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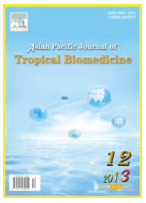

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Bacteriological and pathological studies of egg peritonitis in commercial layer chicken in Namakkal area

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PEER REVIEW

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Comments

The study is well designed and determines the bacterial causes of egg peritonitis in layer chicken. Egg peritonitis due to involvement of various serotypes of *E. coli* and pathological changes in oviduct of affected chicken are demonstrated by the authors. The results will help to design both therapeutic and prophylactic preventive strategies.

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ABSTRACT

Objective: To detect the various bacteriological agents and pathological changes in commercial layer chicken affected with egg yolk peritonitis in Namakkal region of India.

Methods: A total of 6572 layer chicken from 85 commercial farms were subjected for the study, out of which 1715 showed various types of oviduct abnormalities. Among the 1715, 264 birds from six farms were identified as egg peritonitis on the basis of postmortem examination. Trachea, lung, heart blood, liver, peritoneal exudate, oviduct (infundibulum, magnum, uterus) and cloacal swabs were collected from the 264 birds with egg peritonitis lesion for screening of bacterial agents. Signalment, clinical signs and pathological changes were recorded in the affected flocks.

Result: The results of the present investigation indicated that the *E. coli* associated egg peritonitis was responsible for 15.39% of the reproductive tract abnormalities in commercial layers between 21 and 80 week of age. In the affected flocks egg production drop and mortality varied from 3% to 20% and 0.5% to 7.0% respectively. It was noticed during peak egg production (21 to 60 week) and southwest monsoon season (58%). Statistical analysis of age, season and egg production by *Chi* square test of independence revealed highly significant difference. *E. coli* was isolated as a pure culture and concurrent with other bacterial agents in 226 and 38 birds respectively. Among the fifteen *E. coli* serotypes identified serotype O_{166}, O_{64} and O_{111} were predominant. Necropsy examination of affected birds revealed the presence of amorphous or insipissiated yolk material in the abdominal cavity with inflammatory changes in the ovary, oviduct and intestine. Microscopically the oviduct surface epithelium showed degeneration and desquamation, moderate to marked infiltration of inflammatory cells especially heterophils and lymphocytes in various regions and lumen contained serofibrinous exudate, inflammatory and desquamated epithelial cells with bacterial microcolonies. Ovarian follicles revealed hyperemia, degeneration of granulosa cells and infiltration of inflammatory cells. Intestine showed degenerative, necrotic and inflammatory lesion.

Conclusion: The findings of this study showed that the egg peritonitis might be caused by either the translocation of intestinal *E. coli* into the peritoneal cavity or by the movement of cloacal *E. coli* into the oviduct followed by ascension of these bacteria up the oviduct, through the infundibulum, and into the peritoneal cavity. To control the egg peritonitis faecal contamination with *E. coli* should be minimized.

KEYWORDS

Egg peritonitis, Layer chicken, Prevalence, *E. coli*, Pathology

1. Introduction

Namakkal is the most thickly populated poultry zone in India with a layer population of 45 million birds and occupying second place in egg production at national level[1]. The modern strains of commercial layers with the ability to ovulate large numbers of eggs and the sophisticated feeding management strategies to support

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their genetic potential make the birds susceptible to different types of reproductive disorders. The production performance 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[2]. Any disorder that affects the reproductive system will have a great bearing on production potential and incur a heavy loss. 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. Among the various oviduct abnormalities egg peritonitis is a common problem in sexually mature layer chicken.

Egg yolk peritonitis is the inflammatory reaction of peritoneum caused by the presence of yolk material in the coelomic cavity. Yolk material by itself induces a mild inflammatory response and may be reabsorbed by the peritoneum. Since yolk is an excellent growth medium for bacteria, peritonitis may result from secondary bacterial infection leads to secondary ascites and organ inflammation and cause morbidity, mortality and reduced egg production in the affected flocks[3,4]. It is a common cause of sporadic death in layers, as poultry production intensified, however its occurrence has also increased and in some flocks may become the major cause of death and gives the appearance of a contagious disease. In Namakkal poultry zone for the past few years, both the incidence and severity of egg peritonitis have increased and current trend indicate that it is likely to continue and become an even greater problem in the poultry industry. Nevertheless, scientific report on spontaneously occurring egg yolk peritonitis in commercial layer chicken and its economic impact to the layer industry of this region is not available. Therefore, the present study was designed to detect the various bacteriological agents and pathological changes in the layer chickens affected with egg yolk peritonitis.

