrated nuclear DNA was considered a damaged spot.

DISCUSSION

Chemotherapy alone is transitory for control of the severe complications caused by migration of *Toxocara canis* larvae. There is a growing interest in using vaccination with ionizing radiation for control and reduce reinfection (Joy *et al.*, 2017; Selek *et al.*, 2016). In the present work, parasitological results revealed that recovery of *T. canis* larvae in the infected control group was high in both liver and lung tissues which was in agreement with a previous study that showed progressive increase of recovered larvae over the course of infection (Walsh & Haseeb, 2012). However, prior infection triggers a protective response exemplified by a decreased number of larvae with marked infiltration with lymphocytes and esinophils in liver of reinfected subgroup B2. This can be explained by obstruction of larval movement by infiltration of inflammatory cells around it, thus preventing their migration to other organs, such as lung. This was compatible with previous studies, which found that animals which had been infected twice with the same dose of parasites developed strong anti-*Toxocara* immunity and showed reduction in brain larval recoveries. However, this acquired immunity was short-lived (Abo-Shehada *et al.*, 1991; Wnukowska & Dzbeñski, 2008).

A significant decrease in the vaccine control subgroup compared to the control infected and the challenged control infected subgroups indicates that vaccination with attenuated dose exerted a reducing effect on the number of surviving larvae that can reach liver or lung. Previous studies reported that attenuation does not allow most of parasites to complete their cycle. Few of them are able to disseminate to different organs. However, the attenuation makes them non-pathogenic, but could maintain their integrity, antigenicity and increasing immunogenicity. Thus, these larvae found in the vaccine control subgroup are from irradiated eggs, but they are unable to induce patent infection. They can only induce mild inflammatory reactions. This complies with the work conducted by Babayan *et al.* (2005) who reported recovery of the majority of *T. canis* and *T. vitulorum* larvae from liver and lung using 40 Krad gamma radiations while increasing the irradiation dose to 80-160 Krad caused them to remain mainly in stomach and proximal part of small intestine. Therefore, they suggested that this dose disables the larvae from evading host immune system by reducing their motility or killing them.

A high significant reduction in larval recovery in the vaccinated-challenged subgroup coincided with the study of Amin *et al.* (2016) who stated that a marked

reduction in a number of recovered larvae in brain tissues in the vaccinated challenged group has occurred as a result of stimulation of immune response. Live attenuated vaccine has potent immunogenicity since the organisms are still able to behave initially in a similar manner to a natural infection, thereby stimulating the immune system to secrete the immunoregulatory products and induce the cellular activation that would normally occur (Kamiya *et al.*, 1987; Viljoen & Luckins, 2012).

In the present study, the vaccine control and the challenged subgroups revealed amelioration of the severe histopathological alterations that were observed in the control infected group in both liver and lung tissues. This showed a similarity with previous studies that demonstrated an improvement of all histopathological changes in kidney of *T. canis* and liver of *T. vitulorum* infected rats as a result of vaccination with radiation-attenuated infective stage (El-Kabany *et al.*, 2013; Moawad *et al.*, 2015).

NO level in the tissues of the control infected and challenged control infected subgroups demonstrated a significant increase compared to the normal control. This outcome is compatible with different studies that reported a significant increase of expression of iNOs (inducible nitric oxide syntheses) and NO in *T. canis* infected tissues as hepatic cells, in serum and in brain tissues of experimental toxocariasis compared to the normal rats (Ali, 2013; Amin *et al.*, 2016; Fan *et al.*, 2004). On the other hand, a significant reduction in the vaccine control and a high significant reduction in the vaccinated-challenged subgroups compared to the infected control subgroups were detected. This was in agreement with a study of Amin *et al.* (2016) who reported a significant reduction of its level in brain tissues of rats vaccinated with 800 Gy radiation attenuated larvae. However, its level still higher compared to the normal control group as a result of larval migration and inflammatory cell response (Demirci *et al.*, 2006).

