Scholars Journal of Agriculture and Veterinary Sciences (SJAVS) **e-ISSN 2348–1854**
Abbreviated Key Title: Sch. J. Agric. Vet. Sci. **p-ISSN 2348–8883**

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

Incidence and Hematological Study of Trichomoniasis in Domestic and Wild Pigeons in and Around Lahore, Pakistan

Akhtar abbas[1], Hafiz Ishfaq Ahmad[1,2], Muhammad Hassan Saleem[1*], Jawaria Ali khan[1], Muhammad Kamran Ashraf[3]

[1]Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore, Pakistan
[2]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of the Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China
[3]Department of Parasitology, University of Veterinary and Animal Sciences, Lahore, Pakistan

Original Research Article

***Corresponding author**
Muhammad Hassan Saleem

Article History
Received: 10.05.2018
Accepted: 10.08.2018
Published: 30.08.2018

DOI:
10.36347/sjavs.2018.v05i08.004

Abstract: The present study was conducted to determine the incidence of Trichomoniasis and its effect on some blood parameters in pigeons. A total of 120 samples i.e. 60 fecal (Wild n=30, domestic n= 30) and 60 throat swab (Wild n=30, domestic n= 30) were collected during the month of July 2014 to September 2014 from Tollinton Market, Lahore Zoo and Safari park, Lahore. Out of 120 samples examined, 39 samples were found positive for *T. gallinae*. The incidence of *T. gallinae* was (32.5%). The highest rate of infestation by *T. gallinae* i.e. 42.5 % was recorded in the month of September 2014. While in July and August the infestation of this protozoa was 22 % and 32.5 % respectively. The overall infestation rate was 32.5 %. In infected pigeons, there was significant ($P<0.05$) decrease in hemoglobin concentration, number of monocytes and packed cell volume in diseased birds than healthy birds. Likewise, the values of total leukocyte count, lymphocytes and eosinophil were higher significantly ($P<0.05$) in infected pigeons than the healthy ones. While, no significant ($P<0.05$) difference was observed for heterophil count when infected and healthy birds were compared. This study reveals the incidence of Trichomoniasis in their birds especially in pigeons, consequently will help to overcome this disease thus helping them in increasing their income through pigeon farming.
Keywords: Incidence; Trichomoniasis; Pigeon, PCV, Lymphocytes; Eosinophil.

INTRODUCTION

It is very well known that internal parasites cause great loss to the host, by different ways. These parasites live at the expense of host depriving them from the nutrients essential for their growth. Moreover they cause mechanical harm by producing inflammation and tissue damaged. Protozoa inhabiting the digestive tract of birds are responsible for considerable economic losses. Heavy infestation of the parasites affect the health of birds with loss in the body weight, retarded growth, un thriftiness, damage to the gut epithelium, fertility disturbances, emaciation and death especially in young birds [1]. Common name of Trichomonas gallinae is canker, frounce and roup. Predilection site of this parasite is esophagus, crop and proventiculus. It belongs to the family Trichomondidae and class Zoomastigophorasida [2].

This disease is present worldwide. A clonal strain of previously described organism has been recently developed as the cause of widespread disease of birds in Europe and causes a grate economic losses [3]. In Britain, this infectious disease was first described in 2005. This disease causes extraordinary significant mortality in birds which results in decreased population of green finches and passenines [4]. This disease causes large economic loss of avian livestock and also cause problems for wild species of birds. In UK T. gallinae has caused the death of greenfinches [5]. Trichomoniasis was first reported in 2005 in Britain. It was discovered in finches. It caused large scale mortality in finches with population decline [4, 6]. In 2007 this disease is reported in finches in the Canadian Maritime Provinces, southern Fennoscandia and northern Germany [6]. This disease caused high morbidity and mortality in finch population in Britian. It is estimated that about 1.5 million greenfinches which represent the 35% of national population have been died with this disease [6].

The host of T. gallinae is pigeon, turkey, chicken and raptors (hawks, falcons and eagle). As the method of reproduction is concerned it reproduced by longitudinal binary fission. There are no sexual stages and cyst are present in its life cycle. Lesion present in the turkey and chicken are most commonly in the area of crop, oesophagus, pharynx and no lesion are found in mouth [2].

