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Pathological changes and occurrence of infections related to gastrointestinal tract of poultry in Southern Rajasthan

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ABSTRACT

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Poultry is continuously exposed to a wide variety of infectious agents leading to disturbances in Gastro-intestinal system. This study was carried out to study the mortality pattern of poultry and pathological conditions associated with gastrointestinal tract affections in poultry reared in Southern Rajasthan. A thorough postmortem examination of poultry carcasses was done. Samples of heart blood, liver and intestine were taken to isolate microorganisms. Different conditions encountered were Colibacillosis (23.34 %), Salmonellosis (4.59 %), parasitic infections (2.79 %, roundworm and tapeworm), Ascites (1.38 %) other bacterial infections (0.99 %; *Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* infection). Maximum mortality was observed in winter followed by the rainy season. Overall maximum mortality was observed in the 6-12 week age group (27.38 %) and lowest in 0-1 week age group (11.90 %). Congestion, hemorrhages, necrosis, and inflammation were predominant lesions encountered in various internal organs. Collibacillosis and Salmonellosis were the major bacterial infections found in poultry.

Keywords: Bacterial diseases, Colibacillosis, Gastrointestinal tract, Pathological lesions, Salmonellosis

INTRODUCTION

The world poultry industry has grown consistently and major space has been occupied by broiler chicken industry (Yang and Jiang, 2005). The poultry sector has industrialized in many parts of the world but in developing countries, traditional small scale poultry systems play a major role in sustaining livelihoods. Poultry is the principal source of economic and high quality human food. Despite industrialization and significant advances in poultry rearing systems, diseases are still a major constraint for profitable poultry production. Infectious and non-infectious diseases take a heavy toll on all types of poultry enterprises. Due to an emphasis on intensive rearing during the last few decades, a large numbers of birds are predisposed to various diseases, thereby causing economic losses to the farmers.

Amongst the various other systems, the gastrointestinal tract (GIT) is constantly exposed to a wide variety of potentially harmful substances. Functional abnormalities in GIT leads to disturbances with absorption and assimilation of nutrients resulting in decrease in growth, poor feed conversion ratio (FCR) and production losses, thereby severely affecting the health status of broiler farming (Ficken and Wages, 1997). Enteric diseases in poultry leads to disturbances with absorption and assimilation of nutrients resulting in devastating economic effects due to high mortality rates, poor feed efficiency, decreased growth and production (Cooper *et al*., 2013). The etiology of enteric disorders is multifactorial, for instance combinations of viruses, bacteria, immunosuppression and mycotoxins. Affections of digestive system account for high mortality in poultry and the common gastrointestinal tract conditions reported are colibacillosis, salmonellosis, fowl typhoid, coccidiosis, Newcastle disease, necrotic enteritis, and fatty liver syndrome (Hooda *et al*. 2009; Kabir, 2010). These conditions lead to economic losses due to morbidity, mortality, treatment costs and decreased egg or meat production. In Southern Rajasthan poultry farming is mostly under backyard production followed by small range commercial farms. The occurrence of diseases in poultry is less explored in the area. Therefore, the current study was designed to investigate the occurrence and pathology of gastrointestinal tract disorders of poultry in southern region of Rajasthan.

MATERIALS AND METHODS

Study area

The geography of Southern Rajasthan comprise of rocky Aravali mountain ranges and sub-humid, to very humid climatic regions. A survey of different poultry farms of Southern Rajasthan (Udaipur, Dungarpur, Chittorgarh, Rajsamand and Bhilwara districts) was conducted. Age wise and season wise disease occurrence was also determined.

Postmortem examination

Thorough postmortem examination of 84 dead poultry birds (broilers and layers) brought to college for postmortem examination was conducted under aseptic conditions. The diagnosis of different diseases was based

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on the history of the affected birds, clinical signs, postmortem lesions, gross and microscopic examinations and isolation and identification of causative agents.

