STUDY PREVALENCE AND EFFECT OF INTERNAL PARASITES AND HISTOPATHOLOGICAL CHANGES ON COMMON FROGS AT BAGHDAD CITY

M. B. Al-Khamesi I. S. Salman Sh. A. Abid S. M. Ibrahim
Assist. Prof. Assist. Prof. Assist. Lecturer Researcher
Dept. of Bio. Coll. of Sci. for Women- Univ. Baghdad
Shaimaa.awad.abid@gmail.com

ABSTRACT

A total of seventy adult common frogs [sixty infected (85.71%) and ten healthy (14.29%) specimens], were collected from different areas of Baghdad city in more than one locations such as (agriculture lands, river banks, home gardens, ponds, streams and shoel water) from the beginning of September 2016 to the end of March 2017 and examined for parasites, in the aim to study and isolate any parasitic infection from intestine, blood and effect of these parasites on blood, intestine and liver in common frogs. Thus resulting in *Opalina* sp. 17(28.33%), Nematodes (*Strongloides stercoralis*) 14(23.33%), *Balantidium* sp. 14(23.33%), (*Opalina* Sp. & Nematodes) 7(11.66%) and *Hepatozoon* Spp. 8(13.33%). All these parasites which mentioned above not appeared any pathological effect on intestine of infected frogs according to histopathological sections. On the other hand, the current study showed the effect of blood parasites (*Hepatozoon* spp.) in the blood and liver of infected frogs like disappeared of nucleus in red blood cells , in addition to inflammation and infiltration which appeared in histopathological sections of hepatic cells compared with healthy specimens. Finally, we conclude from this study , the parasites which isolates from intestine of infected frogs has a commensalism relationship with its hosts due to not appeared any pathological effect on it, while the blood parasites showed pathological effect on blood and liver of infected frogs.

Key words: amphibians, amphibians and worms, histological sections, protozoa.

دراسة انتشار وتأثير الطفيليات الداخلية والتغيرات النسجية في الضفادع في مدينة بغداد

ميسم بلاسم الخميسي اسراء صكر سلمان شيماء عواد عبد سارة محمود ابراهيم
استاذ مساعد استاذ مساعد مدرس مساعد باحثة
قسم علوم الحياة– كلية العلوم للبنات– جامعة بغداد
Shaimaa.awad.abid@gmail.com

المستخلص

جمعت النماذج والتي ضمنت 70 ضفدع (60 مصابة 85.71% و10 سليمة 14.29%) من مناطق مختلفة لمدينة بغداد ولأكثر من مكان مثل (اراضي زراعية وضفاف الأنهار وحدائق منزلية و برك وجداول و مياه ضحلة) منذ بداية شهر ايلول 2016 وحتى نهاية شهر اذار 2017، بهدف دراسة وعزل الأصابات الطفيلية من الأمعاء والدم وتأثيرها على الدم والأمعاء والكبد لنماذج الضفادع. حيث تم الحصول على طفيليات *Opalina* sp. 17(28.33%), Nematodes(*Strongloides stercoralis*) (14(23.33%), *Balantidium*. sp. 14(23.33%), (*Opalina* sp. & Nematodes) 7(11.66%) and *Hepatozoon* spp. 8(13.33%). جميع الطفيليات المعزولة لم تظهر اي تأثير مرضي على امعاء الضفادع المصابة وفقا للمقاطع النسجية المدروسة، من ناحية اخرى، اظهرت الدراسة الحالية تأثير طفيليات الدم *Hepatozoon* spp. في الضفادع المصابة على الدم والكبد مثل اضمحلال النواة داخل كرية الدم الحمراء اضافة الى حالات الألتهاب والترشيح للخلايا والتي ظهرت في المقاطع النسجية لخلايا الكبد. اخيرا، نستنتج من هذه الدراسة ان الطفيليات المعزولة من امعاء الضفادع المصابة لها علاقة تعايشية معها، لكونها لم تظهر اي تأثير مرضي عليها، بينما اظهرت طفيليات الدم تأثير مرضي داخل كريات الدم وخلايا الكبد للضفادع المصابة.

