Heamatological parameters and cellular morphological characters of frogs *Rana* sp. infected with parasites in Basrah marshlands environmental

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Abstract

The effect of infection with intestinal parasites of frogs *Rana* sp. on heamatological parameters were studied. The blood sample were collected and analyzed, the erythrocytes count (RBC), leukcocytes count (WBC), heamoglobine concentration (HB) and packed cell volume (PCV) were determined. Also the study showed increases in number of monocytes and granular leukocytes in blood of infected frogs when compared with control frogs, Heamatological parameters gaves informations about prognosis of the diseases.

Introduction

Blood cells of frogs are categorized into two main groups erythrocytes red blood cells (RBC) and leukocytes white blood cells (WBC). The erythrocytes (RBC) of frogs are oval, biconvex and nucleated and leukocyte are similar to those of blood of man (Storer *et.al.*, 1972). Monocytes are spherical in shaped and have kidney shaped nuclei is located on one side of cell, neutrophiles are recognized in smears by their characteristic lack of staining.

The nucleus are multi lobed are connected to each other by thin threadlike extensions of the nuclear substance. Eosinophiles are spherical shape, the nucleus is bi-lobed not multi-lobed as in the neutrophiles. Basophiles are also spherical cells, the nucleus is bi-lobed (Arserim and Mermer, 2008).

Quality and quantity of white blood cells (leukocyte) are regarded as a heamatological parameters, used to determine immune response and disease (Tierney *et.al.*, 2004). For example, eosinophils number are thought to be associated with parasitism defense (Kiesecker, 2002).

All non- mammals have nucleated erythrocytes. The presence of nuclei in mammalian erythrocytes is a sign of pathology, but in non- mammals it is normal (Arserim and Mermer, 2008). The aim of this study is to determine the effect of infection with intestinal parasites of frogs *Rana* sp. on its blood parameters.

Materials and Methods

Twenty seven of *Rana* sp. species were captured from Basrah marshlands environmental during March – 2010 and brought to laboratory of Marine Science Center/ Basrah University in plastic aquariums. All frogs were selected with same weight approximately. Frogs were anesthesia with ether to collected 1ml blood sample from heart using 1 ml a syringe. Then put in tube with EDTA to determine heamatological values. White and red blood cells counts were determined by using a standard heamocytometer (Dacie and Lewis, 1984). Heamoglobin rate was determined by using Sahli methods. Packed cell volume (PCV) was determined after the blood transferred to microscopic tubes and centrifuged for 5 min. blood films were prepared, fixed in 95% methanol for 5 min. stained with Leishman's stain (Dacie and Lewis, 1975). Photos were taken under light microscope. After blood collection, frogs were dissected and examined for infection with intestinal parasites. The relative number (percentage) of each white blood cell type was calculated based on the number of cells of that type counted divided by the total number of leukocytes counted (100 fields of view had been examined). One way analysis of variance and Least Significant Differences were used for statistical analysis in the present study (Hill, 1988).

Results

Frogs show variations of infection with intestinal parasites (include: nematode and trematoda), and that lead to variations in heamoglobine, white and red blood cells counts and packed cell volume, (Table 1).

In blood smears from parasitized frogs all type of leukocytes (WBC) lymphocyte, monocyte, basophile, aciophiles and neutrophiles were observed. There are increased in Lymphocytes in the parasitized frogs, (Table 2) and (Figure 1).

Table (1): Blood parameters of frogs *Rana* sp. Infected with intestinal parasites (n= 27)

Heamatological value	**Infected frogs Mean ±SD**	**Control Mean ±SD**
HB (g/d)	5.50± 3.16	8.22±1.00
WBC ($x10^3$ cell/µl)	4.88±1.42	1.42±0.43
RBC ($x10^6$ cel/µll)	1.23±0.21	1.00±0.57
PCV (%)	17.20±9.14	21.20±5.14

Table (2): The number and percentage of white blood cell (leukocytes) differentials count for blood smear of parasitized infected frogs *Rana* sp.(n= 27)

