



A cytopathological study of the role of liver impression as a diagnostic tool in pigeons

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Abstract

The aim of the current study is to investigate any pathological changes which affect local pigeon liver by using liver impression and providing data base for the results of cytological and morphological features of hepatic impressions of local pigeon also to Study the relation between cellular contents and bacterial profiles at those impressions for that purpose about 20 birds of local pigeon were used in current study. the result showed presence of including heterophil 21.53% monocyte 1.52%, eosinophil 1.04%, basophil 0.01%, macrophage 4.01%. RBC 31.9% and vacuolated hepatocyte 4.94%. We also recorded presence of undifferentiated cells 0.19% bacterial infection and parasite infestation of blood protozoa represented by presence of plasmodium parasite inside red blood cell in 4 samples out of 20 samples, G+ Staphylococcus and streptococcus and G- Bacteria coccobacilli as a bacterial. Bacteria including Staphylococci, Streptococci and Coccobacilli were noticed with in different densities between sections, the protozoal parasite as Plasmodium infestation were also detected in 20% of samples We concluded that, the hepatic impression give a diagnostic tool to aim in final diagnosis for inflammatory diseases in pigeons, in addition this impression give a primary idea about bacteria and parasitic infection that can be present in infected pigeons.

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Introduction

The avian liver plays an important role for metabolic processing to several body systems including digestive, hemopoietic and Endocrine; due to these multiple functions it is exposed to infections with different diseases by different ways (1,2). Liver disease of the birds includes numerous cases infectious, non-infectious and congenital anomalies in young birds (3), while infectious diseases of avian liver includes viral, bacterial and parasitic infestations. Viral infection such as psittacine herpes virus which cause (Pacheco's disease) other includes adeno virus, paramyxovirus, reovirus, coronavirus and Rota virus (4). A systemic bacterial infection in birds both G+ and G- bacteria can cause hepatitis, Staphylococcus and Streptococcus spp. are most common G+ (3). Toxoplasma, Leukocytozoonosis, Hemoproteins, *Trichomonas gallinae* are most common

parasitic infestations in the birds (3-6). The aim of the study is to Investigate the cytopathological cases of pigeon liver by using liver impression smears.

Material and methods

Birds

A total of 20 local pigeons were collected and examined for discovering of the pathological cases in the pigeon's liver by using liver impressions smears.

Taking impression

Impression smears prepared from the cut surface of the liver Later, these impressions smears were dried at room temperature, then dried, fixed with methanol and stained with Giemsa stain. Stained slides were examined microscopically to detect cytopathological lesions in the

liver by using oil emersion lens. Some slides showed bacterial cluster the signs (+) applied to referee to 40-50 Bacteria in the field (++) refer to 50-100 Bacteria in the field (++++) refer to $100 \leq$ Bacteria more than 100 in the field (7).

Statistical analysis

Pearson correlation test 2-tailed were made between the score of bacterial densities and percentage ratio of inflammatory cells and hepatocytes in each sample using SPSS program version 19 and under $P \leq 0.05$ significance value.

Results

The results of mean percentage ratio of impression smears of 20 samples of liver. The mean percentage of lymphocyte is 31.44% (Figure 1). Heterophil is 21.53% (Figure 2). Monocyte is 1.52% (Figure 3). Eosinophil is 1.04% (Figure 4). Basophil is 0.01% (Figure 5). Macrophage is 4.01% (Figure 6). RBC is 31.9%, vacuolated hepatocyte is 4.94 (Figure 7). Undifferentiated cell is 0.19% (Table 1). Also, the results showed that there is bacterial infection and parasitic infestation of blood protozoa represented by presence of plasmodium parasite (Figure 8) inside Red blood cell in 4 samples out of 20 samples. While the results of bacterial infections in the liver impressions smears show that there is a G+ Bacterial infection represented by Staphylococcus and streptococcus infection (Figure 9), alone or mixed with G- Bacteria represented by Coccobacilli; The signs (+) (++) (++++) were used to refer to the density of bacteria in the field (Table 2). Intranuclear inclusion bodies were founded in the hepatocyte (Figure 10), intracytoplasmic inclusion bodies were founded. From table 3 the results revealed a significant positive correlation link between each of Staphylococci-Streptococci density, Coccobacilli density and the macrophage percentage ratio in the hepatic impression sections from pigeon with r value =0.47 and r=0.56 respectively.

