

Original Research Article

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Serosurveillance of Infectious Agents Associated with Hydrosalpinx in Commercial Layer Chicken

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ABSTRACT

The present study was undertaken to investigate the prevalence of etiological agents associated with hydrosalpinx in commercial layer flocks for a period of three years (2005 to 2007) by serology. Hydrosalpinx was observed in birds of 41-80 week age group and was accounted to 18.72 per cent of the birds investigated for oviduct abnormalities. 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. Four hundred serum samples were randomly collected from the 17 hydrosalpinx flocks and analysed for the antibody titer against Newcastle disease, Infectious bronchitis and egg drop syndrome-76 virus by Hemagglutination inhibition (HI) test. A commercial indirect enzyme linked immunosorbent assay (ELISA) test kit was used to detect specific antibodies against *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. The HI titre for NDV, IBV and EDS -76 virus was 32 to 128, 32 to 512 and 2 to 8, respectively and in ELISA 100 and 97 per cent of serum samples were positive for MG and MS in the affected flocks. Based on the serology it was concluded that the affected birds might have had individual or combined infection of IB and MG which would have damaged the delicate in fundibular 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.

Keywords

Hydrosalpinx,
Layer chicken,
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Introduction

Namakkal is the most thickly populated poultry zone in India and occupying second place in egg production at national level and it has a layer population of approximately 50 million which produce 35 million eggs per day. The development of modern strains of layer chicken that have been genetically selected for productivity traits will ovulate

more in order to produce more number of eggs during laying period. Such enhanced genetic potential along with the sophisticated feeding management strategies make the bird susceptible to different types of reproductive disorders (Saino *et al.*, 2002). 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 (Leese *et al.*, 2001), however, there is little information available regarding the hydrosalpinx 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. Etiology of hydrosalpinx in commercial layer chicken was not understood. Serological testing is an important tool used in the commercial poultry industry for diagnosis and monitoring of flock health especially in subclinical infections (Barua *et al.*, 2006). The problem was developing gradually over a period of time in the affected flocks. Birds exposed to pathogens develop circulating antibodies that generally persist for several weeks even after the antigens have been cleared. Hence in the present study, the prevalence of etiological agents associated with hydrosalpinx in commercial layer flocks was studied by seroprevalence.

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 a period of three years (2005 to 2007) for the prevalence of hydrosalpinx. 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. Among the 85 flocks 17 showed the hydrosalpinx lesions on postmortem examination. 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 sample collection

Four hundred serum samples were randomly collected from the 17 hydrosalpinx flocks. Two to three milliliter of blood samples were aseptically collected using disposable syringes. Serum was separated by centrifugation at 4000 rpm for 10 minutes. Separated serum samples were stored at -20 °C in 1.5 ml microcentrifuge tubes with air tight cap. Each flock samples were placed in a labeled zip lock bag.

Serum antibody measurement

Serum samples were analysed for the antibody titer against Newcastle disease (Alexander, 1988), Infectious bronchitis (Alexander and Chettle, 1977) and Egg drop syndrome-76 virus (Shakya and Dhawedkar, 1991) by HI test. A commercial indirect enzyme linked immunosorbent assay (Hester Pharmaceuticals Limited, India) test kit was used to detect specific antibodies against *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. The titer value of 0-269, 270-743 and 744 and above were interpreted as negative, suspicious and positive, respectively.

Results and 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 (Solomon, 2002). The present study was carried out to elucidate the etiological agents associated with hydrosalpinx in commercial layer chicken in Namakkal region.

Hydrosalpinx or false layer was recorded in 321 birds from 17 farms and constituted 18.72 per cent of oviduct abnormalities. Affected birds showed cystic dilatation and fluid accumulation in the infundibulum region of the oviduct, ovarian follicles were either normal or atrophied and birds revealed normal depigmentation. The condition was noticed between 41 and 80 wk of age, but more common from 71 wk 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 the affected flocks morbidity, egg production drop and mortality were 1 to 10, 2 to 4 and 0 to 0.5 per cent, respectively. Main economic losses to the farmers are drop in egg production and reduced pricing for culls (Srinivasan et al., 2014).

