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RESEARCH ARTICLE
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Prevalence and pathology of oviduct impaction in commercial white leghorn layer chicken in Namakkal region of India

P. Srinivasan[1], G. A. Balasubramaniam[1], T. R. Gopala Krishna Murthy[2] and P. Balachandran[1]

1. Department of Veterinary Pathology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India;
2. Poultry Disease Diagnosis and Surveillance Laboratory, Veterinary College and Research Institute Campus, Namakkal, India.
Corresponding author: P. Srinivasan, email srinipat2004@yahoo.com
GAB: gabalasubramaniam@gmail.com, TRG: gkmurthy_in@yahoo.com, PB: balaavg@yahoo.co.in
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Abstract

Aim: The avian oviduct is a tubular organ responsible for fertilization, secretion of the components surrounding the yolk and transport of egg in the reproductive tract. Disorders of oviduct may have a great bearing on production potential and incur a heavy loss. A study was undertaken to assess the prevalence and pathological changes of impacted oviduct in commercial white leghorn layer chicken in Namakkal region of India for a period of four years from 2006 to 2009.

Materials and Methods: A total of 5145 carcasses of white leghorn layers, above 20 weeks age from 255 flocks were examined for various oviduct abnormalities. Heart blood, liver and oviduct swabs collected upon necropsy from 45 layer chicken from six flocks with oviduct impaction were screened for bacterial agents. Pooled tissue (trachea, lung, spleen, caecal tonsil, kidney and oviduct) samples from impacted oviduct cases were screened for viral agents. Serum samples collected from affected flocks were screened for Newcastle disease virus (NDV), infectious bronchitis virus (IBV) and egg drop syndrome – 76 (EDS-76) virus by haemagglutination inhibition (HI) and *Mycoplasma gallisepticum* (Mg) and *Mycoplasma synoviae* (Ms) by ELISA. Flock details and pathological changes were recorded in affected flocks to assess the prevalence and impact of oviduct impaction on commercial layer chicken.

Results: The results of the present investigation indicated that the oviduct impaction was responsible for 0.87% of the reproductive tract abnormalities in commercial layers between 21 and 80 wk of age. Egg production drop, morbidity and mortality recorded in the affected flocks were varied from 3 to 8, 0.4 to 1.2 and 0.2 to 0.5 % respectively. The oviduct impaction was commonly noticed above 40 wk old layers and predominantly during colder months. Serum samples collected from three flocks with oviduct impaction were found positive for Mg and Ms infection in ELISA test. *Escherichia coli* was isolated as a pure culture in 29 birds and concurrent with other bacterial agents in 16 birds. Serotyping of *E. coli* isolates revealed that O_{144}, O_{54} and O_{109} were the predominant types. Necropsy examination of carcasses with impacted oviduct showed emaciation, peritonitis, regression of ovarian follicles and distension of oviduct commonly in the infundibulum and magnum region. The exudate was either large mass with concentric layering on cut section or numerous caesous masses with varying size and shape often mixed with creamy or serosangunious fluid. Histopathological examination of oviduct revealed the presence of lymphoid foci in the epithelial layer with marked atrophy and degeneration of mucosal folds.

Conclusions: Impacted oviduct constituted 0.87 % of oviduct abnormality in commercial layer chicken with an overall mortality of 0.5 %. The findings of this study showed that the oviduct impaction might be caused by *E.coli* in concurrence with *Proteous* spp., *Klebsiella* spp., *Streptococcus* spp., and *Mycoplasma*.

Keywords: *E. coli*, layer chicken, mycoplasma, oviduct impaction, pathology, prevalence.

Introduction

The female reproductive tract is commonly referred to as oviduct in birds. The oviduct is a highly convoluted muscular duct, concerned with the transport of the ovum away from the ovary, with fertilization of the ovum and by the deposition of albumen, membranes and shell on to the ovum to form the finished egg [1]. Reproductive diseases in poultry causes high morbidity (35%), mortality (15%) and drop in egg production (40%) [2]. The function of the oviduct could be influenced by a number of disease processes either directly by virtue of the fact that they alter the ability of the lining cells of oviduct to synthesize their integral components or indirectly by generally compromising bird's health. 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 [3].

