Clinicopathological Studies on Gentamicin Toxicity in White Leghorn Commercial Layers

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ABSTRACT
Gentamicin is an effective and economical drug used to control infectious diseases in poultry but is highly toxic and had slow clearance from the body. This study aimed to report three cases of gentamicin toxicity in three White Leghorn (WLH) layer flocks in different poultry producing areas of Pakistan. In first case, gentamicin was injected in a 9000 WLH layer flock @ 10 mg/kg body weight (BW) for seven times during 9-15 weeks for age. In second case, gentamicin was injected in a flock of 7500 WLH layers @ 25 mg/kg BW for four times during 17-18 weeks of age. In third case, gentamicin was injected in flock of 16000 WLH layers @ 22.22 mg/kg BW three times in 20-21 weeks of age. Flock wise mortality was 8.69, 82.63 and 71.86%, respectively. Birds were dehydrated, emaciated and had prominent keel bone. Clinical signs included dehydration, decreased body weight leading to emaciation, decreased feed intake, increased water intake and watery diarrhea. Necropsy revealed prominent keal bone, shrunken muscles swollen kidneys bulging out from bony sockets. Petechial and echymotic hemorrhages were present on heart and skeletal muscles. Liver was enlarged with hemorrhagic streaks on its surface. Microscopically, hemorrhages and acute tubular necrosis was recorded in kidneys. Liver had hemorrhages, cellular infiltration and vacuolar (fatty) degeneration of hepatocytes. From the results, it could be concluded that overdosing and repeated administration of gentamicin was highly toxic to birds.

INTRODUCTION
Gentamicin belongs to aminoglycoside group of antibiotics and is an important therapeutic agent used in poultry and animals. It is used in poultry birds for the treatment of different diseases, e.g., colibacillosis, salmonellosis, etc. (Giurov, 1986). Gentamicin use in Pakistan is common as it is economical and effective. It is used in combination with tylosin, lincomycin and other antibiotics. It is also co-administered with inclusion body hepatitis/hydropericardium vaccine for prevention and treatment of different bacterial diseases in broiler and layers (Khan et al., 2008). Gentamicin in many regions of the world is unofficially administered to day old chicks to prevent early chick mortality (Saleemi et al., 2009). It was retained in muscles and kidneys of turkey birds for 45 and 75 days, respectively which makes it use questionable in birds intended for human consumption (Shem-Tov et al., 2003). Experimental gentamicin toxicity has been studied in day old broiler chicks (Saleemi et al., 2009) and no observable effect level (NOEL) of single intramuscular injection of gentamicin was 10 mg/kg BW. In an experimental study, Khan et al. (2008) found 5-10 mg/kg BW gentamicin I/M injection at the age of 1 and 3 days safe. In this study, we have reported high mortality associated with the treatment of gentamicin in White Leghorn layers.

HISTORY, METHODOLOGY AND RESULTS
Case A
This farm was located in district Vehari of southern Punjab (Pakistan). Total flock size was 9000 commercial WLH layer. Chick mortality up till 10 days of age was
At 8 week of age, ciprofloxacin and tylosin were administered orally for 5 days. After two days of oral therapy birds were injected intramuscularly with gentamicin + Bacolam (Amoxicillin + Colistin) three times on alternate days. Dose rate of gentamicin was 10 mg/kg BW. After three weeks interval (age 12 weeks), gentamicin + Bacolam (Amoxicillin + Colistin) were again injected at the same dose rate for four times on alternate days. During six weeks (starting from 8 week of age) period gentamicin was injected 7 times. Mortality started at 20 days after gentamicin injection at 8 weeks and reached to a maximum at 11th week and remained high up to 14th week of age (Fig. 1). In this flock, overall mortality was 8.69% (Table 1). Morbid birds were depressed, dehydrated and emaciated having prominent keel bone (Fig. 2). Necropsy examination revealed shrunk skeletal muscles that kidneys were hemorrhagic, swollen and bulging out from bony socket. Uric acid crystals were deposited in the kidneys. Liver was swollen, hemorrhagic and friable having light and dark color streaks on its surface. A consistent lesion noticed in many birds was presence of clotted blood and pinpoint hemorrhages on epicardial surface of heart (Fig. 3).

**Case B**

White Leghorn commercial layer pullets of 17 weeks of age were injected gentamicin (10% solution) four times @ 25 mg/kg BW on alternate days during two weeks period at a layer farm, Arifwala, district Sahiwal. Total flock size was 7500. Mortality started 21 days post gentamicin injection. Overall mortality in this flock was 82.63% with a peak during week 21-23 (Fig. 1). Dead birds upon necropsy showed emaciated pectoral muscles, swollen and hemorrhagic kidneys having urates (Fig. 4). Swollen and friable liver and hemorrhages present on epicardial surface were frequent gross lesions.

