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RESEARCH ARTICLE

Hematological and histopathological changes of rat's hearts experimentally infected with protoscoleces

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ABSTRACT

Hydatidosis; is a zoonotic disease caused by *Echinococcus granulosus* and characterized by infiltration of inflammatory cells. This study was investigated the hematological and histopathological changes in the hearts of rats injected with protoscoleces. Rats were injected with protoscoleces collected from either liver of sheep, goats, and cows (from the abattoir of Al-Muthanna province, south of Iraq) or isolated from infected humans from Al-Hussein Teaching Hospital. Sheep protoscoleces showed a significant increase of lymphocytes that refer to the induction of a high response of the immune system in rats. The numbers of WBC, RBCs, and platelets were generally increased in rats injected with protoscoleces isolated from sheep and goats. These changes could refer to the activation of defense mechanisms against the hydatid injected materials. However, the levels of MCV, MCH, MCHC, MPV and PDW were less than normal values. Heart sections of rats injected with protoscoleces isolated from humans showed clear histological changes. While TSP, TGP and TCP exhibited variant histopathological changes such as infiltration of inflammatory cells, pink glass appearance and congestion of arteries. Thus, these alterations can be considered as additional evidence of how the immune response reacts against the injected materials in the heart.

Keywords: Rats; hydatid cysts; heart sections; immunity; hematology.

INTRODUCTION

Cystic echinococcosis is a serious zoonotic disease caused by infection with the metacestode stage of *Echinococcus granulosus*, which is also called a hydatid cyst in humans. It has a widespread distribution around the world (Aziz *et al*., 2011). A total of two mammalian hosts are involved in the life cycle of this parasite; dogs and other canids are served as the definitive hosts. Other mammals with ungulates like sheep, goats, cattle and pigs serving as intermediate hosts (Eckert & Deplazes, 2004), as well as buffaloes, horses, and camels (Aboelhadid *et al*., 2013). The liver and lungs are the most commonly infected organs (Jawad *et al*., 2018; Stoore *et al*., 2018; Ehsan *et al*., 2017). Within these viscera, a unilocular cyst develops and grows at a rate of 1 to 5 cm per year for several years (Siracusano *et al*., 2012). In response to the parasite, the intermediate host produces a layer called the adventitial layer, which is primarily composed of epithelial cells and connective tissue (Sakamoto & Cabrera, 2003). The adventitial layer is the layer that surrounds the hydatid cyst. This adventitial layer is possible to be variable in thickness and show some localized fibrosis as a result of the host immune response, which perceives the cyst as a foreign body (da Silva, 2011). The lumen of the hydatid cyst is filled with a hydatid fluid and is surrounded by two layers of parasite tissue. The innermost cellular layer is known as the germinal layer and is intimately attached to a cellular layer known as the laminated layer, which is in close contact with the adventitial layer. The outermost cellular layer is known as the adventitial layer and is intimately attached to the germinal layer. In the germinal layer, embryonic cells perform the duty of constructing the various components of hydatid cysts (da Silva, 2011; Díaz *et al*., 2011, 2015). The immunology of hydatid disease can be split into two phases: the preencystment phase and the postencystment phase (Rickard & Williams, 1982), which are distinguished by the creation of a laminated layer around the hydatid cyst. Following the swallowing of the egg and the release of the oncosphere, this occurs between 2 to 4-weeks post infection in the intermediate or human host, depending on the species. The susceptibility to infection differed between different strains of mice in the laboratory (Dempster *et al*., 1991) which could show the host susceptibility feature of this parasite.

The cellular immune response in the infected organs (lung and liver) in sheep, cattle, and camels was characterized by the infiltration of inflammatory cells. When comparing

infected and non-infected animals, the pro-inflammatory Th1 cytokine profile was shown to be more prevalent in the infected animals. The humoral immune response, on the other hand, was manifested as a high amount of IgG in infected animals (Vatankhah *et al*., 2019; Abo-Aziza *et al*., 2020).

Eosinophilic cells, macrophages, and lymphocytes play an important role in the body's innate resistance to the metacestode. While initial stimulation of T helper (Th-1) lymphocytes to remove the parasite initiates the inflammatory response in a few weeks, the Th-2 and Treg lymphocytes retain their dominant roles in the T cell population, resulting in the activation and merging of T cells during the inflammatory response. Hydatid cysts are boosted by the immunological environment, which allows the parasite to exert frequent compressive and pathogenic dynamics on the neighboring hepatocytes and vascular compartments (Abo-Aziza *et al*., 2020; Vatankhah, 2019).

The study of pathological and immunological responses of liver hydatidosis was not clearly covered by works of literature. Based on that, a comprehensive comparative analysis of the hematological and histological changes in hearts of rats against protoscoleces isolated from livers of different hosts was investigated in this study. Choosing rats was due to their anatomical, physiological, and genetic similarities to humans (Bryda, 2013), which can show a close picture of the immune responses to protoscoleces in the heart.

MATERIALS AND METHODS

Samples collection

The samples of hydatid cyst were collected from different animals slaughtered in abattoir of Al-Muthanna province, south of Iraq. The human hydatid cysts samples were collected from Al-Hussein Teaching Hospital (the main human hospital in Samawah, the capital city of Al-Muthanna Province). The infected organs (livers) were grossly checked to initiate whether the lesions were hydatid cysts by palpation and inspection.

