ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Clinical and Gross-pathological Diagnosis of Infectious Bacterial Diseases of Layer Chickens in Ajmer region of Rajasthan.

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ABSTRACT

Ajmer district of Rajasthan state showed immense productive potential in the field of poultry sector which if utilized properly can yield good amount of poultry products. Improving poultry status, especially the layer chickens in this area by continuous monitoring of disease conditions, makes it essential to study the causative agents such as bacteria. Intensive poultry production in Ajmer region has made bird stressful leading to decreased resistance for diseases and thus prone to infectious bacterial diseases. This study is an attempt to diagnose various bacterial diseases prevalent in poultry farms through clinical and gross-pathological studies in affected fowls in the selected poultry farms in Ajmer region. Different bacterial diseases prevalent in four selected poultry farms in Rasulpura belt of Ajmer district were thoroughly studied; analyzed and confirmed by regular inspection of fowls for external traits, abnormal behavior of fowls, clinical signs/lesions in body parts, visible symptoms, wounds, changes in color of body organs and skin. Necropsy studies of 12 diseased dead layers, collected from the selected poultry farms were examined for avian clinico-pathology and gross-pathology, indicated predominance of infectious bacterial diseases. Clinical signs included diarrhoea, depression, loss of appetite, nasal discharges, soiled vent, ruffled feathers, labored rapid breathing, reduced egg production, loss of body condition and finally death. Characteristic gross lesions were air sacculitis, egg peritonitis, omphalitis, salpingitis, colisepticaemia, fibrinous perihepatitis and pericarditis revealed severe infection of *E. coli* in fowls. Predominance of different types of bacterial diseases diagnosed from these sample birds were Colibacillosis, Necrotic Enetritis, etc.

Keywords: Perihepatitis, Pericarditis, Colibacillosis, Necrotic Enteritis, Air Sacculitis, Salpingitis, Omphalitis, Egg Peritonitis, Colisepticaemia, Avian Clinico-pathology, Gross-pathology.

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ISSN: 0975-8585

INTRODUCTION

India ranked sixth position in the world in poultry production and the industry is a multi-crore investment and increasingly more competitive with establishment of large number of farms with intensification. This escalation has lead to increased susceptibility to various disease conditions in the birds. Rajasthan has a big poultry industry with over 6.3 million birds out of which the Ajmer district has over 2.5 million birds. In Rajasthan, the egg production during last 10 years has doubled. Ajmer has around 250 poultry farms, the highest number in Rajasthan with 2,000,000 birds of all types, including 1,200,000 layer chickens producing about 700,000 eggs daily (35). The past trends of poultry egg and meat production in Ajmer region suggests that the area had been supporting sizeable production of poultry eggs and meat. However, disease outburst has stucked the vigorous and ideal growth observed once at some historical golden time of poultry which caused a loss of revenue and gradually hampering the growth and finally the closure of some poultry farms in Ajmer. With the remarkable increase of poultry industry in Ajmer and thereby production stress, the poultry flocks have become more susceptible to various infectious and non-infectious disease conditions that caused huge economic loss to the poultry farmers in the form of morbidity, mortality and medication and production loss.

Important bacterial diseases of poultry in Ajmer are colibacillosis and necrotic enteritis (NE) which are responsible for high percentage of morbidity and mortality. Among these, losses due to colibacillosis appears to be one of the bottle necks as it has been recorded regularly in almost all the farms throughout the year. Avian colibacillosis is an infectious disease of birds in which *Escherichia coli* is the primary or secondary pathogens. Infection includes airsacculitis, cellulitis, omphalitis, peritonitis, pericarditis, salpingitis, synovitis and Hjarre's disease (coligranuloma) is caused by *E. coli* (9, 10). *Escherichia coli* is a common pathogen for commercial poultry causing colibacillosis all over the world. The organism affects multiple body system of birds causing various symptoms. It is a major cause of respiratory and septicaemic diseases in broiler chicken causing mortality less than 5% and morbidity over 50% but in layer it affects the reproductive tract resulting failure of egg productivity and infertility (5). *E. coli* infections cause many clinical manifestations such as airsacculitis, pericarditis, septicaemia, anddeath of the birds (19). The infection has also been extended to various parts and organs such as skin, joints, eyes, head, blood, heart, yolk sac, peritoneum etc (41). In the past few years, both the incidence and severity of colibacillosis have increased rapidly, and current trends indicate that it is likely to continue and become an even greater problem in the poultry industry (2). The major disease syndromes in colibacillosis of poultry are yolk sac infection, respiratory disease complex (airsacculitis), perihepatitis, pericarditis, acute septicaemia, salpingitis, peritonitis, synovitis, osteomyelitis, cellulitis, and enteric coligranuloma. Young birds with little resistance to infection will acutely die from septicaemia, but older chickens are often resistant and survive the initial septicaemic lesions (30); however, *E.coli*-associated septicaemic peritonitis in adult laying hens can cause significant mortality (34).