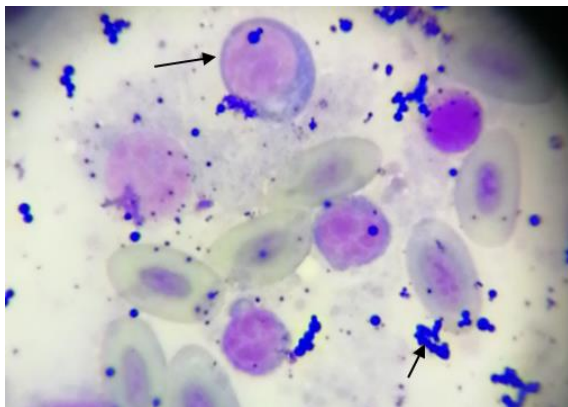


Figure 1: Liver imprint of pigeon showing lymphocytes and staphylococcus infection (arrow). Giemsa stain, 1000x.

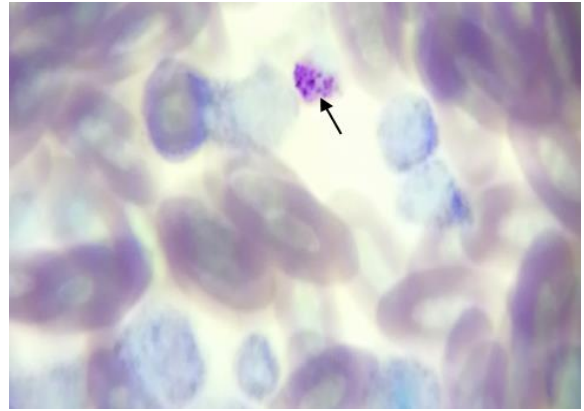


Figure 2: Liver imprint of pigeon showing Bacteria inside heterophil (arrow). Giemsa stain, 1000x.

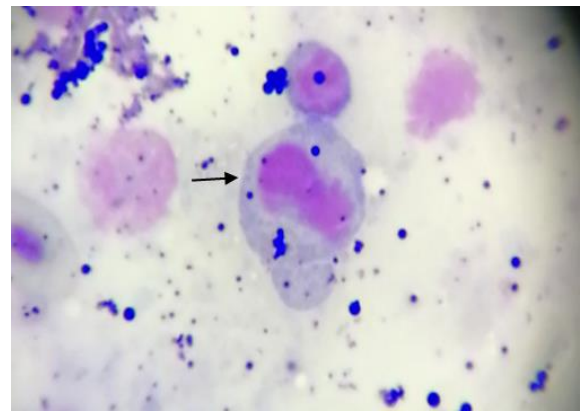


Figure 3: Liver imprint of pigeon showing monocyte and staphylococcus infection (arrow). Giemsa stain, 1000x.

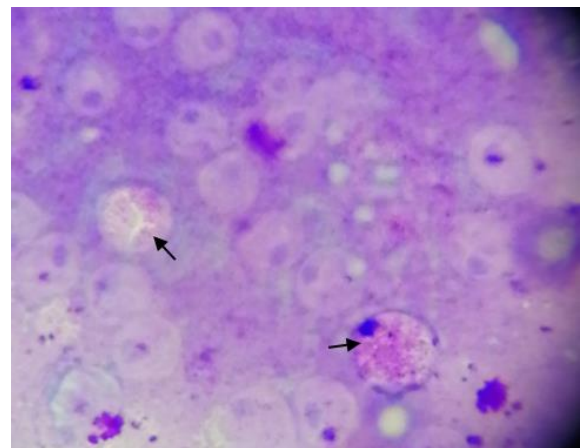


Figure 4: Liver imprint of pigeon showing eosinophil (arrow). Giemsa stain, 1000x.

Table 1: Showing the mean percentage of counted cell in the liver impression slides from local pigeon