The aspiration of cyst contents into sterile tubes was done using clean needles and syringes in the Parasitology Laboratory of the Veterinary Medicine College of Al-Muthanna University. After the fluid collection, the hydatid fluid was aspirated from the cyst using a sterile syringe. Protoscoleces were scraped from the sides of the germinal layer and were transferred into 15 ml test tubes. After that, the tubes were spun down at 2500 ×g for 5 minutes. The supernatant was discarded and the precipitated scolex was stained for the viability test with eosin stain and examined under a light microscope at a magnification of 40x power (Al SeÂ & AlKhaled, 2012). That shows the red protoscoleces were dead, but the green protoscoleces represented the living ones.

In vivo studies

Ethics

The experimental protocol of this study was approved by the Ethics Committee at Veterinary Medicine College of Al-Muthanna University numbered 2 in 12/04/2021.

Animal inoculation

Twenty-five Wistar albino female rats aged 8 weeks, weighting 250 ± 10 gm, were obtained from (Animal Housing Colony of the Veterinary College of Baghdad University). Female animals were stored at room temperature (24° ± 3°C), and humidity (40-70%), with a 12h light/dark period before being used in the experimental procedures. Rats were divided into four separate groups as follows: the first group was injected with human hydatid protoscoleces (THP), second injected with sheep hydatid protoscoleces (TSP), thirds injected with goat hydatid protoscoleces (TGP) and the fourth rat injected with cow hydatid protoscoleces (TCP). All groups were injected with 0.1 ml of protoscoleces intraperitoneally, while control negative rats (NC) were injected with 0.1 mL of normal saline. All the animals were anesthetized by Ether and blood samples were collected after seven days. The blood collection was completed on each rat and used for a hematological examination.

Blood analysis

Rats were killed by inhaling an excessive amount of ether, and blood was collected soon afterwards through a cardiac puncture. Complete blood counts were performed in the AL Hussein Teaching Hospital laboratories using an Avida 2120i machine (Siemens). K3EDTA blood was used for complete blood counts.

Histopathological studies

Rat hearts were collected from all groups and fixed in 10% formalin for hematoxylin and eosin staining (H & E) according to Feldman and Wolfe (2014), then examined under a light microscope. Sections of paraffin blocks were cut to a thickness of 5 m and stained with hematoxylin-eosin (H & E). Sections of the tissue lesions were scanned with Sectra IDS7 (Sectra AB, Linköping, Sweden) to produce digital pictures. Hydatid samples were taken along the same plane as the histological sections, which were cut perpendicular to the lesion's longest side.

Data analysis

Statistical analysis was consistently administered using the GraphPad Prism 8.0.1 version. The data were analyzed by One Sample T- test or ANOVA One Way and Dunnett's multiple comparisons test as a post hock analysis.

RESULTS

In vitro examination

The samples of the liver hydatid cyst were collected from different kinds of animals slaughtered in the Al-Muthanna abattoir. While, infected livers of humans were collected from Al-Hussein Teaching Hospital. The collected infected livers were grossly checked to initially test whether the lesions were hydatid cysts or not by palpation and inspection (Figure 1). The gross lesions were paleness, petechial hemorrhage livers. Some cysts were calcified. The viability of cysts was examined using eosin stain and showed the protoscoleces under the microscope (Figure 2). The fertile cysts were stained with green color. Only fertile cysts were used for the next steps of this project.

In vivo studies

The fertile protoscoleces isolated from animals and infected human livers were intraperitoneally injected into four separate groups. After killing the animals, the blood collection from each group was used for a hematological examination.

Blood analysis

Regarding blood cell count, red, white blood cells and platelets were calculated. Rats injected with liver protoscoleces isolated from sheep and goat showed a significant increase in all types of blood cells compared to the negative group.