Necrotic enteritis is an acute disease caused by *Clostridium perfringens* when proliferates to high numbers in the small intestine and produces toxins responsible for

damaging the intestinal lining (26, 38). It was firstly described by Parish (1961). Infections with *Clostridium perfringens*in poultry can cause several clinical manifestations and lesions include necrotic enteritis, necrotic dermatitis, cholangiohepatitis as well as gizzard erosion. However, subclinical infection can take place too (27, 29, 31). *Clostridium perfringens* may often be a normal inhabitant of the intestinal tract (39) and other factors promote microorganism overgrowth and toxin production in the gut. For example, necrotic enteritis is often preceded by or associated with enteric coccidial infection (1, 38). Also, high levels of wheat or fishmeal in the ration may predispose birds to develop necrotic enteritis (6, 42).

Considering the above facts, the current study was undertaken with a view to diagnose the infectious bacterial disease based on clinical and gross-pathological changes to evaluate the incidence of ubiquitous bacterial diseases in fowls of poultry farms in Ajmer region.

MATERIALS AND METHODS

Regular inspection of birds was done in the 4 selected poultry farms in Ajmer region to detect any bacterial disease affecting poultry birds by viewing the behavior of diseased birds and the external symptoms and traits i.e. clinical signs developed in diseased fowls. The clinical signs were recorded during the physical visit of the affected farms and the farmer's complaints were also taken into consideration.

Necropsy: A total of 12 diseased dead birds (layers) were collected, 3 each from 4 deep litter poultry farms of Rasulpura belt in Ajmer region for performing necropsy to expose the viscera especially liver, lungs, air sacs, ovas, small intestine, large intestine and caeca. The birds were necropsied within few hours of death or collection.

Clinical Diagnosis: Clinical diagnosis was made on the basis of recorded clinical history from farm authority, observed clinicalsigns and gross pathological lesions of the dead and sick birds. The birds were examined systematically and the observed postmortem changes were recorded during necropsy according the procedure described by Calnek *et al.* and Charlton.

Gross-Pathology and Diagnosis of bacterial diseases: All the birds with open viscera were observed pathologically and diagnosed for disease by viewing the lesions on different affected organs of the birds (Table 1). At necropsy, the organs were examined carefully and gross tissue changes were also recorded.

RESULTS AND DISCUSSION

Out of the 12 dead or bacterial cases examined, of which colibacillosis (n = 12), egg peritonitis (n = 7), air sacculitis (n = 5), salpingitis (n = 5), yolk sac infection or omphalitis (n = 5), and necrotic enteritis (n = 4) were diagnosed. Thus, the prevalence of colibacillosis including air sacculitis, egg peritonitis, salpingitis, yolk sac infection, etc. and necrotic enteritis were found significantly high in comparison to other bacterial diseases. The findings revealed that among 12 poultry layers of Ajmer region, the prevalence of *E.coli*infection was 99 percent and that of

Clostridium perfringens was 30 percent in the selected deep litter poultry farms, respectively, as described in our earlier reports (13). These findings revealed that the most prevalent bacterial infection in Ajmer region is the *E.coli* infection, as depicted in our previous studies (13). Details can be seen in Table 1.

Colibacillosis: Almost 12 (99%) of the 12 affected layer chickens were diagnosed as colibacillosis. The most obvious clinical signs regarding abnormal behavior of affected birds in the poultry premises observed were loss of appetite (in appetence or refused to eat), diarrhoea, dyspnoea, dullness, listlessness, severe depression, huddling, droopy head and respiratory distress (rapid labored breathing, coughing, sneezing) (fig. 1, 2, 3 & 10). The birds were found lethargic, dehydrated, reluctant to move, and depressed with poor growth performance (fig. 1, 2 & 3). Few birds were also found to became lazy and remain confined to the corner of the poultry house and showed only reduced or restricted movements (fig. 3). Some birds appeared listless, standing about dejectedly with ruffled feathers, with head and neck of these affected birds drawn into their bodies (fig. 2). Rapid progressing mortality in birds indicated the presence of acute septicaemic form or Colisepticaemia.

The clinical signs of avian colibacillosis in layer birds regarding external symptoms and traits comprised of water diarrhea, which in some cases was seen as blood tinged, yellowish colored droppings, pasting of vent, vent feathers soiled by paste like faeces, ruffled feathers, soiling of cloaca with semisolid cheesy material, nasal discharges, respiratory distress (rapid labored breathing, gasping, coughing, sneezing), persistent anorexia (resulting into emaciation and death of some birds), septicaemia, reduced egg production, reduced weight gain, depressed with distended abdomens, weakness, comb hanging down with no stiffness, occasionally lameness, stunted growth, loss of condition, high mortality, stopped egg laying, and finally death (fig. 1-10). The minute particles of feed and litter were found entangled to the mucous or sticky nasal secretions or “nasal discharge” present on the external surface of nostrils of some fowls (fig. 8). When nostrils of such birds were pressed reverse back, sinuses were found full of white colored pus or “nasal discharge” (fig. 9). Nasal discharges in most diseased birds were seen as an indication of an *E. coli.* infection. The respiratory distress indicated towards the presence of Airsacculitis or Air Sac disease. Heavy mortality in layers and newly hatched chicks indicated the presence of Salpingitis and Peritonitis in adult birds and Omphalitis (Yolk Sac Infection) in chicks, respectively. Stopped egg laying suggested the presence of Egg Peritonitis in layers.