Gross and histopathological examination

Gross pathological examination of GIT comprising of crop, oesophagus, proventriculus, gizzard, pancreas, liver, intestine and cloaca of dead birds were thoroughly examined and the changes observed were recorded. The representative tissue pieces from these organs were collected in 10 percent buffered formalin. The fixed tissues were processed as per standard procedure (Lillie, 1965). The paraffin blocks were prepared and sections were cut at the thickness of 4-5µm with rotary type semi– automatic microtome (Yorco). The paraffin embedded sections were then stained by routine staining method (Luna, 1968) for hematoxylin and eosin stain (Sigma).

Parasitological examination

During postmortem examination, all the parasites visible to the naked eye were collected using a pair of forceps. All recovered nematodes and cestodes were preserved. Cestodes were preserved in 5% formalin with 5% glycerin and Nematodes were preserved in 70% alcohol with 5% glycerin. Formalin preserved nematodes were washed in running tap water and dehydrated before clearing. Nematodes of medium to small size were cleared in lactophenol and mounted in glycerine. All adult worms were identified directly under the microscope (Soulsby, 1982 and Khalil *et al*., 1994).

Bacteriological studies

Isolation of organisms was attempted from the heart blood, liver and intestines of dead birds in the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Navania Vallabhnagar, Udaipur. Samples for bacteriological studies were collected aseptically in sterile cotton swabs. The isolation and identification of bacterial culture was performed as per the culture characteristics and biochemical tests (Cruickshank *et al.*, 1975).

RESULTS AND DISCUSSION

Occurrence of diseases

Different diseases/pathological conditions associated with GIT lesions were colibacillosis (23.34 %), followed by hemorrhages in proventriculus and other organs due to infection and trauma (4.88%), salmonellosis (4.59%), parasitic infections (2.79%), Ascites (1.38%) other systemic infections (0.99% like *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* infection)**.**

The main GIT affections reported in other parts of the country (northern) were colibacillosis, followed by enteritis, hepatitis, coccidiosis, fatty liver and enlargement of the liver (Dadheech *et al*. 2016; Hooda *et al*., 2009; Kurade *et al*. 2001). Borah *et al*. (2017) reported the maximum incidence of omphalitis (13.40%), colibacillosis (11.11%), IBD (10.58%), necrotic enteritis (6.35%), Newcastle Disease (ND) (4.59%), and brooder pneumonia (3.70 %) from birds in Assam. Sangha (2017) in Jammu region reported that occurrence of colibacillosis (24.16%) was maximum followed by that of IBD (7.13%), omphalitis (6.79%), and salmonellosis (5.36%). *E.coli* is an opportunist and colibacillosis mostly occurs as a secondary bacterial infection when host defenses are lowered due to any other viral infection (Saif, 2008). Srinivasan *et al*. (2014) reported a lower (2.35%) prevalence of *S*. Typhimurium in commercial layer chicken in Namakkal (Southern India). Gupta *et al*. (2006) reported coccidiosis and salmonellosis to be the major diseases affecting Meghalaya. Rao *et al.* (2018) reported that in chicken population of Telangana state, the prevalence of *Ascaridia galli* was 43.66%, *Heterakis gallinarum* was 38.03%, *Raillietina tetragona* was 50.72% and *Raillietina echinobothrida* prevalence in the population was 46.48%. The high incidence of these diseases may be attributed to unhygienic conditions that are present at poultry farm premises or due to the supply of contaminated water (Sultana *et al*., 2012). Disease occurrence may be lowered by improving sanitary conditions and adopting effective biosecurity conditions at the farm.

Mortality Incidence

Data regarding the mortality pattern in the affected poultry was obtained from the postmortem examination of carcasses. Mortality percent among the (Table 1) colibacillosis affected flock was highest (10.99%) followed by parasitic infection (10.86%), other infections (10.13%), salmonellosis (3.35%). Study of month-wise distribution of mortality revealed maximum mortality in December (13.09%) followed by August (11.90%), November (10.71%), May (9.52 %), March & September (8.33%), February& July (7.14%). A low mortality was observed during January, April, June & October (5.95%). Colibacillosis was prevalent throughout the year while

Table 1: Mortality pattern of diseases at different farms in Southern Rajasthan

S. No.	Pathological conditions	Total number of birds	Total number affected	Total number of dead birds	Mortality (%) among affected flock
1.	Colibacillosis	48500	34550	3800	10.99
2.	Salmonellosis	14700	7300	245	3.35
3.	Other systemic infection	16560	1480	150	10.13
4.	Parasitic infection	22470	4140	450	10.86

cases of Salmonellosis were encountered in April, May, November December and January month. Age-wise distribution of mortality among poultry revealed overall maximum mortality in the 6-12 week age group (27.38%) and lowest in 0-1 week age group (11.90%).

