



Prevalence of gizzard erosion among commercial layer chicks in the mining area of Karnataka

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ABSTRACT

An epidemiological study was conducted to determine the prevalence of various disease conditions among commercial layer flocks in the mining area of northern Karnataka. Flocks — 3 younger and 9 adult — showing poor performance were chosen from 6 different multiage commercial layer farms in the mining area for the study. Flock details including, breed, age, clinical history, mortality pattern, body weights and egg production were systematically recorded. In addition, ND-HI titers, IBD and IBV ELISA antibody titers and trace elements (iron, manganese, lead and cadmium) concentrations in feed, water and tissue samples, gross and microscopic lesions were studied. The study revealed gizzard erosion with cumulative mortality of 8.72, 8.3 and 16.83% in 3 flocks aged 2, 4 and 5 weeks, respectively. Anorexia, uneven growth, dullness, 26.83–48.19% lower body weights, droopy wings, ruffled feathers, pasty vents and penguin posture were the predominant clinical signs observed. Feed samples revealed Fe, Mn, Pb and Cd at 1,100–1,200, 240–340, 4.8–5.2 and 0.0–0.16 ppm, respectively; however water samples did not reveal any abnormality. The liver, kidney and gizzard tissues had higher levels of Fe, Mn, Pb and Cd. The study indicated that exposure to higher levels of Fe and Pb trace elements induced oxyradical cell injury leading to immunosuppression and gizzard erosion.

Key words: Chickens, Gizzard erosion, Immunosuppression, Mining, Trace elements

Bellary and Koppal districts in Karnataka are major egg producing areas with 6.0 million layer population and 5.35 million eggs per day. During 2005–06, the iron ore mining activities increased in these areas generating enormous dust containing various trace elements and pollute the environment. The poultry farms that exist in the vicinity of mining operations are in constant exposure to mine dust. Prolonged exposure to mine dust results in immunosuppression and vaccination failure in chickens (Muniyellappa *et al.* 2012, 2013). Gizzard erosion was reported in younger birds and several causative factors were implicated. The causes include deficiency of vitamin B₆ (Daghir and Haddad 1981), bacterial infection of the gizzard wall (Fossuma *et al.* 1988), gizzerosine (Tiljar *et al.* 2002), and group I avian adenovirus (Ono *et al.* 2003). Various pathological conditions ranging from inflammatory to neoplastic conditions affecting different organs can occur consequent to immunosuppression in chickens (Muniyellappa 2011). An epidemiological study was taken

up to determine various pathological conditions among commercial layer flocks in the mining area of Karnataka. The present study reports prevalence of gizzard erosion in commercial layer chicks in the mining area.

MATERIALS AND METHODS

Survey work, sample collection and analysis: Six commercial layer poultry farms were randomly identified in the mining area of North Karnataka; 2 each in Bellary, Hospet and Koppal. Flocks — 3 younger and 9 adult — showing poor performance were chosen from these different multiage commercial layer farms. Flock details (including source of chicks, breed and age), history, clinical signs, morbidity, mortality, egg production and details of vaccinations were recorded. Feed and water samples were collected in sterile containers for trace element analysis. Tissue pieces from liver, kidney and gizzard were collected in ice and stored at –20°C till used for the estimation of trace elements. Feed, water and tissue samples were processed and estimated for trace elements by atomic absorption spectrophotometer using procedures described previously (Gowda *et al.* 2003). Blood samples were collected in non-heparinized tubes from each of the affected flock. Serum was separated and stored at –20°C for subsequent analysis. Serum samples were used for measuring vaccination titers for IB and IBD by indirect enzyme-linked immunosorbent assay using commercially

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available ELISA test kits. HI test was performed for determining the antibody titers for ND as described previously (Allan and Gough 1974).

Gross and histopathology: Post-mortem examination was conducted on dead and moribund birds and gross lesions were recorded. Tissue samples from visceral and lymphoid organs were collected and fixed in 10% neutral buffered formalin (NBF). The tissue samples were processed and embedded in paraffin, sections of 4–5 μ thickness were cut and stained with hematoxylin and eosin and examined by light microscopy for histological changes.

