Lymphoid Leukosis in Fayoumi Birds Reared in Countryside

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Abstract

Avian leukosis, an economically important viral disease of laying type birds is not only prevalent in Pakistan but also in other countries. The present study was aimed at describing morbidity, mortality, hatchability and egg production and pathology in naturally occurring cases of avian leukosis in Fayoumi commercial layers in Punjab, Pakistan. The morbidity, mortality, hatchability and egg production were 37.75%, 5.38%, 62.25% and 68.6%, respectively. Clinical signs including emaciation, pale and anemic coxcombs, prominent keel bones, and whitish sclera in affected birds were frequently observed. Postmortem examination revealed extensively enlarged liver that occupied the entire abdominal cavity. From small to large multiple whitish nodular necropsied areas were present on dorsal and ventral surfaces of the liver. Spleen was enlarged with marbling appearance. Bursae were significantly enlarged and significantly enlarged well differentiated multiple whitish nodules were observed cranial to ovarian clutch. Such whitish nodules were also present on intestinal and uterine tube serosal surfaces. Microscopically, entire parenchyma of liver was massively replaced by neoplastic cells. A few healthy hepatocytes were left those were also surrounded by neoplastic cells. Similar cell population was also infiltrating the splenic parenchyma, intestines and uterine tube. The PCR confirmed avian leukosis virus by amplification of specific and conserved fragment gene of 545 bp. The results of present study confirmed the presence of lymphoid leukaosis in Fayoumi egg type birds which is first time reported from Pakistan.

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Keywords: Lymphoid leukaosis; Fayoumi; Egg-type birds; Pathology

Introduction

The economy of Pakistan is agro-livestock based and this sector is playing a pivotal role in rural socio-economic development (Faroqui et al., 2017; Shah et al., 2017). This sector is a source of income for the rural people, thus playing a key role in poverty alleviation (Khan et al., 2017). Livestock sector is contributing to the agriculture value added approximately 58.6% and 11.6% to the overall GDP in Pakistan (Sohab and Jamil, 2017). Among livestock sector poultry industry has its own importance.

Among poultry breeds, Fayoumi breed is tough, compatible to hot climate and very good foragers and layers. Usually matures fast and starts laying by 18-20 weeks while cockerels start crowing 6-8 weeks (Padhi, 2016). Due to these features, this breed is more popular in rural areas of Pakistan. This breed is thought to be resistant to most of the infections, especially viral and bacterial infections, however, some of the viral infections like avian leukosis do infect this breed and hamper its production and performance.

Myeloid leukemia caused by a retrovirus (Avian Leukosis/Sarcoma Group of Retroviruses) is characterized by considerable losses in poultry production throughout the world. In subgroup J viral strains, diversity has been documented from various parts of the world such as North America, Europe and South East Asian countries. Avian leukosis due to J sub type virus has been reported in commercial laying flocks and local chicken breeds in African, Asian, and South East Asian countries including Egypt (Kidary et al., 2015), China (Xu et al., 2004; Chen et al., 2005), Turkey (Venugopal et al., 2000), Taiwan (Wang and Juan, 2002), India (Gopal et al., 2012; Swathi et al., 2012; Sagarika et al., 2017) and Malaysia (Thapa et al., 2004).

Leukosis infects a wide range of birds including captive wild and turkey birds (Dong et al., 2015; Reimusová et al., 2016). Published literature indicated severe economic losses due to ALV-J infection in layer chickens (Gingerich et al., 2002; Xu et al., 2004). The pathological lesions associated with avian leukosis such as whitish nodules, particularly at the visceral organs, serosal surfaces of intestinal coils and uterine tube have been reported in different avian species. These nodules are predominantly consisting of myelocytes/myeloblastic cells with characteristic...
The PCR reaction conducted the necropsy. The impression smears prepared from peripheral blood and spleen, stained with Geimsa stained, did not show any evidence of spirochetes.

