Pathomorphological changes in spontaneous cases of hydrosalpinx in commercial layer chicken

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ABSTRACT

INTRODUCTION
The avian oviduct, is a tubular organ responsible for the secretion of the components surrounding the yolk and transport of the egg. Fully functional and healthy oviduct is essential for both quantity and quality of egg, offspring fitness and maternal transfer of immunoglobulins from mother to offspring. Any disorder that affects the reproductive system will have a great bearing on production potential and incur a heavy loss. Hydrosalpinx (accumulation of fluid in the oviduct) was reported in human being and domestic animals, however on search of literature there was no report in poultry to the best of our knowledge. In recent times, accumulation of fluid in the oviduct is frequently noticed in the laying hens after mid lay (from 40 wk onwards until culling) in Namakkal poultry flocks. It causes economic loss to the farmers in terms of loss in egg production and reduced pricing for culls. The aim of the present study was to find out the prevalence and pathomorphological changes in spontaneous cases of hydrosalpinx in commercial layer chicken encountered during an investigation of oviduct pathology.

MATERIALS AND METHODS
A total of 85 commercial layer flocks, above 20 weeks of age with flock strength of 3,000 to 25,000 birds belonging to White leghorn breed located in Namakkal district, Tamil Nadu, India were investigated for the prevalence of hydrosalpinx for a period of three years (2005 to 2008). Commercial layer birds in this region are maintained for the sole purpose of egg production in an open type houses and are located in close proximity. In most of the farms either cage system or raised floor system of rearing was adapted and fed with commercial or self made layer ration. All the flocks were vaccinated against Marek’s disease, Newcastle disease, infectious bronchitis, infectious bursal disease, fowl pox and infectious coryza according to a standard vaccination schedule. The selected flocks were inspected, records verified and the information regarding breed and strain of chicken, flock strength, age, method of rearing, vaccination schedule, source of feed and water, production performance including time of peak production, percentage of production, production drop and mortality were collected.

Serum antibody measurement: A total of 400 serum samples were collected from flocks affected with hydrosalpinx and analyzed for the antibody titer against Newcastle disease, Infectious bronchitis and Egg drop syndrome – 76 virus by HI test. A commercial indirect enzyme linked immuno-sorbent assay (Hester Pharmaceuticals Limited, India) test kit was used to de-
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tect specific antibodies against *Mycoplasma gallisepticum* and *Mycoplasma synoviae*.

**Etiological identification**: Heart blood and oviduct fluid swabs were collected from dead and ailing birds and screened for bacterial agents. The samples were placed in Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 hours and cultured aerobically in 5% sheep blood agar for isolation of bacteria. Bacterial isolates were identified on the basis of sugar fermentation and biochemical characteristics. Oviduct, spleen, caecal tonsil, kidney and trachea were collected from hydrosalpinx cases and subjected to haemagglutination (HA) test for detection of Newcastle disease virus (NDV), Infectious bronchitis virus (IBV) and EDS-76 virus. The feed samples collected from flocks with oviduct abnormality were analyzed for the presence of aflatoxin.

**Pathological examination**: A detailed post mortem examination was carried out on ailing and dead birds of flocks having hydrosalpinx and gross lesions were recorded. Oviducts were opened carefully and fluid quantity and characteristics were evaluated and a slit along the dorsal longitudinal aspect was made to examine the mucosal surface. Materials for histopathology were collected from different parts of oviduct and fixed in 10% neutral buffered formalin. After fixation, samples were processed by routine histopathological procedures, embedded in paraffin, sectioned at 5 µm thickness and stained with hematoxylin and eosin for histopathological examination. Tissue sections were subjected to indirect immunoperoxidase test (IPT) for detection of IBV, NDV and *Mycoplasma gallisepticum* antigens.

**RESULTS**

Birds with distended abdomen revealed wide spread legs with penguin like posture and all the normal degeneration of layer chicken. On postmortem examination, the condition of the carcass was fair to emaciated. Abdomen was markedly enlarged with fluid. On further examination, infundibular region of the oviduct revealed dilatation with fluid. On opening, creamy white mucus mixed fluid of about 120 to 750 ml with small thin shelled eggs without yolk were observed. The remaining parts of the oviduct were either atrophied or apparently normal depending up on the quantity of fluid. In all the birds, oviduct lumen was patent, since the fluid was moved freely into the different segments of the oviduct, the cases were diagnosed as hydrosalpinx. After removal of fluid, infundibulum appeared stretched and it was very thin. Based on the characteristic gross lesion condition diagnosed as hydrosalpinx. Ovarian follicles were either regressed or apparently normal with follicular hierarchy. Visceral organs showed mild to moderate degree of atrophic changes and congestion.