These observations support the report of Pandey *et al.* and Rahman *et al.* Similar types of findings were described by Calnek *et al.* Chauhan, Vegad and Katiyar, Islam *et al.* and Rakibul Hasan*et al.* The clinical findings of colibacillosis in layer birds recorded in this study are in conformity with the earlier reports of Kaul *et al.* Haider *et al.* and Islam *et al.*, who reported an outbreak of colibacillosis with clinical signs in broiler chicks. The clinical signs observed during the farm visit varied from farm to farm but found to correlatate with previous findings (10, 44).

The pathological lesions of avian colibacillosis are varied and wide, and have been reported to be included omphalitis and yolk sac infection, fibrinopurolent air sacculitis,

fibrinous pericarditis, fibrinous perihepatitis, salpingitis, peritonitis, synovitis or arthritis, avain cellulitis, enteritis, andcolisepticaemia. Necropsy examination of layers died of colibacillosis showed dark, enlarged and congested liver along with swelling and necrotic foci, translucent and thickened caseated air sacs, hemorrhagic enteritis with fluid accumulation in ligated intestinal loops (haemorrhage and mucus in intestine) and omphalitis resulted unabsorbed yolk sacand fluid accumulation in the peritonial cavity of chicks (fig. 11, 12, 13, 14 & 20). Gross lesions included caseous exudation on the respiratory surfaces (caseated air sacs), small white foci on liver surface, abdomen distended with an offensive-smelling caseous material, mis-shapened ruptured ovas or eggs along with thin egg membrane, abdominal cavity filled with yolk, dilated, thin-walled, infected oviduct distended with multiple masses of caseous exudates, septicaemia in liver and intestine, and petechial haemorrhages in the heart, spleen and liver (fig. 11-20). Post-mortem findings revealed cloudy and thickened non-functional air sacs, congested and thickened liver capsule, bunch of mis-shapened ovas with loose texture (similar to grapes), oviduct occluded with masses of yolk, coagulated albumin, shell membranes and fully formed eggs and white, fibrinous, gelatinous and caseous exudates on liver surface (fig. 11, 12, 13, 15, 16& 18). All these lesions indicated the septicaemic form of colibacillosis. The duodenum showed mucus, congestion and haemorrhage (enteritis) and there was haemorrhage in the caecal tonsil (fig. 19 & 20).

All these clinical lesions recorded indicated the presence of an *E. coli* infection. The yellowish material disposed off on caseated, thickened, non-functional air sacs indicated the presence of Airsacculitis or Air Sac disease (fig. 13), as elucidated in our previous findings (13). Yolk debris, inspissated yolk, yellowish fibrinous or purulent material along with large caseated mass or caseous material or milky fluid in the abdominal cavity, together with inflammation and distortion of the ovaries (mis-shapened and ruptured ovas), indicated towards the presence of infection of Salpingitis and Egg peritonitis in the birds (fig. 14, 15, 16, 17 & 18), as described in our earlier reports (13). Unabsorbed yolk material seen as watery, yellow-brown or caseous material often with putrid odor (yolk filled in abdomen cavity) indicated towards the presence of Yolk sac infection or Omphalitis in the birds (fig. 14), as described in our previous studies (13).

These findings of the present study agree with the findings of Nakamura *et al.* (1985) and Rakibul Hasan *et al.* These lesions observed in this study are in conformity with the earlier reports of Kaul *et al.* Calnek *et al.* Haider *et al.* Islam *et al.* and Khaton *et al. E. coli* can cause several disease conditions (5, 12). Young birds with little resistance to infection will acutely die from septicaemia, but older chickens are often resistant and survive the initial septicaemic lesions (30); however, *E.coli*-associated septicaemic peritonitis in adult laying henscan cause significant mortality (personal observation) as in accordance with the findings of Porter. No published inland report on avian colibacillosis is available to compare the results but Sharma and Kaushik and Mahajan *et al.* reported *E. coli* infections as principal disease in broilers in Haryana State, India.

Necrotic Enteritis: Clinical signs described with necrotic enteritis includedruffled feathers, marked depression, in-appetence, watery droppings, diarrhea, huddling, anorexia, and a

sudden rise in flock mortality (fig. 2, 3, 4 & 10) as in accordance with the findings of Long, Gazdzinski and Julian and Porter. The clinical signs appear suddenly; apparently healthy birds may become acutely depressed and die within hours, which corresponded with the studies of Long, Tsai and Tung, Shane *et al.* and Hafez. Some birds showed severe depression, decreased appetite and feed intake, reluctance to move, ruffled feathers and diarrhea, while others showed no visible illness but were associated with temporarily reduced weight gain and impaired feed conversion ratio. Clinical illness in some birds was found very short; often these were just found acutely dead. All these clinical signs indicated towards the infection of *Clostridium perfringens* causing Necrotic Enteritis.