The overall highest mortality was recorded due to colibacillosis (10.99%) followed by parasitic infection (10.86%), other systemic infections (10.13%), and salmonellosis (3.35%). Sangha (2017) also reported colibacillosis (8.33%) to be the major cause of mortality followed by IBD (5.97%) in and around Jammu region. Singh *et al*. (1998) informed that in Punjab major cause of mortality was omphalitis (1.29%) followed by IBD (0.86%), coccidiosis (0.56%), colibacillosis (0.32%) and fowl typhoid (0.23%). However, Mahajan *et al*. (1994) observed that in Hisar (Haryana) fowl typhoid resulted in maximum mortality among chicken followed by colibacillosis, gout, ascites, brooder pneumonia, coccidiosis, and Newcastle disease. Anjaneyulu *et al*. (1998) noted that the major causes of mortality in broilers in Prakasam district of Andhra Pradesh were colisepticaemia (13.4%) followed by IBD (9.1%), coccidiosis (7.0%) and ascites (6.78%). Moreover, Kumar *et al*. (2018) revealed that major causes of mortality in Ranchi, Bihar were chilling and coryza (42.41%) followed by enteritis (12.94%), nephrosis (10.48%), yolk sac infection (7.73%), coccidiosis (6.83%) whereas colibacillosis was responsible for only 5.88% mortality. It indicates that mortality due to colibacillosis is highest all through the country.

Month-wise distribution of mortality pattern depicted that maximum mortality was observed in December (13.09%) followed by August (11.90%) and November (10.71%) indicating the drastic effect of changing and extreme weather conditions in these months. The unfavorable environment can cause stress that could render birds more vulnerable to diseases. Minimum mortality was noted in January, April, June and October months (5.95%) which may be suitable months for rearing poultry in this region. Similarly, Abbas *et al*. (2015) concluded that April to June was safer for the broiler production and January to March for layers as a low incidence of disease was recorded in this period in Pakistan. Baksi *et al*. (2017) observed that fowl typhoid infects the birds highest in monsoon season (20.83%), whereas it was found least in winter (7.42%).

With respect to age, the maximum mortality was observed in 6-12 weeks age group (27.38%), followed by 12-18 weeks (23.80%), > 18 weeks (20.23%), 1-6 weeks (16.66%), and 0-1 week (11.90%), age groups. Colibacillosis and Salmonellosis were noticed in all age groups. However, Bhalerao *et al*. (2013) reported maximum mortality due to *E. coli* infection in birds of 3-4 weeks of age in Haryana state. Kumar *et al.* (2018) observed that starters were more susceptible to diseases like chilling and coryza, yolk sac infection, enteritis, nephrosis, coccidiosis, and colibacillosis whereas growers were affected more by chilling and coryza followed by ascardiasis and enteritis. Adults showed presence of oophoritis, egg bound condition, and egg peritonitis. Baksi *et al*. (2017) recorded that birds aged more than 45 weeks were found affected with *Salmonella* (21.51%) infection. Hooda *et al.* (2009) studied the data pertaining to mortality in poultry between January 2004 and December 2008 in Haryana and revealed that mortality was highest in adult age group (29.1%) and affections of digestive system accounted for highest mortality (30.88%). The main gastrointestinal affections were colibacillosis, enteritis, hepatitis, coccidiosis, fatty liver and enlargement of the liver. These reports suggest that colibacillosis is prevalent in wide geographic range, affects all age groups and causes diseases throughout the year.

Isolation of Bacteria

The present study revealed the presence of different types of bacteria which may be associated with the causation of different GIT disease conditions. The various bacteria isolated from liver, intestine and heart blood of birds were *Escherichia coli* (70.31%), *Salmonella* sp. (12.5%), *Staphylococcus aureus* (9.37%), *Pseudomonas aeruginosa* (6.25%) and *Klebsiella pneumoniae* (1.56%).