no.	L%	H%	MA%	M%	E%	B%	RBC%	VH%	UE%
1	23.18	42.27	4.09	0.45	0.45	0	11.81	17.72	0
2	30.15	23.8	5.29	1.05	0	0	38.95	1.58	0
3	14.94	28.82	4.62	4.27	0	0	33.80	13.52	0
4	16.89	6.84	0	1.36	4.56	0	70.31	0	0
5	15.87	41.26	2.64	12.69	0.52	0	24.33	0.52	2.11
6	25.61	17.76	3.30	0	2.06	0.41	50.41	0.41	0
7	16.72	52.17	4.34	0	0.66	0	11.37	14.38	0.33
8	10.58	17.25	2.35	1.17	1.56	0	67.05	0	0
9	42.06	26.60	3	0.42	2.14	0	12.44	13.3	0
10	46.30	13.42	0.36	0.67	0	0	16.77	17.44	0
11	54.54	8.39	4.19	1.39	0	0	25.87	5.59	0
12	47.94	21.91	6.84	0.68	0	0	18.40	4.10	0
13	49.62	16.29	6.66	1.48	0	0	23.7	2.22	0
14	21.73	8.07	0	1.24	6.21	0	62.73	0	0
15	47.29	10.13	7.43	0.67	0	0	27.70	0	0
16	55.0	14.72	7.75	0	0.77	0	20.15	0.77	0.77
17	14.2	23.8	2.27	0.56	0	0	57.38	0	0
18	55.17	13.79	4.82	1.37	0	0	20.68	4.13	0
19	51.56	16.40	6.25	0	0	0	21.87	0	0
20	47.36	26.84	3.15	0	0	0	22.10	0	0.52
Mean	31.44	21.53	4.01	1.52	1.04	0.01	31.9	4.94	0.19

L: Lymphocyte, H: Heterophil, MA: Macrophage, M: Monocyte, E: Eosinophil, B: Basophil, VH: Vacuolated hepatocyte, UE: Underestimated cell.

Table 2: Showing the signs of bacteria profiles and parasite infestation in liver impression slides from local pigeon

No.	G+ Bacteria	G- Bacteria	Parasite	Others
1	-----	G- bacilli (+)	----	----
2	G+ Staph & Strep (+++)	G- coccobacilli (+++)	----	----
3	G+ Staph Strep (+++)	G- coccobacilli (+++)	----	----
4	-----	-----	Plasmodium+	----
5	G+ Staph & Strep (+++)	G- coccobacilli (+++)	----	4 underentiated cell
6	G+ Staph & Strep (+++)	G- coccobacilli (+++)	----	----
7	G+ Staph & Strep (+++)	G- coccobacilli (+++)	----	1 underentiated cell
8	G+ Staph & Strep (+++)	G- coccobacilli (++)	Plasmodium+	----
9	G+ Staph & Strep (+++)	G- coccobacilli (+++)	----	----
10	G+ Staph & Strep (+++)	G- coccobacilli (+++)	----	----
11	G- coccobacilli (++)	-----	----	----
12	G+ Staph & Strep (+++)	G- coccobacilli (+++)	----	----
13	G+ Staph & Strep (+++)	G- coccobacilli (+++)	----	----
14	-----	-----	Plasmodium+	----
15	G+ Staph & Strep (+++)	G- coccobacilli (+++)	----	----
16	G+ Staph & Strep (+++)	G- coccobacilli (+++)	----	1 underentiated cell
17	-----	-----	Plasmodium+	----
18	G+ Staph & Strep (+++)	G- coccobacilli (+++)	----	----
19	G+ Staph & Strep (+++)	G- coccobacilli (+++)	----	----
20	G+ Staph & Strep (+++)	G- coccobacilli (+++)	----	1 underentiated cell

Table 3: Showing the correlation coefficient values between bacterial densities and cellular percentage ratios in liver impression slides from local pigeons

Bacteria	H	M	L	HE
Staphylococci and Streptococci	0.17	0.47*	0.29	0.01
Coccobacilli	0.18	0.56*	0.42	0.11

H: Heterophil, M: Macrophage, L: Lymphocyte, HE: Hepatocyte. *= significant at 0.05

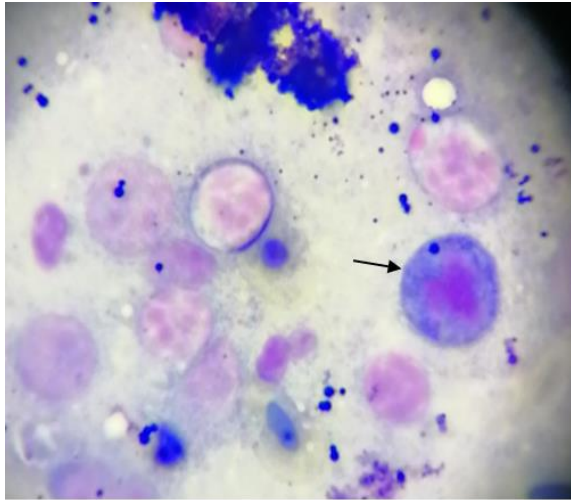


Figure 5: Liver imprint of pigeon showing basophil (arrow). Giemsa stain, 1000x.

