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Immunopathological Studies On Ducks Experimentally Infected With Duck Virus Enteritis And *Salmonella Enteritidis* With Special References to The Effect Of *XPC* Prebiotic

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ABSTRACT

This research was performed to determine the pathogenesis of the Duck Virus Enteritis (DVE) and *Salmonella enteritidis* separately or combined on experimentally infected ducks and evaluating the role of *XPC* prebiotic in reducing these pathological changes observed on the different organs. The experiment was carried out on 80 Muscovy ducks, equally divided into 8 groups. The group (1) was kept as control. Group (2) was administered 1 ml / L from prebiotic *XPC* Diamond V in drinking water from the first day of age till the end of the experiment. Group (3) at 12 days of age infected intramuscularly by (1 ml/duck) Duck Virus Enteritis (DVE) homogenates previously prepared. At the same age (12days) group (4) was infected by *Salmonella enteritides* strain orally. Group (5) was infected by both Duck Virus Enteritis homogenate suspension and *Salmonella enteritides* with the same dose and 12 days age. Groups (6,7and8) were administered 1 ml / L from prebiotic *XPC* in drinking water from the first day of age till the end of the experiment. In the same time groups (6,7and8) infected at 12 day of age by Duck Virus Enteritis organs homogenate suspension, *Salmonella enteritides* and both, respectively with the same dose as groups (3,4 and 5). Tissue samples were collected for viral test and pathological examination when the clinical symptom of the disease appeared. Necropsy was performed and tissue specimen were collected from liver, intestine, esophagus, heart, kidneys, spleen and bursa of Fabricius and fixed in 10% buffered neutral formalin solution for histopathological and immunohistochemistry examination.

The histopathological results revealed congestion in all blood vessels of the most infected groups together with recent thrombus in portal vein of *Salmonella enteritidis* infected group. Degeneration and necrosis with variable degree in addition, inflammatory cells infiltration in different organs of the infected groups were observed. Intranuclear inclusion bodies were seen in the degenerating hepatocytes and in the intestinal epithelium in DVE infected groups. The lesions were alleviated in groups which administered *XPC* prebiotic and infected with Duck Virus Enteritis or *Salmonella enteritides* and both.

It could be concluded that *XPC* prebiotic alleviated the immunological and pathological alteration induced from the experimentally infected ducks by Duck Virus Enteritis or *Salmonella enteritides* infection and both .

INTRODUCTION

Duck virus enteritis (DVE) is an acute, sometimes chronic, contagious viral infection that occurs naturally only in ducks, geese and swans, all members of the family *Anatidae* of the order *Anseriformes. (1).*

Duck virus enteritis or duck plague (DP) is highly lethal in all ages of ducks, which resulted in significant economic losses in commercial duck production *(2)*.

DVE infection of the domestic ducks, swans and geese are characterized by mucosal eruptions of the gastrointestinal tract, internal bleeding (3). Gross lesion is the presumptive diagnosis of the DVE disease in which the histopathologic studies supported the findings. Isolation and identification of the DVE can confirm the diagnosis in the absence of the lesions. DVE virus can be isolated from the liver, cloaca, spleen and bursa *(3,4)*.

Duck plague virus attacks the vascular system, which result in hemorrhage and free blood throughout the gastrointestinal tract. The most prominent lesions were hemorrhagic or necrotic bands circumscribed in the intestine or disk- shaped ulcers. Sometimes there were cheesy raised plaques along the longitudinal folds of the esophagus and proventriculus and on the mucosal surface of the lower intestine. Areas of tissue dead (spots) were also evident in the liver, and hemorrhage on the heart surface of some birds. It is important to recognize that the appearance of lesions may differ somewhat from species to species and not all lesions are present in all birds at all times *(5)*.

Salmonella enteritidis (*S.enteritidis*) is an enteric pathogen that colonizes the intestinal tract in a variety of animals, especially humans and poultry. Each year accounts of millions of gastroenteritis and food borne cases have become a significant public health problem *(6 , 7)*.

China is the biggest country in the raising and consumption of duck in the world, *S. enteritidis* bacillus infection is a severely important infectious disease in duck industry *(8,9)*.

S.enteritidis outbreaks resulted from the consumption of contaminated and undercooked poultry products as eggs and egg- containing products with serious economic and public health problem *(6,9)*.

S.enteritidis infections in poultry are characterized by vascular damage, eruptions as specific locations on the mucosal surface of the gastrointestinal tract, lesions in the lymphoid organs and degenerative sequelae involving the parenchymatous organs *(10-13)*. In susceptible host *S.enteritidis* replicates primarily in the mucosa of the digestive after oral challenge and then spreads to the spleen, liver and various other organs and tissues *(13 ,14)*.