Infected pigeons show wild signs of depression, lose weight, stand huddled with ruffled feathers and may fall over when forced to move. There is an accumulation of greenish fluid present in the mouth and crop containing large number of trichomonads in it. Yellow, necrotic lesions are present in the esophagus and crop [2]. Infection spread to the turkey and chicken by drinking contaminated water. The pigeon and other wild birds are also source of infection, which also use the water source. *T. gallinae* enters in the water through mouth and not from feces of the wild birds. Source of infection is direct contamination because this organism is very sensitive to drying and no cyst are found [2].

Prevalence of *T.gallinae* infection is different in different age of birds. Prevalence increased with the age of nestling [7]. A higher prevalence of Trichomoniasis has been recorded in pigeons in Pakistan season wise prevalence has been recorded to be 43%, being non significantly higher in April (56%) than in March (30%). Trichomoniasis positive cases show a significant decrease in hemoglobin concentration, number of monocyte, packed cell volume, body weight than healthy birds [8]. Looking at the endangered population status of wild and domestic pigeons, the present study was designed to find the incidence of Trichomoniasis in wild and domestic pigeons and to investigate the effects of Trichomoniasis on various blood parameters.

MATERIALS AND METHODS

The present study was designed to study the incidence of trichomoniasis in wild and domestic pigeons and its effect on blood parameters. To study the incidence of *T.gallinae*, a total of 120 samples i.e. 60 fecal (Wild n=30, domestic n= 30) and 60 throat swab (Wild n=30, domestic n= 30) were collected from Tollinton Market, Lahore Zoo and Safari park, Lahore. The fecal sample and throat swabs were examined first directly and then by stained smear [9].

Samples collection

The fecal samples were collected directly from cloaca region of the birds by using an aseptic culture swabs in separate culture tube after dipping it in normal saline and then were examined immediately, in the Medicine Laboratory, University of veterinary and animal sciences, Lahore. Throat swabs sample were collected individually from the pigeons through a swabs and then kept in culture tubes, after dipping it in normal saline to avoid desiccation and were examined immediately.

Sample processing

Fecal samples were examined under microscopic after making smears on glass slide directly. Rub swab on slide after putting a drop of normal saline on it then cover it with cover glass and examined it under low microscope.

Direct Smear Method

In this method, a drop of normal saline was placed on the clean glass slide and the swab which had material stick on it was rub on the slide and thus made a smear on slide and see it under microscope.

Preparation of Wright Giemsa Staining

30 mg of dry wright's stain powder and 30 mg of Giemsa stain powder will be ground in marter with 100ml of absolute methyl alcohol (acetone free). The mixture was allowed to stand for 24 hours before use [10].

Staining of slides

For staining of slides Wright Giemsa stain was used [9]. First made smear on clean glass slide then dry it. Then poured a drop of stain on slide and wait for 1-3 minutes. After it added equal amount buffered distilled water or neutral water (PH 6.6 to 6.8). Allowed mixture to stand for 3-5 minutes then poured it and washed with water thoroughly. Then dried it and examined under microscope.

Hematological studies

Blood samples were collected from both group i.e. infected and healthy pigeon. 5ml disposable syringe was used to collect blood from wing vein of pigeons. One ml of blood was taken from each bird's wing vein, after adding 1% EDTA. Blood samples were examined for total leukocyte count, differential leukocytes count, hemoglobin estimation and packed cell volume.

RESULTS

The present study was conducted to investigate the incidence of *Trichomonas gallinae* in domestic and wild pigeons. To determine the effect of T. gallinae infection on different blood parameter i.e. Hemoglobin estimation, TLC, DLC, PCV % age, Heterophils, Monocytes, Lymphocytes, and Eosinophil. Fecal samples were examined under microscopic after making smears on glass slide directly. Rub swab on slide after putting a drop of normal saline on it then cover it with cover glass and examined it under low microscope (Figure 1).