الكلمات المفتاحية: البرمائيات، البرمائيات والديدان، المقاطع النسجي، الأبتدائيات

***Received:12/1/2017, Accepted:6/3/2018**

INTRODUCTION

Amphibians are an essential part of their ecosystems, can affecting nutrient cycling, as well as they are serving as high quality victim for many species (19). They are notion to be predecessors of all reptiles, frogs, birds and mammals, they are found throughout the world (7) and disease has been concerned as a factor in the decline of its populations worldwide (1).In addition, the other factors such as habitat loss, fragmentation, climate changes, heavy metals, chemical and natural pollution, on the other hand the global warming and increased UV radiation leads to spread of environmental contaminants (6). Two conditions (disease and contaminants) are soon graded as two of the leading hypotheses for the worldwide amphibian decline (4). Frog and amphibian species play important role in ecosystems and have identified as effectual biological indicators of environmental health, for this reason it may have contributed to this exceptional state in species profusion (14). Frogs are transitional animals that inhabits a wide variety of stagnant water, ponds to large lakes and rivers, as well as streams (22). They are act as important for a variation of reasons such as they control populations of insects and they can affected by bacteria, protozoans, haemoparasites, viruses and worms (23). The factors of environment did not effect the infection rate of parasitism in frogs except for rainfall , in addition the parasites needs water for transmission, either when the frog used aquatic intermediate hosts like trematodes, or when the parasites is swimming from one host to another, consequently explaining the high number of parasites during the rainy season (2,24). This study aimed to isolation of parasites from blood, intestine and effect of these parasites on blood, intestine and liver in common frogs in Baghdad city, in addition to studying the effect of these parasites on tissues by histopathological examination.

MATERIALS AND METHOD

1- Frogs collection: A total of seventy adult common frogs were collected from different areas of Baghdad city (Al-Tarmiya, Al-Taji, Al-Mahmoodya, Abu-Ghreeb and Al-Doura) in different locations such as (agricultural lands, river banks, home gardens, ponds, streams and shoal water) from the beginning of September 2016 to the end of March 2017, The specimens were kept in plastic containers, then transports to the laboratory of College of Science for Women. The frogs in the laboratory were placed in the basin with running tap water to avoid dehydration of the frog's skin. Each specimen was anesthetized with ether, then dissected in the lab to open the intestine and searched helminthes, in addition to removed and placed the intestine and liver in 10% buffered formalin in order to fixed these tissues for histopathological study.

2- Preparation of blood smear: Blood specimens were taken from veins and thin blood smears were prepared, air dried, then fixed in methanol and stained with Gimsa stain. All slides were examined under the light microscope (18).

3- Histological study: The specimens were fixed in formalin (10%) for 24 hours at room temperature, then dehydrated in ascending concentrations of ethanol alcohol (70%, 80%, 90%, 95% and 100%) for 45 min. for each concentration. They were cleared in xylene for 45 min. and immersed in a mixture of xylene and paraffin wax (1:1) for 15 min. in oven at 59˚C, then infiltrated with pure paraffin for 1 hour three times. Finally, the specimens were embedded with paraffin by special templates to making a labeled blocks, sectioned by using the rotary microtome at 5µm thickness, the sections were stained Harris's haematoxylin and alcoholic eosin. The slides were mounted with (D.P.X.) and left on the hot plate at 37˚C overnight. Histopathlogical examination cared out using light microscope and photographed by using camera (21).This method occurred in histological laboratory/ College of Science for Women.

Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters . Chi-square test was used to significant compare between percentages in this study (16).

RESULT AND DISCUSSION

The result of current study recorded high infection rate 85.71% (60/70) of common frogs with parasitic infection, compared with non infected 14.29% (10/70) with significant differences $P \leq 0.01$(Table-1-).

Table 1. Prevalence of internal parasites between infected and healthy frogs

Specimens	Numbers	Percentage
Infected	60	85.71
Healthy	10	14.29
Total	70	% 100
Chi-Square	---	** 13.894

Table -2- shows , frequency distribution of study samples which appeared highly prevalence of *Opalina* sp. In 17(28.33%), then Nematodes (*Strongloides stercoralis*) and *Balantidium* sp. In the same numbers of isolation 14(23.33%), after that *Hepatozoon* spp. In 8(13.33%) and (*Opalina* sp. & Nematodes) in 7(11.66%). This result agree with (20).

