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An Outbreak of Infectious Laryngotracheitis in Layer Chickens in Libya

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Infectious laryngotracheitis (ILT) is a highly contagious acute respiratory disease of chickens that may result in high mortality and decrease in egg production. The disease is caused by herpes virus 1 (an alpha-herpes virus). This report deals with an outbreak of infectious laryngotracheitis in layer chickens in Tripoli, Libya.

Materials and Methods

In 2 poultry farms in south of Tripoli, Libya, mortality associated with respiratory signs was recorded in 30 weeks-old layer chickens. The total number of chickens in both farms was 40000 (20000 each). The morbidity was very high (up to 80%) in both flocks. Mortality was high during the first 5 days and reached up to 2.5%. Clinical signs were noted and detailed post-mortem of chickens was conducted. Representative tissue samples were collected from trachea in 10% neutral formalin and processed routinely for histopathology and 5 µm thick paraffin sections were cut and stained with haematoxylin and eosin (Luna, 1968). 10 blood samples were also collected from each flock at 30 and 31 weeks of age. Sera were separated for serological investigations.

The test was carried out using 1% Noble agar dissolved in phosphate buffer saline (pH 7.7). 10 ml of Noble agar was poured into each sterile Petri dish and a pattern of six outer wells surrounding a central well was made using a specially designed metallic bunch. Each well had a 4 mm diameter and 2 mm depth and separated from the adjacent well by a 3 mm distance. The central well of the hexagonal pattern was filled with a reference ILT virus antigen (Animal Health Service, Netherlands), while the five peripheral wells were sued for the test samples and the 6th well contained a reference positive ILT serum. A positive reaction was considered as a distinct clearly visible precipitation line.

Ten-fold serial dilutions were made with sample diluents prior to examination. The ELISA was carried out to detect the presence of antibodies against ILTV, using BioChek antigen Test Kit. The absorbance values were recorded by automated microplate reader at 650 nm (Elx800) from BIO-CHECK (UK) Ltd. The results were expressed as $\log_2$ of the sample to positive ratio (S/P).

Results and Discussion

The clinical signs included moist rales, coughing and gasping. After 5 days, the clinical signs progressed to marked dyspnea with coughing of blood-stained mucous. The clinical signs spontaneously disappeared and a complete recovery was observed on day 10. There was very high morbidity (up to 80%) in both flocks, whereas, the mortality was high during the first 5 days reaching up to 2.5%. Similar clinical signs were reported by Linares *et al.*, (1994) during an outbreak of ILT in broilers in California.

Grossly, the birds showed haemorrhage and accumulation of mucous in the lumen of trachea. Microscopically, syncytial formation and intranuclear inclusion bodies, along with mucous degeneration and necrosis were observed in the tracheal epithelium. There was haemorrhage and infiltration of lymphomononuclear cells in the *lamina propria* and submucosa. The presence of intranuclear inclusion bodies indicated the acute form of the disease as these cannot be detected after 1-5 days post-infection (Guy *et al.*, 1992).

Since the vaccination against ILT is not

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practiced in Libya, these characteristic lesions of ILT were confirmed serologically by AGID and ELISA. At the onset of the clinical signs, all sera tested were negative by AGID and ELISA. However, after one week, two samples from flock 1 and one sample from flock 2 showed positive reaction by AGID. The mean of antibody titers measured by ELISA was 4.2 and 3.5 in flock 1 and 2, respectively after one week. The positive ELISA test is a good indicator of ILT infection (Bauer *et al.*, 1999). Immunity to ILT is mainly cell mediated and a poor correlation has been found between serum antibody titer and immune status of flocks (York *et al.*, 1990). AGID was however, found to be less sensitive than ELISA, but is considered useful for detection of antibodies on a flock basis (Adair *et al.*, 1985).

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Histopathological Studies on Vitamin D_3 Toxicity in Rats

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The present study was designed to elucidate the biochemical and histopathological aspects of vitamin D_3 toxicity in rats.

Materials and Methods

Sixteen adult healthy male Wistar albino rats were randomly divided into 2 groups of 8 rats each (Group A and B). The Group A rats were given vitamin D_3 dissolved in groundnut oil orally at the dose rate 8 mg/kg bw daily. The group B served as control and was administered groundnut oil only. The blood samples were collected by retro-orbital plexus method on day 0 and 6 (or just before dath) in 1% heparin for biochemical analysis.

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The necropsy of dead rats was performed and relevant tissues (kidneys, heart, aorta, lungs, stomach, small and large intestine) were collected in 10% buffered formalin for histopathological studies. The tissues were routinely processed for histopathology and stained with haematoxylin and eosin stain (H&E). Wherever necessary, duplicate tissue sections were stained to confirm calcification with Von Kossa stain (Luna 1968).

The biochemical parameters in plasma were estimated on auto-analyzer by using diagnostic reagent kits (Autopak) supplied by Bayer Diagnostics India Limited, Gujarat, India. The mean values of parameters estimated in plasma