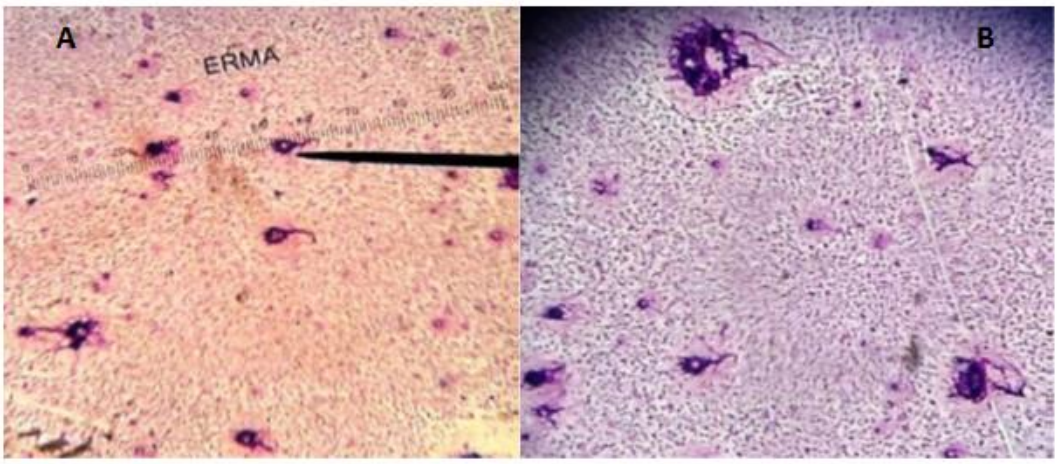

Fig-1: Wright Giemsa stained slide (100X) T. gallinae from wild (A) and domestic (B) pigeons oral cavity

Incidence

To study the incidence of *T. gallinae*, a total of 120 samples i.e. 60 fecal (Wild n=30, domestic n= 30) and 60 throat swab (Wild n=30, domestic n= 30) were collected during July 2014 to September 2014 from Tollinton Market, Lahore Zoo and Safari park, Lahore. Out of 120 samples examined (39) were found positive for T. gallinae. The incidence of T.gallinace was (32.5%). The highest rate of infestation by T. gallinae i.e. 42.5 % was recorded in the month of September 2014. While in July and August the infestation of this protozoa was 22 % and 32.5 % respectively. The overall infestation rate was 32.5 % (Table 1).

Table-1: Month wise prevalence of Trichomonas gallinae in domestic and wild pigeons

Month	Total no. of samples examined	Total no. of positive samples	Infestation
July	40	9	22
August	40	13	32.5
September	40	17	42.5
Total	120	39	32.5

Hematology

For this purpose 15 healthy and 15 infected pigeons were randomly selected and their blood was examined for the following parameters.

Hemoglobin Estimations

The value of hemoglobin of 15 healthy birds was 9.0, 8.5, 11.0, 12.0, 10.0, 11.0, 11.5, 8.0, 9.5, 9.0, 10.0, 11.0, 8.0, 12.0 and 9.5 while the value of hemoglobin of 15 infected birds was recorded as 7.5, 7.0, 7.5, 6.5, 6.0, 5.0, 6.5, 7.0, 6.5, 6.0, 5.0, 7.0, 6.5, 6.0 and 7.0, respectively (Table 2).

Leukocyte count (TLC)

The total leukocyte count of 15 healthy pigeons were as 24000, 28000, 29000, 26000, 26000, 28500, 29500, 27500, 27500, 27000,25000, 28500, 27500, 26000 and 27500 while the TLC of 15 infected pigeons were as 29000, 31500, 31500, 32500, 32500, 32000, 30000, 29000, 33000, 31000, 32000, 29000, 30000, 33000 and 30000, respectively (Table 2). This result shows that infected birds have higher number of leukocytes.

Heterophils count

The heterophils count of 15 healthy pigeons were 4500, 5000, 4500, 4550, 4650, 4750, 4700, 4600, 4700, 4850, 4700, 4550, 4500, 4600 and 4700. While the heterophils count of 15 infected pigeons were 5500, 4800, 5000,4800, 4500, 4000, 4800, 5400,5000, 5500, 4800, 5000, 4800, 5400 and 5000 (Table 3).

Monocytes

The monocytes of healthy pigeons were 1500, 1550, 1450, 1400, 1500, 1400, 1500, 1550, 1400, 1450, 1500, 1500, 1450, 1400 and 1500 while the monocytes of infected birds were 1300, 1250, 1400, 1400, 1250, 1150, 1300, 1400, 1350, 1300, 1400, 1350, 1400, 1200 and 1300. These results show that the infected birds had little bit lower monocytes than the healthy ones Table 3).