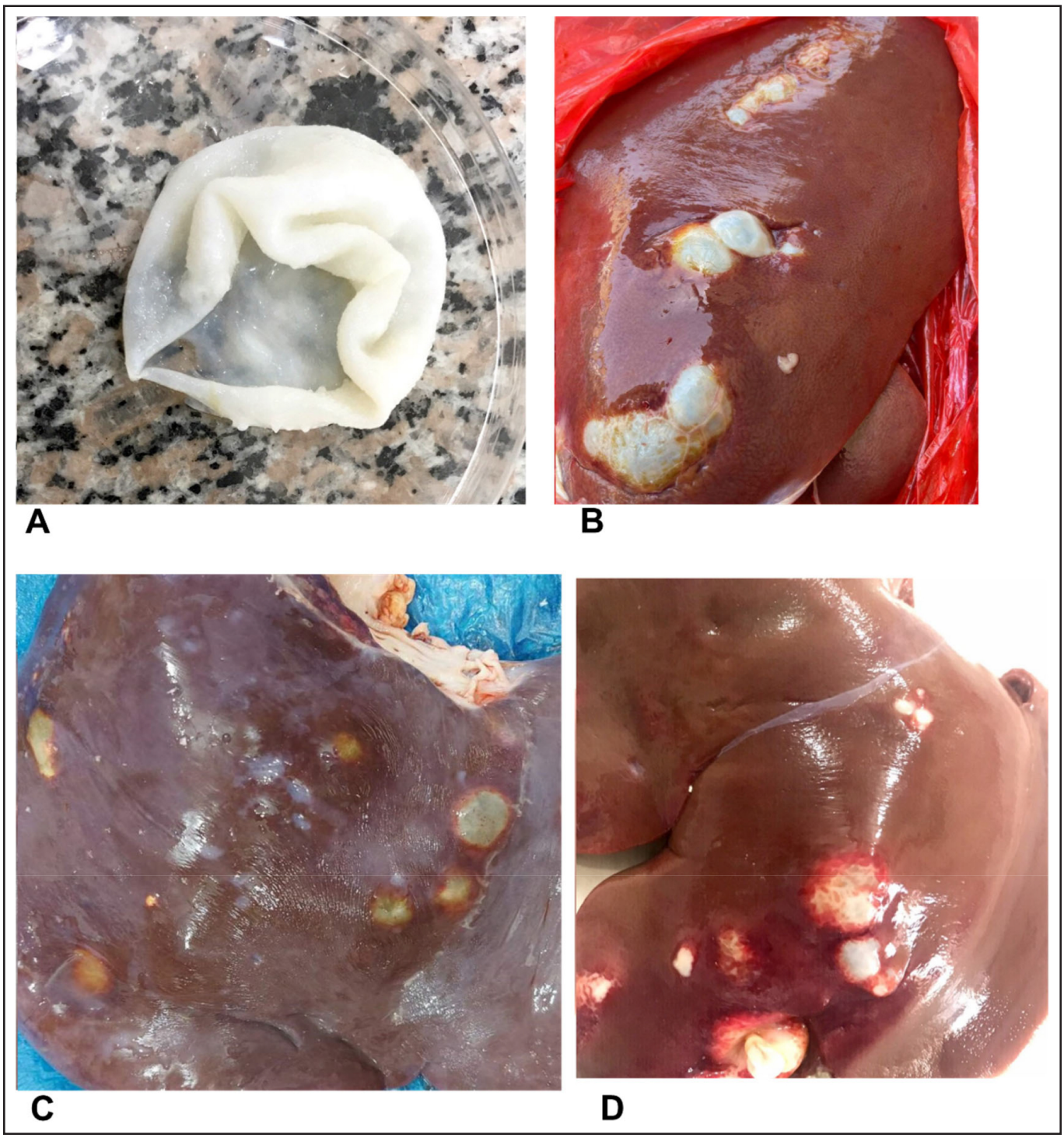

Figure 1. Different hydatid cysts infect livers of different hosts. Different samples of livers were collected from human (A), sheep (B), goat (C) and cows (D).

However, rats injected with liver protoscoleces isolated from cows showed the lowest level of RBCs, WBCs, and platelets (Figure 3).

The blood analysis showed that the protoscoleces isolated from sheep livers induce production higher percentage of lymphocytes in rats compared to the control group (Figure 4). However, lymphocytes were less than the normal levels in the group of rats that were injected with protoscoleces from infected livers of humans and cows. While the changes during injection with protoscoleces isolated from goat livers were not significant. The highest proportion of granulocytes was induced when cow protoscoleces were injected into rats. Human, cow and sheep protoscoleces induced higher levels of other WBCs compared to the negative group.

The RBCs contents were calculated. HBG, MCV and HCT were decreased in the all-tested groups compared to the negative control (Figure 5). Other examined parameters; MCH and MCHC were not significantly different (Figure 6).

Platelet parameters were checked in rats injected with protoscoleces isolated from human, sheep, goat and cows. These parameters are MPV and PDW which do not show significant differences between the examined groups (Figure 7).