Type of WBC	**No. type of WBC**	**% type of WBC**
Lymphocytes	77	47.5
Monocytes	50	30.8
Neutrophils	22	13.5

Eosinophils	15	9.25
Basophils	3	1.8

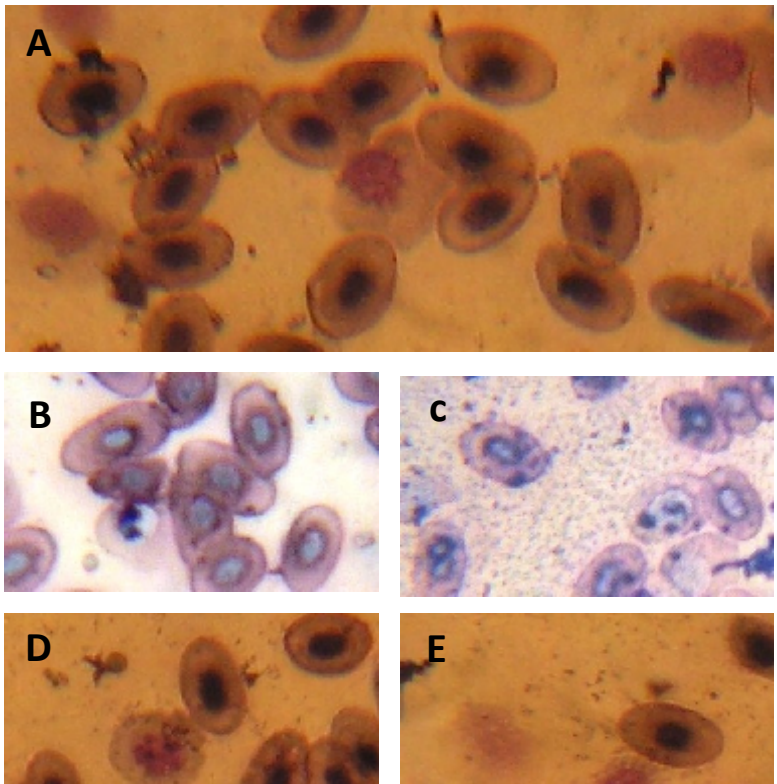

Fig (1): Smears of frog blood cells *Rana* sp. stained with Leishman's stain (A: monocyte / B: Eosinnophils / C: Basophiles / D: Neutrophiles / E: lymphocyte), erythrocyte show in (A,B,C,D,E), (X 40).

Discussion

This present study is the first hematological investigation of frogs infected with parasites. The hematological studies were carried out on various *Rana* species are performed on the blood cell counts (Schermer, 1954 ; Hutchinson and Szarski 1965 ; Arikan, 1989). The erythrocyte (RBC) is ovoidal shape the nuclei are also ovoid and oriented centrally. Variations in the erythrocyte count based on the metabolic activity were reported in amphibians (Goniakowska, 1973 ; Kuramoto 1981).

In this study the white blood cell proportions of infected frogs appeared with the most cell type being lymphocytes (47.5%) of white blood cells followed by monocyte (30.8%), then low numbers of Neutrophiles, Eosinophils and Basophils. Lymphocytes (large and small) are spherical cells, Lymphocytes can be identified in smear by their nucleus is large and usually round and almost the whole cell cytoplasm appear as a thin ring. There are actually two functions, types of lymphocytes one responsible for cell mediated immune response (T- cell) and the other for humoral immunity (B-cell) (Arserim and Mermer, 2008). In recent study, demonstrate suppression of eosinophil numbers by a pesticide causes increased susceptibility to trematode infections in frogs (Kiesecker, 2002)

In Iraq there is no study about effect of parasitic infections on heamatological parameters of the frogs in marshlands environmental. The present results demonstrate the infection of frogs with intestinal parasites (include: nematode and trematoda) and this infection lead to changes in blood parameter. The measurement of heamatological parameters of blood has been

used as a indicator for monitoring the health or diseases of animals (Beaver and June, 1995).