2. Materials and methods

2.1 Flock history

A study on the pathology of egg yolk peritonitis was performed on commercial poultry farms located in Namakkal region of Tamil Nadu. The study period covered three consecutive years (2005-2008). A total of 6 572 layer chickens from 85 commercial layer flocks were utilized for this study. All the flocks were vaccinated according to the standard vaccination schedule including vaccinations against Marek's disease, Newcastle disease, infectious bursal disease, infectious bronchitis, infectious coryza and fowl pox. The flocks were inspected during the period of increased mortality, records were verified and the information regarding breed and strain of chicken, flock strength, 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.

2.2 Necropsy and histopathological examination

The dead birds were surface disinfected and necropsies were performed as per approved procedure[5], and thoroughly examined for gross pathological changes. Peritoneum was examined for its colour, consistency, adhesion and nature of its exudate. Oviduct was removed and opened along its longitudinal axis for examination of internal contents and 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 following the routine histopathological procedures, embedded in paraffin, sectioned at 5 μm thickness and stained with hematoxylin and eosin for histopathological examination.

2.3 Isolation of causative agent

Trachea, lung, heart blood, liver, peritoneal exudate, oviduct (infundibulum, magnum, uterus) and cloacal swabs were collected from dead birds with egg peritonitis lesion for screening of bacterial agents. The samples were placed in brain heart infusion broth and incubated at 37 °C for 24 h and cultured aerobically in sheep blood agar, MacConkey 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[6].

Trachea, lung, spleen, caecal tonsil, kidney and oviduct collected from egg peritonitis cases were subjected to haemagglutination test for detection of Newcastle disease virus (NDV)[7,8], infectious bronchitis virus (IBV)[9] and egg drop syndrome-76 (EDS-76) virus[10]. Serum samples were collected randomly from ten recovered birds from each flock were examined by haemagglutination inhibition test for the presence of antibodies to NDV, IBV and EDS virus and by ELISA for the *Mycoplasma gallisepticum* (*M. gallisepticum*) and *Mycoplasma synoviae* (*M. synoviae*).

To determine the source of infection, materials such as agar plates exposed in poultry house environment, cloacal swabs, water and feed samples were collected from the affected flocks for bacterial examination. Five MacConkey agar plates were exposed at different places of poultry house to determine the environmental microbial load. For cloacal swabs, minimum five apparently healthy birds from each of the six flocks affected with egg peritonitis were collected and processed according to standard procedure as described previously. Pooled water samples (storage tank and at least five nipples from different places of water lines

in the affected flocks) were tested for the presence of *E. coli* in EMBA. Twenty five grams of feed samples collected from each flock was added to 225 mL of brilliant green bile broth and incubated over night at 37 °C, the incubated broth was subcultured on the EMBA and incubated for 24 h at 37 °C and examined for *E. coli*. The *E. coli* isolates obtained were sent to National Salmonella and Escherichia centre, Kasuali, Himachal Pradesh, India for further confirmation and serotyping.

2.4 Statistical analysis

The data on month and age wise occurrence of oviduct abnormalities were collected. The seasons were classified as 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). The age was classified into six groups viz., 21-30 weeks, 31-40 weeks, 41-50 weeks, 51-60 weeks, 61-70 weeks and 71-80 weeks. Analysis of data on age, season, egg production and mortality has been carried out by *Chi*-square test of independence using interactive calculation tool of Pracher[11] to see if there is any significant difference on the manifestation of egg peritonitis in commercial layer chicken.