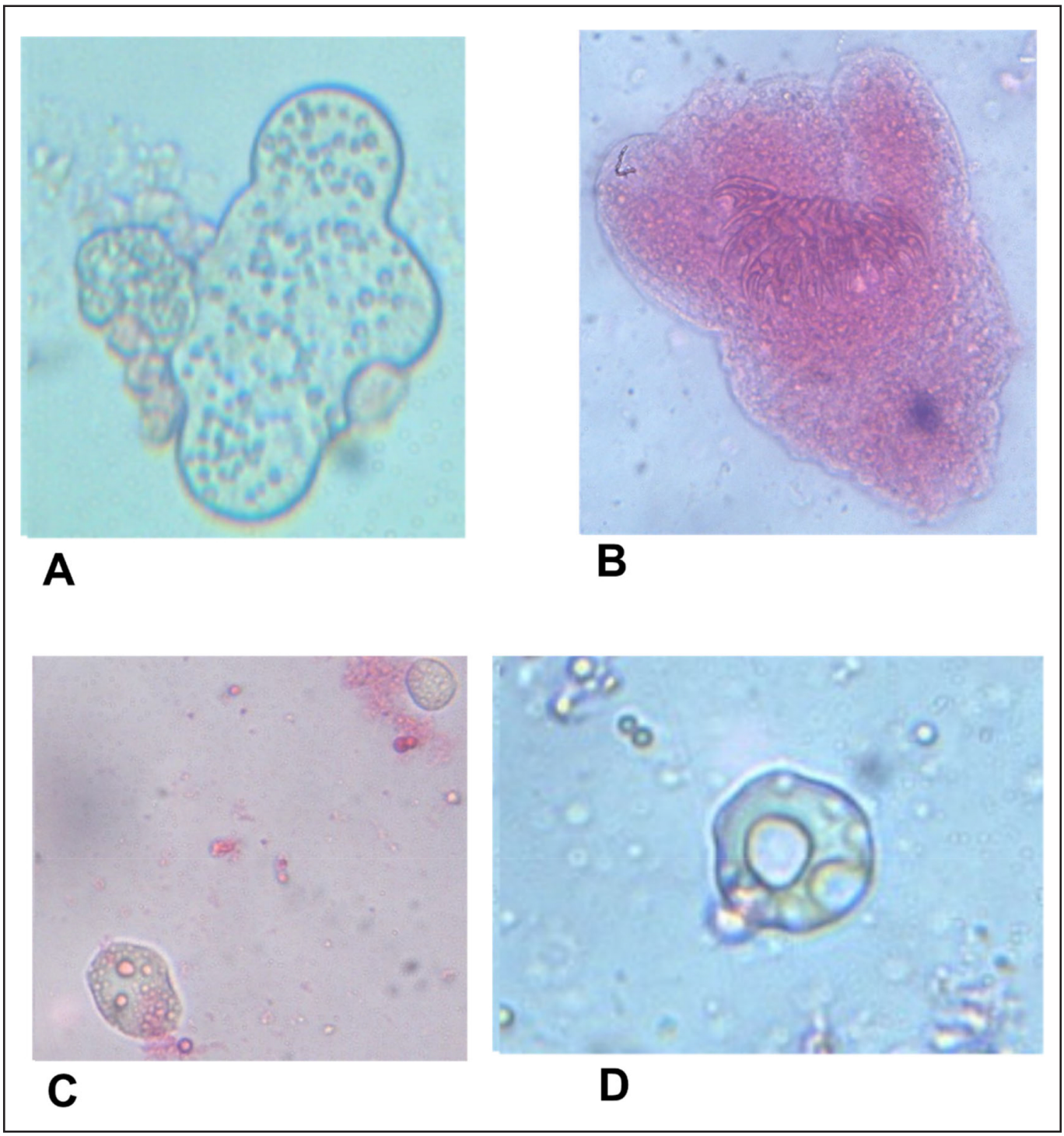

Figure 2. The viability of cysts. Hydatid fluid was collected from different hosts; human (A), sheep (B), goat (C) and cows (D). The samples were stained with eosin then examined under the microscope 400×. The green stained protoscoleces is alive while the red one is dead.

Histopathology results

In vivo pathological experiments involved preparing histopathological sections of rat hearts that were intraperitoneally injected with 0.1 mL of hydatid protoscoleces isolated from different host livers. The heart sections of rats that received liver hydatid protoscoleces isolated from humans were examined under the microscope and showed that the cardiac cells were normally arranged, straight nuclei, and blood vessels filled with blood with no clear lesions (Figure 8A). Sections of rat injected with liver hydatid protoscoleces isolated from sheep revealed glassy pinky materials (Figure 8B). Heart sections of rats that received goat hydatid protoscoleces showed congested coronary arteries which filled with blood cells, in addition to a slight infiltration of inflammatory cells in the myocardium (Figure 8C). The sections from rat hearts inoculated with hydatid protoscoleces isolated from cow livers showed proteinaceous material in the myocardium that was pink and glassy transparent (Figure 8D) where is E, F and G.

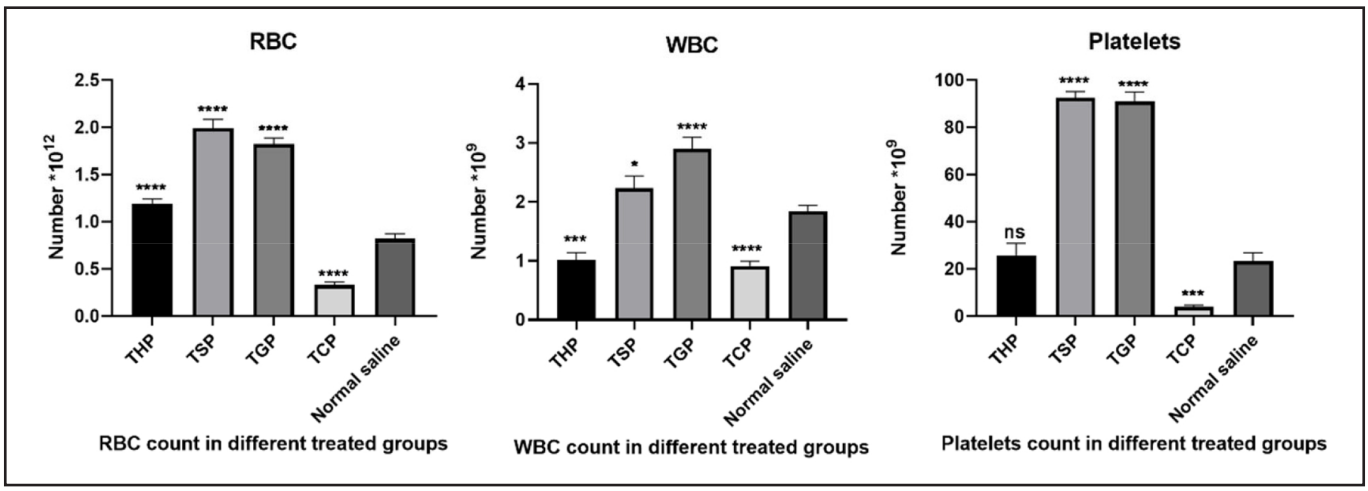

Figure 3. Comparison of blood cell counts in rats inoculated with protoscoleces. Protoscoleces isolated from liver hydatid cysts of human, sheep, goat and cow were inoculated in rats. The results showed remarkedly and significantly increase in RBCs, WBCs and Platelets. The statistical analysis was done using One sample t test, $P \leq 0.05$.