Post mortem lesions in layers died of necrotic enteritis recorded were thickened and inflamed intestine, haemorrhagic ulcers in intestine and lesions on mucosa of intestine (fig. 19 & 20). The intestine was seen dilated with dark offensive fluid and a diphtheritic cauliflower-like membrane that involves the mucosa (fig. 19). Small haemorrhagic ulcers or light yellow spots were observed on the surface of the mucosa, usually in the small intestine, in particular in the jejunum and ileum, and less commonly, the caeca (fig. 20). Lesions were observed on gray, thickened mucosa of jejunum and ileum, indicating towards the infection of *Clostridium perfringens* (fig. 20), as described in our previous studies (13).

Severe inflammation in the duodenum and jejunum is the most predominant finding, but in some instances the entire length of the intestinal tract is involved as in accordance with the reports of Bains, Helmboldt and Bryant, Long *et al.*, Tsai and Tung, Ficken and Berkhoff and Hafez. The intestine is distended, thin walled and filled with gas and contains dark offensive fluid as in accordance with the findings of Broussard *et al.* and Hafez. The mucosa is covered with green or brown diphteroid membrane, which can be easily separated from the lining. Varying degrees of sloughing of the intestinal mucosa could also be observed. As the condition progresses, areas of necrosis can be recognized from outside of the intestine, similar to the findings of Helmboldt and Bryant, Long *et al.*, Balauca, Shane *et al.* and Hafez. Gross lesions are usually restricted to the small intestine, particularly the jejunum and ileum and less often the duodenum and cecum. The intestine is distended by gas and dark brown fluid. A discontinuous to diffuse layer of tan to grey, friable, fibrinonecrotic material (pseudomembrane) is adhered to the mucosa. Rare focal lesions may be observed similar to the observations made by Hemboldt and Bryant, Shane *et al.* and Porter.