Table 2. Frequency distribution of the study samples

Parasites species	Numbers	Percentage
***Opelina* Sp.**	**17**	**28.33%**
Nematodes (*Strongloides stercoralis*)	**14**	**23.33%**
***Opalina* Sp. & Nematodes (*Strongloides stercoralis*)**	**7**	**11.66%**
***Balantidium* sp.**	**14**	**23.33%**
***Hepatozoon* Spp.**	**8**	**13.33%**
Total	**60**	**85.71%**

Table 3- Fig. (2-A), appear that individuals with heavy infections of *Opalina* sp. Compared with other parasites distributed in different locations of study such as ponds and shoel water in (23.53%), then agricultural lands, streams, river banks and home gardens in (17.65% and 11.76%) respectively, this result appeared significant in statistical analysis and agree with Chris T. (9). *Opalina* sp. Are most often found in the intestines and rectum of frogs and toads in addition to other amphibians and reptiles (13). In the spring, when the hosts of the parasites are coupling, *Opalina* will encyst, this condition is expedience for the *Opalina* parasite because its hosts reproduce in water and its progeny begin their lives in water for this reason become infected (5). Furtherly, (Fig. 1-AandB) reveal isolation of Nematodes (*Strongloides stercoralis*) showed highly percentage in streams and river banks in (21.43%), then other locations which mentioned above in (14.29%), this result reach the level of significant in statistical analysis, in addition to agree with (8). As well as, this study appeared isolation of *Opalina* & Nematodes from the same infected specimen in more than one location like shoel water and home gardens in (28.57%), then agricultural lands, ponds and river banks in (14.29%). This result showed highly significant (Table-3-, Fig. 2-C). Most of the Nematode species that infect frogs and toads in a earthly environment, so the transmission of amphibian Nematodes evidence of that there is a relation between soil moisture and the distribution of these parasites in their amphibian host, in addition these Nematodes are mainly present in terrestrial amphibians like toads and less frequently in semi-aquatic and aquatic amphibian species (5). Furthermore, Table -3- Fig. (1-C) shows the prevalence of *Balantidium* showed highly distribution in streams and river banks (21.43%), after that the agricultural lands, ponds, shoal water and home gardens in (14.29%), so this result statistically significant and similar to the results of Lyudmila V. (3). It is sensible to assume that *Balantidium* species inhibiting anura amphibians complete their transferral through cloaca more than oral opening considering their special feeding habits (5). The numbers and genera of protozoa present in an amphibian are very frequently influenced by individual animal dissimilarity in the intestinal tract, such as hydration status, the passing of digesta and pH can have intense effect on the ciliate of the enteric protozoal community (10). Moreover, all the parasites which mentioned above not appeared any pathological effect that are generally recognized when we studies the sections of infected frog intestine, so we suggested that these parasites have a commensalism type relationship with its hosts (Fig. -2- D). This result agree to the result of Goater C. P. (5). On the other hand from this study, *Hepatozoon* spp. (Apicomplexa: Hepatozoidae) were the only blood parasites notice and were found in only eight frogs, so table -3- showed the high numbers of this parasite which isolated from shoal water in (37.50%) followed by agricultural lands and ponds in (25.00%), then streams in (12.50%), this result showed highly significant in statistical analysis. Isolation of this parasites from blood frogs agree with the results of

other researchers Edward C. , Dave Shutler (11,17), *Hepatozoon* spp. (Apicomplexa: Hepatozoidae) are blood parasites and often found in amphibians, transmission of this parasites occurs when the invertebrate host/vector such as mosquitoes infected with mature Oocysts, which containing thousands of sporozoites are consumed by a vertebrate host like amphibians, reptiles and mammals, so the mature sporozoites are released into blood stream and invade erythrocytes (12).

Table 3. Distribution of Parasites according to different locations

Source of Specimens	Parasites Isolated(%)				
	***Opalina*. sp.+ Nem. (*Strongloides stercoralis*)**	***Opalina*. sp.**	**Nematodes. *Strongloides stercoralis***	***Balantidium*. sp.**	***Hepatozoon* spp.**
Agricultural lands	**1 (14.29%)**	**3 (17.65%)**	**2 (14.29%)**	**2 (14.29%)**	**2 (%25.00)**
Ponds	**1 (%14.29)**	**4 (%23.53)**	**2 (%14.29)**	**2 (%14.29)**	**2 (%25.00)**
Streams	**0 (%0.00)**	**2 (%11.76)**	**3 (%21.43)**	**3 (%21.43)**	**1 (%12.50)**
Shoal Water	**2 (%28.57)**	**4 (%23.53)**	**2 (%14.29)**	**2 (%14.29)**	**3 (%37.50)**
River Banks	**1 (%14.29)**	**2 (%11.76)**	**3 (%21.43)**	**3 (%21.43)**	**0 (%0.00)**
Home Gardens	**2 (%28.57)**	**2 (%11.76)**	**2 (%14.29)**	**2 (%14.29)**	**0 (%0.00)**
Total	**7**	**17**	**14**	**14**	**8**
Chi-Square	**** 8.24**	*** 4.75**	*** 4.09**	*** 4.09**	**** 9.62**
(P<0.01) , (P<0.05)***					