Histopathologically, infundibulum revealed atrophy and marked flattening of mucosal folds due to reduction in size of its surface epithelium. The epithelial cells revealed degeneration and necrosis with infiltration of mononuclear cells (Fig. 1). Muscular and serosal layer blood vessels were congested with perivascular cuffing and the muscular layer revealed hyalinization. Magnum showed variable features from apparently normal to severe atrophy of mucosal folds with fibrous tissue proliferation in between the glands. The surface epithelium showed pronounced degenerative changes and sloughing (Fig. 2). Mucosal folds which were decreased in size grossly, showed atrophy of tubular glands at the periphery and secretory activity at center. In few cases, tubular glands showed focal necrosis and cystic dilatation and nuclei of some of the glandular epithelial cells were pyknotic in appearance. Infiltration of mononuclear cells was noticed in the form of lymphoid aggregates or in a diffused manner in the submucosa (Fig. 3). Isthmus showed loss of cilia on the surface epithelium and the cells showed degeneration and desquamation. Inter tubular glands spaces revealed infiltration of mononuclear cells, while the submucosa showed lymphoid aggregates. In few areas, hyperplasia of surface epithelium was also noticed. Uterus showed normal to severe degeneration of the surface epithelium and desquamation of cells in to the lumen. In few birds, hyperplasia of surface epithelium, severe atrophy and necrosis of tubular glands were also noticed. Infiltration of mononuclear cells especially lymphocytes and plasma cells in the form of lymphoid aggregates were noticed in the lamina propria (Fig. 4). Vagina revealed degeneration of mucosal layer and infiltration of mononuclear cells. The corium and folds of vagina contained infiltrating heterophils, lymphocytes along with proliferating fibrous tissue. Serosal blood vessels were congested.

Four hundred serum samples collected from 17 layer flocks with hydrosalpinx were subjected to micro HI test against NDV, IB and EDS – 76. The range of HI titre for NDV, IBV and EDS-76 virus was 32 to 128, 32 to 512 and 2 to 8, respectively. In ELISA, the titer value of 0-269, 270-743, and 744 and above were interpreted as negative, suspicious and positive, respectively. In the present study, all the samples were positive for MG and ninety seven per cent of samples were positive and the remaining three per cent of samples were suspicious for MS antibodies in ELISA. The tissue samples of all the 17 farms showing the lesions of hydrosalpinx were found to be negative for ND, IB and EDS- 76 in HA test. The bacteriological examination of samples also revealed no organisms of etiological significance. In IPT, the tissue sections revealed the presence of IBV and *Mycoplasma* antigens with golden brown fine granular material on the surface epithelium of infundibulum, magnum and uterus regions of the oviduct.
In the present investigation hydrosalpinx was observed in 321 birds from 17 out of 85 flocks investigated for oviduct abnormalities with the prevalence rate of 18.72 per cent. Hydrosalpinx was noticed throughout the year between 41 and 80 wk of age, but more common from 71 wk onwards. In the affected flocks morbidity, egg production drop and mortality were 1 to 10, 2 to 4 and 0 to 0.5 per cent, respectively.

DISCUSSION

The modern strains of commercial layers with the ability to ovulate large numbers of eggs and the sophisticated feeding management strategies to support their genetic potential make the birds susceptible to different types of reproductive disorders. Although it is well known that reproductive disease of poultry results in decreased egg production and increased mortality, avian reproductive pathology is treated rather briefly in literature. The present study was carried out to elucidate the prevalence and pathology of hydrosalpinx in commercial layer chicken in Namakkal region.

Hydrosalpinx was noticed in birds between 41 and 80 wk of age, but more commonly from 71 week of age onwards. In the initial period, the birds may appear normal and difficult to identify the affected birds in the multilayer cage system of management. Due to progressive accumulation of fluid, the birds showed pendulous abdomen with penguin like posture which helped the farmers to recognize the condition very easily. In hydrosalpinx, the drop in egg production was in the range of 2 to 4 per cent and did not reach the expected
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level as against the normal flocks. The morbidity and mortality varied from 1 to 10 and 0 to 0.5 per cent, respectively. Main economic losses to the farmers are drop in egg production and reduced pricing for culs.