Necropsy

Twenty birds from each farm exhibiting the severe clinical signs including anemia, prominent keel bone, leg weakness and hemorrhages in feather follicles were humanly euthanized and postmortem examination was carried out. Morbid organs including liver, kidneys, spleen, lungs, heart, pancreas, ovary, proventriculus, intestine, skeletal muscle, cerebrum and sciatic nerves exhibiting the lesions were collected and preserved in 10% buffered formalin. As the lesions in three flocks were the same, therefore, a common picture of lesions was developed. Gross lesions were recorded.

Histopathological Studies

Morbid organs were collected and preserved as mentioned above to prevent postmortem changes and for fixation. The tissues were completely immersed in formalin solution and kept for 10 days at room temperature for proper fixation. Then tissues were subjected to washing, dehydration in ascending concentrations of alcohol, clearing in xylene, impregnation and embedding in paraffin (Bancroft and Gamble, 2008). Then the tissues were subjected to paraffin sectioning. Briefly, 4-5 µm thick sections were stained with hematoxylin and eosin staining technique (Magouz et al., 2018). Slides were examined for histopathological changes by two pathologists, and if any difference was found, a third pathologist was consulted for opinion.

Molecular Detection

DNA was extracted from liver, spleen and tumor tissue of infected birds using standard phenol chloroform isoamyl protocol (Sambrook and Russel, 2001). For molecular detection and confirmation of virus, DNA was extracted from liver, kidneys, oviduct and spleen of morbid and dead birds. The viral DNA was isolated using TRIZOL® reagent method (Jackwood et al., 2007). The ALV-J-specific primers of avian leukosis virus using F; 5’-GAAGCAGACAAATAGGGACTG-3’ and R; 5’-TTGACCTAGGTTATCCATCTC-3’ were carried out targeting the ALV-J genes and for the confirmation of samples. The PCR was performed using Master Mix (Invitrogen, USA) and following the PCR program as described earlier (Xu et al., 2004). The PCR reaction consisted of deionized water (30 µL), 10x buffer (5 µL), 200 mM each dNTP (2 µL), Taq DNA polymerase (1 µL), primers (2 µL) and DNA template (2 µL) in a total of 42 µL volume. The PCR product was run on 1% agarose gel for electrophoreses and visualized trough Gel Documentation System (Dolphin Doc, USA) (Zahid et al., 2018).
Data Analysis

Data thus collected were subjected to statistical analysis applying the Chi-square test, using the Minitab statistical software package (Anonymous, 2000). The significance level was \( P < 0.05 \).

Results

Physical Parameters

Fayoumi layers morbidity and mortality ranged from 30-45% (with an average 37.75% and 2.27-20% (5.38% average), respectively (Table 1). Morbidity and mortality varied significantly \( P < 0.001 \) among farms. Similarly, egg production and hatchability ranged varied significantly \( P < 0.001 \) among farms (Table 1).

Clinical Signs

Avian leukosis infection rendered various clinical signs such as emaciation with prominent keel bones, lethargy, somnolence, leg weakness and roughened feather were frequently observed in birds of Fayoumi layer present at all the farms. Severe anemic and pale combs were the characteristic feature in affected birds.

Necropsy Findings

The necropsy examination revealed big liver occupied the entire abdominal cavity in most of the infected birds (Fig. 1a). In some birds, whitish solid nodules over the dorsal and ventral surfaces of hens were observed (Fig. 1b). The liver was hard in consistency, borders were sharp (Fig. 1c), color lighter with widespread necrotic areas with whitish appearance were present on the dorsal and ventral surface of the liver (Fig. 1d).

Abdomen of the birds was fully packed with distended intestinal segments and the multiple nodular adenocarcinoma (Fig. 2a). Entire serosal surface of the intestines and uterine tube was covered with numerous well circumscribed encapsulated macro and micro nodules with hard consistency (Fig. 2b). The spleen with marbling appearance was enlarged and cut surface was granular. From the cut surface of spleen, prepared impression smears did not yield any spirochetes but heavy population of neoplastic cells. Bursae were enlarged and appeared as whitish nodular mass (Fig. 2c), similar extension just cranial to the ovarian clutch was also observed (Fig. 2d). Kidneys were markedly swollen, bulges out from bony sockets and some had light grey/white mottled tumors.

