Pathology of Salpingitis in Domestic Fowl

M. Sc. (Vet) Thesis

January, 1971

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I. C. A. R. Junior Fellow

Post-Graduate Department of Pathology

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A Thesis

Submitted to the Magadh University in Partial Fulfilment of the Requirements for the Degree

OF

Master of Science (Vet).

IN

PATHOLOGY

JANUARY 1971

BY

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C E R T I F I C A T E

This is to certify that the Thesis entitled "Pathology of Salpingitis in Domestic Fowl" submitted for the degree of Master of Science (Vet) in Pathology to the Magadh University by Sri Gopala Krishna Susarla embodies the results of work carried out by him under my supervision and guidance.

12. 1. 77.

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A C K N O W L E D G E M E N T S

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C O N T E N T S

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CHAPTER - I

INTRODUCTION

INTRODUCTION

In India, poultry keeping is in vogue since the days of antiquity. The modern Indian breeds of poultry are stated to have descended from the wild jungle fowl (gallus gallus), which had its origin in this country. (Naidu, 1959).

The poultry industry in India received its due recognition due to its economical importance based on nutritive values. Various schemes for the development of this industry have been launched in different parts of the country.

The objective of a well organised poultry farm is optimum production in order to make it profitable commercially. Nevertheless, this industry, like any other livestock industry, is subject to various hazards viz: specific and non-specific diseases which cause heavy mortality leading to economic losses.

Diseases of reproductive system are reported to be responsible for heavy economic loss. Statistical data, collected by Sharma and Singh (1964) during a period of six years, indicated that 44.8% of the total losses at Mathura Poultry Farm were due to diseases of reproductive tract alone.

Salpingitis is one of the reproductive disorders

characterised by inflammation of oviduct. It is variously discussed by different workers as egg-bound, egg-peritonitis, ruptured yolk, impaction of oviduct etc. However, according to Lindgren (1954), all these conditions are the manifestations of the same disease entity. The data so far available are mostly concerned with the indidence of the disease. Only a few reports are available regarding the pathogenesis and pathology of the disease.

Salpingitis may be found either in acute or chronic form. Due to lack of adequate informations regarding the clinical syndromes of this rapidly fatal disease, the acute disease is mainly recognised from autopsical findings which are characterised by inflammatory or hyper-secretory disturbances of the oviduct with or without concurrent presence of peritonitis.

The majority of reports in literature on clinical syndromes of the disease are referable to chronic salpingitis. The symptoms described are loss of appetite and in weight, shrunken and cyanotic comb, partial or complete cessation of egg production, cloacal discharge often with bad smell and the classical "Penguin" or duck sitting posture.

Many workers have incriminated certain predisposing factors viz: breed and age suceptibility, E. coli, harmonal influences etc., Certain physical and physiological stresses caused by factors, such as, high-perches, physical exhaution of the egg-laying apparatus etc. have been incri-

-minated to contribute to this condition.

Many organisms have been isolated from the oviduct of salpingitis cases, putting them as primary or secondary agents. Organisms like Salmonella, Pasturella, Streptococci and E. Coli were isolated. Of these, E. Coli is supposed to be the primary agent which in association with other predisposing factors, precipitates the disease. In India, Gupta and Singh (1963) isolated some strains of E. Coli from egg-peritonitis cases, and typed. They found that only certain serotypes of E. Coli cause this condition. Reports are also available about the relationship of E.Coli to salpingitis as primary agent.

Lindgren (1964), in his work on aetiology of salpingo-peritonitis, found certain post-mortem findings such as high ovarian activity and better nutritional state in naturally occurring cases, and considered that hormonal predisposition was involved in the causation of the disease. He obtained experimental evidences supporting the view that higher level of both progesterone and oestrogen but not alone predisposed the bird thereby enhancing the pathogenicity of the E. Coli.

However, the role of infection by bacteria including E. Coli as a causative agent in the reproductive disorders of domestic fowl has been subjected to controversial discussions. Several authors (Litzke, 1934: Moore and Martin, 1944 : Kaunker and Moser, 1954 and Munker, 1955)

noted frequent coincidence of E. Coli infection and salpingitis, but believed bacteria to be secondary or agonal invaders without any primary importance. Glantz, Narotsky and Bubash (1962) frequently isolated E. Coli from cases of salpingitis and concluded that certain serotypes of these bacteria were evidently important complicating factors.

A few attempts to reproduce salpingitis in fowl are reported in literature. Twissleman (1939) was unable to reproduce the disease by inoculating six 4-months old pullets with E. Coli isolated from the naturally occurring disease. Later, Gross and Seigel (1959) successfully reproduced the disease by simultaneous inoculation of E. Coli and sterile egg-yolk intra-peritoneally. Sharma (1964), made successful attempts in the above way.

In the present study, the incidence of salpingitis was studied in relation to season, age, breed and other factors in an organised poultry farm. An attempt was made to reproduce this condition experimentally in normal birds. Further, attempts were also made to induce the infection in sexually immature chicks that were pretreated with oestrogen and progesterone, using E. Coli as infective agent to investigate the interrelationship of intra-oviducal E. Coli infection and certain endocrine factors in domestic fowls.

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CHAPTER - II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

1. HISTORY :

Prior to 1900, only sketchy reports are available in the literature on 'salpingitis' as a condition associated with reproductive disorders in poultry. In 1870, Dr. E. Crisp in London exhibited some of reproductive organs of hens showing that malformed and imperfect ova in the hen generally arose due to inflamed condition of the oviduct. For 3 decades, the conception of the disease remained the same since very little was done in this direction. Litzke (1934), while reviewing European literatures observed as follows :-

(i) The condition "egg-bound" is a clinical concept denoting symptoms of difficulties in oviposition. The aims of the most of the approaches to the problem are curative treatment of the condition.

(ii) A very wide variety of factors are incriminated as the causes of "egg-bound". Most of them are external generally mechanical or traumatic as well as secondary infections. Some workers attribute infection to be the primary cause.

(iii) Salpingitis is one of the causes of egg-bound condition, next to intraabdominal tumors. This evidently means that salpingitis is the primary lesion. (Fleischhauer, 1926).

(iv) According to Breig (1919), salpingitis is the main disease entity and the egg-bound condition is one of its symptoms. Schutt (1930) called the condition as "Eileitererkrankung" of fowl, which means the salpingitis.

In American literature, a very special and complex nomenclature has been used in the descriptions of this disease. To illustrate this, Thompson and Dodson (1922) placed a total of 422 cases of reproductive disorders under six different categoris. These were ovarian tumor, ruptured oviduct, egg-bound, salpingitis, prolapsed oviduct and peritonitis. All these, according to current European concept, can be grouped under salpingitis.

Gross and Seigel (1959) brought new aspects on the disease by reproducing it by inoculation with E. Coli using sterile yolk intraperitonially as an adjuvant.

Where as Domermuth and Gross (1962) produced this condition in day old chickens by injecting with P.P. L.O. via the yolk sac.

According to available records, the difference in conception of this disease entity in the old world and new are however only superficial. Interesting to note is that, that there are no earlier reports on this disease, in Indian literature, which signifies, the need to work a lot on this line.

2. ETIOLOGY :

(a) Specific factors :-

Whether the disease salpingits was of primary or secondary nature remained a debatable subject for some time in past. Latter, working on the etiology of the disease, various workers have discussed the role of micro-organisms in association with predisposing factors.

TeBennepe (1924), while working on etiology of salpingitis referred to several species of micro-organisms as causative agents.

According to Schimdt (1929), Mass (1923) had diagnosed salpingitis for the first time in an epedemic form and termed it "Mass-fowl epedemic". On bacteriological examination, micro-organisms were isolated. However, he attached no etiological significance to these organisms although the bacteriological and other findings had indicated their role in the causation of the disease.

Heironymi and Bittner during the years 1925-1926 reported salpingitis in domestic fowls in an epidemic form and attributed trematodes - Prosthogonimus species as the etiological agent of the disease.

Eber (1930) advocated that salpingitis is one of the causes of "egg-bound" condition. The inflammatory condition is a consequence of mishapen or otherwise defective eggs, which obstruct the oviduct and cause irritation

and inflammation.

Litzke (1934) reported the relationship of S. pullorum to salpingitis in fowls. S. pullorum infection was found by cultural and serological methods in 20% of thirty nine fowls suffering from inflammation of the oviduct.

Edwards and Bull (1937) isolated haemolytic streptococci, belonging to group 'C' of Lancefield from inflamed oviduct, peritoneal exudate and internal organs. The portal of entry was thought to be genital tract.

Jull (1938), while describing the clinical picture of bird suffering from salpingitis, reported his failure in isolating organisms from diseased birds.

Beach (1940) reported isolation of E. Coli in pure culture from several cases of egg-peritonitis, although most of the egg-peritonitis cases examined bacteriologically proved to be sterile. The significance of this organism in causation of egg-peritonitis could not be ascertained. He also pointed out that unless the etiological agent was investigated and known, it was difficult to formulate preventive measures against this condition.

Moore and Marten (1944), on bacteriological examination of autopsy materials of 274 (16.1%) of 1051 hens having reproductive disorders, obtained bacterial cultures from 44.5% of them. The work was done to investi-

-gate the possible role of micro organisms in the causation of diseases of female genital tract. The organisms isolated were Staph. aureus and Staph. albus 61.3%; E. Coli 26.7%. Others such as B. Subtilis, Str. Viridans, haemolytic streptococci, Past. aviseptica and Salmonellae were found in the remaining 12% cases. Pathogenicity tests with the isolates resulted in illness or death in 54.9% of cases. They attributed no etriological significance to the isolated organisms, since no significant results were obtained on comparative bacteriological examination of infected and non-infected groups of birds.

Romanoff and Romanoff (1949) stated that Prosthogonimus macrorchis after getting into the oviduct resulted in salpingitis.

Kjos-Hanssen (1950) isolated shigella-like organisms in pure culture from oviducts of 24 out of 51 hens suffering from egg-peritonitis. The organisms resembled with cloacal bacteria present in many of normal hens.

Rungerford (1951) stated that Salmonella Pullorum occassionally caused salpingitis in chickens resulting in haemorrhagic discharge from the oviduct. Salpingitis also occured from "Vent glut" in which infection ascended from the vent to the oviduct. He stressed the role of coliform organisms in infection of oviducts which subsequently resulted in omphalitis epidemic in hatching chicks.

Hall (1953) felt that salpingitis out breaks might be caused due to S. Pullorums infection, which inturn resulted in peritonitis due to rupture of the infected ovarian follicles. Salpingitis also occured in immature pullets. He further advised to discard the birds affected with salpingitis due to S. Pullorum in order to avoid the constant source of pullorum disease in chicks.

Munker (1955) examined infected oviducts of 30 hens and isolated aerobic micro-organisms in pure or mixed culture. This included E. Coli, S. gallinarum, Micrococci, Streptococci, Pasturella, Proteus and Pseudomonas from 25 cases. He incriminated S. gallinarum as the primary etiological agent.

Advisory staff poultry world (1956) described egg-peritonitis as the most common disease and opined that carriage of pullorum disease and fowl typhoid were some of the conditions which led to this condition.

Arnall (1961) found 13 cases of peritonitis out of 26 cage birds had reproductive disorders. Presence of free egg material in the peritonial cavity or retention of normal or abnormal eggs in the oviduct resulting in its rupture were the common findings in most of the cases. Bacteriological examination of the abdominal fluid revealed E. Coli in one case only. He considered egg-peritonitis associated with adhesions as a serious conditions as it did not allow

the removal of degenarated material from the abdominal cavity even on abdominal irrigation. The condition resulted in toxemia.

Vior (1961) found tuberculous lessions in the oviduct of hens and demonstrated avian type of Myco. tuberculosis in shells, yolks and embryos of their eggs.

Glantz et al. (1962) typed serologically 72 strains of E. Coli isolated from cases of salpingitis. Most of the strains belonged to 'O' groups 2a. The serotype 2a : 1 : NM was the most common. He also isolated the serotype 78: K : 4 from a flock affected with salpingitis. Strains with O groups 13, 15 and 111 a, c were occassionally obtained. E.Coli having O agglutinations 17 and 18 were also isolated from inflamed oviducts.