A total of 400 sera samples collected from 17 layer farms showing the signs of hydrosalpinx were subjected to micro HI test against ND. The HI titre of 64 and above was considered as positive in the present study. The ranges of HI titre against ND are presented in Table 1. The HI titre ranged from 8 to 128. Ninety per cent of birds showed HI titre of 32 and above. Among these, 40.5 per cent showed a HI titre of 64 and above. Raghul *et al.*, (2006) 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 8 to 128 was within the normal range due to vaccination (Srinivasan *et al.*, 2012).

In the present study all the 400 serum samples were subjected to micro HI test against IB. The HI titre of 64 and above was considered as positive in the present study. The ranges of HI titre against IB are presented in Table 2. The HI titre ranged from 32 to 512. Ninety per cent of birds showed HI titre of 64 and above. The average minimum and maximum age of layer flocks surveyed in this investigation was 21 and 80 wk. The detection of antibodies in serum against IB would arise a question whether these antibodies are due to the vaccination or true infection. 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, and 16th wk of age. Kleven (1981) 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.

All the 400 serum samples were subjected to micro HI test to detect the presence of EDS-76 viral antibodies. The HI titre of 8 and above was considered as positive in the present study. The range of HI titre against EDS-76 is presented in Table 3. All the

samples revealed the antibody titre of 8 or below. The samples were collected mostly from birds of above 40 wk of age showing the lesions of hydrosalpinx. The serum antibody titre of 8 and below should be considered as negative due to the presence of nonspecific HI

antibodies to haemagglutinating adenoviruses (Calnek, 1978). 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.

Table.1 Newcastle disease serum antibodies in hydrosalpinx cases

S.No.	HI Titre	Number of samples	Per cent of positivity
1	0	-	-
2	2	-	-
3	4	-	-
4	8	10	02.5
5	16	30	07.5
6	32	198	49.5
7	64	115	28.75
8	128	47	11.75

Table.2 Infectious bronchitis disease serum antibodies in hydrosalpinx cases

S.No.	HI Titre	Number of samples	Per cent of positivity
1	0	-	-
2	2	-	-
3	4	-	-
4	8	-	-
5	16	-	-
6	32	40	10.00
7	64	71	17.75
8	128	139	34.75
9	256	94	23.5
10	512	56	14.0

Table.3 Egg drop syndrome - 76 serum antibodies in hydrosalpinx cases

S.No.	HI Titre	Number of samples	Per cent of positivity
1	0	148	37.0
2	2	164	41.0
3	4	72	18.0
4	8	16	4.0

Table.4 ELISA titre for *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) in hydrosalpinx cases

	ELISA titre			Number of samples positive	Per cent of positivity
	0 - 269	270 - 743	744 and above		
MG	-	-	102	102	100
MS	-	3	99	99	97

A total of 102 random sera samples (6 per farm) taken from 17 poultry farms, showing the signs of hydrosalpinx were screened for the presence of antibodies against MG and MS using synbiotic ELISA kit. The ELISA titre of 270 to 743 was fixed as probable and 744 and above as positive for both the MG and MS. In the present study, all the samples (102/102) were positive for MG and ninety seven (99/102) per cent of samples were positive and the remaining three per cent of samples were suspicious for MS antibodies. The ELISA titre against MG and MS are presented in Table 4. In Namakkal poultry belt, there is no vaccination programme against MG and MS. This leaves us with the conclusion that the antibodies detected by ELISA in the hydrosalpinx cases are primarily due to field infection. 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.

The source of infection to oviduct is probably through mechanical transfer from abdominal airsacculitis (Domermuth and Gross, 1967). This conclusion was derived from the fact that infection does not spread to oviduct if the organisms (*Mycoplasma*) was injected intracardially and air sac infection development is also slow than it did in the air sac injected chickens, thereby allowing immunity to develop to the point where salpingitis could no longer occur. *Mycoplasma gallisepticum* (MG) generally

enter the host via the respiratory tract (except for in-ovo infections) and upper airways and trachea are preferred sites of infection for most strains of MG. Therefore in hydrosalpinx cases a significant respiratory disease would have existed in all the chickens and persisted for longer duration.

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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