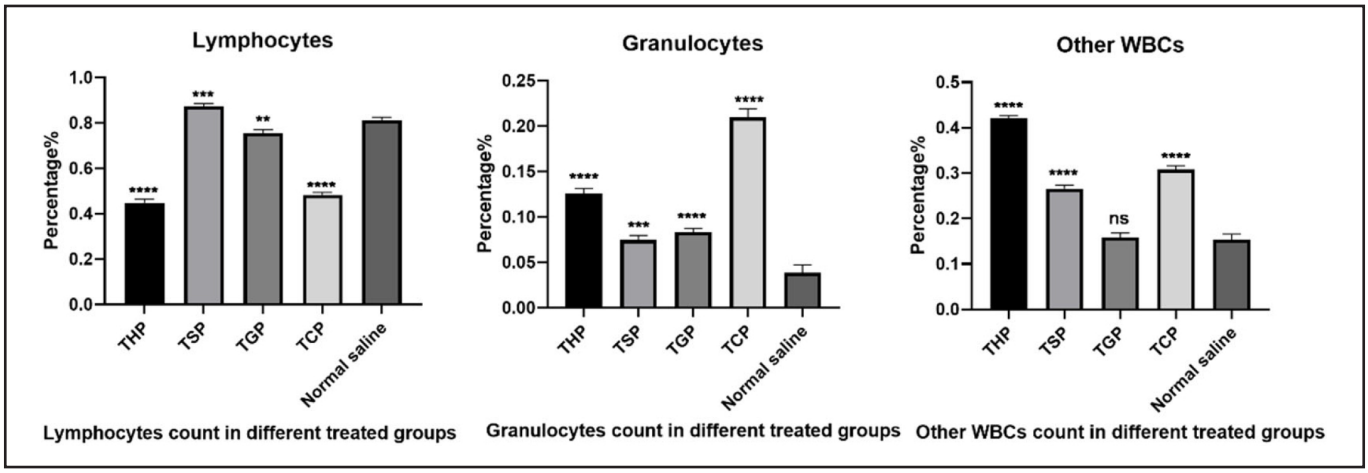

Figure 4. WBC count in Protoscoleces injected rats. Protoscoleces isolated from humans, sheep, goat and cows were injected into rats. Blood was collected and WBCs were calculated. The recorded values were statistically analysed using the One Sample T test. Other WBCs include less frequently occurring and rare cells correlating to monocytes, eosinophils, basophils, blasts and other precursor white cells that fall in a particular size range.

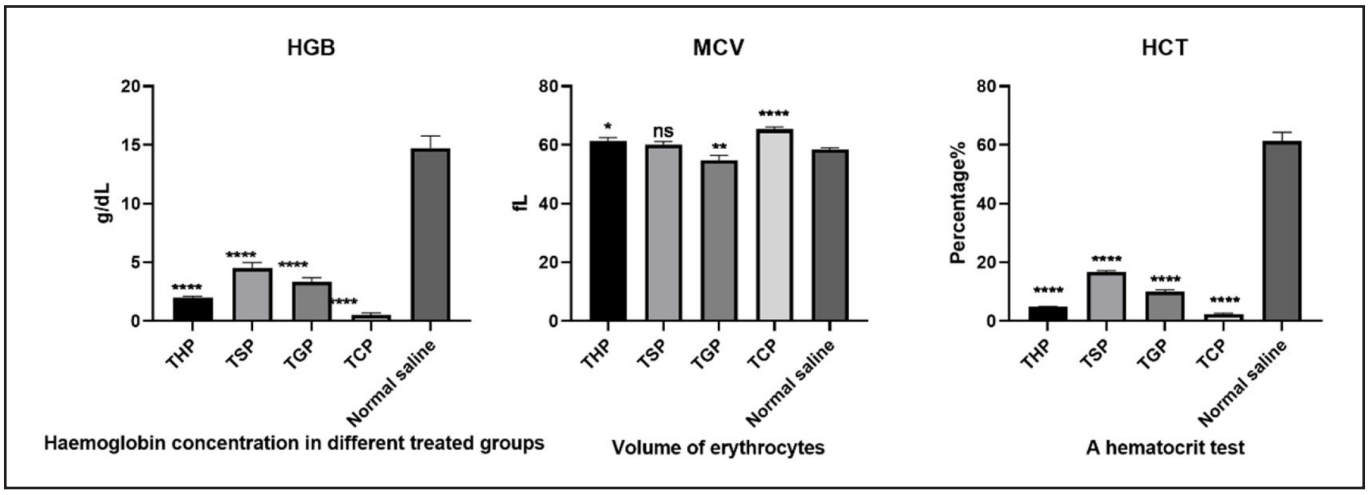

Figure 5. RBC content count in Protoscoleces injected rats. Protoscoleces isolated from human, sheep, goat and cows were injected into rats. Blood was collected and the RBC's associated parameters were calculated. The recorded values were statistically analysed using the One Sample T test. HGB is hemoglobin concentration in blood of injected groups. HCT stands for A hematocrit test measures the proportion of red blood cells. MCV is the volume of erythrocytes.