Mixed infections

The mixed bacterial infections recorded were, *E. coli* along with *Salmonella* infection. *E. coli* serotypes were found along with *Pseudomonas aeruginosa* infection. *E. coli* were found along with *Staphylococcus aureus*. *E. coli* infection was also found along with parasitic infections (roundworm & tapeworm). In addition, *Klebsiella pneumoniae* was found along with *Salmonella* Typhimurium & parasitic infections (round worm & tape worm).

Pathological studies

Pathological lesions associated with various bacterial infections were as follows:

Colibacillosis: Clinical signs observed in birds of the affected farms were anorexia, dullness, depression, and drooping of the head, neck and wings, ruffled feathers and labored breathing. Mixed infections of *E. coli* were observed along with *Salmonella* spp, *Pseudomonas aeruginosa*, *Staphylococcus aureus* & Nematodes. It was also found along with ascites. The gross lesions observed were the presence of thick fibrinous layer on all the visceral organs particularly on heart and liver **(**Fig. 1). In some cases, the fibrinous layer was also present on kidneys and spleen. In some cases, there was the presence of small necrotic foci on the liver and spleen. The purulent exudate was observed in the lumen of proventriculus & gizzard in some cases. Hemorrhagic spots in the mucosa with slimy mucinous exudates in

the lumen of the intestine were observed in some birds. In some birds, the congested and consolidated lung was observed. In young chicks, infection in the yolk sac, (Omphalitis) was observed. Microscopically, fibrinous perihepatitis was characterized by the accumulation of fibrin strands and infiltration of heterophils, congestion and hemorrhages in blood vessels and hepatic sinusoids. Liver also revealed multifocal areas of degeneration, and necrosis. The lesions recorded in the intestine were degeneration, necrosis, and desquamation of villi along with heterophilic infiltration. Spleen revealed multifocal necrosis and depletion of lymphocytes in the white pulp, along with the presence of hemosiderin. Severe pericarditis characterized by the accumulation of fibrin and infiltration of heterophils, myocarditis, and fragmentation of myocardial muscle fiber was noticed**.** In most of the cases, lungs revealed congestion and hemorrhages and perivascular edema.

Fig. 1: Thick fibrinous layer on heart, liver and peritoneum along with adhesions with each other (*E. coli infection*)

The gross lesions observed in colibacillosis were similar to those observed by others (Bhalerao *et al.*, 2013; Sangha, 2017). Similar lesions have been described in experimentally induced *E. coli* infection in chicks by Kumari and Gupta (2014). Colibacillosis was found to be associated with omphalitis in young birds similar to the earlier reports (Bhalerao *et al.*, 2013).

Salmonellosis: The liver was firm, dark red and blood oozed out from the cut surface. Most cases revealed hepatomegaly and splenomegaly along with multiple pale necrotic foci on the surface of the liver (Fig. 2) and spleen (Fig. 3). In some cases, the fatty liver condition was present. The enlarged gall bladder was present in most of the cases. In a few cases, a white nodule on myocardium and distortion of shape of heart was observed. Lungs were congested. Catarrhal enteritis was evident in some cases characterized by mucous exudate on the mucosal surface in the lumen of the intestine. Kidneys appeared congested and enlarged with few necrotic foci. Microscopically, congestion, hemorrhages, necrotic foci and infiltration of heterophils was observed in liver, spleen, heart, proventriculus and kidneys (Fig. 4, 5). Mild depletion of lymphoid tissue was observed in the bursal follicles along with inter follicular fibrosis. Postmortem lesions noticed in *Salmonella* sp. infections were in accordance with the findings of earlier reports (Nazir *et al.*, 2012 and Srinivasan *et al.*, 2014).

Fig. 2: Presence of hemorrhages and necrotic area on the surface of liver in Salmonellosis.

Fig. 3: Enlarged spleen showing multiple pale foci of necrosis in *S. Typhimurium* infection.

