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Evaluation of Hematological, Biochemical and Pathological Changes in Squabs Naturally Infected with Pox Virus

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Abstract: Pigeon pox is a disease caused by virus belonging to the poxvirus group. It is a worldwide disease. The infection occurs by contact with diseased birds or through mechanical transmission of the virus by biting insects. A total of twenty squabs (2-4 weeks old) were naturally infected with pigeon pox. PCR and histopathological examination confirmed the presence of pigeon pox virus. Bollinger inclusion bodies were appeared in histopathological examination. Erythrogram results at the end of 1st week from pox lesions appearance revealed a significant decrease in erythrocytic count, PCV and Hb concentration with development of macrocytic hypochromic anemia. Leucogram revealed leucopenia, lymphopenia and heteropenia during the first week post appearances of skin nodules and leucocytosis at the end of the third week. Proteinogram showed a significant decrease in total protein and hypoalbuminemia beside a significant hyperglubulinemia. Increase in the activities of aminotransferases (AST and ALT) with significant increase of serum uric acid and creatinine level at the end of 1st week. While, squabs that kept for two weeks with adding multivitamins 1ml/3litres, showed improvement in these parameters toward the normal healthy one. It could be concluded that pigeon pox virus infection causes anemia and disturbances in liver and renal functions.

Key words: Pigeon · Pox · Virus · PCR · Anemia · Leucocytosis

INTRODUCTION

Pigeon pox is a disease caused by a virus belonging to the poxvirus group, which induce the pox disease in many species. It is a worldwide disease. The infection occurs by contact with diseased birds or through mechanical transmission of the virus by biting insects. The avipox virus subgroup includes a number of closely related viruses such as fowl pox, pigeon pox and canary pox [1, 2]. Generally the virus carried between birds through saliva possibly through shared feed and water. The virus can gain entry through minute breaks in the skin or mouth lining (often caused by being pecked) or transmitted by mosquitoes through bites. Pigeon pox disease causes economic losses due to poor body condition and poor flight endurance. The disease is seldom life-threatening and is often more of a nuisance in racing pigeons as it results in a break in the training program. Nodular lesions were visible both on head, neck, under the wings and around the cloacae [3]. Obviously, the resulting lesions will appear on featherless areas of the body, e.g. on the eyelids, around the beak and occasionally elsewhere on the body. The lesions begin as small papules and gradually advance to a wart-like thick dark scab. Eventually the scabs will fall off and complete healing generally takes place within four weeks of infection [4]. Although pox vaccines are available for use in the latter, no commercial products are available to actively immunize pigeons [5]. There are many aspects of pigeon pox disease that remain unknown so the aim of the present study was to investigate clinical, hematological, biochemical and pathological findings in pigeon, in which natural pox infection were observed.

MATERIALS AND METHODS

Experimental Design: A total of twenty squabs (2-4 weeks old) were obtained from private owner after the onset of appearance of nodular lesions suspected to be pox virus infection. Squabs were Kept for two weeks with adding multivitamins (LiquiVit*Strong: Liquid Multivitamin-Concentrate (Biochem Zusatzstoffe/

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Germany) Composed of Vitamins A, D3 and E) to drinking water as supportive treatment at dose 1ml/3litres. Samples were collected at the end of (1st week) and (3rd weeks) post appearance of nodular lesions. Another 10 healthy squabs of the same age were used like normal control.

Blood Samples: Two blood samples were collected from wing vein. The first blood sample was collected in EDTA tube and used for hematological studies. The second blood sample was collected without anticoagulant for separation of serum biochemical analysis [6].