Histopathology

Histopathological examination of the tissue sections prepared from avian leukosis virus affected tissues showed complete organization destruction. Masses of specialized cells, i.e., myelocytes with large nuclei with peripheral location and cytoplasm was studded with eosinophilic granules were present in the affected tissues (Fig. 3a). In the affected areas, monstrous macrophages were seen engulfing the necrotic cells (Fig. 3b).

The liver sections were studded with neoplastic cells, parenchyma of liver was destroyed while few healthy hepatocytes were trapped in islands of hepatocytes and putting pressure on hepatocytes thus becoming atrophied.

### Table 1: Morbidity, mortality, egg production and hatchability of Fayoumi layers as a result of avian leukosis

<table>
<thead>
<tr>
<th>Farm</th>
<th>Morbidity</th>
<th>Mortality</th>
<th>Egg Production</th>
<th>Hatchability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>I (n = 6000)</td>
<td>1800</td>
<td>30</td>
<td>225</td>
<td>3.75</td>
</tr>
<tr>
<td>II (n = 11000)</td>
<td>4400</td>
<td>40</td>
<td>250</td>
<td>2.27</td>
</tr>
<tr>
<td>III (n = 3000)</td>
<td>1350</td>
<td>45</td>
<td>600</td>
<td>20.0</td>
</tr>
<tr>
<td>Total (n = 20000)</td>
<td>7550</td>
<td>37.75</td>
<td>1075</td>
<td>5.38</td>
</tr>
<tr>
<td>Chi-Sq Value</td>
<td>112.778</td>
<td>1215.833</td>
<td>55.988</td>
<td>55.389</td>
</tr>
</tbody>
</table>

First two farms are from Toba Tek Singh while third one from Faisalabad. Data analysis by Chi-square test and df in each case was 2 and \( P \leq 0.001 \).
(Fig. 4a and b). Cells of myeloblastic series with prominent nucleus and less cytoplasm invaded liver parenchyma. There was necrosis of hepatocytes along with diffuse and focal infiltration of uniform sized lymphoid cells, lymphoblasts, and round mononuclear cells. Mitotic figures were frequent in infected tissues. Hyperplasia of the bile ducts with massive infiltration of the mononuclear cells in the portal areas around the blood vessels and obliteration of the sinusoidal spaces were also observed. The epithelial linings of most of the bile ducts were degenerated and necrotic. Portal veins were engorged with erythrocytes.

The tissue sections from affected spleen showed less population of lymphocytes and increased tumor cells in both the red pulp and the white pulp destroying the normal histological arrangement of splenic cells.

All layers of intestines were invaded by neoplastic cells (Fig. 5a and b). Inter-villus spaces were also infiltrated with neoplastic cells. Villi became denuded with the sloughing of epithelial cells along with hemorrhages and necrosis. There was massive population of neoplastic proliferation in the form of clusters with higher number of mitotic figures destroying the bursal follicular pattern (Fig. 5b). In the heart, multifocal aggregates of myelocytes were observed in the myocardium and covered by the pericardium.

Ovary sections were characterized by invasion of normal tissue by homogenous population of lymphoblasts, myeloblastic cells/myelocytes in the stromal layer underneath the granulosa cells. Moreover, well circumscribed embedded mass of metastatic adenocarcinoma among the ovarian clutch, thick tunica muscularis with onion layer...
pattern arrangement (Fig. 6a and b) was observed. Different stages of cavernous hemangiomas were recorded in ovary sections (Fig. 6b). A few normal follicles were also seen (Fig. 6a).

**Molecular Diagnosis**

The PCR confirmed avian leukosis virus by amplification of specific and conserved fragment (ALV-J-specific primers H5/H7) gene. The amplicon of ALV-J yielded 545bp PCR product (Fig. 7).

**Discussion**

Avian leukosis causes severe economic losses in the layer, broiler breeders and backyard poultry. In spite of successful eradication programs and extensive control measures, avian leukosis due to subgroup J virus induces huge losses to poultry industry throughout the world (Fadly and Smith, 1999; Malkinson et al., 2004; Thapa et al., 2004). Various reports are available about the incidence of avian leukosis in various regions of the world (Wang and Juan, 2002; Chen et al., 2005; Sun and Cui, 2007; Gopal et al., 2012).