3. Results

On postmortem examination, the condition of the carcass was fair to good, crop was filled with feed materials and the pectoral muscles were dark red in appearance. The most prominent lesion was rupture of ovarian follicles (Figure 1) and presence of amorphous yolk material in the peritoneal cavity (Figure 2), more commonly attached to the surface of ova and the serous surface of the intestines and oviduct through strands (Figure 3). In few cases peritoneal cavity contained watery yellowish foul smelling fluid of about 10 to 15 mL together with inflammation and distortion of the ovaries (Figure 4). In chronic cases abdominal cavity contained cream to yellowish and brown insipissiated yolk material which varied from small to large in quantity and accumulated in the form of cyst (Figure 5). On opening, varying sizes of yolk materials were noticed (Figure 6), however, the quantity of exudate in the peritoneum was never as large as that seen in the dilated oviduct, and rarely associated with distension of the abdomen. Oviduct serosal blood vessels were congested and the mucosa revealed albuminous exudate with moderate congestion of its folds (Figure 7). Intestine showed mucus exudate and congestion of mucosa especially in duodenal and Jejunal regions. The peritoneum was thickened and yellowish to dark brown in appearance (Figure 8) and none of the affected bird showed the typical lesions of colisepticasemia or other pathological changes in visceral organs.

Figure 1. Egg peritonitis: abdominal cavity showing the ruptured yolk material.

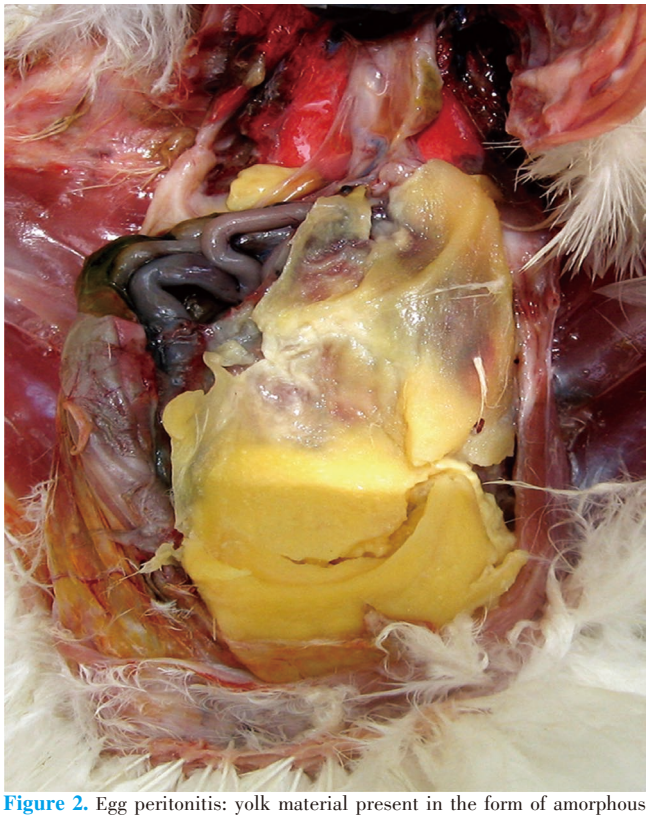

Figure 2. Egg peritonitis: yolk material present in the form of amorphous material.

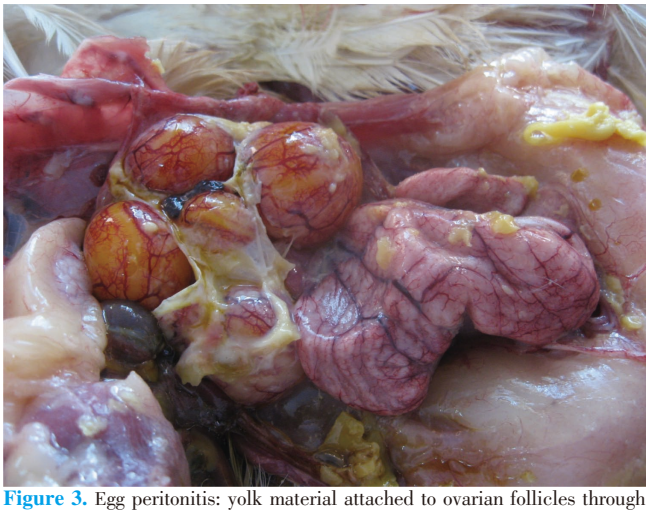

Figure 3. Egg peritonitis: yolk material attached to ovarian follicles through strands.