Confirmation of the Disease (Polymerase Chain Reaction-PCR): DNA was extracted from 20 mg of cutaneous lesion using Gene JETTM genomic DNA Purification Kit (Fermentas) following the manufacturer's instructions. The APV specific PCR was performed using a primer pair as described before [7] for amplification of fpv167 gene; Forward primer: 5□-CAGCAGGTGCTAAACAACAA-3□ and reverse primer: 5□-CGGTAGCTTAACGCCGAATA-3□). The amplification reaction was carried out under the following temperature profile: 2 min at 95°C (initial denaturation) followed by 35 cycles of 35 s at 95°C (denaturation), 30 s at 55°C (annealing) and 1 min at 72°C (extension) and cycle of final extension at 72°C for 10 min. The amplified PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide.

Hematological Studies: The erythrocytic count was performed using the improved Neubaur hemocytometer with Natt and Herrick solution as diluting fluid according to the method described by Natt and Herrick [8]. The hemoglobin was estimated by cyanmethemoglobin method (after centrifugation) [9]. Packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were determined [10].

Biochemical Analysis: The serum activities of ALT and AST were determined [11]. The serum total protein, albumin, globulin level and later fractions were measured by electrophoresis [12]. The serum uric acid level [13] and serum creatinine level were estimated [14].

Statistical Analysis: The data obtained from this investigation were statistically analysed using one way ANOVA (f) test [15]. Means at the same column followed by different letters were significantly different and the highest value was represented with the letter (a).

Histopathological Examination: Small specimens from the skin nodules were collected from infected pigeons and then fixed in 10 % neutral buffered formalin for 48 h then washed overnight under running water. The samples were blocked in hard paraffin and then cut into sections of about 5 µ thickness, then stained with haematoxylin and eosin (H&E). The sections were mounted with Canada balsam and covered with cover slide to be ready for histopathological examination [16].

RESULTS AND DISCUSSION

Pigeon pox is a worldwide disease. It occurs by contact with the diseased birds or through mechanical transmission of the virus by biting insects. The disease is more frequent in spring and summer when there are large numbers of vectors and when contacts between pigeons are frequent because of training and competitions [17].

On examination, squabs appeared emaciated in nature. Several coalescing, round, yellowish, rough and firm masses were found at the head, eyelids, beak and the mouth on un-feathered parts (Figs. 1-2) and sometimes were superficially ulcerated. Histopathological examination of skin nodules revealed presence of vacuolated cytoplasm and single, round, dense eosinophilic intracytoplasmic inclusions (identified as Bollinger bodies) that is pathognomic lesions for pox viral infection. In addition to crust covering the surface, hyperplastic epiderm and feather follicle and dermal lymphoid aggregations (Figs 3-4) which agreement with Mohan and Fernandez [18]. Moreover PCR examination confirms the presence of pigeon pox virus (Fig. 5).

There are no data about the effect of pigeon pox virus on hemogram. Erythrogram results at the end of the first week post appearances of skin nodules (Table 1) revealed a significant decrease in erythrocytic count, PCV and Hb concentration with development of macrocytic hypochromic anemia in the infected pigeons. This may be a signs of regenerative anemia [10]. Leucogram of pigeon pox infected squabs (Table 2) revealed leucopenia, lymphopenia and heteropenia at the end of the first week post appearances of skin nodules that could be attributed to viral infection [19]. While the recorded leucocytosis at the end of third week from appearance of skin lesions was due to the appearance of significant heterophilia and monocytosis. Such increase in the phagocytic cells (heterophil and monocytes) could be due to tissue damaged and secondary bacterial infection [20].