The present study describes the gross and microscopic lesions induced by naturally occurring ALV-J infections in Fayoumi laying birds in Pakistan. At postmortem examination, extensive liver enlargement with multiple nodular and raised areas on entire surface of the liver and splenomegaly was observed. Cut surface of the spleen showed marbling appearance. In Chinese commercial layer chicken due to naturally occurring avian leukosis infection, swollen kidneys along with hemorrhagic thymus, muscle and glandular stomach are described (Wang and Juan, 2002; Xu et al., 2004). In present study, extensive metastasis in liver, spleen and thymus were also reported in ALV-J infection in chickens (Pandiri et al., 2009; Payne and Nair, 2012) and avian leukosis virus fowl glioma (Hatá et al., 2005). Nodular tumors due to this infection have also been reported in turkeys infected with acute transforming ALV-J strain 966 (Venugopal et al., 2000). Enlarged lymphoid nodules in the bursa Fabricius are considered pathognomonic for lymphoid leukosis.

In the present study, histopathologically liver revealed extensive neoplastic cells infiltrating in the parenchyma along with cells of myeloblastic series having with projecting nucleus, scant cytoplasm. Neoplastic cells were also seen in red pulp and the white pulp of the spleen. Similar neoplastic cells infiltrated in various layers of intestines, similarly uterine walls were extensively infiltrated with neoplastic cells. Infiltration of neoplastic cells could be due to mutational changes in the β-cells of the follicles of the bursa of Fabricius which might result in switch on the oncogenes and inhibiting the tumor suppressor genes by viral genome. It could also be due to the enhanced production of oncogenic proteins and enhanced cell proliferation or growth promoting Ras PI (3) and m-TOR signaling along with their effectors.
including raf, ERK family (Fung et al., 1983). Moreover, uniform population of lymphoblast with large vesicular nuclei and basophilic cytoplasm were infiltrating in infected tissues. Previously, the diffuse and distinct nodular pattern of such types of tumors has been reported (Gopal et al., 2012).

The tissue sections from infected livers revealed extensive loss of hepatocytes and bile duct hyperplasia with infiltration of solid sheets of lymphoblast. Similarly, other organs showed massive infiltration of tumorous cells in the hepatic parenchyma, blood vessels and myeloid areas (Venugopal et al., 2000; Gingerich et al., 2002). Previously, in the bursa Fabricius, lymphoid follicles at early stages showed proliferations of lymphoblasts and infiltration of differentiated myelocytes and erythroblasts due to ALV-J infection in turkeys (Venugopal et al., 2000; Payne and Nair, 2012). Recently, from Pakistan, avian leukosis infection in commercial egg laying birds without symptoms/clinical signs has been reported (Akram et al., 2012).

The confirmation of avian leukosis virus infection, in the present study, was carried out using PCR. The amplicon of ALV-J yielded 545bp PCR product. Previously, avian leukosis virus infected birds with tumors in oviduct, liver, spleen and kidneys have also been confirmed (Xu et al., 2004). Previously, various techniques including immunohistochemical localization, amplification of the genome through PCR, nested PCR and sequence analysis of PCR products of avian leukosis virus have been used for confirmation of the virus (Stedman et al., 2001; Davidson and Borenshtein, 2002; Zeng et al., 2014). Evidence of variation in susceptibility of avian leukosis within different breeds of chickens and layers is available (Smith et al., 1998; Bacon et al., 2004).

This is the first ever study about the naturally occurring avian leukosis caused by ALV-J virus in commercial Fayoumi layer in Pakistan. Further investigations are suggested regarding evolutionary relationships between different species of virus through nucleotide analysis and construction of phylogenetic trees. The presence of the leukosis in inbred laying birds brought a new challenge to the poultry industry and may inflict significant economic losses. Therefore, virus screening and elimination should be made to eradicate the disease in the region.

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