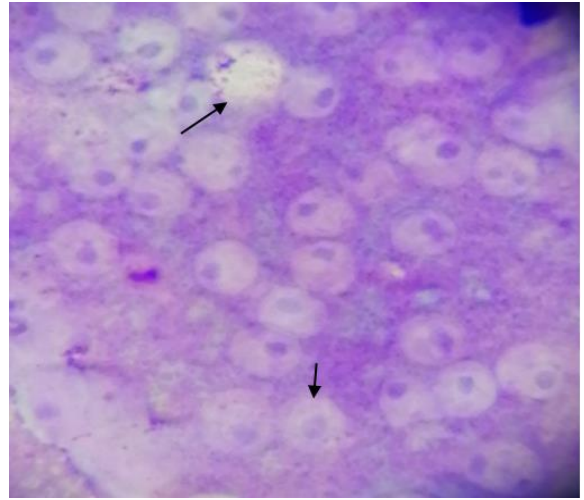


Figure 7: Liver imprint of pigeon showing sloughed and vacuolated hepatocyte (arrow). Giemsa stain, 1000x.

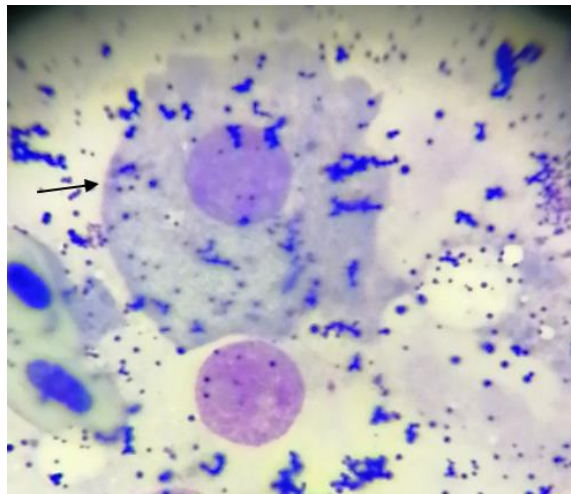


Figure 6: Liver imprint of pigeon showing macrophage lymphocyte bacteria (arrow). Giemsa stain, 1000x.

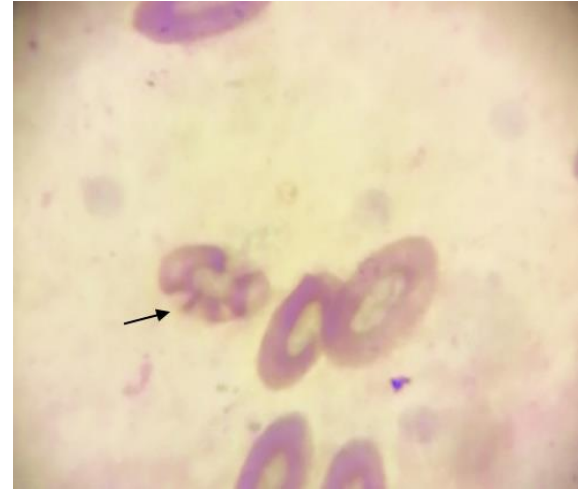


Figure 8: Liver imprint of pigeon showing plasmodium parasite inside RBC (arrow). Giemsa stain, 1000x.

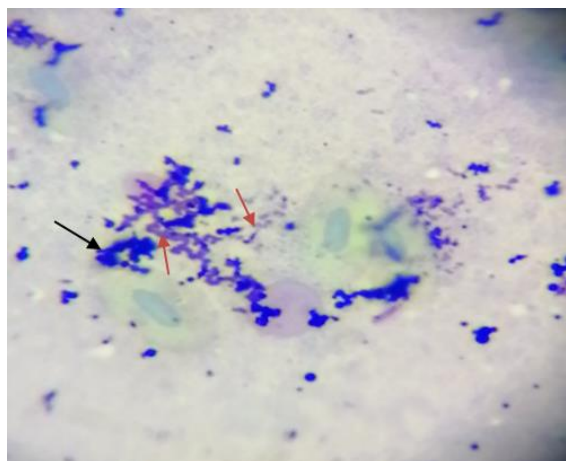


Figure 9: Liver imprint of pigeon showing staphylococcus (arrow) and cocci (arrow). Giemsa stain, 1000x.