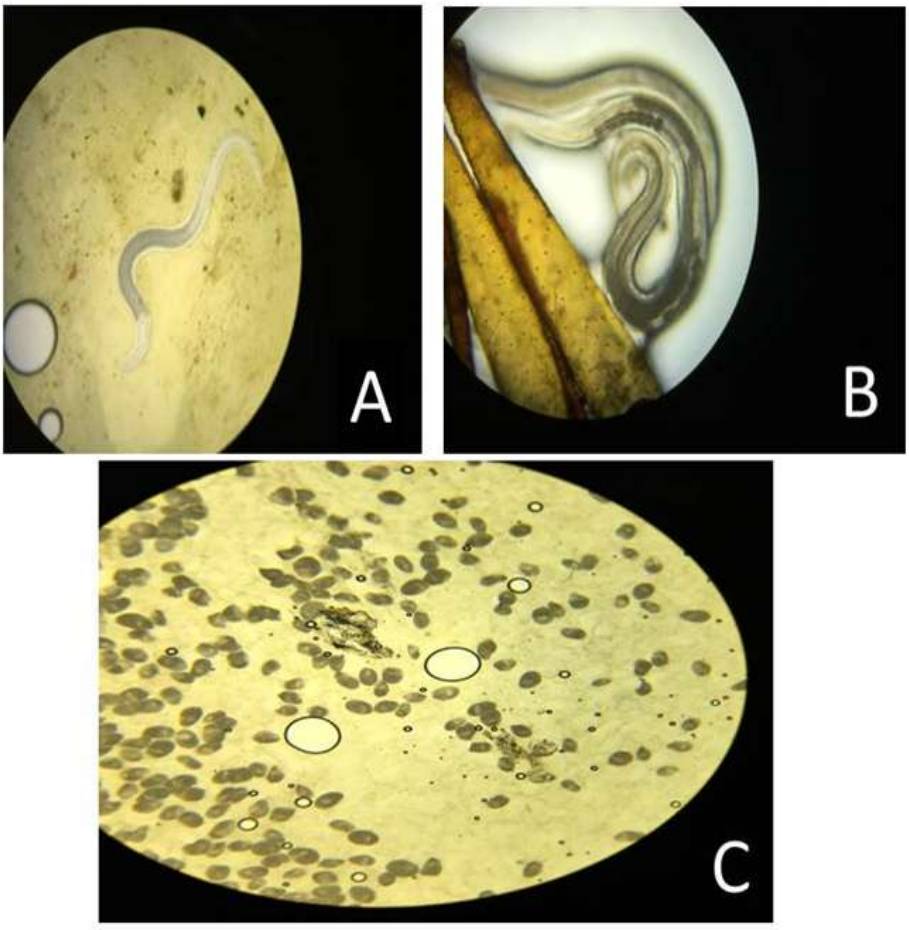

Fig.1. (A & B) Nematodes(*Strongloides stercoralis*)(10X & 40X), C – *Balantidium* sp. (in intestine of frogs)(10X), stained with Iodine stain