Rao et al. (1962) described peritonitis with yellowish caseous exudate in the peritoneal cavity, ruptured ovum, and inflamed ovaries as the invariable post-mortem findings in chickens affected with ornithosis. Peritoneum revealed petechial haemorrhages. Such post-mortem lesions were confused with either chronic fowl cholera or CRD complicated with egg-peritonitis.

Jacobs et al. (1962) found Toxoplasma gondi in ten pools of oviducts out of 124 pools examined. Each pool consisted of 10 oviducts or ten ovaries from hens having macroscopically normal viscera.

Gupta (1963) isolated 17 strains of E. Coli from egg-peritonitis cases in chickens. Fifteen of these strains proved to be pathogenic for mice. Serologically, they belonged to 'O' groups, O_{18}, O_{64}, O_{9}, O_{17}, O_{88}, O_{23}, O_{111}, O_{57} and $O_{?}$. Serotype O_{8} on intraperitonial inoculation proved to be pathogenic for chicks also.

Sharma (1964), while working on pathology of female genital tract and egg-peritonitis, isolated E.Coli from 8 cases. In his experiment, he also assesed the role of E. Coli in causing egg-peritonitis in chicks given simultaneously egg-yolk through intraperitoneal route.

Lindgren (1964) studied the possible role of E. Coli infection in naturally occuring cases of salpingitis. He observed that the co-existance of E. Coli and salpingitis was too often (43% of 1784 salpingitis cases examined) to be loosely leaned off as a mere casual incidence.

Nicolet and Fey (1965) isolated pure cultures of Past. Haemolytica frequently in spring from young hens with salpingitis. In pathogenity tests with one strain, the LD_{50} in mice could be increased about fifteen-fold by simultaneous injection of egg-yolk, half diluted with broth. The strain was pathogenic for day old chicks through inoculation in to yolk sac; but no death occured when it was injected inraperitoneally or intratracheally; 12 hens at the cessation of

laying were inoculated intraperitoneally, subcutaneously, intravenously or into the oviduct and killed two months later. Post-mortem, histological and bacteriological findings were given, the organism was recovered from pharynx and trachea in all 12 cases but in none from the oviduct which was histologically normal. It was concluded that P. haemolytica plays an important role in salpingitis of fowls but pre-disposing factors were necessary for its causation.

Kohlert (1968) examined specimens from 237 laying hens affected with acute salpingitis with or without peritonitis. From 128 specimens (55%), pasturella-like organisms were isolated; E. coli was recovered from 71 (29.9%) cases, while the remaining specimens yielded Aerobacter aerogenes. Because of their biochemical properties, the pasturella like organism was considered to constitute a distinct species for which the name Pasturella Salpingitidis was proposed. The organism was pathogenic for day old chicks by intraperitoneal or intramuscular inoculations.

Ball et al. (1969) made comparisons of the reproductive tracts of five groups of turkey hens -(i) mycoplasma free, (ii) conventionally reared disease free (iii) experimentally infected with Mycoplasma meleagridis (iv) experimentally infected with CELO virus and (v) birds from flocks with infertility. They suggested that lymphoid nodules and plasma cell infiltrations were not the normal structures in the tract and that the degree of change indicated the severity of infection [redacted]

[redacted] rather than the aetiological agent involved.

The works of the authors reviewed above are mainly related to isolation of different micro-organisms from naturally occuring cases in relation to salpingitis. Some of theauthors, whose work is reviewed below, have tried to reproduce the condition experimentally in normal birds, to asses the role of micro-organisms in causation of the disease.

Broadfoot et al. (1956) exposed several groups of chicks of similar age, to the field strain of virulent infectious bronchitis virus in order to study its effect on the female genital tract of the fowls. These chicks were infected at intervals of three or four days during the 1st 18 days of life. Among the six age groups, 52 non-layers were examined which on post-mortem examination were found to have short non-patent oviducts with varying quantity of of free yolk in the body cavity of 33 hens. Oviduct was found to be mainly affected while the ovary remained active. They believed that the activity of ovaries was probably enhanced resulting in the formation of a large number of immature ova.

Gross (1956) inoculated several groups of chicks of varying age groups with cultures of E. Coli alone or together with 'W' strain of CRD agent. Salpingitis occur as one of the manifestation of this infection which

was probably caused due to bacteraemia. The organisms remained alive in the oviduct for a long period.

Gross (1957) further observed frequent involvement of oviduct following experimental infection of left great abdominal airsac with certain strains of E. Coli. He also described the gross and histopathological changes in the oviduct.

Sevoian and Levine (1957) described inflammatory and glandular distention in oviducts in seven month old birds infected with bronchitis virus experimentally.

Gross (1958) produced salpingitis experimentally and reported the probablity of direct extension of E. Coli infection from left greater abdominal airsacs to the oviduct through the attached position of meso-salpinx. It was found that the infection of the oviduct lasted for a period of more than five months and probably resulted in permanent malfunction of the oviduct. He further described the association of salpingitis with CRD particularly when the latter was complicated with E. Coli.

Gross and Seigel (1959) produced egg-peritonitis experimentally with simultaneous injection of egg-yolk and E. Coli intraperitoneally or per-vaginum. They conducted two identical experiments on 84 laying birds. The lesions of peritonitis produced experimentally were as severe as those found in natural cases. On the basis of these experiments,

as well as the findings of Cole and Nutt (1953), they discussed the pathogenesis of egg peritonitis and observed E. Coli present in the faecal matter were deposited in the vagina which entered the peritoneal cavity through the oviduct. In natural cases, the egg yolk in the peritoneal cavity became contaminated with E. Coli and caused peritonitis. Free- yolk in the peritoneal cavity occured when ova after ovulation were not engulfed by the infundibulum but dropped into the abdominal cavity. On rupture of vitelline membrane, yolk which in normal course would be absorved without any detertiment unless contaminated, get dispersed in the peritoneal cavity and cause peritonitis due contaminated by E. Coli.

Domermuth and Gross (1962) conducted three experiments and produced salpingitis by inoculation of culturesof M. gallisepticum. From the experiment, they postulated that salpingitis occure by mechanical transfer of M. gallisepticum from yolk sac or airsac to oviduct.

Igysite (1963) isolated Toxoplasma gondii from albumen of two eggs laid by two hens infected with a high dose (one million per bird) of organisms by intramuscular route. One of these hens was found to have lymphocytic infiltration in subglandular region of oviduct.

Sharma (1964) stressed the role of E. Coli isolates and free egg-yolk in producing egg-peritonitis.

Lindgren (1964) infected healthy birds under different experimental conditions per vagina or intraperitoneally with E. Coli isolated from natural cases of salpingitis. He found that young chicks were relatively resistant to infection. He stressed that the predisposing factors play a deciding role in the causation of the disease.

(b) Non-specific factors :-

(1) Salpingitis in relation to season:-

Several authors have reported that the incidence of salpingitis in fowl varies with season.

Te Rennepe (1924), found that the incidence of the disease increased in spring season.

Schutt (1930) found an increase in the incidence of salpingitis and total mortality during May through August and regarded this due to defective management during the breeding season when the farm staff were too busy in incubating and hatching eggs, caring for baby chicks and to give the layers proper care.

Dudley, Dobson and Gordon (1941), whose data showed similar preponderance of salpingitis cases during April through August, regarded that salpingitis was caused by exhaution of the egg-laying apparatus following intensive production. This was attested by the fact that the death

from salpingitis increased during the latter half of laying season.

Hays (1949) in his study on mortality among 6 - 18 months old pullets observed that mortality rate was low from September through February, later getting on high from March to June when it is reached its maximum.

Brunsen and Godfrey (1952) found the incidence of salpingitis to reach its peak in June which was coincident with high spring production. They believed that the rise in incidence was due to wear and tear of production. The period of next highest incidence was October through December following the attainment of sexual maturity. The latter nevertheless varied from year to year.

Ogle (1938) reported his data on adult hen mortality but did not enumerate the causes. He found the heaviest mortality during the quarter between April and June, when normally in Newyork State birds would be having the advantage of milder temparature and considerably more sunshine. The lowest rate of loss was between October and December.

Lindgren (1964) in his extensive work on salpingitis in domestic fowl observed a characterestic seasonal variation in the incidence of salpingitis with peaks during the late spring and around the turning of the years. He found similar correlation between egg-production and season

as determined by purchase figures from the Swedish Egg Marketing Association and monthly average number of hours of sunshine per day. & Peaks of egg production were always ahead of peaks of availability of sunlight and salpingitis incidence.

Informations on these lines are not available in the Indian literature as their works are mainly refered to etiology of the disease.

(ii) Salpingitis in relation to breed:-

Stafseth and Boyd (1929) studied the incidence of salpingitis in relation to breeds in poultry. He observed that the highest incidence of the disease was in WLH birds (19.4%). The incidence among RIR, Plymouth-rock and White Wyandottes was of the same order (11 - 14%).

Bryant and Jhonson (1944) gave data on mortality and its causes in two strains of WLH birds numbering 1099 and 1021 pullets respectively and reported a high incidence of peritonitis due mostly to secondary infection resulting from decomposed broken yolks in the abdominal cavity. The authors claimed that the difference in losses from peritonitis between two strains was highly significant.

Darcel et al. (1952) reported significant difference in the suceptibility of breed to salpingitis. He found that 12% of all mortality in barred plymouthrocks and 27% in brown leghorns were due to salpingitis.

However, Lindgren (1964) who examined natural cases of salpingitis statistically failed to find association of breed disposition to this condition. He pointed out that the distribution of breeds was very skewy.

(iii) Salpingitis in relation to age:-

The literature on age factors is very little. Jordan (1956) opined that age may be the one of the factors, that predisposes the bird to the disease.

Whereas Lindgren (1964) found out that birds died from salpingitis during the pullet year have an average age of 392 days. He also found out that there is a highly significant negetive correlation between hatching dates and age at death in salpingitis, that does not exist for other diseases. He considers that this fact is taken to indicate that season in itself is the determining factor in the seasonal incidence of salpingitis and not age or the duration of egg production preceding death.

(iv) Salpingitis in relation to harmones:-

Lindgren (1964) [redacted] carried out infection experiment in sexually immature pullets under various harmonal treatments. He stressed the role of oestrogen and progesterone in the defence mf mechanism of oviduct.

A disease is considered professional when it is directly involved with its productive performance. In this connection, it has been found that the incidence of salpingitis is directly involved with high egg-production. Quite a few authors have reported on this line, and have correlated the incidence of the disease with high production.

Schutt (1930), working on etiology of salpingitis opined that diseases of the oviducts of hens with maximum production may righly be regarded as professional disease. During active production, the hens are exposed to considerable strain due to exaggarated metabolism not infrequently leading to physiological derangement. During this period, the reproductive organs are based with maximum physiological burden. Even a slight departure from the optimum conditions of breeding and management will cause injury to manifest in the organs.

Bushnell and Twiehans (1945) mentioned that "egg-bound" frequently occured in pullets and occassionally in adult ones. Salpingitis or presence of an abnormally large size egg in the oviduct resulted in this condition. Affected birds visited the nests frequently and remained rest less.

Lindgren (1964) found that mortality from salpingitis has been shown to be positively correlated with a high pullet year egg weight and a relatively high egg-

However, no such information is available in the Indian literature due perhaps to non-availability of proper data in either organised or rural flocks. In order to assess the losses for this economically important disease, to determine the specific and non-specific etiological agents and to elucidate the pathogenesis and pathology of this malady, it is essential that poultry breeders are educated and statistical data are properly recorded for study.

C H A P T E R - III

MATERIALS AND METHODS

MATERIALS AND METHODS

1. INCIDENCE OF SALPINGITIS IN POULTRY:

The study on the incidence of salpingitis was made on data collected from the post-mortem records maintained in this department from April, 1968 to November, 1970. This also included the data collected by author in course of autopsy examination during the current year. The incidence was studied in relation to the season, age, and breed of the affected birds. The birds belonged primarily to the Central Poultry Farm, Patna.