The modern strains of commercial layer chicken with their genetic potential to lay more number of eggs during laying period make them susceptible to different types of reproductive tract disorders [4]. Prevalence of different reproductive tract abnormalities in commercial layer chicken was reported as 26.10% [2]. Oviductal derangements such as atrophic oviduct [5], cystic oviduct [4] and persistent right oviduct [6] are reported in commercial layer chicken and cause reduction in egg production and increased

Table-1: Flock details and antibody titer in layer chicken affected with oviduct impaction.

Age	Flock size	Production %		Morbidity%	Mortality%	HI Titer (GMT)			ELISA titer	
(week)		Actual	Expected			NDV	IBV	EDSV	MG	MS
25	6000	88	92	0.5	0.2	891.4	168.9	3.6	<269	<269
44	17000	90	93	0.4	0.3	168.9	73.3	4.8	<269	<269
57	12000	83	89	0.6	0.4	512	97.0	2.8	<269	<269
60	15000	80	88	0.8	0.4	588.1	111.4	3.2	>744	>744
68	10000	79	84	1.0	0.5	222.9	128.0	4.0	>744	>744
72	7000	76	81	1.2	0.5	111.4	42.2	3.4	>744	>744

mortality.

Oviduct impaction occurs from accumulation of eggs or egg material within the oviduct and is usually a consequence of chronic salpingitis [7]. Oviduct impactions may also occur due to excessive production of albumin or mucin, in patients with cystic hyperplasia or inspissated egg material in the magnum. An incidence of oviduct impaction was reported in companion birds resulting in life threatening symptoms and high mortality [8]. Modern commercial layers are highly prolific and susceptible to oviduct impaction, however it was rarely diagnosed and reported [9] and their etiological factors were not investigated. To ensure persistent and maximum production in poultry flocks, it is imperative to investigate the oviduct impaction in order to understand its prevalence, nature and significance of this disorder.

The present study was planned to find out the prevalence of oviduct impaction associated mortality in layer chickens in Namakkal zone of India.

Materials and Methods

Ethical approval: Samples were collected after permission from the Institutional Animal Ethics Committee.

Flock history: The study was carried out over a period of four years (2006 to 2009). A total of 5145 carcasses of white leghorn layers, above 20 weeks (wks) of age belonging to 255 commercial poultry flocks situated in and around Namakkal district of Tamil Nadu state, India were examined for various oviduct abnormalities. The flocks showing oviduct impaction were inspected and information regarding shed capacity, flock size, cage size, strain of chicken, cage management and hygiene, feed additives, vitamins-mineral supplements, treatment given and egg production traits were collected from the owners of the farms. To study the seasonal variations in the incidence of the oviduct impaction the whole year was divided into four seasons namely summer (March, April and May), south west monsoon (June, July and August), north east monsoon (September, October and November) and winter (December, January and February). According to the age, layers were grouped into six as 21-30 wks, 31- 40 wks, 41-50 wks, 51- 60 wks, 61-70 wks and 71-80 wks.

Pathological examination: The dead birds surface disinfected and necropsies were performed as per approved procedure [10]. The abdomen and cloaca were examined for signs of distension and inflammation respectively. The carcasses were thoroughly examined for gross pathological changes including inflammatory signs and presence of exudates in the peritoneal cavity, ovary, oviduct or all of them. Oviduct with impaction was removed and opened along its longitudinal axis for examination of mucosal surface and internal contents for its size, shape and quantity. Materials for histopathology were collected from different parts of impacted oviduct and fixed in 10 % neutral buffered formalin. After fixation, samples were processed by following the routine histopatho-logical procedures, embedded in paraffin, sectioned at 5 µm thickness and stained with hematoxylin and eosin for histopathological examination.

Isolation of causative agent: Heart blood, liver and oviduct swabs were collected from dead birds with impacted oviduct for screening of bacterial agents. The samples were placed in Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 h and cultured aerobically in Brian heart infusion agar (BHIA), Mac Conkey's agar and eosin methylene blue agar (EMBA) for isolation of bacteria. Bacterial isolates were identified on the basis of their morphology, growth characteristics, sugar fermentation and biochemical characteristics [11]. The *Escherichia coli* isolates obtained from impacted oviduct cases were sent to National Salmonella and Escherichia centre, Kasuali, Himachal Pradesh, India for further confirmation and serotyping.