CONCLUSIONS

Considering the higher mortality rates in layers of selected poultry farms and external traits and symptoms developed in sick layers and abnormal behavior of diseased layers and clinical signs and postmortem lesions in necropsied layers, collectively caused by *Escherichia coli* and *Clostridium perfringens,* colibacillosis and necrotic enteritis may be considered as a threat to the layer industry in Ajmer region. The present study should be extended further to microbiological research work to confirm the results and histopathological studies to evaluate the effects of these microorganisms on cell and tissue integrity of affected fowls. Therefore, these disease problems can be checked by adopting sound management, good sanitation and

judicious selection of antibiotic based on further antibiogram studies which is recommended and should be carried out as future research work. Such high mortality in layers and high frequency of the incidence of infectiousbacterial diseases in layers pointed towards the illiteracy of poultry farmers in Ajmer region especially of remote and village areas and unawareness of scientific methods and technology used by them in poultry industry.

Table-1: Prevalence of infectious diseases in the dead layer birds of deep litter farms collected for necropsy.

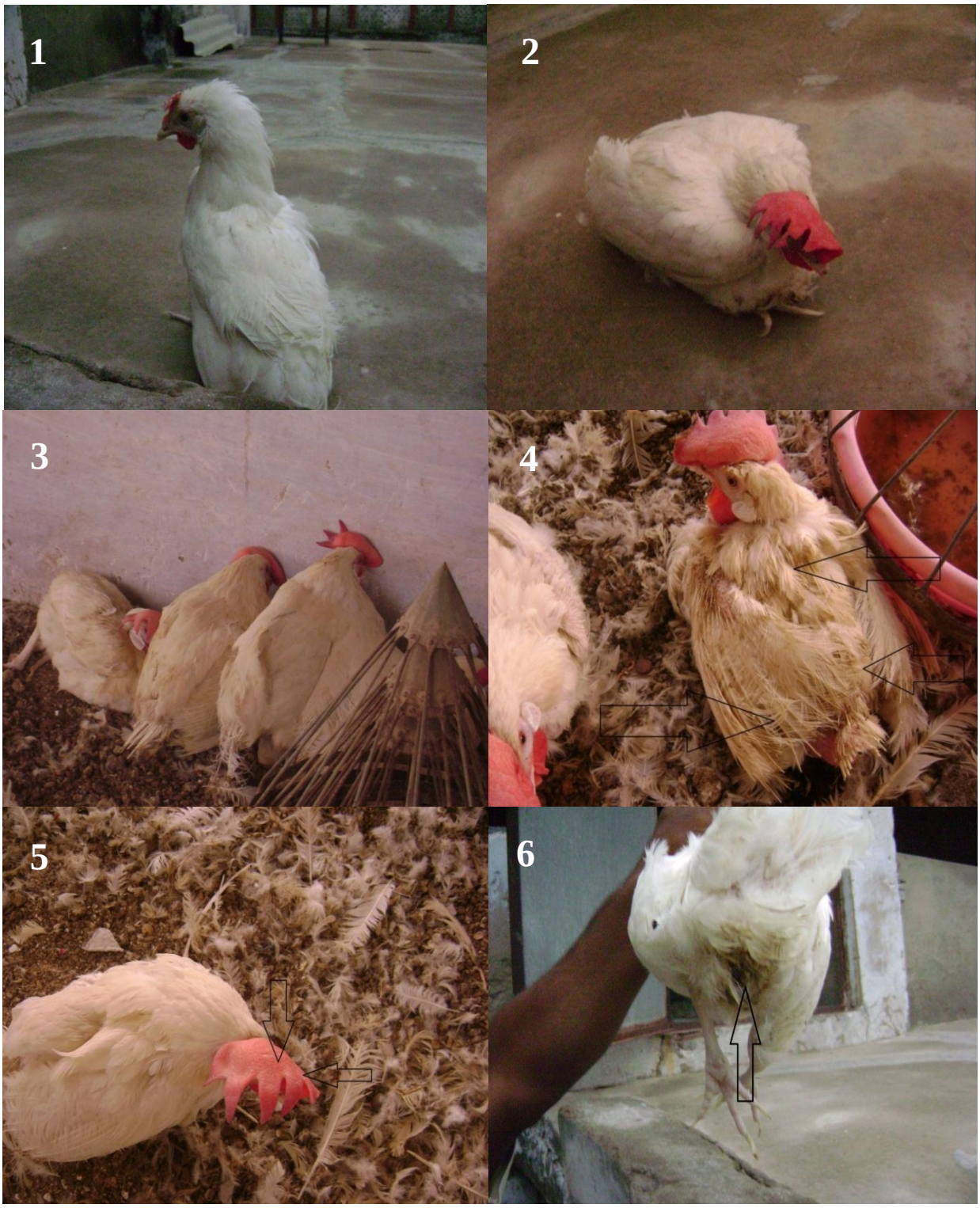
Bacterial Diseases	Birds Diagnosed (n = 12)	Poultry Farms				Overall Prevalence among tested cases (percent)
		A	B	C	D	
Colibacillosis	12	3	3	3	3	99
Egg Peritonitis	7	2	1	2	2	60
Air Sacculitis	5	2	1	1	1	50
Salpingitis	5	1	1	1	2	50
Omphalitis	5	2	1	1	1	50
Necrotic Enteritis	4	1	2	1	0	30