RESULTS AND DISCUSSION

Occurrence of gizzard erosion and clinical signs: Gizzard erosion was recorded in 3 flocks aged 2, 4 and 5 weeks with a cumulative mortality of 8.72, 8.3 and 16.83% respectively. Nakamura *et al.* (2002) have reported variable mortality rates ranging from 4 to 50% in chickens with gizzard erosion. Anorexia, uneven growth, low body weights, dullness, droopy wings, ruffled feathers, pasty vents and penguin posture (Fig.1) were the predominant clinical signs observed in the affected birds. The mean body weights were 26.83–48.19% lower than the standard. The uneven growth and lowered body weights were attributed to the anorexia due to the gizzard erosion and immunosuppression. Heavy metals may have interfered with the nutrient absorption thereby reducing the nutrient availability to chicken leading to in reduction in body weights. Penguin posture observed in the present study could be attributed to the pain caused due to lesions in the gizzard consequent to Pb toxicity.

Trace elements in feed water and tissue samples: In the present study, the levels of Fe, Mn, Pb and Cd in feed samples were 1,100–1,200, 240–340, 4.8–5.2 and 0–16 ppm respectively. The feed Fe and Mn levels in poultry farms were higher compared to recommended level (40 ppm Fe and 60–80 ppm Mn) in the layer feed (Reddy and Rao 2001). The feed Pb level was in the range of 4.8 to 5.2 ppm; while, the feed Cd level was lower than 0.16 ppm. Effects of water constituents on performance of layers and broilers were studied (Coetzee *et al.* 2000). Water samples from the mining area did not show any abnormal mineral level compared to earlier studies (Vohra 1980, Coetzee *et al.* 2000).

Fe, Mn, Pb and Cd levels observed in liver, kidney and gizzard tissue samples from chicks with gizzard erosions are presented in Table 1. The liver Fe level was higher compared to previous studies on dry weight basis (Kienholz *et al.* 1974). The kidney and gizzard Fe concentrations were

lower compared to the previous findings in chickens (Kienholz *et al.* 1974). The liver Mn levels were lower and the kidney Mn concentration was higher compared to previous findings in chickens (Kienholz *et al.* 1974). The Pb concentration in liver, kidney and gizzard tissues was higher compared to the previous findings in chickens (Teofila *et al.* 2005). Similarly, the Cd concentration in liver, kidney and gizzard samples was also higher compared to the previous findings in chickens (Teofila *et al.* 2005). Muniyellappa *et al.* (2012) demonstrated that feeding of mine dust fortified feed to chickens resulted in increased relative organ weights and accumulation of trace elements in visceral organs. In the present study the higher tissue concentration of trace elements could be due to the continuous exposure to environmental contaminants through feed in the mining environment.

Serum antibody levels: The mean ($\pm$ SD) HI titers ($\log_2$) observed in the 3 flocks ranged from 3.92 $\pm$ 0.91 to 7.85 $\pm$ 0.66 suggesting a protective level of HI antibody titer (Mazija *et al.* 2009) could be due to the booster vaccinations. IBD geometric mean titer (GMT) and coefficient of variation (CV) were in the range of 12–3448 and 23.31 and 120.54. Similarly, IB GMT and CV were 1742–3848 and 40.63–69.50 respectively. IBD antibody titers indicating either low maternal antibody levels and distributed randomly in the flock or poor response to vaccination. While, the IBV antibody titers indicating protective titers and presence of uniformly distributed antibodies in the flock (Leerdam and Kuhne 2009) but the vaccination response is poor. The poor vaccination response in the present study could be due to suppression of immune cells activity by oxidative attack consequent to exposure to mine dust. Prolonged exposure to high levels of trace elements, particularly lead and cadmium, leads to immunosuppression (Muniyellappa *et al.* 2012).

Gross and histopathology: Gizzard showed discoloured, rough and thickened lining (Fig. 2) with erosions, more frequently around the duodenal orifice (Fig.3). Microscopically, increased corrugations (Fig.4) thickened koilin layer with multifocal fissures (Fig.5), vacant spaces and diffuse erosions along with masses of coccoid bacteria were observed (Fig.6). In addition, severe haemorrhages, multifocal colonies of bacteria in sub mucosa and muscularis along with degeneration, necrosis and infiltration of inflammatory cells were also recorded in the present study. The bursa, thymus and spleen were atrophied and histologically showed mild to moderate lymphoid tissue depletion and diffuse haemorrhages. Our gross and

Table 1. Mean Fe, Mn, Pb and Cd levels (ppm) in liver, kidney and gizzard samples