2. EXPERIMENTAL STUDIES:

(i) Objective and plan of the experiment:-

It will appear from the review that natural salpingitis cases if not complicated with any specific diseases, are encountered in sexually mature hens. In order to determine the role of these sex hormones, in predisposing the suceptible birds to salpingitis. two groups of birds, sexually immature pullets and hens, to be described latter were selected for experimental infection. The pullets were divided into three groups for treatment with oestrogen, progesterone and oestrogen + progesterone together, prior to infection. The adult birds which were sexually mature were not treated with these harmones. The details about the procedure of hormone treatment are described in appropriate section.

(ii) Birds :-

Ten pullets and 3 sexually mature hens each of WLH and RIR breeds, were used. They were obtained from poultry unit of "Hariana Cattle Breeding Farm, Dumraon". The pullets were of the age group of 16-20 weeks while the hens were aged, 8 months. They were maintained on poultry ration obtained from Central Poultry Farm, Patna, water, and greens were supplied ad libitum. They were free from intestinal parasites as determined by examination of their fecal samples.

(iii) Pre-infection hormonal treatment :-

As stated earlier, the experimental pullets numbering 14 were divided into 3 groups for treatment with (1) oestrogen (2) progesterone and (3) progesterone + oestrogen. Besides, 6 pullets were left untreated as control. Of these 6 pullets, 4 were inoculated only with the cultures without pre-infection treatment with harmone, while two were left as untreated control. This is shown in the table below:-

TABLE NO. 1.

Plan of pre-infection hormonal treatment

PLAN OF PRE-INFECTION HORMONAL TREATMENT

Oestrogen		Progesterone		Oestrogen + Progesterone		Controls			
						Sterile nutrient broth only		Culture only. No harmone	
WLH	RIR	WLH	RIR	WLH	RIR	WLH	RIR	WLH	RIR
59	70	57	67	54	66	64	71	51	68
52	65	60	61	56	69	-	-	55	58
				53	63				

N.B. :- The figure indicate the bird number.

The oestrogen and progesterone commercially known as "VETESTEROL" and "PROGESTIN" were the products of May and Baker and Organan respectively, purchased locally. The oestrogen and progesterone were administered intramuscularly to the respective groups of birds separately each in the doses of 2.5 mgm. daily for a period of fifteen days. The 3rd group of six birds received both the products on either side similarly for fifteen days. The hens were not treated with any harmone.

(iv) Experimental infection :-

(a) Strain :-

For experimental infection, a virulent strain of E. coli isolated by the author from a natural case of acute salpingitis in poultry was used. The pathogenicity of this isolate was confirmed by inoculating 0.1 ml. of an 18-hour old broth culture intra-peritoneally into chickens and mice. They died within 18 hours, and the organisms were isolated from heart blood, liver, spleen, and peritoneal fluid on plain agar as well as Eosin-Methylene Blue agar plates in pure culture.

(b) Preparation of inoculum :-

The E. coli strain was grown in nutrient broth aerobically at 37°C for 18 hours. The viable count of organisms present in broth was determined by 10-fold serial dilutions on agar plates. The number of organisms in the

culture was found to be 6.4×10^{10}/ ml.

(c) Method of inoculation:-

As reported earlier, 18 pullets and six hens were used for the purpose of artificial infection. Each pullet received a dose of 1 ml. of the broth culture of E. coli containing 6.4×10^{10}/ ml. as described above. The culture was inoculated per vaginum into the shell-gland in the doses of 1 ml. for the pullets and 5 ml. for hens. The procedures described by Lindgren (1964) were followed for experimental infection in all the groups of experimental birds.

TABLE NO. 2

Plan of experimental infection of mature laying hens.

PLAN OF EXPERIMENTAL INFECTION OF MATURE LAYING HENS.

Particulars	Bird numbers				Date of inoculation
Infected with E. coli.	78	73	80	81	17.8.70
Controls (Sterile nutrient broth only)	72	75			17.8.70

First, the small finger was inserted into the vagina through cloaca and the inoculum was dropped beyond

the utero-vaginal orfice with the help of a specially modified goat teat canula attached to a syringe. The passage leading to the shell gland was ascertained by the finger which, evinced resistance while passing through the utero-vaginal orfice. After inoculation the birds were observed for 1 hour, regurgitation of inoculum did not occur in any of them.

(v) Post-infection observations :-

(a) Clinical :-

Prior to infection, all the experimental birds were observed closely for any sign of disease. They were included in the experiment, when they were found clinically free from any disease. Further, body weight and body temperature of these birds were also recorded prior to infection.

After artificial infection, the birds were further examined for clinical sign of disease with particular reference to salpingitis. Particular attention was paid to recording of drowsiness, incoordination of gait and duck sitting posture, thermal response, cloacal discharge etc.

(b) Serological response :-

Slide agglutination test was done on the serum samples of birds collected at the time, when they were sacrificed. For this purpose, the infecting strain of E. coli was used as antigen. The agglutination reaction was

recorded and graded according to the degree of reaction as follows :-

✓✓✓	=	Strongly positive.
✓✓	=	Moderately positive.
✓	=	Positive.
✓	=	Doubtful.
—	=	Negative.

(vi) Haematological studies :-

In order to draw information regarding the establishment of infection, the infected as well as control birds were examined for total and differential count.

(a) Differential count :-

Smears were prepared with blood obtained from the wing-vein and stained by modified wright's stain. Two hundred leucocytes were counted in each smear following the battlement system (

(b) Total count :-

It is well recognised that procedures of counting blood cells of mammals are not suitable for avian blood. Of the three methods available, (i.e. indirect, semi-direct and direct methods) semi-direct method (Wisemann's method) as described by Coffin (1953) was followed in the present study. With this procedure, the leucocytes were stained intense pink in contrast to light pink nucleated red blood cells.

Wiseman's diluent:

Phloxine	-	50 mgm.
Formalin	-	5 c.c.
Ringer's solution.	-	95 c.c.

The erythrocyte pipette was filled to 0.5 mark with blood and rest of pipette was filled to 101 mark with Wiseman's diluent. The pipette [redacted] kept in refrigerator overnight to obtain maximum staining of the cells. The following morning, the red blood cells were counted in 80 small squares and as the blood was diluted 200 times, the number of red cells counted in 80 squares was multiplied by 10,000 to give the number of R. B. C. per cubic millimeter of blood.

The number of acidophilic granulocytes was counted in the entire ruled area of the haemocytometer. A differential count in a properly stained with blood smear was performed to obtain proportion of heterophils, eosinophils in percentage. The total number of leucocytes was calculated according to the following formula.

Total leucocytic count :- $\frac{\text{No. of acidophilic cell count} \times 10 \times \text{dilution factor} \times 100}{\% \text{ of acidophilic cells in blood smear} \times \text{number of square millimeters in the hemocytometer.}}$

(viii) Isolation of E. coli from experimental cases:-

The experimental birds were sacrificed at varying

intervals between 48 hours and 15 days.

For re-isolation of E. coli, approximately 1 gm. of tissue from the oviduct was tirturated in nutrient broth and inoculation made on to surface of McConky agar and Eosin-Methylene blue agar plates either direct as well as after enrichment over night in nutrient broth. The identification of isolates resembling culturally and biochemically E. coli was made following the procedures laid down in Bergys manual

(viii) Gross and histopathological studies:-

All the experimental birds either dead or sacrified post-infection were autopsied and gross lesions were recorded. The oviduct was divided into 5 segments i.e. infundibulum, isthmus, magnum, uterus and vagina. A slit was made along the oviduct and tissues showing lesions were fixed between two pads of cotton soaked in 10% formaline for about 1 - 2 hours. Later, they were transfered as such in specimen bottle containing the fixative.

Apart from above, 30 out of 120 oviducts showing gross lesions of salpingitis which were collected during the study of the disease were also selected, and fixed similarly, for histopathological studies. Paraffin embedded sections were cut at 6 - 7 microns and stained by haematoxylin-eosin (Harris), vangieson's methods.

CHAPTER - IV

RESULTS

R E S U L T S

INCIDENCE OF SALPINGITIS :

As stated in the previous section, the incidence of salpingitis was studied on the data available in the autopsy records of the pathology department of this College from 1968-1970 as well as those collected by the author in course of this investigation during the current year. During this period, the incidence of the disease ranged between 10% and 37.5% with an overall percentage of 25.6. Seemingly, the incidence was found to be the lowest around 10% in 1968 which rose to 37.5% in 1970. The results are shown in Table No. III.

T A B L E NO. III.

Incidence of salpingitis in poultry at Central Poultry Farm, Patna.

Year	Total number of adult hens autopsied	Number of salpingitis cases found.	Percentage.
1968	674	68	10.1
1969	601	198	32.9
1970	512	192	37.5
Total.	1787	458	25.6

The data on the incidence of salpingitis were studied in relation to the breeds of poultry. It can be seen from Table No. IV that the average indidence among RIR and WLH was 47.2% and 47.8% respectively. On the other hand, the percentage among the Black Austrolps was 5.0% during this year. The year-wise break-up of the incidence shows that among the RIR, the percentage of salpingitis was between 35.3 and 51.0 while in WLH it remained between 40.7 and 64.7. The Austrolops which were raised during the current year only had the incidence of salpingitis to the extent of 12.0% only.

T A B L E NO. IV.

Incidence of salpingitis in relation to breeds at Central Poultry Farm, Patna.

Year	Total no. of salpingitis cases.	RIR		WLH		Black Austrolops	
		No. of salpingitis cases	Percent	No. of salpingitis cases.	Percent	No. of salpingitis cases.	Percent
1968	68	24	35.3	44	64.7	-	-
1969	198	101	51.0	97	49.0	-	-
1970	192	91	47.4	78	40.7	23	12.0
Total.	458	216	47.2	219	47.8	23	5.0

It would appear from Table No. V that maximum number of salpingitis cases occurred in poultry in the age group of 6 to 8 months, irrespective of their breeds and advancing age. The incidence gradually declined in both WLH and RIR but their percentages hugged closely in the particular age group.

T A B L E NO. V.

Incidence of salpingitis in relation to age, at Central Poultry Farm, Patna.

Age group	WLH		RIR		Black Austrolops	
	No. of salpingitis cases.	Percentage of salpingitis.	No. of salpingitis cases	Percentage of salpingitis.	No. of salpingitis cases.	Percentage of salpingitis.
6 to 8 months.	112/458	24.4	107/458	24.36	23/458	5.0
8 to 12 months.	80/458	17.46	93/458	20.3	-	-
12 to 18 months.	27/458	5.9	16/458	3.5	-	-

N. B. :- Numerator indicates number of cases of salpingitis within the age group.

Denominator indicates number of deaths due to salpingitis.

Further it was also observed that the percentage of mortalities due to salpingitis was the highest (53.94%) during winter i.e. from November to February while it was the lowest (19.32) during the monsoon season i.e. July to October.

T A B L E NO. VI.

Incidence of salpingits in relation to season at Central Poultry Farm, Patna.

Year	Total no. of cases of salpingitis	Number of positive cases					
		Winter		Summer		Monsoon	
		Number of salpingitis cases.	Percentage	No. of salpingitis cases.	Percentage.	No. of salpingitis cases.	Percentage.
1968	68	15	22.2	19	27.9	34	50.0
1969	198	123	62.1	48	24.3	27	13.6
1970	192	109	56.7	56	23.9	27	14.1
Total.	458	247	53.94	123	26.86	88	19.3

N. B. :- Winter = November to February.

Summer = March to June.

Monsoon = July to October.

T A B L E NO. - VIIA

Clinical data of experimental hens.