Antibody detection: Trachea, lung, spleen, caecal tonsil, kidney and oviduct collected as pooled sample from impacted oviduct cases were subjected to haemagglutination (HA) test for detection of NDV [12, 13] IBV [14] and EDS-76 virus [15]. Serum samples were collected randomly from ten birds of flocks affected with impacted oviduct and examined by haemagglutination inhibition (HI) test for the presence of antibodies to NDV, IBV and EDS-76 virus and by ELISA (Hester Pharmaceuticals Limited, India) for the *Mycoplasma gallisepticum* (Mg) and *Mycoplasma synoviae* (Ms). The ELISA titer value of 0-269, 270-743, and 744 and above were interpreted as negative, suspicious and positive for Mg and Ms infection respectively. Serum samples collected from the same age group of birds which are clinically normal were utilized as control birds.

Statistical analysis: Analysis of data on egg production, morbidity and mortality in different age groups of flocks affected with impacted oviduct was carried out by using chi square test to see if there is any

Table-2: Bacterial agents isolated from impacted oviduct of layer chicken.

Organism	Positive birds	% of positivity
E. coli	29	64.45
E. coli + *Proteous* spp.	6	13.33
E. coli + *Klebsiella* spp.	5	11.11
E. coli + *Streptococcus* spp.	5	11.11

Table-3: Frequency pattern of *E.coli* serotypes isolated from impacted oviduct of layer chicken.

Organism	Positive birds	Percentage
O_3	05	11.11
O_{12}	04	08.89
O_{54}	09	20.00
O_{88}	05	11.11
O_{109}	08	17.78
O_{118}	03	06.67
O_{144}	11	24.44

Figure-1: Oviduct impaction: Affected bird showing emaciation, prominent keel bone and distension of abdomen.

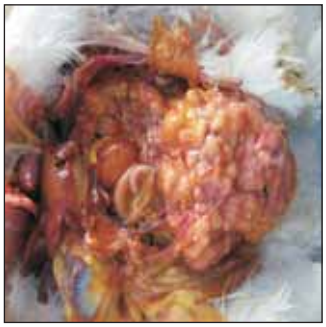

Figure-2: Oviduct impaction: Diffuse peritonitis and adhesion of visceral organs in abdominal cavity.

Figure-3: Oviduct impaction: Abdominal cavity occupied by a markedly distended oviduct associated with atrophy of visceral organs.

Figure-4: Oviduct impaction: Moderate distension of infundibulum and magnum, atrophy of the remaining parts of oviduct. Ovarian follicles were showing atretic changes.

significant difference on the manifestation of impacted oviduct in commercial layer chicken.

Results

The antibody titre for NDV, IBV, EDS-76, Mg and Ms were shown in Table-1. The geometric mean antibody titer for NDV, IBV and EDSV in the affected flocks ranged for 111.4 to 891.4, 42.2 to 168.9 and 2.8 to 4.0 respectively. In the present study, thirty serum samples (50%) collected from three flocks with oviduct impaction were positive for Mg and Ms and the remaining 50 % were negative for both Mg and Ms antibodies. Sixty control serum samples were negative for Mg and Ms antibodies.

Among the 45 birds with impacted oviduct lesions, *E. coli* was isolated as a pure culture in 29, whereas in the remaining 16 birds *E. coli* was isolated along with other bacteria (Table-2). *E. coli* was identified based on lactose fermenting, pink colour round, smooth and glistening colonies on Mac Conkey's agar, black colonies with metallic sheen on EMB agar, indole production at 44ºC, gas production in Eijkmann test and acid and gas production in differential sugar fermentation tests. Other bacterial organisms *viz., Proteous* spp., *Klebsiella* spp., and *Streptococcus* spp., were identified based on their cultural and biochemical characteristics. Different serotypes identified from impacted oviduct cases are presented in Table-3. Serotypes O_{144}, O_{54} and O_{109} were predominantly present in the impacted oviduct cases. Necropsy examination of the affected flocks did not reveal any lesion indicating NDV, IBV and EDS-76 viral infections. Tissue samples collected for virological examination was found to be negative in haemagglu-tination test against NDV, IBV and EDS-76 virus.