Arikan (1989) noted that the leukocyte count varies depending on species, season, sex, nutritient conditions and some physiological conditions such as diseases. The infection with parasites caused an increase in number of monocytes and other leukocytes (Dias *et.al*., 2007), blood leukocytes, especially granulocytes and monocytes could destroy pathogenic organisms. Eosinophils are generally thought to be involved in the innate immune response to parasites (Kiesecker, 2002).

It is well known that defence mechanisms in frog play an important role in all stages of parasitic infestations. The changes in fish physiology caused by *Dolops carvalhoi* a fish louse infestation and the results obtained indicate that a mild infection can lead to important osmoregulatory disturbances in hosts (Dias *et.al*., 2007).

This study indicated that the infection with parasites had effect on heamatological values and increased in number of some leukocytes. There are limited studies were performed on the heamatological studies (Arikan, 1989).

References

Arikan, H. (1989). Investigation on *Rana ridibunda* (Anura, Ranidae) populations aspect of blood cells, (in Turkish). Turk. J. Zool., 13: 54-59.

Arserim, S.K. and Mermer, A. (2008). Hematology of the Uludag frog *Rana macrocnemis* Boulenger, 1885 in Uludag National Park(Bursa, Turkey). J. Fish.& Aqu. Sci., 25(1): 39-46.

Beaver, P.C. and June, R.C.(1995). Animal agents and vector of human diseases. 5th ed. Lea and Febiger: Philadelphia : 24-26.

Dacie, J.V. and Lewis, S.M. (1975). Practical Haematology 5th. ed., Churchill Livingstone. London, Pp. 628.

Dacie, J.V. and Lewis, S.M. (1984). Practical hematology, ghurch ill Livingston(ed,) selecto printing Co. ltd., New Yuork, Pp. 445.

Dias, M.T. ; Moraes, F.R. ; Onaka, E.M. ; Rezende, P.C.B.(2007). Changes in blood parameters of hybrid tambacu fish parasitized by *Dolops carvalhoi* (Crustacea, Branchiura), a fish louse. Vet. Arhiv, 77(4): 355-363.

Goniakowska, L. (1973). Metabolism, resistance to hypotonic solutions, and ultrastructure of erythrocytes of five amphibian species. Acta. Biol. Crac., Ser. Zool. 13: 225-236.

Hill, A.B. (1988). A short textbook of medical statistics, 11th ed., Edward Arnold, London. Pp. 298.

Hutchison, V.H. and Szarski, H. (1965). Number of erythrocytes in some amphibians and reptiles. Copeia 3: 373-375.
Kiesecker, J. M. (2002). Synergism between trematode infection and pesticide exposure: a link to amphibian deformities in nature? Proceedings of the National Academy of Sciences 99:9900–9904.
Kuramoto, M. (1981). Relationships between number, size and shape of red boold cells in amphibians. Comp. Physiol., 69: 771-775.

Schermer, S. (1954). Die Blutmorphologie der laboratorium stiere, Barth.
Storer, T.I. ; Usinger, R.L. ; Stebbins, R.C. and Nybakken, J.W. (1972). General zoology, 5th ed., McGraw-Hill Book Co., New York, Pp. 899.

Tierney, K.B., Farrell, A.P. and Kennedy, C.J. (2004). The differential leucocyte landscape of four teleosts: *Juvenile Oncorhynchus Kisutch,Clupea pallasi, culaea inconstans* and *Pimephales promelas.* J. Fish Biol., 65: 906-919.

المعايير الدموية والصفات الشكلية الخلوية لخلايا دم الضفادع جنس *Rana* sp. ألمصابه بالطفيليات في بيئة اهوار البصرة

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الخلاصة

تم دراسة تأثير الاصابه بالطفيليات المعوية للضفادع جنس ***Rana* sp.** على المعايير الدموية. أظهرت الدراسة تغيرات في تعداد خلايا الدم الحمر والبيض ومستوى الهيموغلوبين وحجم الدم المضغوط. كذلك أظهرت الدراسة زيادة في عدد الخلايا الاحاديه وخلايا الدم البيض الحبيبية للضفادع المصابة عند مقارنتها مع ضفادع السيطرة، وان المعايير الدموية أعطت معلومات حول تزايد المرض.