Days at infection.	78		73		80		81		72		75	
	T	W	T	W	T	W	T	W	T	W	T	W
1	2	3	4	5	6	7	8	9	10	11	12	13
-7	107.6	1.76	107.6	1.73	107	1.45	106	1.99	107	1.18	107.8	1.43
-6	106.	1.76	107.6	1.73	107	1.45	107	1.99	107	1.18	107.8	1.43
-5	106	1.8	107.6	1.73	107.8	1.45	107	1.99	107	1.18	107.6	1.43
-4	107	1.8	107.6	1.7	107.	1.45	107	1.99	107	1.18	107.6	1.43
-3	107.6	1.8	107.6	1.73	107	1.45	107	1.99	107	1.18	107	1.43
-2	107.6	1.8	107.6	1.73	107	1.45	107	1.99	107	1.18	107	1.43
-1	107.6	1.8	107.6	1.73	107	1.45	107	1.99	107	1.18	107	1.43
0	107.6	1.8	107.6	1.73	107	1.45	107	1.99	107	1.18	107	1.43
1	108	1.8	108.6	1.73	110	1.45	110	1.99	107	1.18	107	1.43
2	109	1.8	-	-	110	1.45	110	1.99	107	1.18	107	1.43
3.	108	1.8	-	-	109	1.45	110	1.99	107	1.18	107	1.43
4.	108	1.8	-	-	108	1.45	109	1.99	107	1.18	107	1.43
5	107	1.8	-	-	107	1.5	107	1.99	107	1.18	107	1.43
6	107	1.8	-	-	107	1.5	107	1.99	107	1.18	107	1.43

Cont'd Table No. VIIA.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
7					107	1.5	107	1.99	107	1.18	107	1.43		
8					107	1.5	107	1.99	107	1.18	107	1.43		
9					107	1.5	107	1.99	107	1.18	107	1.43		
10					107	1.5	107	1.99	107	1.18	107	1.43		
11					107	1.5	107	1.99	107	1.18	107	1.43		
12					107	1.5	107	1.99	107	1.18	107	1.43		
13					107	1.5	107	1.99	107	1.18	107	1.43		
14					107	1.5	107	1.99	107	1.18	107	1.43		
15					107	1.5	107	1.99	107	1.18	107	1.43		

T = Temparature in degrees F.

W = Body weight in Kgs.

CONTROLS

Days at infection	CE					RIR								
	WLH			55		58		64		68		71		
	59			W	TT	W	T	W	T	W	T	W		
	T	W		31	32	33	34	35	36	37	38	39		
1	2	3												
-7	107.6	1.2		1.06 .2	1.05 107.6	1.30 107.2	1.30 107.6	1.3 107.6						
-6	107.6	1.2		1.06 .2	1.05 107.6	1.3 107.6	1.34 107.	1.3 107.6						
-5	107.6	1.		1.06 .4	1.05 107.6	1.35 107.2	1.3 107.6	1.3 107.4						
-4	107	1.		1.06 .4	1.05 107.6	1.3 107.2	1.35 107	1.3 107.4						
-3	107	1.		1.06 .4	1.05 107.6	1.3 107.2	1.35 107	1.3 107.4						
-2	107	1.		1.06 207	1.05 107.2	1.3 107	1.35 107.6	1.2						
-1	107.6	1		1.06 .4	1.05 105.6	1.3 107.2	1.35 107	1.3 107.6						
0	107.6	1		1.06 .4	1.16 107.6	1.3 107.2	1.35 107	1.32 107.6						
1	108.7	1		1.16 .6	1.07 107.2	1.35 107.6	1.35 107	1.32 107.6						
2	108.4			1.06 .4	1.16 107.6	1.30 107.2	1.38 107	1.32 107.6						
3	108			1.06 107.6	1.16 107.2	1.3 107	1.35 107.6	1.32						
4	107.8			1.16 .6	1.3 107.2	1.35 107.2	1.35 107.6	1.32 107.6						
5	107.8			1.06 .4	1.16 107.6	1.3 107.2	1.35 107.	1.32 107.6						
6.	107.8			1.06 .4	1.16 107.6	1.3 107.2	1.35 107	1.32 107.4						
7	107.8			1.06 .4	1.38 107.6	1.38 107.2	1.35 107	1.32 107.4						

	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
						PROGESTERONE (ii)							OESTROGEN-PROGESTERONE (iii)											CONTROLS											
		RIR				WLH				RIR			WLH					RIR						WLH						RIR					
		70		65		57		60		67	61		54		56	53		66		69		63		51		55		58		64		68		71	
		T	W	T	W	T	W	T	W	H	T	W	T	W		T	W	T	W	T	W	T	W	T	W	T	W	T	W	T	W	T	W	T	
	.05	107	1.28	107	1.45	Died		107	1.31	Died	107.6	1.25	107.6	1.11	Died	107.6	1.11	107.6	1.12	107.6	1.51	107.	1.38	107.4	1.47	107.2	1.06	107.6	1.05	107.2	1.30	107.6	1.30	1007.6	1.
	1.05	107	1.28	107.8	1.45			107.6	1.33		107.6	1.23	107.6	1.11		107.6	1.11	107.4	1.12	107.6	1.51	107	1.35	107.4	1.47	107.2	1.06	107.6	1.05	107.6	1.3	107.	1.34	1007.6	1.
	1.05	107	1.28	107.2	1.45			107.6	1.56		107.6	1.23	107.6	1.11		107.6	1.11	107.4	1.12	107.6	1.51	107	1.38	107.4	1.47	107.4	1.06	107.6	1.05	107.2	1.355	107.6	1.3	1077.4	1.
	1.05	107	1.28	107.2	1.45			107.6	1.37		107.6	1.28	107.6	1.11		107.6	1.11	107.4	1.12	107.6	1.51	107	1.35	107.4	1.47	107.4	1.06	107.6	1.05	107.2	1.3	107	1.35	1077.4	11.
	1.05	107	1.28	107.2	1.45			107.6	1.33		107.6	1.28	107.6	1.11		107.6	1.11	107.4	1.12	107.6	1.51	107	1.38	107.4	1.47	107.4	1.06	107.6	1.05	107.2	1.3	107	1.35	107.4	11.
6	1.05	107.2	1.25	107	1.45			107.6	1.33		107.8	1.28	107.6	1.11		107.6	1.12	107.4	1.12	107.6	1.51	107	1.38	107.4	1.07	108	1.06	207	1.05	107.2	1.3	107	1.35	107.6	1.
.6	1.05	107.4	1.25	107.2	1.45			107.6	1.35		107.4	1.28	107.6	1.11		107.	1.12	107.4	1.12	107.6	1.51	107.6	1.35	107.4	1.47	107.4	1.06	105.6	1.05	107.2	1.3	107	1.35	107.6	1.
.6	1.05	107.2	1.28	107.2	1.45			107.6	1.33		107.6	1.28	107.6	1.11		107.6	1.12	107.4	1.12	107.6	1.51	107	1.38	107.4	1.47	107.4	1.06	107.6	1.16	107.2	1.3	107	1.35	107.6	11.
.6	1.06	107.2	1.38	107.5	1.5			107.6	1.33		107.6	1.25	107.8	1.11		107.6	1.12	108.8	1.12	109.8	1.51	107	1.38	107.4	1.47	107.6	1.16	107.2	1.07	107.6	1.35	107	1.35	107.6	1.3
.6	1.07	107.	1.5	107	1.5			107.6	1.53		107.6	1.25	107.8	1.12		107.6	1.12	108.8	1.12	109.8	1.5	107	1.38	107.4	1.47	107.4	1.06	107.6	1.16	107.2	1.30	107	1.38	107.6	1.3
.6	1.07	107.2	1.28	107.2	1.5			107.6	1.33		107.6	1.25	107.8	1.11		107.6	1.12	108.8	1.12	110	1.51	107	1.38	107.4	1.47	107	1.06	107.6	1.16	107.2	1.3	107	1.35	107.6	1.32
.8	1.07	107.2	1.32	107.8	1.5						107.8	1.28	107.8	1.11		107.6	1.12	108.8	1.12	110	1.51	107	1.38	107.4	1.47	107.6	1.16	107.2	1.3	107.2	1.35	1007.6	11.35	107.6	1.32
.6	1.07	107.2	1.33	107	1.5						107.8	1.28	107.8	1.11		107.6	1.12	108.8	1.12	108	1.51	107	1.38	107.8	1.47	107.4	1.06	107.6	1.16	107.2	1.3	107.	1.35	107.6	1.32
.8	1.07	107	1.33	107.2	1.5						107.5	1.28	107.8	1.11		107.6	1.12	108.8	1.12	110	1.51	107	1.38	107.6	1.47	107.4	1.06	107.6	1.16	107.2	1.3	107	1.35	107.4	[illegible]
6	1.68	107.4	1.3	107.2	1.5								107.8	1.11		107.6	1.12	108.8	1.12	110	1.51	107	1.38	107.6	1.47	107.4	1.06	107.6	1.38	107.2	1.38	107	1.35	107.4	[illegible]

able No. VIIB

4	5	6	7
107.6	1.08	107	1.
107.6	1.08	107	1.
107.6	1.08	107	1
107.6	1.08	107	1
107.6	1.08	107	1
107.6	1.08	107	
107.6	1.08	107	
107.6	1.08	107	

P.S. :- T = Tem
W = Bod

Details	Total RBC count in millions/ c m.m.	Total WBC in thousands/c m.m.	Differential Heteroophils.	Lymphocytes.	Monocytes	Eosinophil	Basophils.

TABLE NO. IX.

Haemotological observations of pullets following experimental infection with *E. coli*.

Details	OESTROGEN (i)				PROGESTROME (ii)				OESTROGEN-PROGESTERONE (iii)						CONTROLS					
	59	52	70	65	57	60	67	61	54	56	53	66	69	63	51	55	58	64	68	71
Total RBC count in millians/ c m.m.	3.3	2.4	2.3	2.1	-	2.0	-	2.2	2.8	-	2.2	3.2	3.3	2.8	1.9	2.4	2.6	2.3	2.1	2.1
Total WBC in thousands/c m.m.	39	24	20	18	-	20	-	24	27	-	18	40	38	29	30	17	18	25	24	18
Differential count: Hetero-cyphils.	45	25	28	35	-	38	-	30	35	-	30	40	52	33	35	38	39	30	35	35
Lympho-cytes.	50	63	65	60	-	59	-	62	54	-	60	53	38	62	55	56	54	62	60	58
Monocytes.	2	8	6	4	-	3	-	7	8	-	7	3	8	4	9	4	5	7	4	-
Eosinophils.	3	3	1	1	-	-	-	1	2	-	2	4	2	1	1	1	1	1	1	6
Basophils.	-	1	-	-	-	-	-	-	1	-	1	-	-	-	-	1	1	-	-	1

T A B L E NO. - VIII

Haemotological observations of hens following experimental infection with E.coli.

Details		Total R.B.C. in millians/c m.m.	Total W.B.C. in thousands /c m.m.	Differential count (%)				
				Hetero-phils	Lympho-cytes.	Mono-cytes	Eusin-ophils	Baso-phils.
	73	4.0	30.0	62	33	4	1	-
	78	4.2	42.5	59	35	2	3	1
	80	3.8	35.1	60	36	3	1	-
	81	2.6	20.5	55	39	3	2	1
Controls	72	2.2	18	35	55	8	1	1
	75	2.5	30	28	60	9	2	1
Normal values	Daiger and Davies (1946).	3.0	25	33	45	15	3	4
	Seagar (1953).	2.9	27	34	54	5	5	2

CLINICAL SYNDROME :

In the present study, the clinical symptoms were observed both in natural as well as in experimentally infected birds.

In naturally occurring cases, the birds showed clinical signs viz. weakness of legs, shrunken cyanotic combs, general depression. Tendency to remain in "Duck-like posture" was the characterestic symptom (Figure No.1). The cloacal region was soiled and calcarious deposits were observed around the vent (Figure No. 2). However, their general condition of health remained fair.