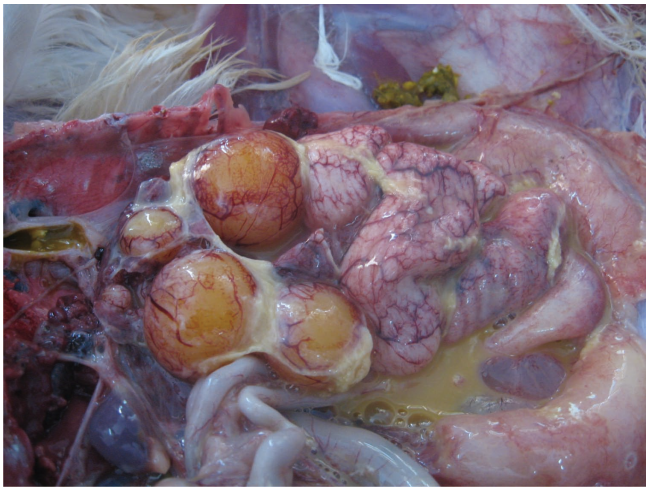

Figure 4. Egg peritonitis: Peritoneal cavity containing watery yellowish fluid with inflammatory changes in ovary and oviduct.

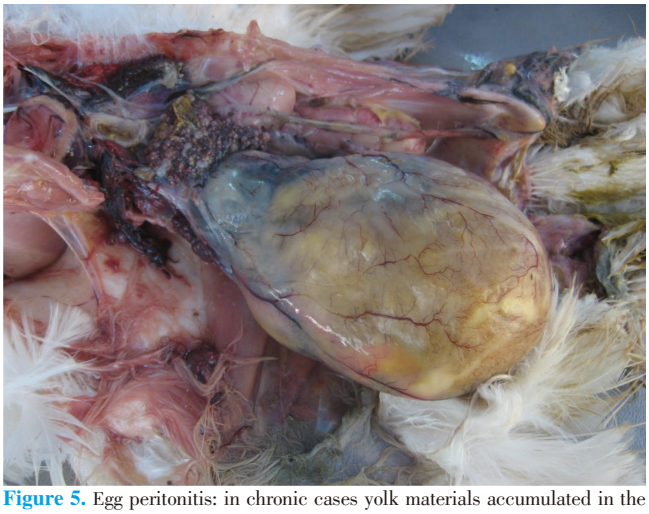

Figure 5. Egg peritonitis: in chronic cases yolk materials accumulated in the form of cyst.

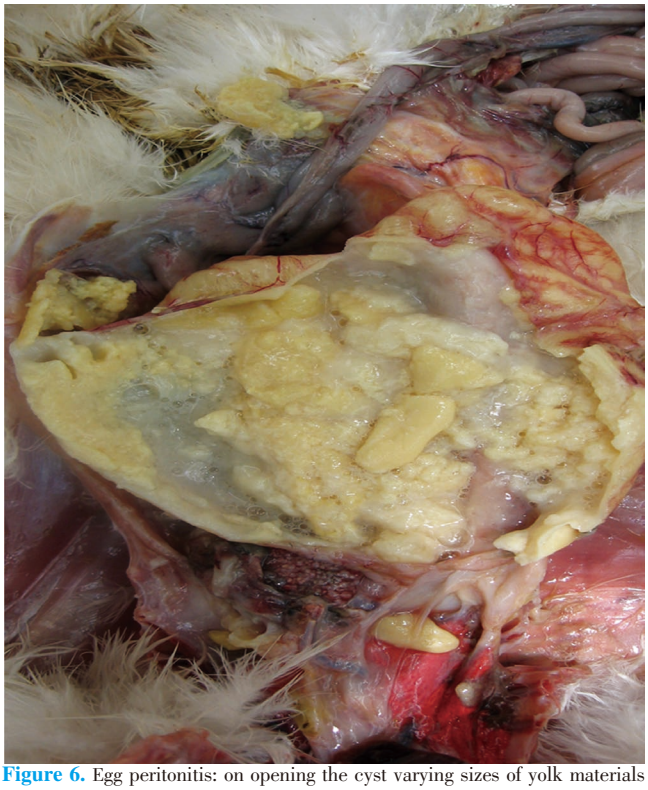

Figure 6. Egg peritonitis: on opening the cyst varying sizes of yolk materials were noticed.