**Case C**

A total of 16000 commercial WLH layer (Sheikhupura) at 20 weeks of age were intramuscularly administered gentamicin @ 22.2 mg/kg BW. The same treatment was repeated three times on alternate days. Mortality started three weeks after the treatment. Mortality increased gradually from week 24 and remained high during weeks 25-27 (Fig. 1). Overall mortality in this flock was 71.86% (Table 1). Birds were depressed, emaciated having lesser feed intake and more water intake. The postmortem examination of dead birds revealed emaciation, swollen and hemorrhagic kidneys with urates. Liver was swollen and friable having streaks of light and dark color, hemorrhages were also present on epicardial surface (Fig. 3).

**Microscopic lesions**

In all three cases, liver and kidneys were collected during postmortem examination, fixed in 10% neutral buffered formalin and processed for histopathological examination by paraffin embedding method. Sections of 5 µm thickness were cut and stained with hematoxylin and eosin (Bancroft and Gamble, 2007).

Kidneys of all birds from three cases had condensed and shrunken nuclei of epithelial cells of convoluted tubules indicating acute tubular necrosis. In some birds these changes were more severe involving extensive areas of tubular necrosis (Fig. 5). Glomeruli were congested having pinkish material in the urinary spaces. At few places congestion was also present in renal parenchyma. Liver of birds showed vacuolar degeneration characterized by vacuoles of different sizes present in the cytoplasm of hepatocytes. These vacuoles were clear in appearance, round in shape and had sharp boundaries suggesting fatty change (Fig. 6). Individual cell necrosis of hepatocytes was present. The congestion was present throughout the parenchyma of liver. At many places cellular infiltration was also present.

**Fig. 1:** Mortality pattern in WLH layers in three flocks (Case A-C).

**DISCUSSION**

There is scanty information available about toxicopathological effects of gentamicin in chicken. Experimentally produced pathological effects of gentamicin have been reported in growing cockerels of 5-6 weeks of age (Khan et al., 2008), day old broiler chicks...
(Saleemi et al., 2009), growing broilers (Javed et al., 2008) and owls (Bauck and Haigh, 1984). In these studies the safe dose of gentamicin has been reported as 10 mg/kg BW in day old broilers (Saleemi et al., 2009) and growing al., 2009) and growing White Leghorn cockerels (Khan et et al., 2008).

Table 1: Overall mortality recorded in White Leghorn layer due to gentamicin treatment

<table>
<thead>
<tr>
<th>Case</th>
<th>Age of flock (weeks)</th>
<th>Total flock</th>
<th>Mortality No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8-15</td>
<td>9000</td>
<td>782</td>
<td>8.69</td>
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<tr>
<td>B</td>
<td>19-25</td>
<td>7500</td>
<td>6197</td>
<td>82.63</td>
</tr>
<tr>
<td>C</td>
<td>23-29</td>
<td>16000</td>
<td>11497</td>
<td>71.86</td>
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<tr>
<td>Overall</td>
<td></td>
<td>32500</td>
<td>18476</td>
<td>56.85</td>
</tr>
</tbody>
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Fig. 2: Photograph showing prominent keel bone with smaller pectoral muscles in layer birds repeatedly injected with gentamicin.

Fig. 3: Photograph showing hemorrhages on heart surface and swollen liver.

Fig. 4: Photograph showing swollen and congested kidneys with urates.

Fig. 5: Photomicrograph of kidneys showing karyorrhectic changes indicating acute tubular necrosis and congestion. H&E. 400X.

Fig. 6: Photomicrograph liver showing vacuolar degeneration, individual cell necrosis and congestion. H&E. 400X.
Gross and histopathological alterations observed in these cases were similar to those reported in experimental studies in birds (Khan et al., 2008; Saleemi et al., 2009), humans (Bourguignon et al., 2010; Beger et al., 2010) and other animals (Faraq et al., 1996; Lakshmi and Sudhakar, 2010). In present cases another persistent finding was hemorrhagic lesions on epicardial surface. No such lesions have been reported by previous workers. The variation in mortality of the birds in the reported cases could be due to the doses of gentamicin administered over multiple times and age of the birds. However, the pattern of mortality in all three cases was similar to each other. In the present case studies all the flocks were administered higher than safe dose level of gentamicin (10mg/kg BW) and the treatment was repeated many times. Gentamicin is a known nephrotoxic antibiotic for human (Ali, 1995; Selby et al., 2009; Lv et al., 2010), farm animals (Godber et al., 1995), rats (Bibu and Jor, 2010; Ozaki et al., 2010; Yaman and Balikci, 2010; Manali et al., 2011) and pet birds (Bird et al., 1983). It was assumed that repeated injections of gentamicin might have resulted in the severe nephrotoxicity in these cases. The history of these multiple overdose injection of gentamicin, gross and histopathological lesions and pattern of mortality had close resemblance with those of earlier reported experimental studies. These findings along with the absence of any other clinically apparent toxicity or infection were the basis of diagnosis of gentamicin toxicity in these cases.

It has been concluded from findings of present field and previous experimental studies that depending upon the type of birds, age, dose and frequency of treatment, gentamicin clinical toxicity starts after 2-3 weeks of administration and may continued upto five weeks. It is suggested that gentamicin should not be administered above the safe dose level (10 mg/kg BW) and its repeated use should be avoided.

REFERENCES