In experimental layers, the above symptoms were observed to varying extent of severity. The legweakness and cessation of egg-production formed the prominent symptoms. Within 26-48 hours after experimental infection, the layers showed slight cloacal discharge. This wasmx at first clear, later turning white in course of 4 -5 days. This persisted until the birds were either sacrificed or were dead. The duck-like posture was observed in all the 4 infected layers. This appeared concoitittently with the rise of temperature in adult birds and disappeared when the temperature became normal. These symptoms were not seen in any of the control hens. Of the experimental pullets, only 59 and 66 and 69 showed signs of infection marked by mild rise of temperature only.

Apart from the above, records in respect to rise of temperature, and body weight were maintained. In the layers group, all the birds (Numbers 73, 78, 80, 81) showed rise of temperature varying between 108°F and 110°F (Table No. VIIA) whereas the control (Number 72 and 75) did not show departure from the normal range of 107 to 107.6°F. Among hormone pretreated pullets, the rise in temperature was found in bird No. 59 in group (i) and 66 and 69 in group (iii), ranging from 108°F to 110°F. The pullets in the remaining groups including the controls did not show any rise of temperature. The temperature returned to normal within an average period of 3 days (Table No. VIIB).

The weight records of both pullets and hens when compared with the control did not reveal any loss in weight gain during the experimental period (Table No. VII A & B).

HAEMOTOLOGICAL OBSERVATIONS :

Haematological studies were made on all the experimentally infected birds. For this, blood samples were collected from the wing vein just before they were sacrificed at different intervals after infection. These included examinations for total R.B.C. and W.B.C. and differential

counts. The haematological data obtained are presented in the Table No. VIII and IX for hormone treated pullets and layers respectively.

Among the layer group, 3 out of 4 birds (number 73, 78, 80) showed a significant rise in their total R. B. C. and W. B. C. counts, ranging between 3.8 to 4.2 millions/C. mm. and 30 to 42.5 thousand/C. mm. respectively. The number of heterophils rose high between 55 and 62% in all the four infected birds (number 73, 78, 80 and 81) which also recorded concomittent fall in the percentage of lymphocytes.

Among the hormonally pretreated pullets, only two (numbers 66, 69) in the group (iii) and No. 59 in group (i) showed rise in total R.B.C. and W.B.C. counts, as well as in the percentage of heterophils.

The haematological studies on natural cases could not be made.

SEROLOGICAL RESPONSE :

The slide agglutination test was performed on serum samples of all the experimental pullets and layers on the day they were sacrificed. Among the layers group, birds number 80, 81, which were tested on the 15th day after infection, proved positive for E. coli agglutinin while the controls and the remaining two hens tested within

6 days after infection did not show agglutinin in their sera.

Among the immature pullets, only one bird number 69 (group iii) tested on the 15th day after infection gave strong positive reaction for E. coli agglutinins. One pullet number 66 (group iii) also showed doubtful reaction for serum agglutinin when tested on the 6th day following infection. All others including the uninfected controls did not show the presence of serum agglutinin.

ISOLATION OF E. coli :

Natural cases : -

E. coli was isolated from 4 cases of naturally occurring salpingitis out of 35 samples tested. These were identified on cultural and biochemical tests as stated elsewhere.

Experimental cases :-

All the experimental birds, both pullets and hens including the controls, were subjected to bacteriological examinations after they were sacrificed for re-isolation of E. coli from the oviducts. Among the immature pullets, only 3 out of 18 experimentally infected pullets (numbers 59, 66, 69) yielded E. coli on bacteriological examinations while the rest failed to show the infecting organism in their

oviducts. Among the layers, all the artificially infected 4 hens (numbers 73, 78, 80, 81) yielded E. coli on cultural tests. The control hens did not show up the organisms. The results of isolation and their correlation with gross lesions and serological response are shown in Table No. X.

TABLE NO. X.

Correlation between gross leisons, serological response and reisolation of E. coli from experimental pullets and hens.

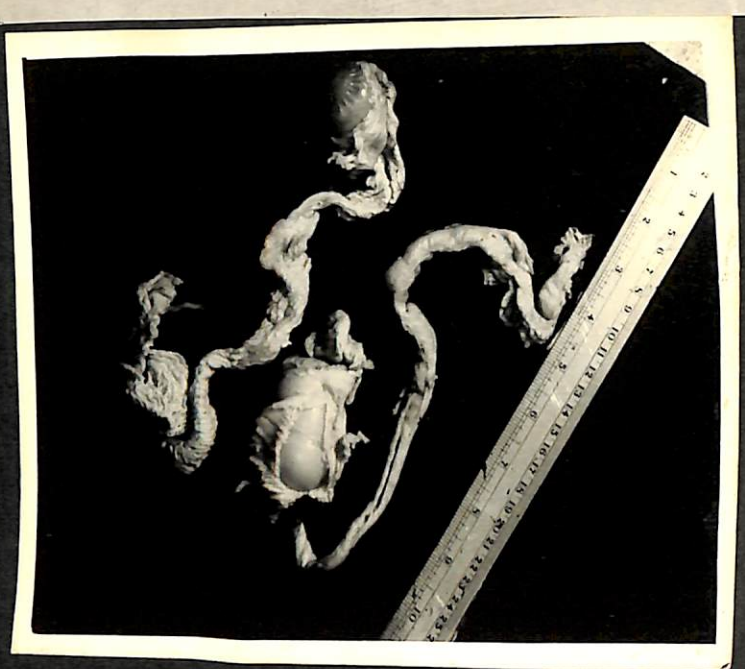
Groups	Bird nos.	Interval between infection and sacrifice.	Gross lesions	Serological response.	Re-isolation of E. coli.
1	2	3	4	5	6
i.	52	15	No	-	No
	59	7	No	-	Yes
	65	15	No	-	No
	70	15	No	-	No
ii.	57	-	No	-	No
	60	4	No	-	No
	61	6	No	-	No
	67	-	No	-	No

Cont'd. Table No. X.

1	2	3	4	5	6
111.	53	15	No	-	No
	54	7	No	-	No
	56	-	No	-	No
	63	14	No	-	No
	66	16	Yes	≠	Yes
	69	15	Yes	≠≠≠	Yes
Control (culture only no hormone).	51	15	No	-	No
	55	15	No	-	No
	58	15	No	-	No
	68	15	No	-	No
Control (sterile broth only, no hormone)	64	15	No	-	No
	71	15	No	-	No
Layers (no hormone).	73	2	No	-	Yes
	78	6	Yes	-	Yes
	80	15	Yes	≠≠	Yes
	81	15	Yes	≠≠≠	Yes
Control (no hormone).	72	15	No	-	No
	75	15	No	-	No

Fig. 3 : Picture showing oviduct and adjacent viseral organs, showing spilled cheesy yolk like material in a natural case of salpingitis.

Fig. 4 : Natural cases showing lodgement of shell-less eggs, in the uterus portion of the oviduct.



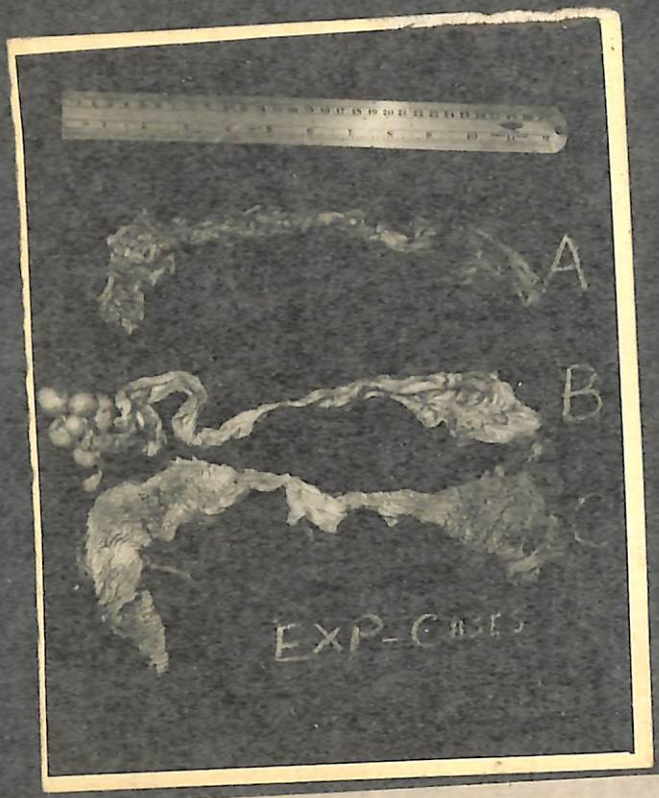


Fig. 7 : Picture showing oviducts of experimentally produced salpingitis cases : A. Bird No. 80 = engorgement of blood vessels on the serosa. B. No. 81 slight engorgement with active ovary. C. No. 78 showing petechiae in mucous membrane.

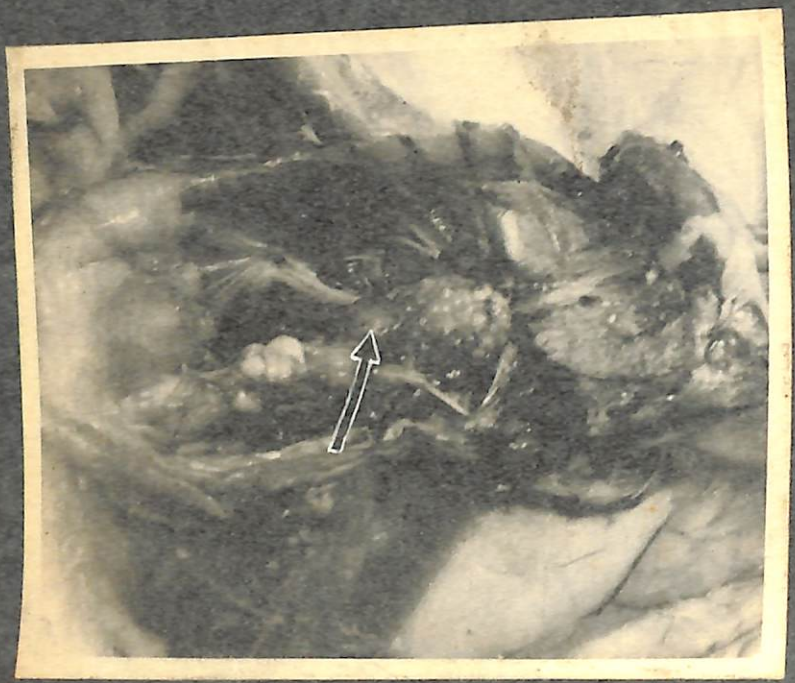


Fig. 5 : A chronic case of natural salpingitis with egg-concretions in uterus portion.