Table-2: Blood Parameters of Healthy Birds tested in this study

Sr#	Hb (g/dl)	TLC (103/µl)	Heterophils (103/µl)	Monocytes (103/µl)	Lymphocytes (103/µl)	Eosinophil (103/µl)	PCV (%)
1	9	24000	4500	1500	16000	450	30
2	8.5	28000	5000	1550	15500	430	28
3	11	29000	4500	1450	14500	470	29
4	12	26000	4700	1400	16000	300	30
5	10	26000	4650	1500	15500	430	30
6	11	28500	4750	1400	15000	420	28
7	11.5	29500	4700	1500	16500	470	30
8	8	27500	5000	1550	16000	450	28
9	9.5	27500	4900	1400	15500	430	26
10	9	27000	4850	1450	15000	450	30
11	10	25000	4700	1500	15500	400	28
12	11	28500	4850	1500	16500	450	26
13	8	27500	4500	1450	16000	400	28
14	12	26000	4800	1400	17000	420	32
15	9.5	27500	4700	1500	16500	450	28

Table-3: Blood Parameters of Infected Birds tested in this study

Sr#	Hb (g/dl)	TLC (103/µl)	Heterophils (103/µl)	Monocytes (103/µl)	Lymphocytes (103/µl)	Eosinophil (103/µl)	PCV (%)
1	7.5	29000	5500	1300	19000	650	18
2	7	31500	4800	1250	19500	680	18
3	7.5	31500	5000	1400	17500	600	28
4	6.5	32500	4800	1400	19000	780	20
5	6	32500	4500	1250	18500	700	24
6	5	32000	4000	1150	17500	650	20
7	6.5	30000	4800	1300	18000	750	22
8	7	29000	5400	1400	18000	700	22
9	6.5	33000	5000	1350	17500	600	20
10	6	31000	5500	1300	18500	550	26
11	5	32000	4800	1400	18500	620	20
12	7	29000	5000	1350	18000	500	22
13	6.5	30000	4800	1400	18500	700	20
14	6	33000	5400	1200	18000	400	24
15	7	30000	5000	1300	19500	650	20

Lymphocytes

The lymphocytes of healthy pigeons were 16000, 15500, 14500, 16000, 15500, 15000, 16500, 16000, 15500, 15000, 15500, 16500, 16000, 17000 and 16500 while the lymphocytes of infected birds were 19000, 19500, 17500, 19000, 18500, 17500, 18000, 18000, 17500, 18500, 18500, 18000, 18500, 18000 and 19500. These results show that the infected birds have higher value of lymphocyte than the healthy ones.

Eosinophil

The number of eosinophil of healthy pigeons were 450, 430, 470, 300, 430, 420, 470, 450, 430, 450, 400, 450, 400, 420 and 450, while the unhealthy birds had the number of eosinophil were 650, 680, 600, 780, 700, 650, 750, 700, 600, 550, 620, 500, 700, 400 and 650. These results shows that the infected birds have slight increase in the number of eosinophil than the healthy ones.

Packed Cell Volume (PCV %)

The Packed cell volume percentage (PCV %) in healthy birds range from 26 to 32, while the range in infected birds was 18 to 26, which is below than healthy ones.

DISCUSSION

The present study was conducted to investigate the incidence of Trichomonas gallinae in domestic and wild pigeons. To determine the effect of T. gallinae infection on different blood parameter i.e. Hemoglobin estimation, TLC, DLC, PCV %age, Heterophils, Monocytes, Lymphocytes, and Eosinophil.

Incidence

To study the incidence of *T. gallinae*, a total of 120 samples i.e. 60 fecal (Wild n=30, domestic n= 30) and 60 throat swab (Wild n=30, domestic n= 30) were collected during July 2014 to September 2014 from Tollinton Market, Lahore Zoo and Safari park, Lahore.

Out of 120 samples examined (39) were found positive for *T. gallinae*. The incidence of *T. gallinae* was (32.5%). The highest rate of infestation by T. gallinae i.e. 42.5 % was recorded in the month of September 2014. While in July and August the infestation of this protozoa was 22 % and 32.5 % respectively. The overall infestation rate was 32.5 %.