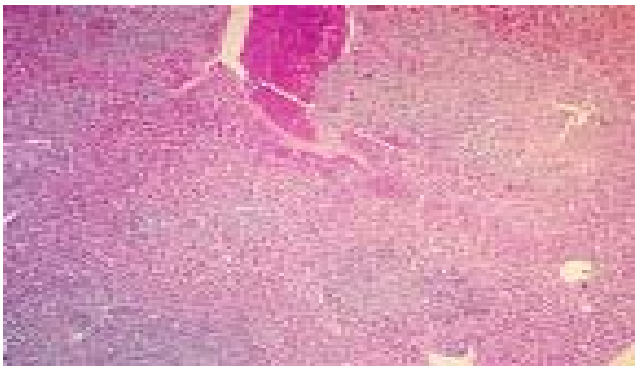

Fig. 4: Liver- Congestion, hemorrhages, multiple necrotic foci and infiltration of heterophils. H & E (100X)

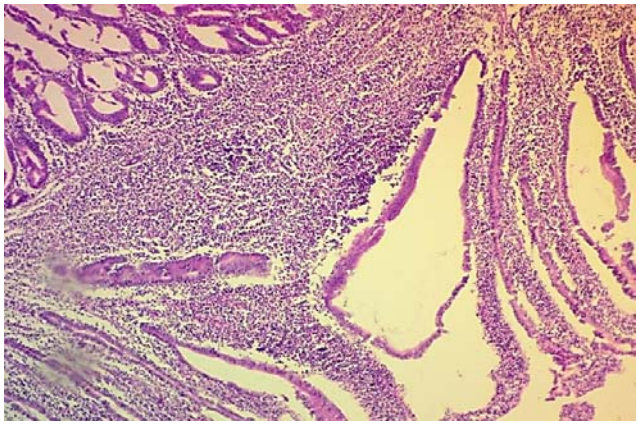

Fig. 5: Proventriculus- Proventriculitis characterized by mucosal degeneration, necrosis and thickening of mucosa due to severe infiltration of heterophils and few macrophages. H & E (100X)

Pseudomonas aeruginosa Infection: Congestion was observed in trachea, lungs, heart and intestines and liver. Mild fibrinous perihepatitis and pericarditis were seen. In a few cases, suppurative enteritis characterized by thick mucus on the intestinal mucosa was evident. Mild fatty changes and necrosis was present in liver. Necrosis and desquamation of the mucosal epithelium were observed in the duodenum (Fig. 6). The cases of *Pseudomonas aeruginosa* were encountered in both young and adult birds and mostly in monsoon season in the months of August and September. This may be because this organism is usually associated with a humid environment and contaminates soil and water (Saif *et al*. 2008). Gross and histopathological observations were similar to those recorded by others (Kebede, 2010 and Shukla and Mishra, 2015).

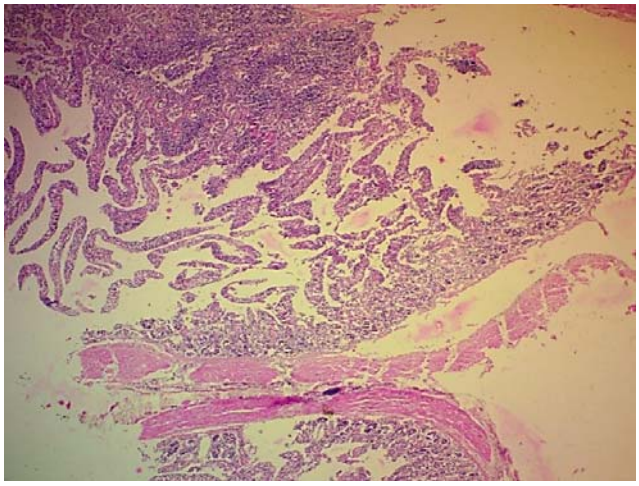

Fig. 6: Intestine- Necrosis and desquamation of the mucosal epithelium, broken villous processes and suppurative exudate in the lumen. H & E (100X)