In this field study, birds with distended abdomen revealed wide spread legs with penguin like posture and all the normal depigmentation of layer chicken. On necropsy examination, infundibulum region of the oviduct was dilated with accumulation of 120 to 750 ml creamy white mucus fluid along with thin shelled eggs and the remaining parts of the oviduct were either atrophied or normal. The oviducts of all affected birds were patent, ovarian follicles were either regressed or normal. Landman et al., 11 and Bano et al., 12 reported that the variant (QX) strain of infectious bronchitis virus infection at young age can produce permanent damage to the genital tract leading to cystic oviduct at later stage termed as false layer syndrome. In the present study specific etiological agents could not be detected, however the lesions are similar to that of earlier observation 11,12.

In the present investigation, oviduct of affected birds revealed a range of histopathological changes. Reduction in the size of mucosal folds and severe atrophy of surface epithelium were noticed in all the parts of oviduct. This might be due to pressure atrophy caused by the accumulating fluid. Degeneration and desquamation of surface epithelium, atrophy of tubular glands and the formation of submucosal germinal centers were the predominant microscopic findings in the different regions of oviduct. Degenerative and inflammatory changes observed in the various regions of the oviduct suggest that, the infectious agent would multiplies on the surface epithelium and damage the mucosal layer of oviduct. The lymphoid nodules and plasma cells found in the different segment of oviduct are not normal structure as they are found in few to negligible numbers in healthy birds, which are exposed to a minimum level of pathogenic microorganisms. This assumption would also tend to agree with findings in gnotobiotic mammals 13, in which the lymphoreticular tissue of the intestinal wall is very poorly developed, but after live microflora become established, progressive series of changes occur, leading towards the conventional normal morphology. As the level of exposure increases, there is an increase in numbers of lymphoid foci and plasma cells, and also in the extent of perivascular cuffing in the subserosal and intermuscular connective tissue.

The antibody titre for NDV in the affected flock was ranged from 32 to 128. Raghu 14 observed that, a HI titre of 32 to 64 was sufficient to protect the oviduct from NDV induced direct damage. In Namakkal area, the vaccination against Newcastle disease using mesogenic and killed vaccines was performed at 16 - 18 wk of age, followed by revaccination regularly at every three months intervals after 40th wk. Hence, the antibody titre found in this study i.e., from 32 to 128 was within the normal range due to vaccination 15. The antibody titre against IBV was ranged from 32 to 512. Various regimens have been employed in Namakkal for field vaccination programmes to confer protection in chickens against IBV, however in the layer flocks with hydrosalpinx cases, vaccination with live virus against IBV was performed on 1st, 5th, 12th, and 19th wk of age. Kleven 16 observed that humoral immune response against MG decline rapidly after vaccination (Mycoplasma vaccination was done between 9th and 12th wk) and hardly be detected after approximately 25 wk. Similar situation may occur in IB also this leaves us the conclusion that the antibodies detected by HI in the present study were primarily due to natural infection. The HI antibodies against EDS - 76 viruses was 8 or below. The serum antibody titre of 8 and below should be considered as negative due to the presence of nonspecific HI antibodies to haemagglutinating adenoviruses 17. Absence of clinical signs and histopathological lesions suggestive of EDS - 76 further support the above findings that hydrosalpinx probably was not due to EDS - 76. In ELISA, the sera samples revealed 100 per cent positivity (titer above 744) for MG and 97 per cent positivity (titer above 744) for MS. In Namakkal poultry belt, multiple age groups of birds are maintained in open house system of management which might have attributed to persistent infection with mycoplasma leading to positive reaction in ELISA.

In the present study, positive reaction in IPT was noticed against IBV and Mycoplasma in and on the surface epithelium of infundibulum, magnum and isthmus. Despite the finding of positive reaction in immunohistochemical techniques, etiological agents could not be detected in this organ. This might be due to neutralization of the agents (IBV and MG) by antibody present in the organs when they were emulsified. Infection with other causative agents was not confirmed by serological, bacteriological, histological and immunohistochemical examination, the accumulation of fluid in the oviduct might be due to IB infection. Hence, the affected birds might have had individual or combined infection of IB and MG which would have damaged the delicate infundibular fimbriae leading to adhesion and impairment of the normal fluid movement within the blocked oviduct resulting in development of characteristic cyst like lesion. However, further molecular studies are required to confirm the etiological agents in hydrosalpinx cases.

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REFERENCES