The prevalence of *T. gallinae* in birds is different in the different parts of world. It is may be due to the different ecological conditions. The highest rate of infestation by *T. gallinae* i.e. 42.5% was recorded in the month of September 2014. These results were correlate with the study that was conducted in Lahore, Pakistan from where a total of 100 samples from the pigeons (50 wild and 50 domestic) were collected during the months of March and April 2005. Higher prevalence ($P<0.05$) was recorded in wild pigeons (60%) than in domestic pigeon (26%). The overall prevalence recorded was 43%, being non significantly higher in April (56%) than in March (30%) [8]. These results were also correlate with the study that was done in Bangladesh. During July 2007 to 2008, he examined three hundred pigeons in which 202 were positive for T. gallinae. Adult pigeons having age more than 3 months were comparatively more affected (75%) than the squab having age less than 30 days (72.1%) and the younger aged between 30 days to 90 days (64.7%). Significance ($P<0.01$) of T. gallinae infection were low in summer (48.4%) than rainy (69.8%) and winter (69.3%) seasons [11].

The overall positive percentage of T. gallinae of 32.5% correlate with other study that was done in Spain, in which 91 hunter-harvested Common Wood Pigeons Columba palumbus from northern (n = 30) and southern (n = 61) Spain during the winter period. He examined the pigeons by using three different methods i.e. direct inspection for the presence of lesions, direct microscopic observation and culture. The positive percentage for the presence of *T. gallinae* was 34.2%. This prevalence was significantly higher in adult Wood Pigeons than in the juvenile group, and prevalence was significantly lower in birds sampled from the north [12]. The highest rate of infestation by *T. gallinae* i.e. 42.5% was recorded in the month of September 2014. These results correlate with another study that was done on UK. This study indicate that *T. gallinae* is a major threat to an endangered endemic, the pink pigeon. They examined that the population of columbids act as a reservoirs of Trichomonas gallinae. 296 birds were examined. Prevalence of T. gallinae was 44.3% [13].

These results are in accordance with the study that was conducted on the prevalence and pathological lesion of Trichomonas gallinae in pigeons in the northeast part of Iran. Total 418 Samples were collected from oral cavity of pigeons and were examined by using Giemsa's stain between April 2008 and June 2009. Prevalence of *T. gallinae* was 37.32% [14]. But these result are not in accordance to the study that was conducted on Trichomonas gallinae in pigeons (*Columba domestica*) in Isfahan, Iran. Total 100 pigeons were examined from January to June 2010. Throat swabs and blood samples were taken from all birds. After making smear and Giemsa staining, these sample were examined. Out of 100 Oropharyngeal swab smears 57 (57%) were positive for *T. gallinae* [15]. Similarly these results were not in accordance to the study that was conducted on prevalence of Trichomonas gallinae in northern goshawks from the berlin area of northeastern Germany. In total, 65.1% of the northern goshawk nestlings were culture positive for *T. gallinae* [7]. The results of my study do not correlate the result of this study that was conducted in Spain. In this study a total 274 pigeon samples were examined. After analyzing the culture 612 samples were positive. So prevalence of T. gallinae was 44.8% in pigeons [16]. These variations with the present study are due to many factors. It may be due to climate conditions, geographical difference, rate of infestation, seasonal difference, resistance of the host, difference in feeding habitats, age of birds and difference in housing conditions.

Hematology

In the present study 15 blood samples from naturally infected pigeons were examined for hematological studies to know the changes in blood picture due to T. gallinae and also see the 15 blood samples of healthy pigeons which were already declared -ve carrier of *T. gallinae* after laboratory confirmation to see the clear difference between both groups.

Hemoglobin Estimation

The results of present study show that the values of hemoglobin estimation in infected birds are lesser than that of healthy birds. It may be due to these protozoa. Statistical analysis showed that there is significant difference ($P<0.05$) between the healthy and infected birds. The present observations correlates with the study conducted on the prevalence of trichomoniasis and its effect on some blood parameter and weight gain. In that study two groups were made, each group were consisted of ten birds. Group 'A' were nominated of a disease birds that was affected with trichomoniasis and group 'B' were nominated of a healthy birds that was negative for this disease. Two ml blood was taken from wing veins of each bird from both group 'A' and group 'B'. Some blood parameters like hemoglobin (Hb) concentration, total leukocyte count (TLC), heterophilis, monocytes, lymphocytes, eosinophil and packed cell volume (PCV) were measured by using typical procedures, which was defined in studied [17]. Affected pigeons were anemic due to lessened Hb concentration and there was a significant ($P< 0.05$) decrease in Hb concentration and packed cell volume in diseased pigeons [8].