n = total number of layers diagnosed for bacterial infections.

Table 1 represents the data regarding the prevalence of different infectious bacterial diseases in the dead layer birds of deep litter farms which were sacrificed and diagnosed for the confirmation of a particular disease.

ISSN: 0975-8585

FIGURES 1-10: Observation of layers in the selected deep litter poultry farms revealing the peculiar external traits and symptoms and abnormal behavior of diseased layers as clinical signs providing the indication about the occurrence of a specific disease.



ISSN: 0975-8585

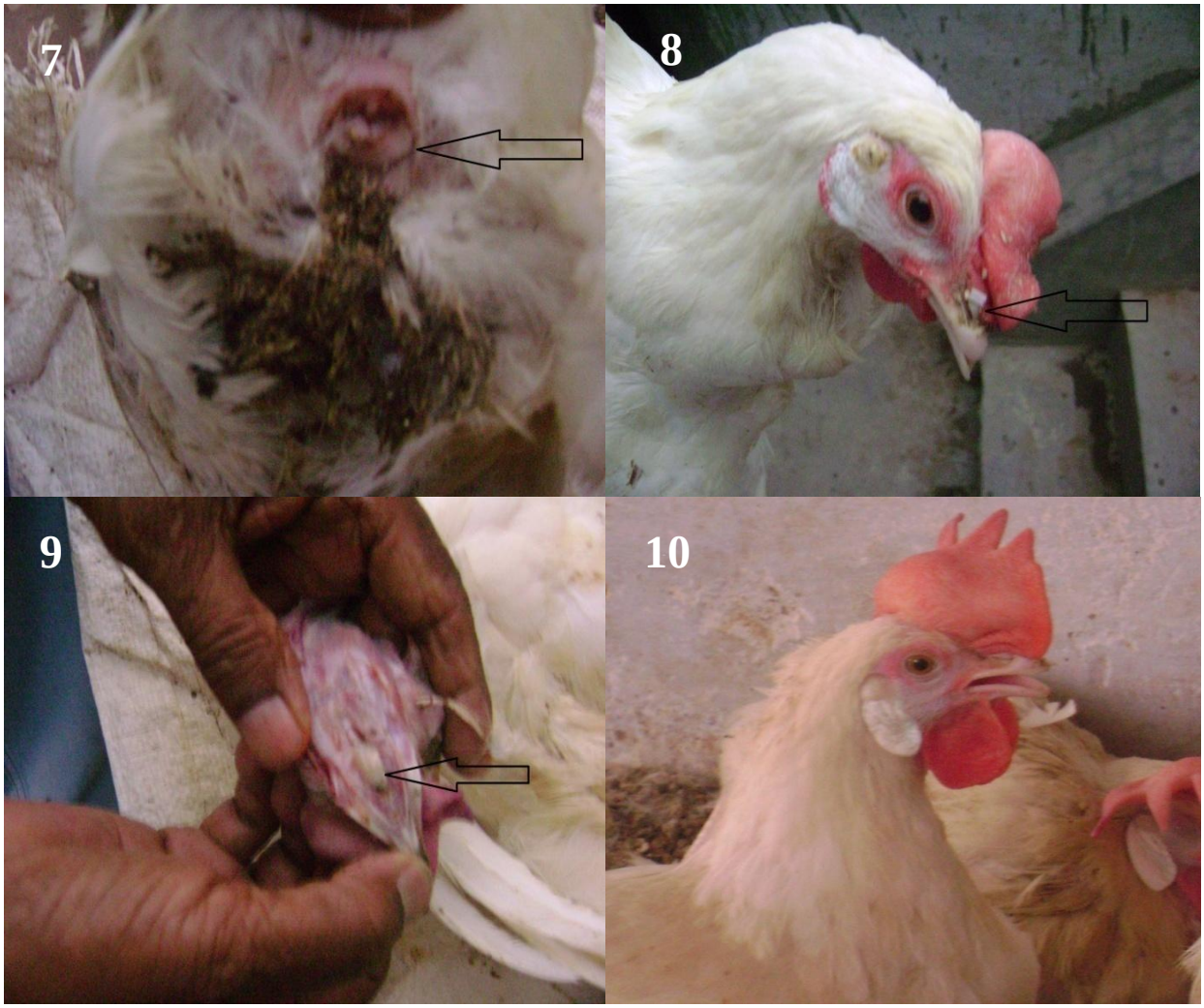


Fig. 1 represents a weak, dull, lethargic and reduced weight bird. **Fig. 2** represents a dull, lethargic bird under depression standing about dejectedly, along with its head and neck drawn into its body. **Fig. 3** represents some lazy birds depressed with distended abdomens confined to the corner of the poultry house with reduced or restricted movements, along with a tendency to huddle. **Fig. 4** represents a diseased bird with ruffled feathers. Arrow heads in **Fig. 5** shows the comb of a diseased bird hanging down with no stiffness. Arrow heads in **Fig. 6** shows vent feathers of a diseased bird soiled by paste like faeces. Arrow heads in **Fig. 7** shows the pasty vent of a diseased bird. Arrow heads in **Fig. 8** shows minute particles of feed and litter entangled to the mucous or sticky nasal secretions or "nasal discharge" present on the external surface of nostrils of a diseased bird. Arrow heads in **Fig. 9** shows white colored pus or "nasal discharge" in the sinuses of a diseased bird by pressing its nostrils reverse back. **Fig. 10** represents labored rapid breathing and gasping (respiratory distress) in a diseased bird.