On post mortem examination, birds affected with oviduct impaction showed emaciation, prominent keel bone and marked distension of abdomen (Figure-1). Diffuse peritonitis and adhesion of abdominal viscera were noticed (Figure-2). The impacted mass was expanded to the point that it filled most of the body cavity, in which the abdomen was wider and more pendulous and the abdominal viscera showed marked atrophic changes (Figure-3). Oviduct showed mild (Figure-4) to marked (Figure-5) distension in all region, however it was more common in the initial parts especially infundibulum and magnum and the remaining parts of were either normal or atrophied. Distribution of impacted mass in different region of the oviduct is presented in Table-4. On opening the oviduct lumen was occluded by 100 to 500 gm of inspissated albumin and yolk materials which adhered to the mucosal surface. On cross-section, impacted mass showed the layered appearance (Figure-6) with partially formed eggs (Figure-7). Less frequently, the impacted mass contained numerous caseous granules of varying size and shape (Figure-8) with or without turbid or blood-tinged fluid (Figure-9). Affected oviduct wall was thin and the mucosal folds showed mild congestion. In persistent right oviduct the right one showed impaction (Figure-10) and the left one contained yolk and albuminous debris. In some cases impaction due to numerous partially formed eggs were noticed (Figure-11) along with the presence of thin shelled eggs in abdominal cavity. The ovarian follicles appeared either normal or atretic depending upon the size of the impacted oviduct. Histopatholo-

Table-4: Distribution pattern of lesions (%) in various regions of oviduct

Oviduct region	Number of birds	Percentage
Infundibulum (I)	10	22.22
Magnum (M)	05	11.11
Isthmus (Is)	04	08.89
Uterus (U)	01	02.22
I+M	09	20.00
M+Is	04	08.89
I+M+Is	04	08.89
M+Is+U	01	02.22
I+M+Is+U	02	04.45
All region	05	11.11

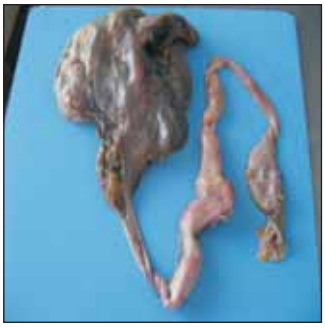

Figure-5: Oviduct impaction: Marked distension of infundibulum and mild distension of portion of magnum and isthmus.

Figure-6: Oviduct impaction: Impacted yolk and albuminous mass with concentric rings.

Figure-7: Oviduct impaction: Impacted mass containing partially formed eggs.

Figure-8: Oviduct impaction: Showing numerous caseated masses of varying size and shape.

Figure-9: Oviduct impaction: Showing blood tinged fluid and caseous masses in the infundibulum region.

Figure-10: Oviduct impaction: In double oviduct the right one contained impacted mass and the left one having yolk and albuminous materials.

Figure-11: Oviduct impaction: Numerous partially formed eggs in the isthmus region of the oviduct.

gically, affected oviduct showed lymphoid foci in epithelial layer and marked atrophy and degeneration of mucosal folds.

Out of 5145 carcasses form 255 layer flocks investigated for various types oviduct abnormalities, 45 birds from six flocks showed impacted oviduct with an overall prevalence rate of 0.87 %. The production drop ranged from 3 to 8 % below the standard level of egg production was noticed in different age groups of birds. The morbidity and mortality varied from 0.4 to 1.2 and 0.2 to 0.5 % respectively (Table-1). Chi square test for independence revealed no significant ($P>0.05$) difference on egg production, morbidity and mortality at different age groups of layer flocks affected with oviduct impaction. Among the six flocks examined, highest prevalence of impacted oviduct was noticed above 40 wks old layers (83.33%). Season wise analysis revealed higher prevalence rate (66.67%) during winter season.

Discussion

Impaction of oviduct in commercial layer chicken was diagnosed based on the presence of egg or egg materials in the oviduct on necropsy examination and laboratory investigation [7]. In the present study, thirty serum samples collected from three flocks were positive for Mg and Ms antibodies in ELISA. Commercial layer chickens are not vaccinated against mycoplasmosis (Mg and Ms) in Namakkal region. This leaves us with the conclusion that the antibodies detected by ELISA are primarily due to field infection [16]. The remaining 30 samples from three flocks were negative for Mg and Ms antibodies since these flocks were treated with antibiotics (tiamulin and tertracycline). Trawinska *et al*. [17] also observed low percentage of positivity for Mg and Ms in serological monitoring cobb line repro-

ductive hens with ELISA and stated that the use of antimycoplasmal agents eliminated the serum positive titer of Mg and Ms. The commercial layer flocks were regularly vaccinated against Newcastle disease and infectious bronchitis at 60-75 days intervals during laying period after 40 wk of age. Hence the antibody titre against NDV and IBV found in this study was within the normal range due to vaccination [5]. The HI titre against EDS -76 was 2.8 to 4. The HI titre of 8 and below should be considered as negative due to the presence of nonspecific HI antibodies to haemagglutinating adenoviruses [18].