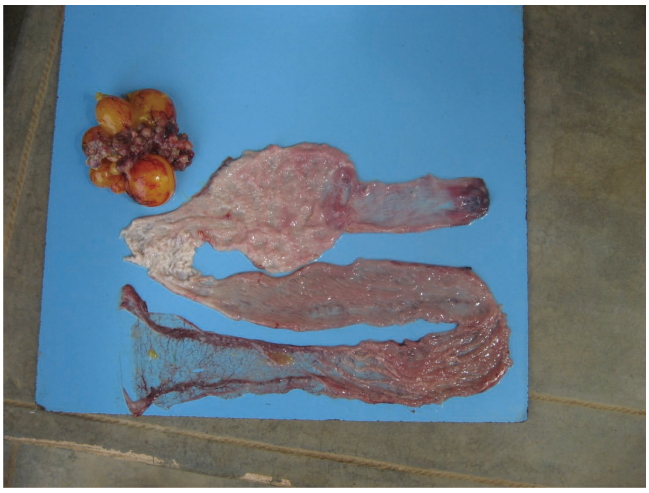

Figure 7. Egg peritonitis: Oviduct mucosa showing moderate congestion.

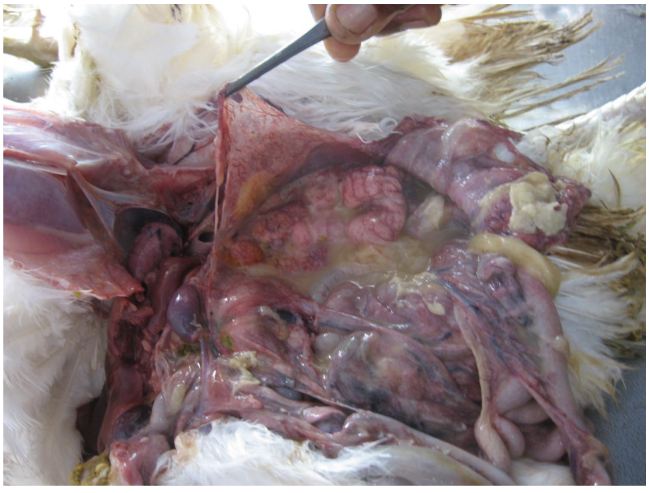

Figure 8. Egg peritonitis: Peritoneum was thickened with yellowish brown in appearance.

Microscopically, oviduct surface epithelium showed degeneration, matting of cilia and desquamation. Moderate to marked infiltration of inflammatory cells especially heterophils and lymphocytes in the lamina propria and muscular layers of various regions of the oviduct were observed. The lumen of the oviduct revealed desquamated epithelial cells, serofibrinous exudate with bacterial microcolonies and inflammatory cells. The glandular epithelium showed degenerative and necrotic changes. Ovarian follicles revealed hyperemia, degeneration of granulosa cells and infiltration of leukocytes. Intestine showed the degeneration, necrosis and desquamation of the mucosal epithelium with infiltration of leukocytes in the submucasa and dilated mucosal glands.

Among the 264 birds with egg peritonitis lesions, *E. coli* was isolated as a pure culture in 226, whereas in the remaining 38 birds *E. coli* was isolated along with other bacteria (Table 1). The *E. coli* organisms were identified based on lactose fermenting pink coloured round, smooth and glistening colonies on Mac Conkey's agar, black metallic sheen colonies on EMB agar, indole production

at 44 °C, gas production in Eijkmann's test and acid and gas production in different sugar fermentation tests. Other bacterial organisms viz., *Proteous sp.*, *Klebsiella sp.*, *Staphylococccus sp*., and *Streptococcus* sp., were identified based on their cultural and biochemical characteristics. In egg peritonitis cases *E. coli* organisms were isolated from peritoneal cavity, oviduct and clocal swabs. Poultry house environment and cloacal swabs (25 out of 30) revealed the presence of *E. coli* organisms, whereas the water and feed samples were negative for *E. coli* in all the six flocks with egg peritonitis lesions. Different serotypes identified from egg peritonitis cases are presented in Table 2. Among the serotypes O_{166}, O_{64} and O_{111} were predominantly present in the egg yolk peritonitis 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 also found to be negative in haemagglutination test against NDV, IBV and EDS-76 virus. Moreover, this was confirmed in the serological tests also, since all sera were positive for Newcastle disease virus and Infectious bronchitis virus as a result of vaccination. The haemagglutination inhibition titer for EDS- 76 and ELISA value for *M. gallisepticum* and *M. synoviae* were found to be negative.