Regarding caspase-3 which plays central roles in execution-phase of apoptosis and is considered a common molecule of all metabolic pathways of programmed cell death (Zhou *et al.*, 2013), there was a significant increase of its level in the control infected group compared to the normal control one. Previous studies reported that parasitic infection causes tissue injury that result in apoptosis which exhausts host immunity (Bienvenu *et al.*, 2010; Zakeri, 2017). An increased activity of Caspase-3 and apoptotic cells was found in astrocytes treated with *Toxocara canis* excretory-secretory antigens (Chou & Fan, 2018). Caspase-3 activation may be an important factor in the apoptotic pathway in other parasitic diseases as in early heterophyiasis (Ashour *et al.*, 2014). Shalan *et al.* (2018) reported congestion, thickening of arterioles, inflammatory infiltrate and gliosis associated with marked immunohistochemical expression of caspase-3 in brain tissues in the infected control group of a mouse model.

Vaccinated group showed a significant reduction which coincides with study recorded that the level of caspase-3 in the irradiated metacestode of *E. granulosus* resulted in a significant change compared to the infected control (Alam-Eldin & Badawy, 2015). Irradiated parasite might causes cellular apoptosis which are very effective activators of T cells and stimulators of dendritic cells which is a potent method of inducing protective immunity (James, 2005).

Regarding molecular studies, the control infected group showed more comet cells with a significant increase in tail moment, tail DNA%, and tail length representing DNA damage in both liver and lung tissues. This coincided with previous studies employing comet assay reported DNA damage in hepatocytes infected with *Toxoplasma gondii* and in *Plasmodium* infected RBCs. It was explained that a marked release of NO from activated macrophages as a result of infection can be metabolized by auto-oxidation forming peroxynitrite which is potentially toxic to DNA (Pen *et al.*, 2003).

The vaccine control and vaccine challenged subgroups revealed a significant reduction % DNA damage, tail moment and tail length. More or less, similar results were recorded by Amin *et al.* (2016) who revealed a significant decrease in tail moment, tail

DNA%, and tail length in comet assay in brain tissue of the irradiated *T. canis* larva group, with more reduction in the vaccinated-challenged group.

CONCLUSION

Vaccination with 800 Gy gamma radiation-attenuated *Toxocara canis* infective stage produces an enhanced immunogenicity against reinfection. A marked amelioration of all histopathological, biochemical and molecular changes in liver and lung tissues that occurred as a result of infection could be detected. This can drive insights to the future design of a useful tool for vaccine production.

Conflict of interest
The authors declare that they have no conflict of interest.

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REFERENCES

Abo-Shehada, M.N., Al.-Zubaidy, B.A. & Herbert, I.V. (1991). Acquired immunity to *Toxocara canis* infection in mice. *Veterinary Parasitology* **38**: 289-298.

Alam-Eldin, R.Y. & Badawy, A.F. (2015). Destructive effect of gamma irradiation on *Echinococcus granulosus* metacestodes. *Parasitology Res*earch **114**: 3145-3150.

Ali, A.M. (2013). Study of histological and physiological effects of *Toxocara cati* larvae infection in experimentally infected white rats. *International Journal of Current Microbiology and Applied Sciences* **2**(8): 49-59.

Amin, M.M., Hafez, E. & AbdRaboo, M. (2016). Assessment of Radiation-Attenuated Vaccine or Thyme Oil Treatment on Controlling DNA Damage and Nitric Oxide Synthesis in Brain of Rat Infected with *Toxocara canis*. *Arab Journal of Nuclear Sciences and Applications* **94**(2): 199-210.

Archana, M., Bastian, D., Yogesh, T.L. & Kumaraswamy, K.L. (2012). Various methods available for detection of apoptotic cells – A review. *Indian Journal of Cancer* **50**(3): 274-283.

Ashour, D.S., Othman, A.A. & Radi, D.A. (2014). Insights into regulatory molecules of intestinal epithelial cell turnover during experimental infection by *Heterophyes heterophyes*. *Experimental Parasitology* **143**: 48-54.