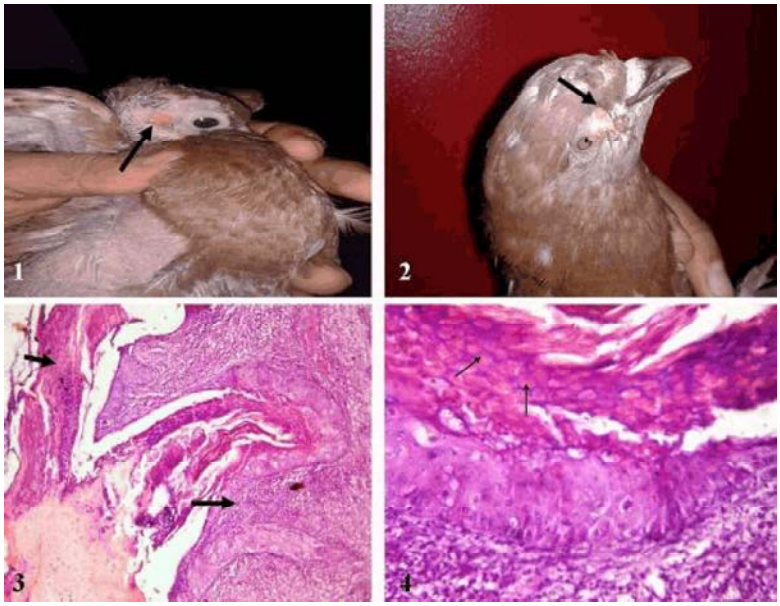

Fig. 1: Squabs showing skin nodule on head
Fig. 2: Squabs showing skin nodule on eyelid
Fig. 3: Skin of pigeons, showing crust covers the surface. hyperplastic epiderm and feather follicle. Dermal lymphoid aggregations., H&E, X300.
Fig. 4: Skin of pigeons, showing hyperplastic feather follicle containing large intracytoplasmic inclusion bodies (Bollinger bodies), H&E, X1200.

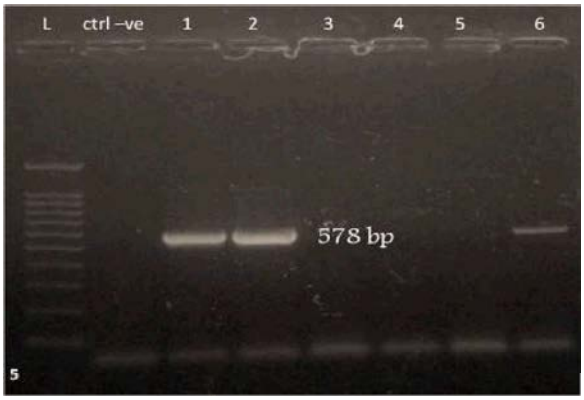

Fig. 5: PCR, pigeon pox virus

Table 1: Erythrogram of normal and infected squabs with pox virus after 1 and 3 weeks (mean values ±SE)

	Parameters					
Groups	RBCs (×10^6 µl)	Hb (g %)	PCV (%)	MCV (fl)	MCH (pg)	MCHC %
Healthy squabs	2.98^a ±0.26	10.98^a ±0.32	35.40^a ±0.33	118.74^b ±1.46	36.83^a ±1.09	31.01^a ±0.81
1st week pox infection	2.41^b ±0.22	7.96^c ±0.16	32.66^b ±0.33	135.54^a ±0.65	33.05^b ±0.59	24.39^c ±0.47
3rd week pox infection	2.90^a ±0.08	9.66^b ±0.17	34.90^a ±0.25	121.54^b ±4.22	33.53^b ±0.93	27.71^b ±0.59

RBC red blood corpuscles Hb hemoglobin
PCV packed cell volume MCV mean corpuscular volume
MCH mean corpuscular hemoglobin
MCHC mean corpuscular hemoglobin concentration

Table 2: leukogram of normal and infected squabs with pox virus after 1 and 3 weeks (mean values ±SE)

	Parameters				
Groups	WBCs ($X10^3$ / µL)	lymphocyte ($X10^3$ / µL)	Heterophil ($X10^3$ / µL)	Monocyte ($X10^3$ / µL)	Esinophilis ($X10^3$ / µL)
Healthy squabs	12.75 ^{b}±0.14	7.03 ^{a}±0.13	4.84 ^{b}±0.05	0.29 ^{b}±0.04	0.48 ^{a}±0.02
1st week pox infection	9.29 ^{c}±0.13	5.32 ^{b}±0.12	3.27 ^{c}±0.04	0.36 ^{b}±0.03	0.27 ^{b}±0.03
3rd week pox infection	14.49 ^{a}±0.60	7.86 ^{a}±0.28	5.54 ^{a}±0.29	0.54 ^{a} ±0.05	0.44 ^{a}±0.05