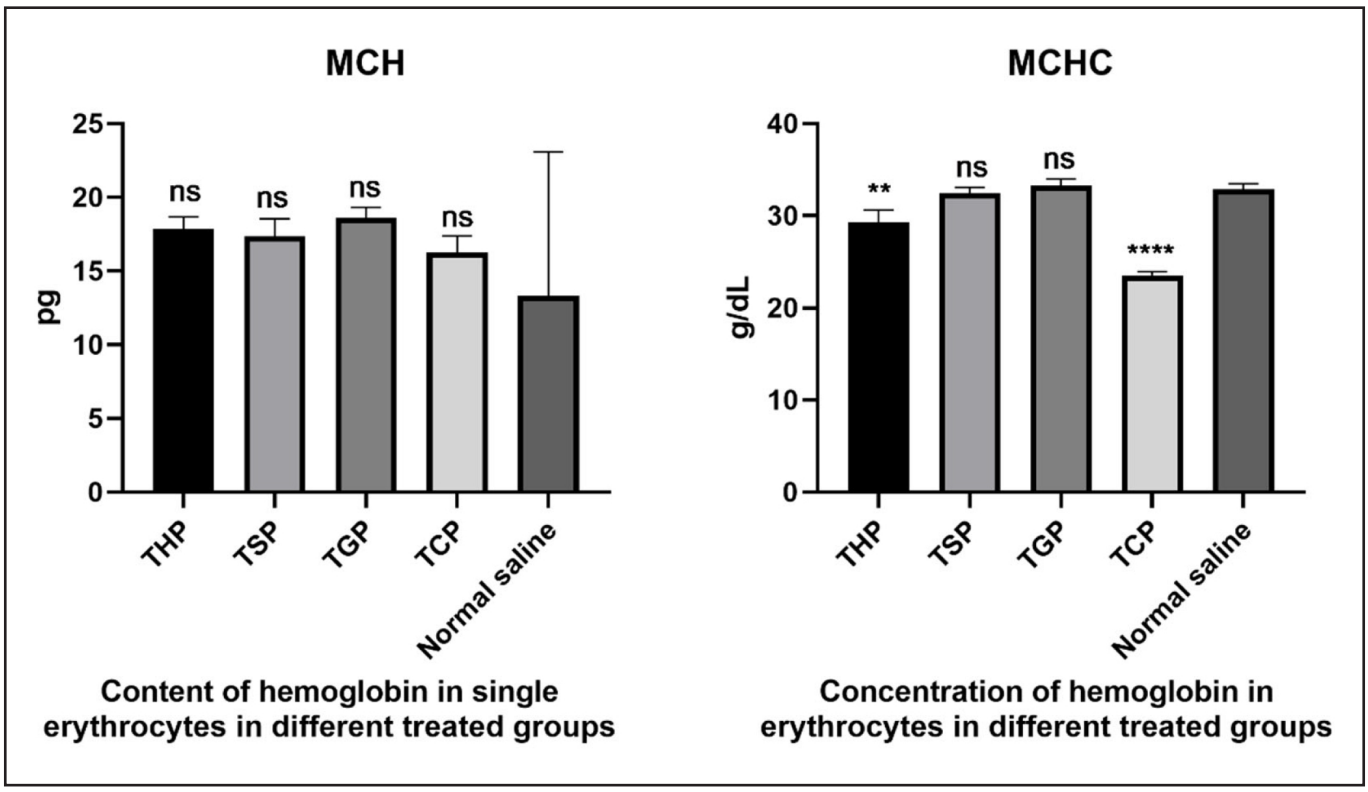

Figure 6. Hemoglobin parameters in injected rats. Protoscoleces isolated from human, sheep, goat and cows were injected into rats. Blood was collected and the Hemoglobin's associated parameters were calculated. The recorded values were statistically analysed using the One Sample T test. MCH means the content of hemoglobin in single erythrocytes in absolute units. MCHC is the concentration of hemoglobin in erythrocytes.