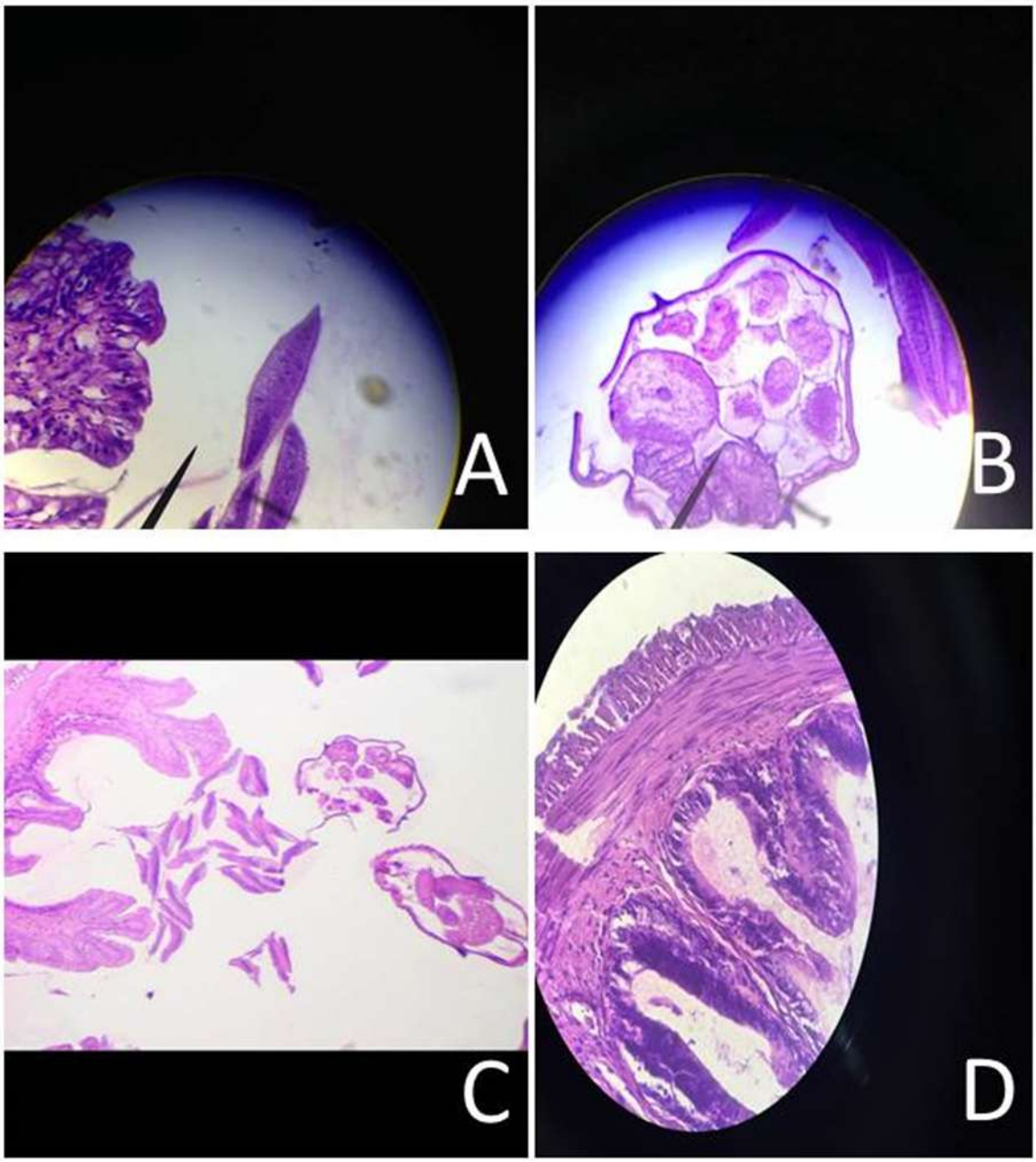

Fig. 2. Cross section in infected intestine not appeared any pathological effect on it, A- *Opalina* sp.(40X) , B- Section in Nematodes(*Strongloides stercoralis*)(40X), C- *Opalina* sp. & Nematodes(*Strongloides stercoralis*)(10X) , D- Cross section of intestine in healthy frog (40X), (H & E. stain).

Fig.-3-(AandB)shows *Hepatozoon* spp. Were present inside red blood cells of infected frogs and disappearance of nucleus observed in frogs infected with this parasites compared with healthy specimens (Fig. -3-, C), thus might be due to the effect and the action of the parasites on the blood cells.

Fig. -3- D shows the effect of *Hepatozoon* spp. On the liver cells of frogs, which produced pathological lesions mainly in the liver with subacute to chronic inflammation and infiltration, those lesions be similar to granuloma in liver which correlate with cell – mediated immune response, this result agree with the results of Rungsipipat A. (15).