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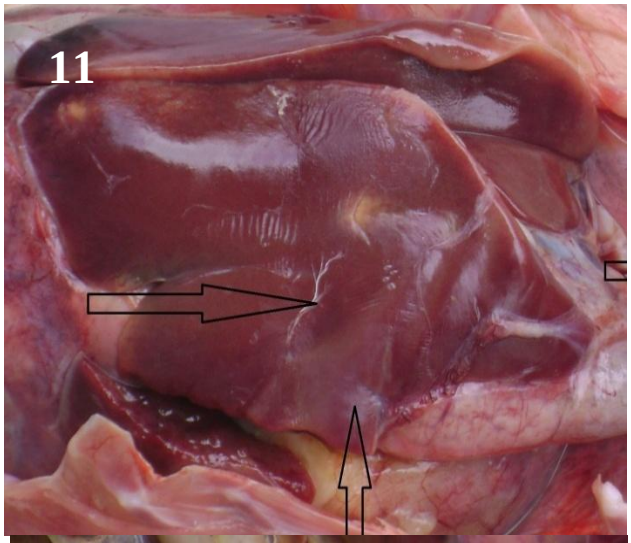
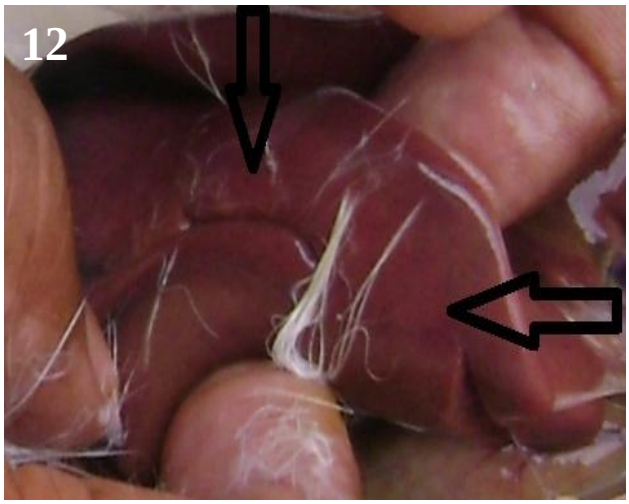
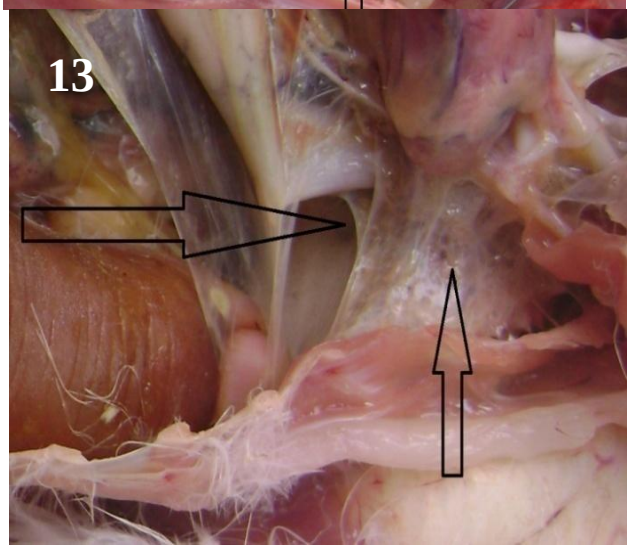
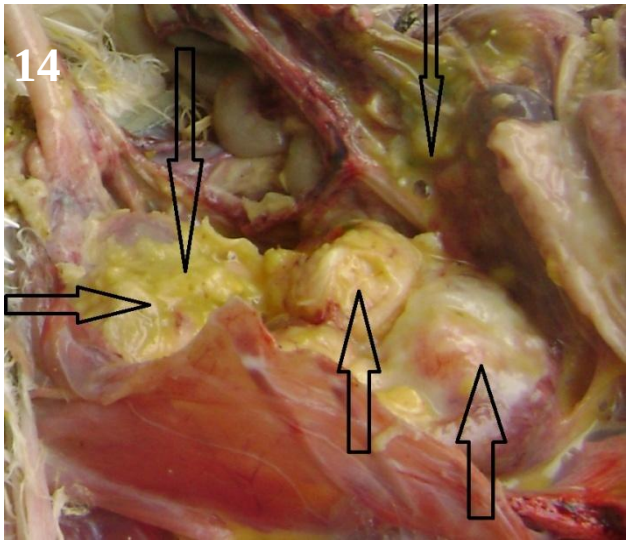
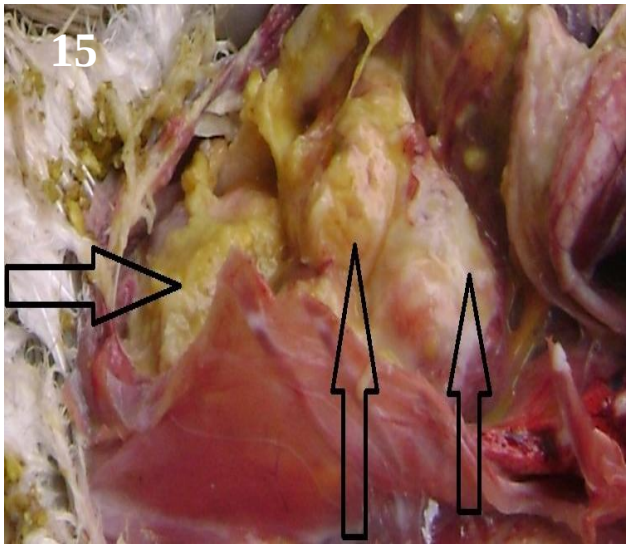
FIGURES 11-20: Necropsy of diseased birds showing characteristic clinical signs and lesions on affected organs and diagnosis of the related disease.