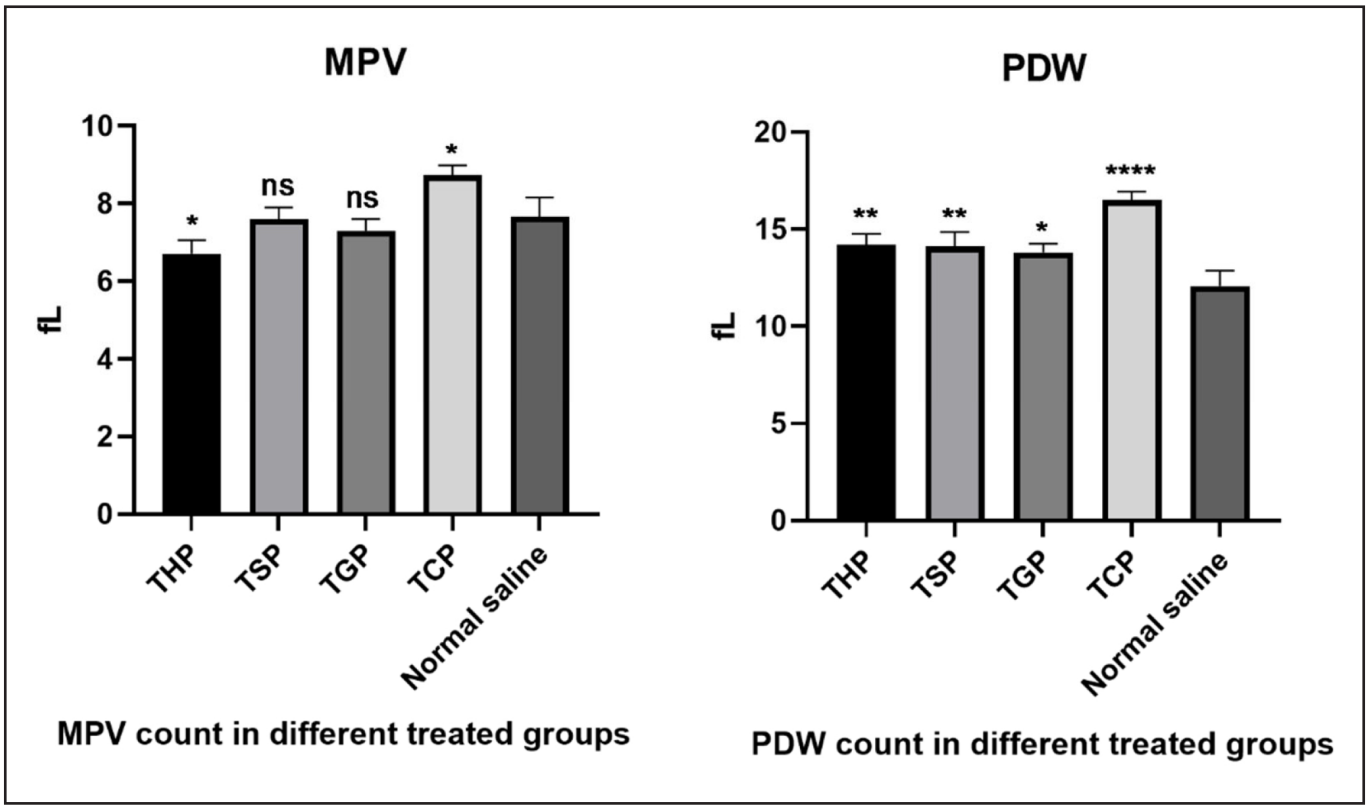

Figure 7. Platelet parameters count in Protoscoleces injected rats. Protoscoleces isolated from human, sheep, goat and cows were injected into rats. Blood was collected and the platelet associated parameters were calculated. The recorded values were statistically analysed using the One Sample T test. MPV and PDW do not show significant differences between the groups. MPV is platelet volume. PDW is the relative width of platelet distribution in the volume index of the heterogeneity of platelets.