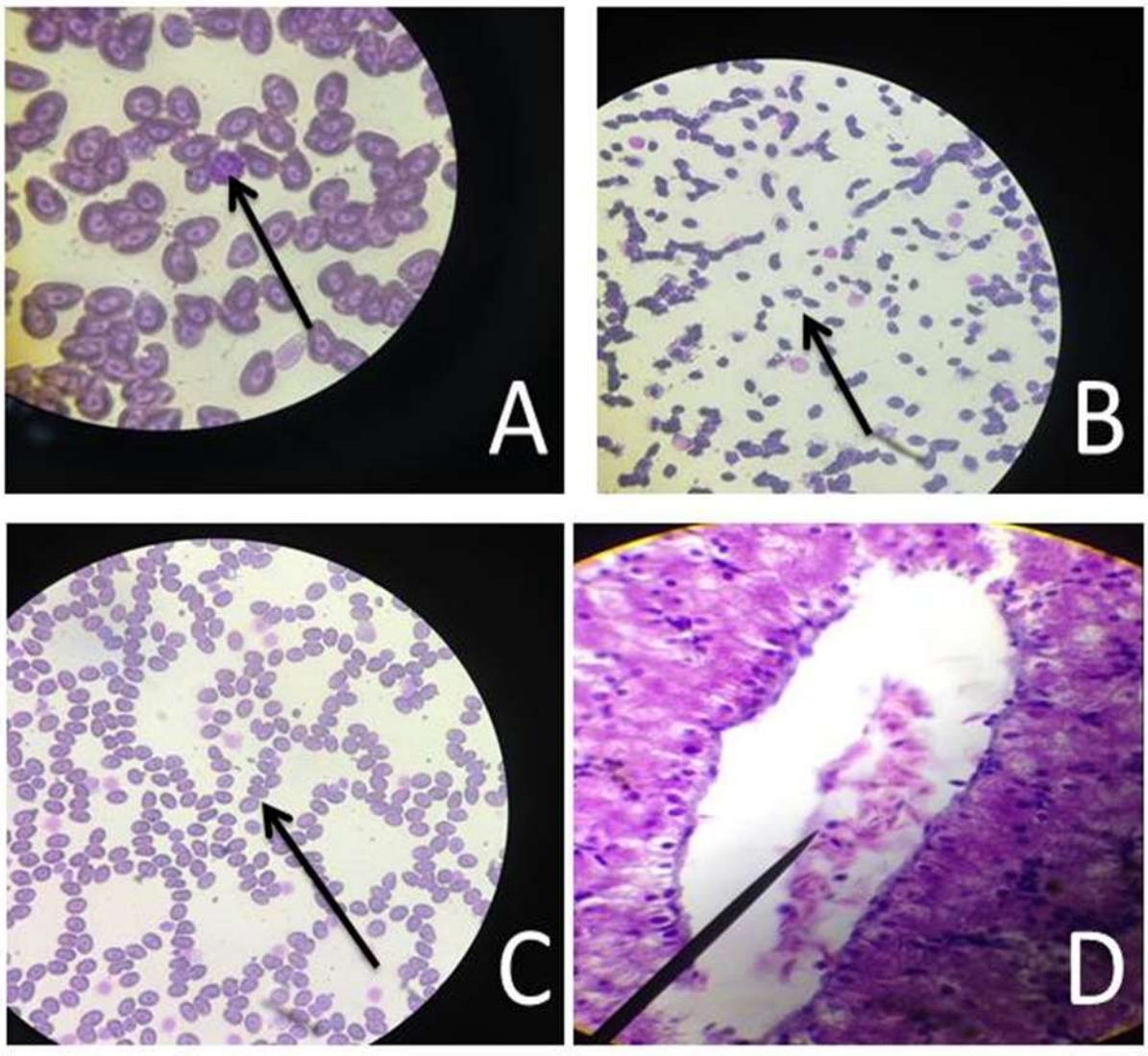

Fig. 3. peripheral blood smear stained with Gimsa stain, showed A- Meront stage of *Hepatozoon* spp. (40X), B- Disease of infected RBCs(disappeared of nuclei)(10X), C- Blood smear of healthy frogs(10X), D- Effect of parasites in liver cells of infected frogs(40X).

In conclusion, the parasites found in the present study live commensally in the intestine of frogs, therefore there is no pathological effect appeared in intestine of infected frogs, while the blood parasites showed the effect on red blood cells and pathological lesions in the liver of infected frogs.

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