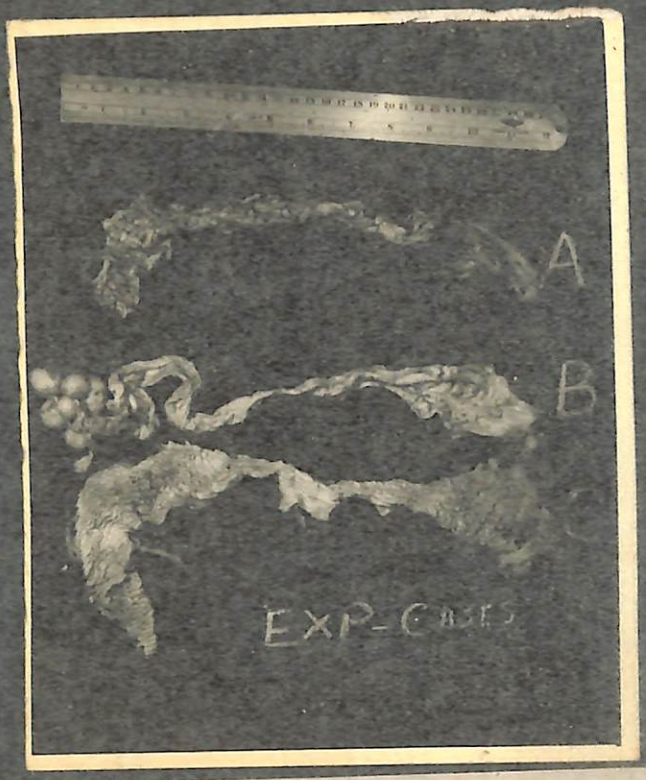
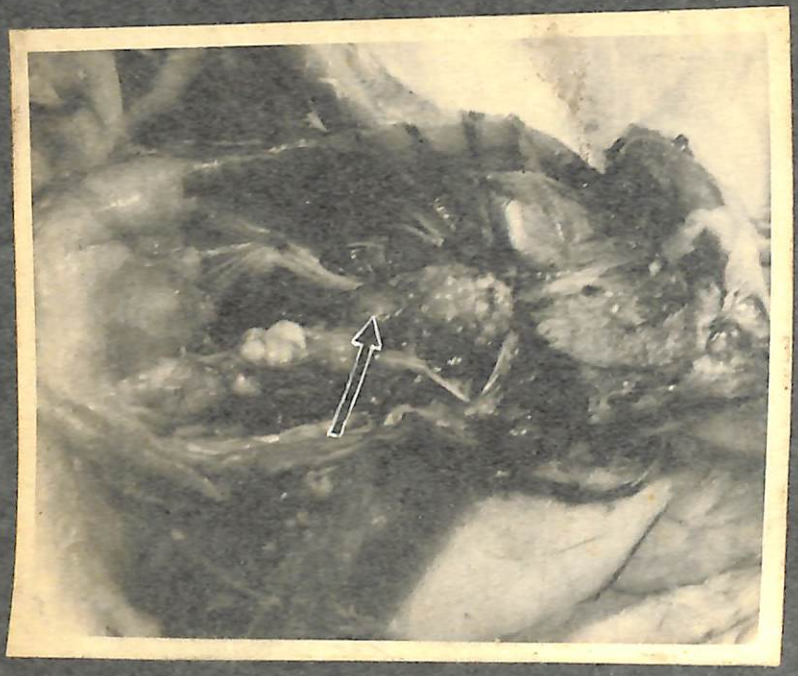


Fig. 7 : Picture showing oviducts of experimentally produced salpingitis cases : A. Bird No. 80 - engorgement of blood vessels on the serosa. B. No. 81 slight engorgement with active ovary. C. No. 78 showing petechiae in mucous membrane.



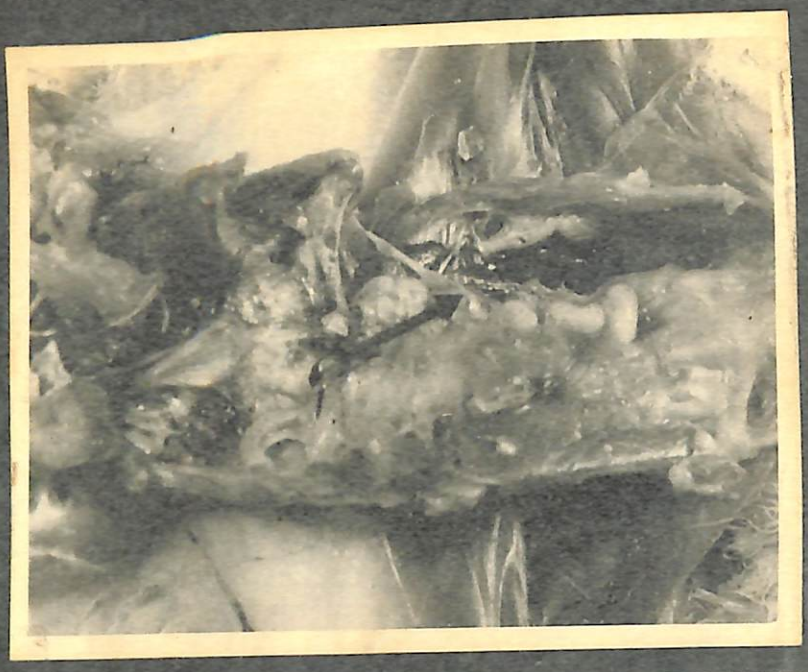


Fig. 9 : Picture showing deposits of cheesy like material on peritonium in Bird No. 66 of group iii.

Fig. 10 : Oviduct of Bird No. 69 showing deposits of cheesy material in the mucous membrane.

GROSS PATHOLOGY :

Natural cases :-

In the present study, a total of 192 natural cases of salpingitis was found during the current year out of 512 examined by the author. All except (20 cases). showed lesons viz. hyperemic conditions of the oviduct and cheesy exudates coating the peritoneum and the oviducts (Figure No. 3). The oviductal lumen of some of the cases showed lodgement of egg or a mass of concretions (Figure No. 4 and 5). Only a few showed hyperemic changes on the serosa of the ducts with or without the lodgement of egg in their lumen (Figure No. 6). In majority of cases, the ovaries were in functionally active stage, showing on an average 6 - 10 mature follicles of peanut size. In a few cases, the follicles were ruptured and the yolk was split smearing the peritoneum and the adjacent abdominal organs.

Experimental cases :-

The experimental birds were sacrificed, at different intervals ranging from 48 hours to 15 days, following artificial infection. The controls as well as those killed at 48 hours did not show any gross lesion.

Among the layers, bird number 78 showed half-formed egg in the uterus, which when opened showed petechiae in the mucous membrane as well as of magnum

(Figure no. 7c). In bird number 80 and 81 (Figure no. 7 a and b), the serosa showed engorgement of blood vesels but the mucous membrane did not show any gross pathological change.

Among the immature pullets, birds number 66 and 69 (group ii), which were the only one that showed lesions of experimental salpingitis revealed caseous deposits, yellowish white in color, on the oviductal mucosa. The deposit also coated the peritoneum and adjacent abdominal organs. Apart from this, bird number 69 also showed several matured follicles, not infrequently showing tendency to float in the peritoneal cavity (Figure number 8).

HISTOLOPATHOLOGY : natural cases.

In majority of cases, the infundibulum showed short columnar or cuboidal epithelium densely infiltrated with heterophils containing pleomorphic nuclei. The mucosal glands were few in number but they were filled with mucous secretions. The submucosa revealed extensive edematous changes and contained large amounts of fibrin. The heterophilic infiltration also affected the submucosae as well as the muscular layer (Figure number 11).

The magnum showed the epithelial cells in various stages of degeneration at places the ductal lumen was filled with exudate containing several degenerated cells and heterophils. The submucosal glands were numerous in number,

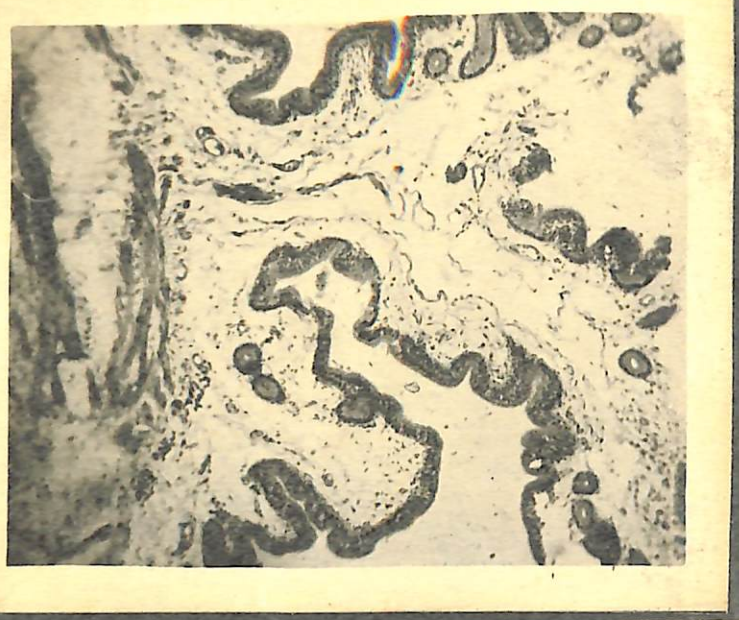
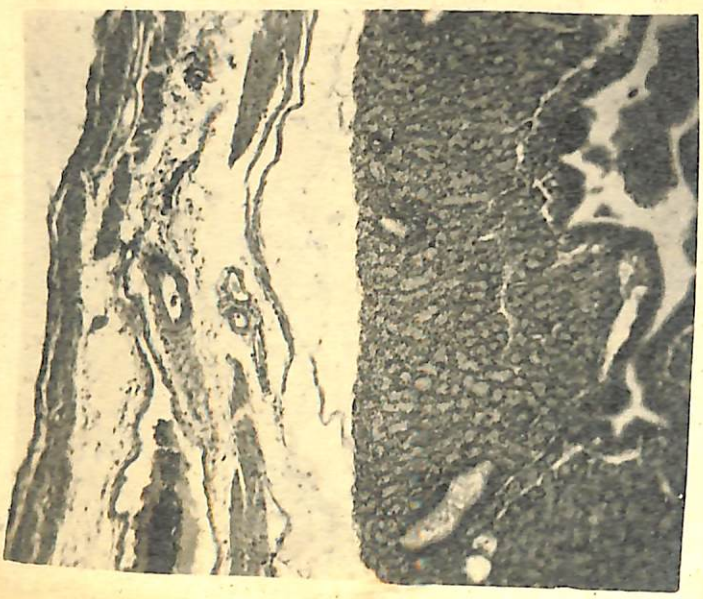


Fig. 11 : Infundibulum showing infiltration of heterophils with hyperactive mucous producing glands, submucosa showing extensive edema with fibrin with heterophilic infiltration. H&E x 100.

Fig. 12 : Magnum of a natural salpingitis case showing degenerative changes in the epithelial cells, exudate in ductal lumen, glandular hyperplasia, edema in muscular layer, with moderate infiltration of heterophils and dilation of blood vessels. H&E x 100.



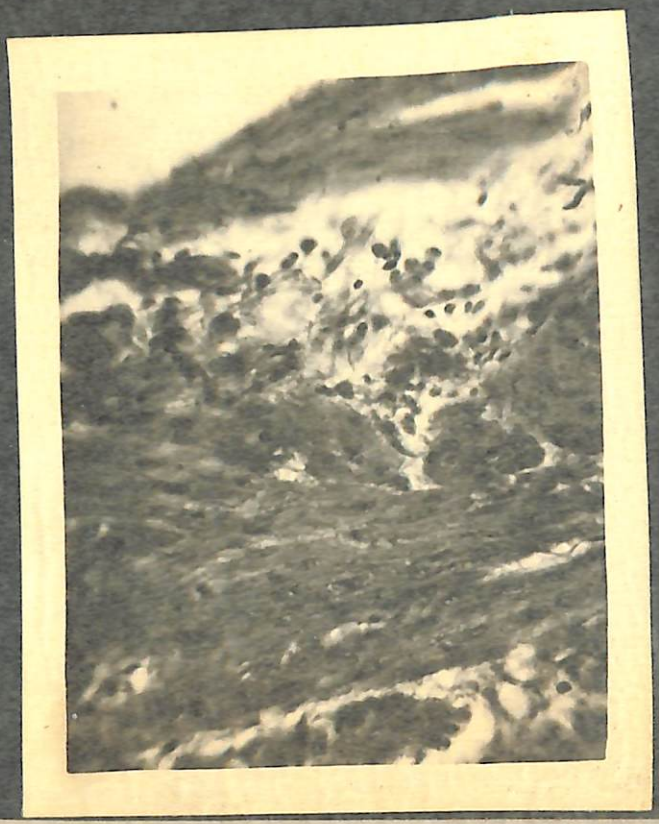
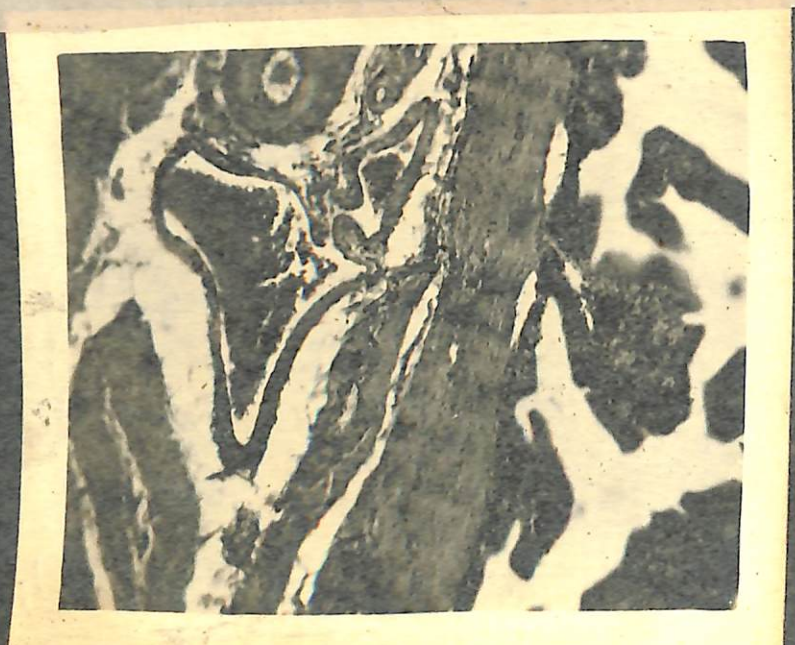


Fig. 13 : Magnum of a natural salpingitis case showing extravassation of erythrocytes, moderate infiltration with plasma cells. H&E x 400.