Flock ID (Age in weeks)	Liver (n=12 samples)				Kidney (n=12 samples)				Gizzard (n=12 samples)			
	Fe	Mn	Pb	Cd	Fe	Mn	Pb	Cd	Fe	Mn	Pb	Cd
KAF1(2)	245.00	11.50	15.50	1.25	187.50	10.02	14.75	1.25	102.50	6.45	14.00	1.37
KAF2(4)	490.00	5.97	55.72	1.81	995.00	19.27	13.75	2.87	400.00	10.05	20.25	2.97
KBF1(5)	695.00	9.35	14.00	1.57	505.00	6.57	17.00	2.65	410.00	8.17	15.25	2.17

microscopic findings were similar to the previous observations in natural outbreaks of gizzard erosion in commercial layer flocks (Fossuma *et al.* 1988, Sairam *et*



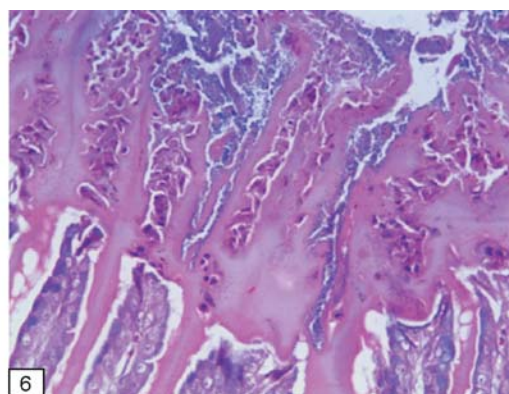
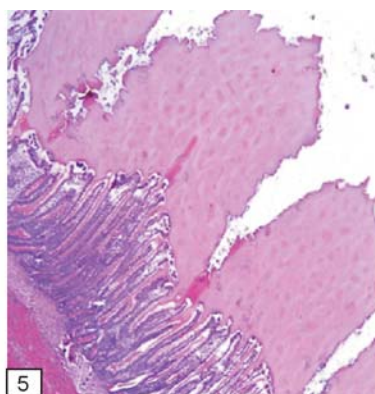
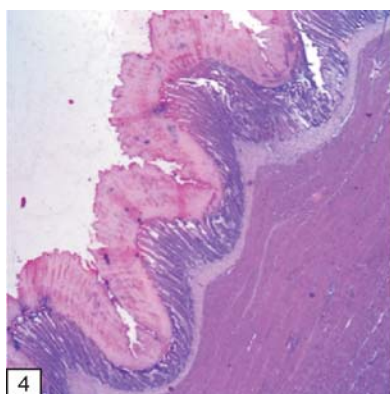
Fig. 1. Two-week old commercial layer chicks exhibiting dullness, droopy of wings and ruffled feathers with sitting on hock joints or penguin posture.

al. 2002). Several infectious and non-infectious agents are implicated for gizzard erosion in poultry. In the present study the gizzard erosion could be attributed to ingestion of high levels of trace elements. In an experimental study, gizzard erosion similar to field observations was observed in chicks exposed to mine dust at 1.5 and 3.0% level in feed (Muniyellappa 2011). The inflammatory response observed in gizzard could be due to cell injury mediated by metal generated oxyradicals and peroxidation of lipid membranes of mitochondria, microsomes and peroxisomes (Valko *et al.* 2005). Further, consequent to trace element induced immunosuppression (Rada 2008, Muniyellappa *et al.* 2012, 2013) might have resulted in poor growth mortality due to secondary bacterial infections.

Gizzard erosion was recorded in 3 younger commercial layer flocks aged between 2 and 5 weeks with a cumulative depletion of 8.3 - 16.83% in the mining area. Anorexia, uneven growth, lower body weights, droopy wings ruffled feathers pasty vents and penguin posture were the predominant clinical signs. Lower vaccination response to IB and IBD was recorded. Tissue samples showed higher levels of Fe, Mn, Pb and Cd. Occurrence of gizzard erosion was attributed to immunosuppression consequent to exposure to high levels of trace elements particularly Fe and Pb in the mining area.



Figs 2–3. **2.** Gizzards from 4-week-old commercial layer chicks showing discoloured, thickened, and rough koilin lining. **3.** Gizzard from 2-week-old commercial layer chick showing rough and thickened koilin lining with erosions predominantly around duodenal orifice.



Figs 4–6. **4.** Section of gizzard from 4-week-old layer chick showing increased corrugations and multiple fissures. H&E $\times 40$. **5.** Section of gizzard from 4-week-old layer chick showing increased thickening of koilin layer with multiple deep fissures and degeneration and necrosis of glandular tissue. H&E $\times 40$. **6.** Section of gizzard from 5-week-old layer chick showing multifocal severe erosions, deep fissures and degeneration in the koilin layer along with colonies of bacteria. H&E $\times 400$.

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