Babayan, S.A., Attout, T., Vuong Phat, N., Goff, L.L., Gantier, J. & Bain, O. (2005). The subcutaneous movements of filarial infective larvae are impaired in vaccinated hosts in comparison to primary infected hosts. *Filaria Journal* **4**: 3.

Bienvenu, A.L., Gonzalez-Rey, E. & Picot, S. (2010). Apoptosis induced by parasitic diseases. *Parasites & Vectors* **3**: 106.

Bories, P.N. & Bories, C. (1995). Nitrate determination in biological fluids by an enzymatic one-step assay with nitrate reductase. *Clinical Chemistry* **41**(6): 904-907.

Chieffi, P.P., Del Guercio, V.M., Ueda, M. & Mello, L.B. (1981). Im-portância de *Rattus norvegicus* capturados no município de São Paulo, SP, Brasil, comohospedeirosparatênicos de *Toxocara canis* (Ascaroidea, Nematoda). Revista do Instituto *A dolfo Lutz* **41**: 89-91.

Chou, C.M. & Fan, C.K. (2018). Significant apoptosis rather autophagy predominates in astrocytes caused by *Toxocara canis* larval excretory-secretory Antigens. *Journal of Microbiology, Immunology and Infection* **18**: 30223-8.

Chung, L.Y., Fang, B.H., Chang, J.H., Chye, S.M. & Yen, C.M. (2004). The infectivity and antigenicity of *Toxocara canis* eggs can be retained after long-term preservation. *Annals of Tropical Medicine and Parasitology* **98**: 251-260.

Demirci, C., Gargili, A., Kandil, A., Cetinkaya, H., Uyaner, I., Boynuegri, B. & Gumustas, M.K. (2006). Inhibition of inducible nitric oxide synthase in murine visceral larva migrans: effects on lung and liver damage. *Chinese Journal of Physiology* **49**: 326-334.

Durante, W., Johnson, F.K. & Johnson, R.A. (2007). Arginase: A critical regulator of nitric oxide synthesis and vascular function. *Clinical and Experimental Pharmacology and Physiology* **34**(9): 906-911.

El-Kabany, H. (2013). Biochemical and histopathological studies on liver of rats infected with non irradiated and/or Irradiated *Toxocara vitulorum* eggs. *Journal of Radiation Research and Applied Sciences* **6**: 137-154.

Engvall, E. & Perlmann, P. (1971). Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin *G. Immunochemistry* **8**(9): 871-874.

Fan, C.K., Lin, Y.H., Hung, C.C., Chang, S.F. & Su, K.E. (2004). Enhanced inducible nitric oxide synthase expression and nitrotyrosine accumulation in experimental granulomatous hepatitis caused by *Toxocara canis* in mice. *Parasite Immunology* **26**(6-7): 273-281.

Galvin, T. (1964). Experimental *Toxocara canis* infections in chickens and pigeons. *Journal of Parasitology* **50**(1): 124-127.

James, E. (2005). Apoptosis: Key to the Attenuated *Malaria* Vaccine? Editorial Commentary *Journal of Infectious Diseases* **191**(10): 1573-1575.

Joy, A.T., Chris, O.I. & Godwin, N.C. (2017). Toxocariasis and Public Health: An Epidemiological Review Article. *Global Journal of Infectious Disease and Clinical Researches* **3**(1): 28-39.

Kamiya, M., Ooi, H.K. & Nomura, T. (1987). The effect of radiation on the viability and migratory ability of second-stage larvae of *Toxocara canis* in mice. *Veterinary Parasitology* **24**(1-2): 87-92.

Kleine, A., Springer, A. & Strube, C. (2017). Seasonal variation in the prevalence of *Toxocara* eggs on children's playgrounds in the city of Hanover, Germany. *Parasites & Vectors* **10**: 248-255.