Klebsiella pneumoniae Infection: Necropsy examination revealed hepatomegaly along with multiple pale hard necrotic areas in all the liver lobes (Fig.7). Necrotic nodules were also present in heart & it was congested (Fig. 8). Spleen revealed enlargement and presence of multiple necrotic foci. Intestine contained tapeworm and roundworm in the lumen. Severe congestion & hemorrhages were noted in large intestine & cloaca in mixed infections**.** The kidney was pale in colour & multiple pale necrotic foci were evident**.** Microscopically, liver revealed multiple abscesses along with hemorrhages, congested sinusoids, and degeneration and necrosis of hepatocytes**.** Intestine revealed congested mucosa, necrosis & desquamation of villi and necrosis of mucosal glands along with infiltration of heterophils. In spleen, there was the presence of necrotic foci and focal depletion of lymphocytes in the white pulp. In the lungs, mild fibrin accumulation surrounding the blood vessels was noticed and alveoli were infiltrated with erythrocytes & leucocytes**.**

Fig. 7: Presence of multiple pale necrotic foci, congested and enlarged liver in *Klebsiella pneumoniae* infection

Fig. 8: Heart- Presence of dense nodules and congestion in myocardium in case of *Klebsiella pneumoniae* infection

This bacterium was also isolated from a bird with tapeworm infection in the intestine. Unhygienic conditions increase the incidence of both *Klebsiella* and parasitic infection in birds. Similar findings have been reported by Hossain *et al*. (2013).

Staphylococcus aureus Infection: Necropsy examination revealed the presence of hemorrhages and congestion throughout the gastrointestinal tract, liver and spleen. Enteritis characterized by necrosis, rupture of villi & heterophilic infiltration was observed in duodenum and caeca**.** In addition, mild fibrin deposition and heterophils infiltration was found on serosa**.** Lungs were showing congestion, mild hemorrhages and presence of mild heterophils around the blood vessel. Lymphocytic depletion with reticular cells hyperplasia was observed at focal areas. Congestion and degeneration of tubules was also noticed**.**

Ascites and hemorrhages: The cases of ascites syndrome revealed excessive accumulation of straw-coloured fluid in the abdominal cavity, mild congestion and enlargement in the liver, duodenum, trachea, and oesophagus. Histologically, mild degenerative and necrotic changes were present in hepatocytes along with infiltration of leukocytes around the portal triad. Intestine and spleen revealed congestion. Hemorrhages were noticed near the junction of proventriculus and gizzard and also diffusely in the whole mucosa of the proventriculus. In few cases large clots of blood were found in the abdominal cavity and around liver surrounding the whole lobes of liver. Rupture of liver was found to be the cause of internal bleeding. Pale and fragile liver and kidneys were noticed. These changes seen were similar to earlier reports (Bhalerao *et al*., 2013; Brar *et al.*, 2017; Sangha, 2017). Cases in which hemorrhages were found in proventriculus may be suspected for NCD (Bhutia and Singh, 2016 and Kumar *et al.,* 2016). Lesions observed in internal hemorrhages found in visceral organs of birds were similar to those seen by Parimala (2003) in cases of fatty liver haemorrhagic syndrome and by Sangha (2017).

Pathological lesi ons associated with parasitic infections

Nematodes and Cestodes were found during postmortem examination of birds showing signs of diarrhoea along with emaciated body condition. Diarrhoea was noticed in most of the cases depicted by soiling of the vent with faeces. *Ascaridia galli* worms were found in proventriculus, gizzard and intestine. Tapeworms (*Raillietina* spp.) were found in the lumen of intestine. Severe congestion and hemorrhagic enteritis was another common feature observed during the postmortem examination. The mucosa of the intestine was thickened**.** Congestion and mild hemorrhages were noticed in proventriculus and gizzard. Intestine revealed severe congestion, hemorrhages and necrosis of villi and mucosal glands along with infiltration of heterophils and macrophages. Similar findings have been reported by others (Alam *et al*., 2014; Dutta *et al*., 2013). The presence of parasitic infections indicates the improper managemental conditions and infrequent cleanouts at poultry farms.

CONCLUSION

It is concluded that colibacillosis and salmonellosis were the most common diseases in poultry in Southern Rajasthan.

Competing Interest

The authors declare no conflicts of interest.

Ethical approval

No live bird was used in the study, only dead birds were used for examination and sampling. So, ethical approval was not required from Institutional Animal Ethics committee for animal experimentation. However, the study was approved by the Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India (Approval No. RAJUVAS/PGS/2018/122 dated 16/10/18).

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