Table 1
Bacterial agents isolated from egg peritonitis in layer chicken.

Organism	Positive birds	Percent of positivity(%)
E. coli	226	85.61
E. coli+*Proteous* sp.	7	2.65
E. coli+*Klebsiella* sp.	6	2.27
E. coli+*Staphylococcus* sp.	11	4.17
E. coli+*Streptococcus* sp.	14	5.30

Table 2
Frequency of occurrence of different *E. coli* Serotypes in egg peritonitis.

S. No.	Serotypes	Number of birds	Percentage
1	O5	12	4.55
2	O6	16	6.06
3	O8	19	7.20
4	O20	16	6.06
5	O29	16	6.06
6	O64	31	11.74
7	O75	19	7.20
8	O83	24	9.09
9	O89	12	4.55
10	O96	9	3.40
11	O104	18	6.81
12	O111	28	10.61
13	O119	8	3.03
14	O166	32	12.12
15	Rough	4	1.52

Out of 6572 birds, 1715 from 85 farms showed various types of oviduct abnormalities. Among the 1715, 264 birds (15.39%) from six farms were identified as egg peritonitis on the basis of postmortem lesions. Affected flocks showed sudden raise in mortality (0.5% to 7.0%) without any premonitory signs except the drop in egg production to the tune of 3% to 20%. The drop in egg production among the six farms differed highly significant (x^2 value=17.09, *df*=5) whereas the mortality was non-significant (x^2 value=8.841, *df*=5). Age wise analysis on the occurrence of egg peritonitis revealed highest incidence at 51-60 weeks (31.1%) followed by 31 to 40 (24.6%), 41 to 50 (21.6%), 21 to 30 (11%), 61 to 70 (8.0%) and 71 to 80 (3.0%) weeks and they showed highly significant difference (x^2 value=92.0, *df*=5) among the different age groups. Season wise analysis on the occurrence of egg peritonitis showed highest incidence during southwest monsoon (58.0%) followed by summer (17.4%), winter (16.6%) and northeast monsoon (8.0%) and they differed highly significant (x^2 value=156.136, *df*=3).

4. Discussion

The results of the present investigation indicated that the *E. coli* associated egg peritonitis was responsible for 15.39% of the reproductive tract abnormalities in commercial layers between 21 and 80 weeks of age. Sporadic deaths in adult laying birds could be attributed to egg peritonitis and the percentage of bird loss from these conditions average one per cent in a year. Bandyopadyay and Dhawedkar[12] reported 11.25% of salpingioperitonitis. The higher percentage of incidence in this study might be attributed to poor management practices and occurrence of more virulent *E. coli* serotypes in the affected farms. The incidence of the egg peritonitis was noticed throughout the laying period, however more common during the peak production *i.e.*, 21-60 weeks of age. The birds at peak of production are more susceptible due to stress imposed by the stage of lay. As long as the intestinal mucosal barrier is intact, the normal microflora of bird is likely to inhibit the translocation of pathogenic *E. coli* from the intestine to the blood stream and organs. When these barriers are damaged, possibly by the stress of coming to lay and peak production, the pathogenic bacteria may invade and cause peritonitis[13]. In the egg peritonitis affected farms the drop in egg production and mortality varied between 3 and 20 and 0.5% and 7% respectively. Qu *et al*.[14] reported 5.5% mortality and 10% to 20% drop in egg production with *E. coli* infections in egg type layers reared in cage. Zenella *et al*.[3] reported 5% to10% mortality due to *E. coli* infection with no pronounced signs, suggesting that the infection may be there but couldn't be easily detected until regular tests are performed for its proper diagnosis. The

situation leading to mortality with no pronounced clinical signs will be more critical as it wound result in heavier losses of reduced egg production prior to the investigations. Egg peritonitis was recorded in all the four seasons of the year with significantly higher rate during southwest monsoon (58.0%). Lambie *et al*.[15] also reported higher incidence of *E. coli* infection during rainy season.