Lombard, M., Pastoret, P.P. & Moulin, A.M. (2007). A brief history of vaccines and vaccination. *Revue Scientifiqueet Technique* **26**(1): 29-48.

Ma, G., Holland, C.V., Wang, T., Hofmann, A., Fan, C., Maizels, R. & Hotez, P.J. (2018). Human toxocariasis. *The Lancet Infectious Diseases* **18**: 14-24.

Matsuura, K. & Kurokawa, M. (2016). Metabolic Regulation of Apoptosis in Cancer. *International Review of Cell and Molecular Biology* **327**: 43-87.

Moawad, M.A., Amin, M.M. & Hafez, E.N. (2015). Role of ionizing radiation on controlling kidney changes in experimental infection with *Toxocara canis*. *Assiut Veterinary Medical Journal* **61**(147): 87-94.

Muller, P. (2019). The comet assay: ready for 30 more years. *Mutagenises* **33**(1): 1-7.

Muro, A. & Arellano, J. (2010). Nitric Oxide and Respiratory Helminthic Diseases. *Journal of Biomedicine and Biotechnology* **10**: 1-8.

Nilsson, B.O. (1999). Biological effects of aminoguanidine. *Inflammation Research* **48**(10): 509-515.

Pen, B.W., Lin, J., Lin, J.Y., Jiang, M.S. & Zhang, T. (2003). Exogenous nitric oxide induces apoptosis in *Toxoplasma gondii* tachyzoites via a calcium signal transduction pathway. *Parasitology* **126**(6): 541-55.

Selek, M.B., Karagoz, E. & Baylan, O. (2016). Toxocariasis: a review. *Medicine Science* **5**(4): 1063-1067.

Seo, H.S. (2015). Application of radiation technology in vaccines development. *Clinical and Experimental Vaccine Research* **4**(2): 145-158.

Shalan, F.H., Elbadry, A.A., Afifi, A.F., Beshay, E.V.N., El-Refai, S.A., Metwaly, H.G., Ahmedy, E., Badr, E.A., Al-Sharaky, D.R. & Sadek, G.S. (2018). Mesenchymal stem cell as a cure for brain damage induced by chronic *Toxocara canis* infection in an experimental mouse model. *Journal of Infectious Diseases and Therapy* 6.

Shalini, S., Dorstyn, L., Dawar, S. & Kumar, S. (2015). Old, new and emerging functions of caspases. *Cell Death Differentiation* **22**(4): 526-539.

Team, R., Bohler, A. & Iersel, V.M. (2017). Intrinsic Pathway for Apoptosis (Homo sapiens). *Proteomics* **11**(18): 3598-3613.

Vasou, A., Sultanoglu, N., Goodbourn, S., Randall, R.E. & Kostrikis, L.G. (2017). Targeting Pattern Recognition Receptors (PRR) for Vaccine Adjuvantation: From Synthetic PRR Agonists to the Potential of Defective Interfering Particles of Viruses. *Viruses* **9**(7): 186-202.

Viljoen, G.J. & Luckins, A.G. (2012). The role of nuclear technologies in the diagnosis and control of livestock diseases – a review. *Tropical Animal Health and Production* **44**(7): 1341-1366.

Walsh, M. & Haseeb, M.A. (2012). Reduced cognitive function in children with toxocariasis in a nationally representative sample of the United States. *International Journal of Parasitology* **42**(13-14): 1159-1163.

Wnukowska, N. & Dzbeñski, T.H. (2008). Studies on the development and role of some cell-mediated immune responses in experimental toxocarosis in mice. *Acta Parasitologica* **53**(2): 205-210.

Zakeri, A. (2017). Helminth-induced apoptosis: a silent strategy for immunosupression. *Parasitology* **144**(13): 1663-1676.

Zhou, X., Zhao, Y., Zhou, R. & Zhang, H. (2013). Suppression of *E. multilocularis* hydatid cysts after ionizing radiation exposure. *PLoS Neglected Tropical Diseases* **7**(10).