CONCLUSION

There is a significant ($P<0.05$) difference between the means of Hb, PCV, TLC, Monocytes, Lymphocytes and Eosinophil. While there is no significant ($P\geq0.05$) difference between the means of heterophils. It is anticipated that outcome of this work will provide the veterinarian and pigeon farmers a guide line about the common occurrence of Trichomoniasis and will help them to overcome this disease thus helping them in increasing their income through pigeon farming.

ACKNOWLEDGEMENT

Authors are thankful to anonymous reviewers for critical review and their valuable comments for the improvement of manuscript.

Conflict of Interest

Authors declare that there is no conflict of interest.

REFERENCES

1. Urquhart G, Armour J, Duncan J, Dunn A, Jennings F. Veterinary helminthology. Vet Parasitol. 1996, 2 122-125.
2. Taylor MA, Coop RL, Wall RL. Veterinary parasitology Third edition page. 2007, 460-461, 652-653.
3. Ganas P, Jaskulska B, Lawson B, Zadravec M, Hess M, Bilic I. Multi-locus sequence typing confirms the clonality of Trichomonas gallinae isolates circulating in European finches. Parasitology. 2014, 141 (05): 652-661.
4. Lawson B, Cunningham A, Chantrey J, Hughes L, Kirkwood J, Pennycott T, Simpson V. Epidemic finch mortality. Vet Rec. 2006, 159 (11): 367-367.
5. Robinson RA, Lawson B, Toms MP, Peck KM, Kirkwood JK, Chantrey J, Clatworthy IR, Evans AD, Hughes LA, Hutchinson OC. Emerging infectious disease leads to rapid population declines of common British birds. PLoS one. 2010, 5 (8): 12215.
6. Lawson B, Robinson RA, Neimanis A, Handeland K, Isomursu M, Agren EO, Hamnes IS, Tyler KM, Chantrey J, Hughes LA. Evidence of spread of the emerging infectious disease, finch trichomonosis, by migrating birds. Ecohealth. 2011, 8 (2): 143-153.
7. Krone O, Altenkamp R, Kenntner N. Prevalence of Trichomonas gallinae in northern goshawks from the Berlin area of northeastern Germany. J wild dis. 2005, 41 (2): 304-309.
8. Saleem M, Khan M, Chaudry A, Samad H. Prevalence of trichomoniasis in domestic and wild pigeons and its effects on hematological parameters. Pak Vet J., 2008, 28 (2): 89-91.
9. Coles EH. Veterinary Clinical Pathology Third edition, 1980, .Sounder's Company, Philadelphia USA.
10. Neimanis AS, Handeland K, Isomursu M, Ågren E, Mattsson R, Hamnes IS, Bergsjo B, Hirvela-Koski V. First report of epizootic trichomoniasis in wild finches (family Fringillidae) in southern Fennoscandia. Avian diseases. 2010, 54 (1): 136-141.
11. Begum N, Mamun M, Rahman S, Bari A. Epidemiology and pathology of Trichomonas gallinae in the common pigeon (Columba livia). Journal of the Bangladesh Agricultural University. 2008, 6 (2): 301-306.
12. Villanua D, Hofle U, Perez-Rodriguez L, Gortázar C. Trichomonas gallinae in wintering common wood pigeons (Columba palumbus) in Spain. Ibis. 2006, 148 (4): 641-648.
13. Bunbury N, Bell D, Jones C, Greenwood A, Hunter P. Comparison of the InPouch TF culture system and wet-mount microscopy for diagnosis of Trichomonas gallinae infections in the pink pigeon (Columba mayeri). J Clin Microbiol. 2005, 43 (2): 1005-1006.
14. Borji H, Razmi G, Movassaghi A, Moghaddas E, Azad M. Prevalence and pathological lesion of Trichomonas gallinae in pigeons of Iran. J Parasit Dis. 2011, 35 (2): 186-189.
15. Nematollahi A, Ebrahimi M, Ahmadi A, Himan M. Prevalence of Haemoproteus columbae and Trichomonas gallinae in pigeons (Columba domestica) in Isfahan, Iran. J Parasit Dis. 2012, 36 (1): 141-142.
16. Sansano-Maestre J, Garijo-Toledo MM, Gomez-Munoz MT. Prevalence and genotyping of Trichomonas gallinae in pigeons and birds of prey. Avian. Path. 2009, 38 (3): 201-207.
17. Rehman H, Abbas S and Lohahet N. Laboratory Manual of Physiology, 2003, Vol. 1. Society of Veterinary Physiology, Lahore, Pakistan.