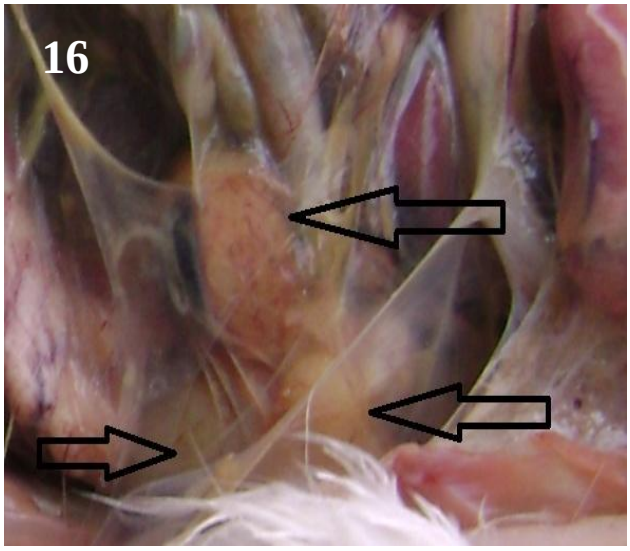




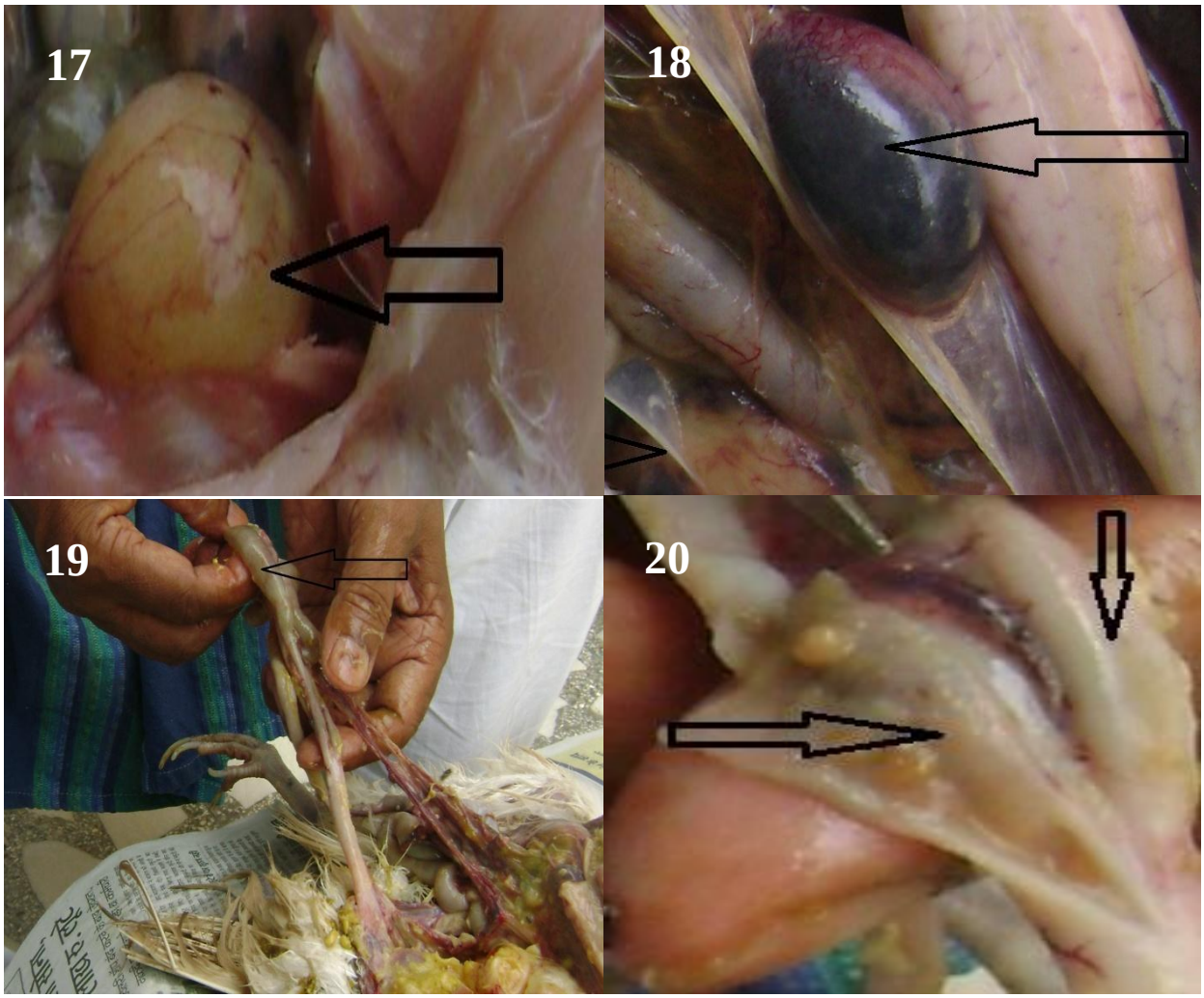








ISSN: 0975-8585



Arrow heads in **Fig. 11** shows small, white necrotic foci and white, fibronous, gelatinous and caseous exudates on liver surface, along with swelling. **Fig. 12** shows the presence of dark, enlarged and congested liver. Arrow heads in **Fig. 13** shows the presence of thickened, translucent, non-functional caseated air sacs along with disposed yellowish material due to an *E.coli.* infection. **Fig. 14** represents the yolk filled in the abdomen, which oozes out from the eggs or ovas. The unabsorbed yolk filled in abdominal cavity was seen with abnormal color and consistency. **Fig. 15** represents the inflammation and distortion of the ovaries seen as a bunch of mis-shapened and ruptured ovas (loose in texture) in ovary. **Fig. 16** represents mis-shapened and ruptured ovas of a bunch like ovary which can be seen from the covering of caseated and translucent air sacs. **Fig. 17** represents mis-shapened and ruptured ovas or eggs, along with thin and ruptured egg membrane. **Fig. 18** represents represents dilated, thin-walled, infected oviduct distended with multiple masses of caseous exudates, masses of yolk, coagulated albumin, shell membranes and fully formed eggs. **Fig. 19** represents the thickened, dilated and inflamed intestine. **Fig. 20** represents haemorrhagic ulcers or light yellow spots on the surface of gray, thickened mucosa (characteristic gross lesion) of small intestine indicating the presence of *Clostridium perfringens*.

ACKNOWLEDGEMENT

We greatfully acknowledge the technical assistance of Dr. V. K. Mishra, Retired Govt. Veterinary Doctor, Ajmer in performing necropsy of birds and his invaluable advice and encouragement in preparing the manuscript.

ISSN: 0975-8585

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