Fig. 14 : Uterus: Heterophilic infiltration degenerative and necrotic changes in epithelium and severely congested blood vessels. H&E x 100.



some of them being dilated forming tubules. These changes were suggestive of glandular hyperplasia. Their lumen was stuffed with secretions. In the submucosa, [redacted] edema was evident which affected the integrity of the muscular layers. In the muscular layer, there were moderate number of heterophils and the blood vessels were dilated (Figure number 12). In a few cases, the muscular layer was also infiltrated with moderate number of lymphocytes and extravasated erythrocytes. Besides, moderate infiltration with plasma cells was also found in the muscular year (Figure number 13).

In the uterus, the papillae formed by the surface epithelium showed very heavy cellular infiltration primarly consisting of heterophils. The epithelial cells showed degenerative and necrotic changes. The muscular layer was disintegrated longitudinally due to severe edematous changes which contained severely congested blood vessels and thickened arteries. The edematous space showed acute congestion and thickened arteries. Cellular response was minimal in this zone. (Figure Number 14).

Experimental cases:

Among experimental pullets, as stated earlier, only two birds (Numbers 66, 69) of group iii had shown gross lesions characterized by cheesy deposits on the peritonium and the visceral organs and the presence of several mature follicles.

Among the layers, gross lesions were found in 3 out of 4 hens. There were evidences of secretary hyper - activity of mucosal glands laiden with mucous secretions. Besides, cellular infiltrations predominantly by heterophils and moderate number of plasma cells. The space between the mucosa and lamina propria was widely seperated by edematous fluid, but was devoid of any cellular activity. The capillaries in the muscular layer has slightly congested (Figure 15).

In the magnum region, the edematous changes were more marked in the submucosab, which contained large amounts of fibrin. These changes also affected the muscular coat leading to disintegration of muscular bundles (Figure 16). Further more, the mucosal glands were hypertrophied and their lumen greatly dilated. The glandular lumen were predominantly empty. There was heavy infiltration of glandular epithelium with plasma cells (Figures 17 & 18).

In the uterus the lining epithelium were tall columnar in nature, in which mitosis well marked. This

as well as the submucosae were heavily infiltrated with plasma cells, lymphocytes as well as heterophils (Figure 19).

Microscopically the magnum of birds numbers 66 and 69, the lining epithelium consisted of columnar cells containing frequently two or more nuclei of varying sizes. Some of the nuclei were hyper-chromatic occassonally showed evidence of mitosis. In the submucosa there were foci of cellular infiltration consisting predominantly of heterophils, a few lymphocytes and plasma cells. The mucosal glands were found in large number which were also hypertrophied. The glandular lumen was filled with secretions. Some of the dilated glands formed tubular structure of various sizes and were empty. There were focal infiltrations predominatly with hetrophils a few lymphocytes and plasma cells. The submucosa was showed edematous changes which also affected the muscular coat. In the latter the blood vessels were congested but devoid of cellular infiltration (Figure 20).

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C H A P T E R - V

DISCUSSION

D I S C U S S I O N

Affections of the female genital tract of poultry have been held responsible for affecting the economics of poultry industry in most countries. Of the various diseases, salpingitis, is supposed to be one of the major factors causing decreased egg production, malformation of eggs, together with high morbidity and mortality rate amongst laying hens.

INCIDENCE IN GENERAL :

Salpingitis in poultry is known to be widespread in most countries of the world. This has been reported to occur in varying proportion with the development of poultry industry despite the optimum level of poultry management and hygiene achieved in the European and western countries. This reported to the extent of 36.4% in U.S.A. (Beach, 1940) 13.84% in Canda (Weaver, 1930), 19% to 25.21% in Netherland (TeKennepe, 1924), 3.6 to 9.09% during the years from 1950 to 1954 in England (Coles, 1955) and 22.7% in Scotland (Matheson, 1933). In India, Sharma (1964) reported various disorders of reproductive tract and found salpingitis and egg-peritonitis to the tune of 9.4% and 29.35% respectively.

In the present investigation incidence of salpingits in poultry increased from 10% in 1968 to 37.5 %

in 1970, with the overall percentage around 25.6. The lower percentage of incidence in the year 1968 may be attributed to the fact that the present data include only the incidence from April to December, (the data from January to March were not available) thus missing the cases that may have occured during early winter of this year. As it will appear from the review of literature, no systematic study seems to have been made in India to determine the incidence, the etiologic factors, the pathogenesis and pathology of the disease. It is essential that proper records are maintained in regard to the aspects of the disease in order to appreciate precisely the extent of problem in this country. The data in the present study related to the mortality figures obtained at the Central Poultry Farm, Patna. This is a production farm where recognised procedures of management and disease control are followed. The overall percentage of the incidence recorded as 25.6% at this farm and progressive increase in mortality during last 3 years, give an insight to the magnitude of the problem prevalent in the rural poultry flocks.

INCIDENCE OF SALPINGITIS IN RELATION TO BREED:

On perusal of the data setout in Table IV, it will be clear that there was no breed predisposition to salpingitis since the overall percentage of incidence in both RIR and WLH birds was the same (47%). Also the incidence of the disease in these two breeds of poultry

during 1969 for which the data were available for the entire period of the year was also similar (50% and 49% respectively). These observations are in accord with those of Stafseth and Boyd (1929); Darcel et al. (1952). Lindgreen (1964) also did not find any evidence of breed susceptibility to salpingitis. He concluded that there was possibility of strain rather than breed pre-disposition of a flock to the disease. However, the difference in the percentage of incidence of salpingitis in RIR and WLH breeds during the year 1968 was well marked (35.3%, 64.7% respectively). Further more, some differences in the percentage was also noticed during the year 1970 (47.4% and 40.7% respectively). The difference in the percentage of incidence in these two breeds during 1968 and 1970 may be ascribed to the fact that complete data for these two years were not available. For 1968, the data were available from April 15 to December 31 only. Similarly, for 1970 the incidence was studied on informations collected from January to November only. It may well be that these differences in the susceptibility of RIR and WLH breeds during these two years were due to non-availability of data for the winter months which seems to be the most vulnerable season predisposing the birds to salpingitis. In the present study, seasonal pre-disposition was found to be responsible for high incidence of the disease during winter. This will be discussed in appropriate section. The data about the incidence of the disease among Black Astrolorps were meagre. Therefore, it was not possible

to arrive at any conclusion regarding the susceptibility of this dibreed to salpingitis.

INCIDENCE OF SALPINGITIS IN RELATION TO AGE:

Age susceptibility to the disease has been confirmed by several workers in the past. Schutt (1930) reported that the disease affects mostly the birds at the times of maximum egg production. This view was endorsed by Lindgren (1964) who stressed that the disease was pronouncedly one of the young laying pullet. This investigation has also revealed relatively high percentage (24%) of incidence of salpingitis in layers of both RIR and WLH breeds in the age group of 6 to 8 months. With the advancing age the incidence gradually declined. Thus, amongst the birds aged 8 - 12 months, the incidence in them ranged between 17 - 20% while in birds aged 12- 18 months the percentages were approximately 6 and 3 respectively.

INCIDENCE OF SALPINGITIS IN RELATION TO SEASON:

Extensive studies have been made on the seasonal variation of the incidence and mortality due to salpingitis. Schutt (1930) observed high incidence of the disease during the breeding season (May - August) and attributed this due to defective management during this period when the farm staffs were too busy incubating and hatching eggs, and found little time to give the layers proper care.

Dudley et al. (1941) also made similar observations and suggested that the high incidence of the disease following intensive production during April - August was due to exhaution of egg-laying apparatus. Thus, it is clear that in their studies, the incidence of the disease was high during May - August which were the active breeding season for their flocks.

The present study has shown approximately two - fold increase in the incidence (54%) during winter (November - February) in comparison to that found during summer (27%). The incidence during Monsoon period (July-October) was reletively low (19%). It may be pointed out here that at the Central Poultry Farm, Patna as well as at other production farms in this State, new hatches are introduced into the flock around the month of February - March. The new crops attain the laying age by the following winter which is the period of maximum production. Therefore, it is reasonable to assume that the higher incidence of the disease during winter, which is the maximum laying season in this state, has a direct bearing on the breeding season of the flock. Other predisposing factors such as physiological exhaution of the laying apparatus and defective management at the time of intensive production have already been discussed.

CLINICAL SYNDROMES :

In natural cases, the early symptoms of acute salpingitis are usually missed due to short course of the disease. It is only after the disease has passed on to chronicity that the disease is diagnosed.

In the present investigation, the symptoms most frequently encountered were leg-weakness, shrunken and cyanotic combs, and duck-like posture. The cloacal region was soiled with foetid discharge and calcarious deposits were present around the vent. The latter signs can be unescapably attributed to the chronic course of the disease.

In the present study, all the 4 adult hens showed the first sign of salpingitis within 24 to 48 hours marked by clear odourless cloacal discharge turning white in course of 4 to 5 days. Besides, there was mild rise of temperature the day following experimental infection and this persisted for 3 to 5 days. The cloacal discharge was coincident with thermal reaction and leg-weakness. From these symptoms, it is reasonable to conclude that initially the disease begins with an acute course which, if left unattended, results in complete cessation of egg-production and death.

In experimental pullets Number 66 and 69, which were the only ones, that showed gross lesions of salpingitis suffered from mild temperature begining 24 hours following

SEROLOGICAL RESPONSE :

The presence of E. coli agglutinin in the serum of birds is considered to be a good indication of established infection. Gross (1956) reported experimental evidences to substantiate the view that, although 'O' serological titre was not a reliable indication of imonunity, it was a reliable indication of infection following exposure to live E. coli organisms.

As report earlier, salpingitis was reproduced experimentally in only two out of 6 expemimental pullets of group iii (Oestrogen - Progesterone treated), whereas, the disease could be reproduced in 3 out of 4 sexually matured hens (Numbers 78, 80 and 81). It can be seen from Table X that only one (Number 69) out of two pullets and two out of three layers (Numbers 80 and 81) that had shown gross lesions of salpingitis proved positive on slide agglutination test. The discrepancy between the gross lesion and serological response may be explained due to short interval between experimental infection and the serological test. The pullet Number 66 (group iii) which showed gross lesion was sacrificed and its serum tested for the presence of agglutinin on the 10th day after exposure to E. coli, but the slide agglutination test proved doubtful for serum agglutinin. Similarly, amongst the layer group, hen Number 78 when sacrificed on the 6th day

ISOLATION OF E. coli :

The etiological association of E. coli with salpingitis has been hitherto subjected to some controversial discussion. It has beenheld that the bacteria including E. coli are secondary invaders. However, several authors have noted frequent coincidence of E. coli infection in salpingitis. Moor and Marten (1944), isolated E. coli from about 26.7% of cases. Glantz et al. (1962) reported frequent isolation of E. coli in salpingitis cases and concluded that the certain serotypes were evidently important complicating factors. Lindgren (1964) reported a very high percentage (43.3%) of recovery of E. coli from cases of salpingitis while in other reproductive diseases, the rate of recovery was low (3.9%). Thus he concluded that E. coli had a too intimate association with salpingitis to be neglected in the etiological investigation of this disease.