WBCs white blood cell

Table 3: Proteinogram of normal and infected squabs with pox virus after 1 and 3 weeks (mean values ±SE)

	Parameters					
Groups	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	α globulin (g/dl)	β globulin (g/dl)	γ globulin (g/dl)
Healthy squabs	3.66 ^{a}±0.02	1.31 ^{a} ±0.03	2.35 ^{c} ±0.02	0.68 ^{b} ±0.01	0.72 ^{a} ±0.01	0.95 ^{c} ±0.03
1st week pox infection	3.45 ^{b} ±0.04	0.99 ^{b} ±0.03	2.45 ^{b} ±0.03	0.75 ^{a}±0.01	0.59 ^{b} ±0.01	1.11 ^{b} ±0.03
3rd week pox infection	3.48 ^{b} ±0.04	0.93 ^{b} ±0.02	2.54 ^{a} ±0.03	0.72 ^{a} ±0.01	0.61 ^{b} ±0.01	1.21 ^{a} ±0.02

Table 4: Some serum enzymes, creatinine and uric acid of normal and infected squabs with pox virus after 1 and 3 weeks (mean values ±SE)

	Parameters			
Groups	AST (U/l)	ALT (U/l)	Creatinine (mg/dl)	Uric acid (mg/dl)
Healthy squabs	64.42^c±0.66	18.47^c ±0.50	0.75 ^{c} ±0.01	4.38 ^{c} ±0.04
1st week pox infection	89.43^a ±0.91	29.05^a ±0.68	1.04 ^{a} ±0.02	6.36 ^{a}±0.15
3rd week pox infection	79.52^b ±1.29	26.63^b ±0.57	0.89 ^{b} ±0.02	5.79 ^{b} ±0.08

AST Aspartate aminotransferase, ALT Alanine aminotransferase

Proteinogram (Table 3) showed a significant decrease in total protein at the end of the first week post appearances of skin nodules. Such decrease in the total protein was due to hypoalbuminemia. The significant hypoalbuminemia could be due to lowering of feed intake and disturbed metabolism of the liver [21]. The results recorded a significant hyperglubulinemia; this increase was due to increase of alpha and gamma globulins. The significant increase in alpha globulin was attributed to it is acute phase protein that typically increase with inflammation and tissue injury [22]. While the significant increase in gamma globulin was due to the response of the body to the antigenic stimuli which led to sensitization of B lymphocyte for production of antibodies [23].

There are no available literatures about the effect of pigeon pox virus on hepatic function. The increased activities of aminotrasnferases (AST and ALT) at the end of the first week post appearances of skin nodules (Table 4) were associated with hepatocellular damage [6]. The present work showed a significant increase in the serum AST and ALT activities in the diseased group. This may be due to the degenerative changes, induced by pigeon pox virus in the liver [24].

The present work showed that the pigeon pox virus damaged the renal tissue as clarified by the biochemical investigation. There are no data about the effect of pigeon pox virus on renal functions. The recorded renal damage was reflected by significant increase of serum uric acid and creatinine level at the end of the first week post appearances of skin nodules. Hyperurecemia, in birds, occurs with starvation, gout, massive tissue destruction and renal diseases [25]. Some investigators think that the serum creatinine may become elevated in birds with renal diseases, but less reliably than the uric acid [26].

The biochemical parameters of infected squabs improved significantly at the third week, compared with that of the first week measurement [4].

CONCLUSION

It could be concluded that pigeon pox virus infection causes anemia and disturbances in liver and renal functions.

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