Various bacteria have been reported to cause primary or secondary reproductive tract infections in commercial layers [19]. The *E. coli* and other bacterial organisms *viz., Proteous* spp., *Klebsiella* spp., and *Streptococcus* spp., were identified from impacted oviduct cases based on their cultural biochemical charecteristics [11]. *E. coli* was isolated as a pure culture and concurrent with other bacterial agents in 29 and 16 birds respectively. Many investigators have previously isolated pathogenic bacteria such as Mg, *E. coli*, *Proteus* spp., *Klebsiella* spp., *Pasteurella* spp., *Streptococcus* spp., and *Staphylococcus* spp., from lesions in the peritoneum and reproductive tract of hens [19-23]. *E. coli* causes transitory immunosuppressive effect in chicken and makes the bird susceptible to opportunistic bacterial agents [24].

The source of infection to oviduct is probably through mechanical transfer from abdominal air sacculitis. Domermuth *et al*. [25] reported that infection could not spread to oviduct if the organisms (Mycoplasma) was injected intracardially and air sac infection development was also slow than it did in the air sac injected chickens, there by allowing immunity to develop to the point where salpingitis could no longer occur. 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 impacted oviduct cases a significant respiratory disease would have existed in all the chickens and persisted for longer duration.

Among the different serotypes identified from impacted oviduct, serotypes O_{144} , O_{54} and O_{109} were predominant, however none of the serogroup was belongs to the systemic form serogroups such as O_1, O_2 and O_{78} [26, 27] . The occurrence of a specific serotype and its role in disease production depends upon the health status of the birds, climatic conditions, geographical situations and managemental strategies. Healthy laying hens have *E. coli* within the cloaca but not in the oviduct. *E. coli* is a normal inhabitant of the chicken intestinal tract with up to 10^6 of these bacteria per gram of intestinal contents. Approximately 10 to 15% of intestinal *E. coli* is considered to be potential pathogens. This might indicate that the oviduct impaction causing *E. coli* organisms arise from the intestinal sources [28].

On necropsy examination, carcasses with impacted oviduct showed emaciation, peritonitis, regression of ovarian follicles and distension of oviduct. The exudate was either large mass with concentric layering on cut section or numerous caesous masses with varying size and shape often mixed with creamy or serosangunious fluid. Distension and accumulation of exudate was more commonly noticed in the infundibulum and magnum region of oviduct which occupied the entire abdominal cavity. It might have compressed the intestine and visceral organs and impeded their functions leading to atrophy of visceral organs. Histopathologically, presence of lymphoid foci in the epithelial layer with marked atrophy and degeneration of mucosal folds is suggestive of chronic nature of the problem [29, 30].

Out of 5145 birds from 255 flocks, 45 birds from 6 flocks revealed impaction of the oviduct which constituted 0.87 % of total oviduct abnormalities observed in the present study. This was contrary to Bonia *et al*. [31], they observed 5% in Kalinga brown breeds. The lower prevalence rate in this study might be due to preventive medication with antibiotics (tiamutin and tetracycline) at regular intervals and adequate hygienic conditions on the farms. In the affected flocks egg production drop, morbidity and mortality ranged from 3 to 8, 0.4 to 1.2 and 0.2 to 0.5 % respectively. The results concurred with the observations of Valsala and Sivadas [32], Batra and Singh [9] and Keymer [33]. On flock examination birds look healthy, but due to chronic pathologies of the ovary or oviduct, they stop laying eggs. Mortalities from reproductive pathologies are rare, and in most of cases, are caused by other complications, such as acute and chronic peritonitis [21, 34, 35] and salpingitis [30].

Conclusion

It can be concluded from this study that among the reproductive tract disorders, oviduct impaction contributed 0.87% of oviduct abnormalities in commercial white leghorn layer chicken. Existence of persistent respiratory infection in chicken would predispose to oviduct impaction. Chronic infection of oviduct by *E. coli* and *Mycoplasma* might be the cause for the occurrence of oviduct impaction.

Authors' contributions

The present research is the part of PS's Ph.D. research work. PS conceived and implemented the work. PS drafted the manuscript and analysed the data. GAB was the major research supervisor. PB given valuable suggestions to carry out the study. TRG critically reviewed and revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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