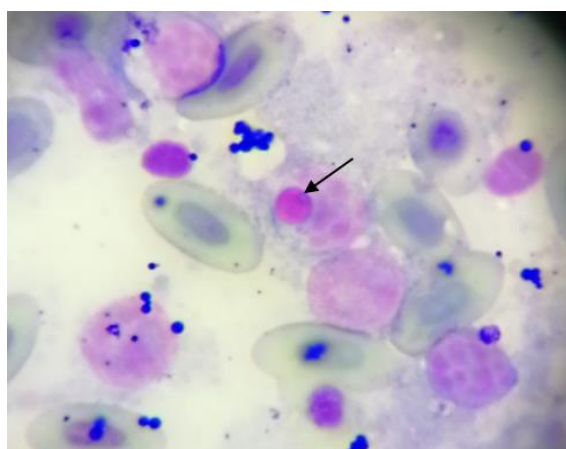


Figure 10: Liver imprint of pigeon showing intranuclear inclusion body (arrow). Giemsa stain, 1000x.

Discussion

Our results showed that there is infiltration of inflammatory white blood cells with all its types such as lymphocyte, monocyte, heterophil, eosinophil and macrophage the presence of all these type of cells in impressions indicate to chronic inflammatory reaction is due to different bacterial infections with G+ and G- bacteria and that is in agreement with (8-10). Also, our results show presence of eosinophil in the impressions and this is due to parasitic infestation with blood protozoa (plasmodium). Inside the red blood cells and this is in agreement with (11-13). In our results presence of RBC within liver parenchyma and this is normal because the liver is regarded as a haemopoietic system and this is in agreement with (14,15). Our results showed presence of vacuolated hepatocyte due to accumulation of fat droplet in its cytoplasm as a reason of

metabolic problems of fat sin the body and this is in agreement with (11,16).

The presence of G+ bacteria such as staphylococcus and streptococcus and G- bacteria such as coccobacilli in our results indicates to infection with this bacterium. in pigeon and this may be act as a predisposing factor for generalized enterococcus and this in agreement with (17,18). G+ bacteria are normally present as a microflora in the body and liver and this is in agreement with (19,20). Our results showed intra nuclear inclusion body in the necrotic hepatocyte and infiltration of lymphocyte in the liver parenchyma indicates to viral infection with adeno virus and this is in agreement with (21-23). While presence of intra cytoplasmic inclusion body in our results indicates to infection with chlamydia and this is in agreement with (24).

Conclusion

We concluded that, the hepatic impression gives a diagnostic tool to aim in final diagnosis for inflammatory diseases in pigeons, in addition this impression gives a primary idea about bacteria and parasitic infection that can be present in infected pigeons

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Conflict of interest

None of the authors has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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دراسة مرضية لدور الطبعات الكبدية كوسيلة تشخيص في الحمام

زهراء عادل النعيمي و أيمن عبدالله الحياي

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الخلاصة

هدف الدراسة هو التعرف على التغيرات المرضية التي تصيب الكبد في الحمام المحلي وذلك باستخدام الطبعات أو المسحات الكبدية، وكذلك دراسة العلاقة بين محتوى هذه الطبعات لأنواع مختلفة من الخلايا والمحتوى البكتيري لتلك الطبعات، وتم استخدام حوالي ٢٠ طائرا من الحمام المحلي لهذا الغرض، وأظهرت النتائج وجود الخلايا المتغايرة بنسبة ٢١,٥٣%، وخلايا وحيدة النواة بنسبة ١,٥٢%، والحمضات بنسبة ١,٠٤%، والقاعدية عند ٠,٠١%، والخلايا البلعمية هو ٤,٠١%، خلايا الدم الأحمر بنسبة ٣١,٩%، والخلايا الكبدية المجوفة ٤,٩٤%، كما تم تسجيل خلايا غير متميزة بنسبة ٠,١٩%، كما أظهرت النتائج وجود عدوى بطفيلي الدم البلازموديوم داخل الخلايا الحمراء في اربع عينات من اصل عشرين عينة وبنسبة ٢٠%، كما أظهرت الطبعات وجود العدوى ببكتيريا المكورات العنقودية والسبحية موجبة الكرام، والإصابة أيضا بالعصيات المكورة السلبية الكرام، واستنتجنا من ذلك بان الطبعات الكبدية ممكن أن توفر وسيلة تشخيصية تساعد في التشخيص النهائي للأمراض الالتهابية التي تصيب الحمام فضلا عن ذلك فان تلك الطبعات تعطي فكرة أولية عن البكتيريا والطفيليات التي تصيب الحمام المحلي.