The *E. coli* and other bacterial organisms viz., *Proteous sp.*, *Klebsiella sp.*, *Staphylococccus sp*., and *Streptococcus* sp., were identified based on their cultural biochemical charecteristics[6]. *E. coli* was isolated as a pure culture and concurrent with other bacterial agents in 226 and 38 birds respectively. *E. coli* causes transitory immunosuppressive effect in chicken and makes the bird susceptible to opportunistic bacterial agents[16]. *E. coli* organisms were isolated from peritoneal cavity, oviduct and clocal swabs in all the dead birds with egg peritonitis lesion and poultry house environment and cloacal swabs of all the affected flocks. Among the different serotypes identified from egg peritonitis cases serotype O_{166}, O_{64} and O_{111} were predominant, however none of the serogroup was belongs to the systemic form serogroups such as O_1, O_2 and O_{78}[17,18]. 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 had *E. coli* within the cloaca but not in the oviduct or peritoneal cavity. *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. It is believed that pathogenic *E. coli* from the normal intestinal microflora are the source of infection for the oviduct and ultimately, the peritoneal cavity[4]. Necropsy examination and virological examination of tissue samples of the egg peritonitis affected flocks were found to be negative for NDV, IBV and EDS-76 infections, possibly indicate that these infections did not play a role in the pathogenesis of peritonitis in the investigated flocks. Results of the present study indicates that pathogenic *E. coli* can induce peritonitis lesions without the presence of predisposing factors[19].

Necropsy examination of affected birds revealed the presence of yolk material in the peritoneal cavity and it adheres to the surface of ovarian follicles, serosal surface of intestine and oviduct through strands. The colour of the exudate in chronic cases varied from cream to yellow and dark brown and the quantity varied from small to large, however the amount was never as large as that seen in the dilated oviduct to cause distension of abdomen[20,21]. Microscopically the oviduct surface epithelium showed degeneration and desquamation, moderate to marked infiltration of inflammatory cells especially heterophils and lymphocytes in various regions of the oviduct. The lumen of the oviduct revealed serofibrinous exudate with bacterial microcolonies, inflammatory and desquamated epithelial cells[22]. These changes could be due to direct damaging effect of endotoxins elaborated by *E. coli*[23]. *E. coli* can produce diseases becoming attached with mucosal epithelia and another form by invasion to the mucosal epithelia. The recorded lesions of intestine were in the form of degeneration, necrosis and desquamation of mucosal epithelia associated with inflammation indicates the involvement of enteroinvasive type of *E. coli* organisms in the present investigation[24].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Production performance of modern strains of commercial layers is influenced by reproductive disorders due to various diseases. Egg peritonitis is considered to be one of the leading causes of reproductive disorders and results in economic loss in the poultry industry. This manuscript determines the causative organisms of egg peritonitis in commercial layer in Namakkal area.

Research frontiers

The present research work focus on the prevalence of egg peritonitis caused by various serotypes of *E. coli*. The work depicts the pathological changes in the oviduct caused by enteroinvasive type of *E. coli* organisms.

Related reports

E. coli causes immunosuppressive effect in chicken and makes the birds susceptible to opportunistic pathogens. When intestinal barriers are damaged, the pathogenic bacteria may invade and cause peritonitis.

Innovations and breakthroughs

This study utilized standard microbiological and pathological techniques to study the egg peritonitis. Bacteriological agents and pathological changes were documented with good quality of illustrations which are useful for Avian medical practitioners.

Applications

In this study, the relationship between egg peritonitis and age, season, mortality and drop in egg production has been investigated. This research assumes significance as this information can be used to design to prevention strategies for peritonitis.

Peer review

The study is well designed and determines the bacterial causes of egg peritonitis in layer chicken. Egg peritonitis due to involvement of various serotypes of *E. coli* and pathological changes in oviduct of affected chicken are demonstrated by the authors. The results will help to design both therapeutic and prophylactic preventive strategies.

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