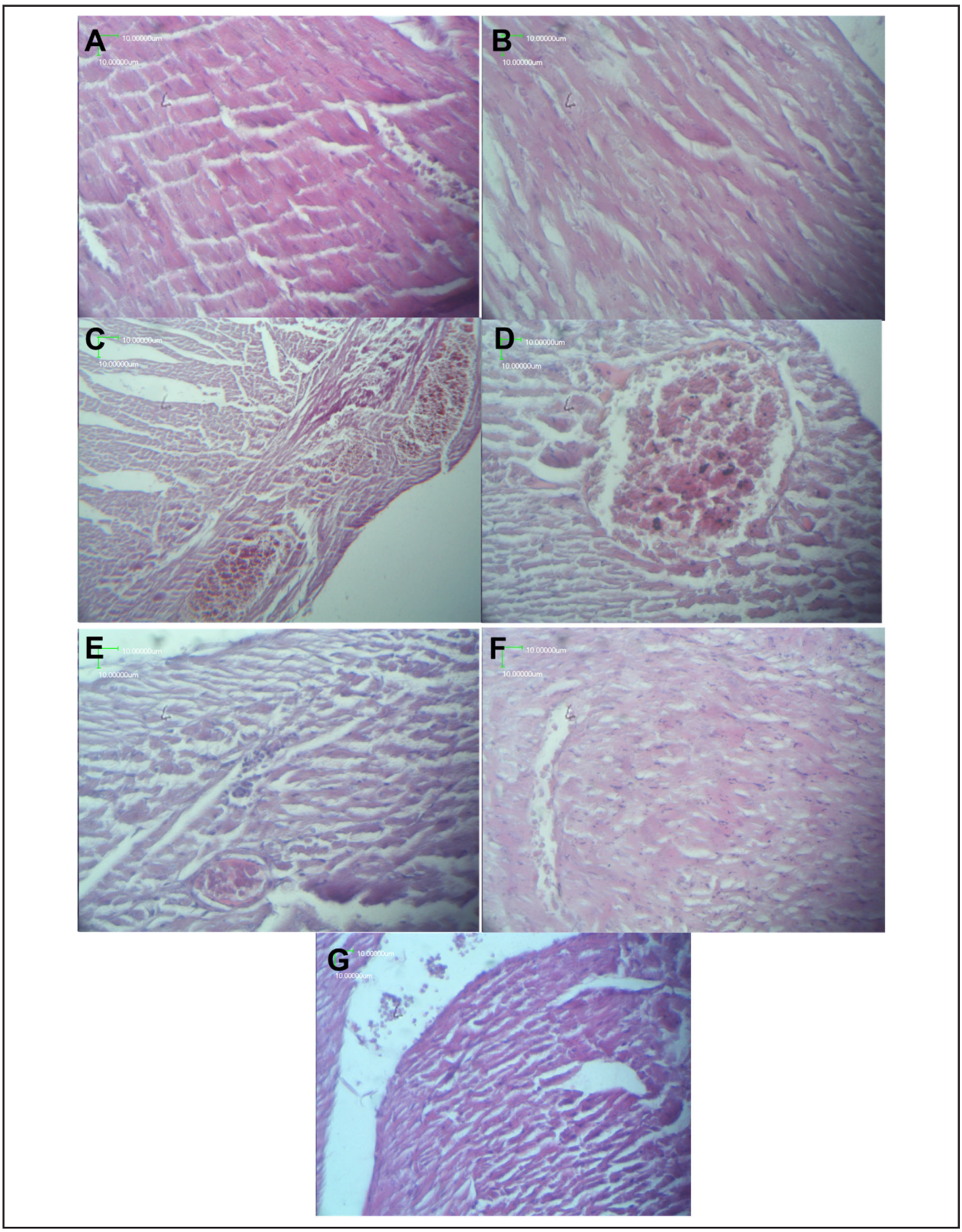

Figure 8. Histopathological sections of rat hearts intraperitoneally injected with 0.1 ml of hydatid protoscoleces isolated from different hosts. The hydatid protoscoleces were isolated and intraperitoneally injected in rats. Different heart sections were examined under the microscope as the following: A: A heart section of rats received human hydatid protoscoleces showed straight nuclei of cardiac cells and blood vessels filled with blood. B: A heart section of rats received sheep hydatid protoscoleces showed glassy and pinky materials related to immune activation. A heart section of rats received goat hydatid protoscoleces showed coronary arteries congested and filled with blood cells (C) and (D). There is a slight infiltration of inflammatory cells in the myocardium in the latter experiment (E). A heart section of rat received cow hydatid protoscoleces showed material in the myocardium that was pink and glassy transparent (F). A heart section of rat received normal saline showed normal cardiac tissue without lesions (G). All of the sections were H&E stained and examined at a magnification of 400× [with the exception of figures C (100×) and D (400×)].

DISCUSSION

More research is needed to look for pathological and immunological responses in the heart during protoscoleces infection. Study of such changes could be useful to clearly understand the specific immune response required against the cystic echinococcosis. Besides, to determine the effective antigens of the protoscoleces that can induce the high protection response for the production of future vaccines. Injection of fertile protoscoleces in rats can show more realistic changes that could reflect the host interactions with the injected materials. Regarding hematological changes, lymphocytes were significantly increased when hepatic protoscoleces isolated from sheep were injected into rats (Figure 4). These changes could refer to the induction of the immune system.

Blood parameters were examined in this study. It was shown that the numbers of WBC, RBCs, and Platelets were generally increased in rats injected with sheep and goat protoscoleces. The increase in WBCs can be related to the induction of a defense mechanism against the injected hydatid materials, which could stimulate the bone marrow to produce a greater number of WBCs.

It is becoming increasingly clear that red blood cells (RBCs) play a crucial role in modulating the innate immune response. The chemokines, nucleic acids, and pathogens in the bloodstream are bound and scavenged by erythrocytes (Moraitaki *et al*., 2010). However, (Moraitaki *et al*., 2010) found that RBC levels were not different during pulmonary hydatidosis. The high lymphocyte percentage was induced when the protoscoleces isolated from sheep livers were injected into rats (Figure 4). Infiltration with lymphocytes and multinucleated giant cells indicates that the immune response is closely related to cyst viability (Hidalgo *et al*., 2019). MCH and MCHC were measured and slight differences were found between positive and negative control groups (Figure 6), therefore, that parameters were kept under normal levels and these results were similar to previous studies (Moraitaki *et al*., 2010).

Platelet's parameters were measured in rats injected with protoscoleces isolated from humans, sheep, goat, and cows. MPV and PDW are the measures that did not indicate statistically significant differences between the groups that were evaluated (Figure 7). The platelet count in rats injected with sheep and goat liver protoscoleces, on the other hand, was higher than in the negative control. It has been previously published that platelet have cytotoxic effects against parasites either *in vivo* or *in vitro* (Fajardo & Rao, 1971; Polack *et al*., 1991; Ladhani *et al*., 2002). According to these studies, platelets may be implicated in the transmission of infectious diseases to other individuals.

The hydatid protoscoleces were isolated and intra-peritoneally injected in rats. Different heart sections were examined under the microscope, which showed that the heart sections of rats that received human protoscoleces did not show clear histological changes. However, sheep, goat and cow hydatid protoscoleces showed variant histopathological changes (Figure 8). Injection of hydatid materials is associated with infiltration of lymphocytes and plasmatic cells, fibroblasts, macrophages, giant multinucleated cells, and eosinophils (Hidalgo *et al*., 2019). In addition, the recorded changes refer clearly to the role of immune response against injected materials, which in turn explains that the heart is also another part of the body that is involved in immunity to hydatidosis.

CONCLUSION

The hematological and histological changes detected in the hearts of rats that had been injected with hydatid protoscoleces showed presence of immune response against the injected materials. There is a considerable rise in lymphocytes, indicating that the immune system was stimulated to a high level in rats infected with sheep protoscoleces. In the same way, WBC, RBC, and platelet counts were frequently enhanced in rats injected with both sheep and goat protoscoleces. This can ensure that the defensive mechanism against hydatid materials was activated. However, hematological tests revealed low normal levels of MCV, MCH, MCHC, MPV, and PDW. Heart sections of rats injected with THP demonstrated distinct histopathological alterations. While TSP, TGP, and TCP exhibited clear histopathological alterations such as inflammatory cell infiltration, pink glass appearance, and arterial congestion. These changes can explain why the heart is worth studying as a vital organ of the body involved in hydatidosis immunity.

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Conflict of interest

The author declares that they have no conflict of interests.

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