In the present study, only 4 (11.4 %) out of 35 samples of oviducts collected from natural cases of salpingitis yielded E. coli. A small recovery rate of this organism in this study in contrast to higher rates of other workers may well be due to the limited number of specimens subjected to bacteriological study. More number of cases could not be examined due to advanced post-mortem changes and also because this was not within

the scope of this study.

In the present experiment, those pullets (Number 66, 69) and hens (Numbers 78, 80, 81) that showed gross lesions also revealed E. coli in their oviducts. Although the number of positive isolates apparantly seems to be low to be considered significant, the isolation of the organisms from all the cases of experimental salpingitis tends to suggest its importance in the causation of the disease. None of the pullets that failed to show lesions following infection as well as uninfected controls showed E. coli.

From Table X, it may be noted that apart from the above birds, the pullet Number 59 (group 1) and the hen Number 73 (layers) also proved positive for E.coli on bacteriological tests although they did not developed clinical or gross evidence of salpingitis. These two birds were also serologically negative for E. coli agglutinin. However, it may be pointed out here that the former was sacrificed on 7th day and the latter on the 2nd day following the infection. Therefore, it is reasonable to believe that in the layer Number 73, which was sexually matured, serological response and gross lesion did not develop owing to short interval between infection and sacrifice. It is probable that this bird may have developed the disease, had it lived for some more days. This view is supported by the fact that E.coli

was isolated from the oviduct of this bird. On the other hand, it was difficult to predict the fate of the pullet Number 59 despite the fact that E. coli was isolated from the oviduct, had this been allowed to live for some more time. It is well to remembr that this bird belonged to group i i.e. oestrogen pretreated group only. In the absence of adequate level of progesterone, it may be that the inapparent infection may have died out spontaneously without causing the disease.

GROSS PATHOLOGY:

In general, the gross lesions in natural cases were strongly suggesting of acute salpingitis characterized by hyperemic condition of the oviduct and cheesy exudate coating the oviductal mucosa as well as the adjacent peritoneum. This may be taken to suggest the hypersecretory activity of the ductal glands in the acute stage of the disease. The lodgement of egg in the duct may be due to muscular atony of the duct which was not able to expel the eggs. In a few cases, evidence of chromic salpingitis exemplified by the presence of mass of concretions in the ductal lumen was noticed.

In the experimental cases, the hens that showed gross lesions of salpingitis also suggested the acute course of the disease. In them, the prominent lesions were engorgement of blood vesseles, petechial haemorhage on the

mucous membrane of the uterus and magnum. These findings are in agreement with Lindgren (1964) who reported that the lesions of experimental salpingitis were not distinguishable from those occuring in natural cases.

Among the pullets, the lesions in two birds that became experimentally infected were also similar to those found in natural cases. Therefore, this suggests that the early stage of the disease in natural cases which involves clinical, thermal, and gross lesions of acute salpingitis are usually missed, and only chronic cases are metwith on the autopsy table.

HISTOPATHOLOGICAL OBSERVATIONS:

In general, the histopathological changes in both natural and experimental cases of salpingitis were similar. The changes consisted primarily of hyperemia and edema. The degree of cellular reaction varied in different cases. Broadly, the histopathological observations were in agreement with those of Lindgren (1964), Biswal and Morril (1954).

The vascular changes affected almost all the layers and all the portions of the oviduct. In contrast to the observation of Lindgren (1964), inflamtory reaction, chiefly hyperemia and edema, were more marked in natural than in experimental cases (Figures11, 12 and 14). On the other hand, the cellular reaction was

more pronounced in the experimental than in natural cases. This consisted of focal aggregation of heterophilic leucocytes, moderate lymphocytes and strong plasma cell reaction.

In natural cases, the lumen of duct glands was found largely filled with secretion in contrast to the experimental cases which showed dilated but empty glandular lumen. The degree of glandular proliferation was found to be coincident with the degree of cloacal discharge. The pullet Number 69 did not show cloacal discharge and the microscopic changes were suggestive of only glandular hyperplasia without much evidence of hyper-secretory activity. Therefore, it is believed that the amount of cloacal discharge and in consequence calcareous deposition, are largely dependent upon extent of secretory activity of mucosal glands.

The reports on the histopathological study of the disease are scarce in the literature. One of the reasons for the lack of this information may be the difficulty in obtaining fresh materials for this study since the oviductal epithelium quickly undergoes post-mortem autolysis. In order to obtain accurate information on this aspect of the disease, it is necessary to maintained a small flock of layers for experimental purposes. In the present study, fresh specimens of clinically affected

birds were obtained from the poultry dressing plant through the courtesy of the farm manager.

ROLE OF OESTROGEN AND PROGESTERONE IN CAUSATION OF THE SALPINGITIS:

In the present experiment, salpingitis was reproduced in 3 out of 4 adult hens, and in 2 (Numbers 66 and 69) of six pullets of group iii (oestrogen - progesterone treated) only. None of the pullets of the other groups including the controls developed the disease.

This is a common observation that salpingitis is a disease of sexually matured laying hens. Only a few sketchy attempts have been made to investigate the predisposing effects of oestrogen and progesterone in the pathogenesis of this disease. Lindgren (1964) observed that sexually immature normal chickens are relatively resistant to intra-oviductal E. coli infection. The administration of oestrogen alone does not materially alter the suceptibility of pullets to the disease, although it may bring about partial maturation of the oviduct. On the other hand, if pretreatment with oestrogen is combined with pretreatment with progesterone, the suceptibility to E. coli infection in immature birds is changed to the same level as that in mature laying hens. He further substantiated this observation by significantly

higher E. coli counts in the combined oestrogen-progesterone pretreated group than in normal immature pullets or in oestrogen or progesterone treated group alone. Although, further studies are needed to confirm this observation un-equivocally, ~~with~~ the reproduction of the disease in pullets pre-treated with both oestrogen and progesterone (group iii) tends to support the observation of Lindgren (1964). It is essential that studies be further extended on hypophysectimized adult laying chickens under varied harmonal pretreatments. The hypophysectimized hens will be deprived of heavy endogenous hormones. As a consequence, they may presumably become as refractory to the disease as the immature pullets, while further administration of exogenous oestrogen and progesterone to hypophysectomized layers may demonstrate decisevely the predisposing role of these harmone. Similarly, pretreatments with oestrogen and progesterone invaried concentrations may show their priming effect enhancing their suceptibility to infection.

It is reasonable to believe that vaginal route of infection is probably more natural but perhaps not very accurate for experimental purposes. In the latter, it is difficult to rule out the carriage of infection to oviduct from the faecal sources. Further, the manipulation of utero-vaginal orfice with finger at the time administering inoculum may possibly convey the infection

to the oviduct from external sources. Also, the regurgitation of inoculum due to peristalisis may vitiate the response and add experimental errors. However, in the present study, regurgitation of inoculum and spontaneous infection in control birds given sterile broth only did not occur. Therefore, such errors did not seem to have antagonised the usefulness of the technique employed in the present study. Lindgren (1964), apart from the vaginal route, used direct intra-isthmal route post-laparotomy with satisfactory results. This could not be tried in this study due to lack of desired facility. More work is needed to improve upon the techniques of experimental infections which may throw light on the pathogenesis and pathology of the disease.

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CHAPTER - VI

SUMMARY

CHAPTER - VI

SUMMARY

S U M M A R Y

This thesis deals with an investigation undertaken to study the incidence and pathology of salpingitis in domestic fowls. The work consisted of two parts, (1) survey on the incidence of natural salpingitis in relation to various predisposing factors viz. predisposition to breeds, age, and seasons etc.; (2) an experimental study to determine the role of E. coli in the causation of the disease under hormonal stresses with particular reference to oestrogen and progesterone.

A survey made on the incidence of the disease during last 3 years at the Central Poultry Farm, Patna showed a gradual increase in the incidence ranging between 10% and 37% with an overall incidence of 25.6%. There was no evidence to suggest breed predisposition to salpingitis studied in RIR and WLH birds. The overall incidence in these two breeds were 47.2% and 47.8% respectively.

There was a close correlation between the incidence and the age of the birds affected. The young laying birds aged between 6 and 8 months were most succeptable to the disease (24.3%). With the advancing age, the birds showed gradually increasing resistance to natural infection. This was borned out of the observations that in the laying birds between the age group of 8 - 12

months, the incidence ranged between 17 and 20% while those layers aged between 12 and 18 months showed the incidence between 3.5 to 5.9% only.

The incidence of the disease showed a characterestic seasonal variation with the peak occuring in winter. There was a two-fold increase (53.9%) in the incidence of the disease during the winter months (November - February) in comparison to that (26.8%) met with during the summer (March - June). During the monsoon (July - October), the incidence was found to be low (19.3%).

Clinically, the disease was characterised by shrunken and cyanotic combs, cloacal discharge, general depression, leg-weakness leading to "duck-like" posture and death. Apart from the above, in experimental cases the onset of the disease was marked by moderate rise in temparature and cessation of egg-production.

An attempt was made to produce experimental salpingitis in sexually matured laying hens and young immature pullets aged about 16 - 20 weeks by inoculating approximately 6.4×10^{10}/ml. E. coli isolated from a natural case of salpingitis per vaginum. The pullets were pre-treated with oestrogen and progesterone either singly or in combination. Three out of 4 matured laying hens developed salpingitis while only 2 out of 6 young

pullets pretreated with oestrogen - progesterone (group iii) developped the disease. None of the pullets pretreated with either oestrogen or progesterone alone including controls developed salpingitis.

The haematological study performed on cases of experimental salpingitis revealed a moderate increase in the total erythrocytes and leucocytes counts, as well as a rise in percentage of heterophilic leucocytes. The haematological response was coincident with the thermal reaction.

The slide agglutination test performed to detect the presence of E. coli agglutinin in the sera of experimentally infected birds indicated strong correlation between infection and serological response. Only those birds that developed experimental salpingitis showed E. coli agglutinins in their sera. The results further showed that the presence of serum agglutinin was a reliable index of infection but not of immunity. The results of serological test further demonstrated that the pathological changes begin to develop before the appearance of agglutinin in the serum.

In the present study, E. coli was isolated from the oviducts of 4 out of 35 naturally occuring cases of salpingitis examined bacteriologically. On the other

hand, E. coli was recovered from all the cases of experimental salpingitis.

There was a perfect correlation between the results obtained on serological and bacteriological tests and development of gross lesions of salpingitis in experimental cases.

The gross lesions of salpingitis occuring in natural and experimental cases were indistinguishable. In essence, the lesions consisted of hyperemic condition of the oviduct, and cheesy exudate coating the oviductal mucosa as well as the adjacent visseral organs and peritoneum. A few cases showed lodgement of shell-less egg in the uterus and the presence of concretions in the different regions of the duct.

The histopathological changes were broadly similar in both natural and experimental cases of salpingitis. These were characterized primarily by vascular response, chiefly hyperemia and edema which affected all most all the layers and all the portions of the oviduct. The vascular response was more marked in natural than the experimental cases. The cellular reaction consisted of focal aggregation of heterophils, a moderate number of lymphocytes and numerous plasma cells. This was more pronounced in the experimental than in natural cases.

Pre-treatment with either oestrogen or progesterone alone prior to experimental infection with E. coli did not alter the susceptibility of young sexually immature pullets to salpingitis, When pretreatment with oestrogen was done concurrently with the pretreatment with progesterone, the susceptibility to E. coli infection was found to have changed to the level of mature laying hens. Evidences were obtained to support the view that these two hormones when used together exert the priming effect enhancing the susceptibility of the young immature pullets to the disease.

Various suggestions have been offered for the furtherance of this study which may resolbe some of the complex problems related with the pathogenisis and pathology of salpingitis of domestic fowls.

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