A prebiotic was defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and activity of intestinal microflora *(15,16)*. However, use of this type of additives has a beneficial effect on; the production animals equalization, reduce mortality and morbidity and lower treatment costs *(17-20)*.

Prebiotics affects on bifidobacteria proliferation and reduce harmful microorganism proliferation, increase animal performance, remove harmful enzymes and toxic metabolites, lower blood cholesterol level, lower blood pressure, prevent the processes of carcinogenesis and affect on synthesis of vitamins B_1, B_2, B_6, B_{12}, folic acid and nicotinic *(20 - 22)*.

The present work was carried out to study the pathological and immunological alteration of the DVE and *S.Eneritides* separately or combined on the different organs of ducks and evaluate the role of prebiotic *XPC* in reducing the pathological changes induced by the infection.

MATERIAL AND METHODS

Drug: (Prebiotic)

XPC Tm Liquid: Manufactured by Diamond V Mills. Inc. Cedar. Rapids. Lowa. U.S.A.52407. Net volume: 1L. Concentrate Product For Manufacturers For Livestock & Poultry Food.

Preparation of virus for experimental infection

Liver, spleen and kidney tissue have been collected from infected ducks showing clinical sings of Duck virus enteritis (confirmed with PCR performed in Central Laboratory for Evaluation of Veterinary Biologics) homogenized in saline containing 2000 iu/ml Penicillin and 200mcg/ml Streptomycin. These organs were pooled then ground in a Tenbroeck tissues grinder (20% W/V), then centrifuged at 3000 rpm for 15 minutes. After centrifugation the clear supernatant fluid from sample was extracted and preserved at -20 C until used *(23)*.

Production of duck Plague antiserum

Anti- Duck Virus Enteritis antiserum was prepared in rabbits. The viccinal strain was used to inoculate rabbit. There were 2 inoculations using adjuvant (Montnide ASA50 1:1) with virus and third with virus alone without adjuvant. The animals were monitored for Duck Virus Enteritis antibody using serum neutralization test (SNT). Serum was harvested when titer were high. This hyper immune serum was used in immunohistochemistrey *(24)*.

Bacterial Strain

Salmonella enteritidis (*S.enteritidis*) strain obtained from Avian Disease Department-Animal Health Research Institute- Dokky-Egypt.

Preparation of *S. enteritidis* anti-serum

The primary antiserum was prepared according to the method described by *(25)*. Adult New Zealand rabbits were inoculated 3 times with 0.25, 0.5 and 1ml 0f *S.entritidis* strain (approximately 7×10^9 cfu/ml) via ear vein at 5- day intervals. Blood samples were collected from the rabbits on day 15, after the last injection, and sera were separated and stored at -20°c. Serial dilutions (log) of the primary antiserum- from 1/2 to 1/256 were made to obtain optimal primary antibody titers. This primary antiserum was used in immunohistochemistry.

Experimental Design

A total number of eighty Muscovy ducks one-day old of both sex were obtained from Commercial hatchery weighting from (35-50gm). Ducks were maintained in isolation units in a biosecure animal building and fed a commercial duck diet adlibitum, cloacal and tracheal swaps were collected from ducks for bacteriological examination to sure that all ducks found to be negative from bacterial infection. Ducks were randomly divided into 8 equal groups. Group (1) was kept as control (negative control); group (2) was administered 1 ml /L (recommended dose of the product company) from prebiotic *XPC* Diamond V in drinking water from the first day of age till the end of the experiment. At 12 days of age group (3) was infected by 1 ml/duck intramuscularly from organs homogenate suspension previously prepared containing (100 ID_{50}) from Duck Virus Enteritis (DVE). At 12 days of age group (4) was infected by *S. enteritidis* strain at a dose 4×10^5 CFU/bird orally according to *(13)*. Group (5) was infected with both DVE suspension and *S. enteritidis* strain at the same age and dose as groups (3, 4). Groups (6,7and 8) were administered 1 ml/L from *XPC* prebiotic in drinking water from the first day of age till the end of the experiment and infected by DVE suspension intramuscular at the same age and dose as group(3). Group (7) was infected by *S.enteritidis* strain orally at the same age and dose as group (4). Group (8) was infected intramuscular and orally by both DVE suspension and *S. enteritidis* strain at the same age and dose as group (5).

Preparation of tissues from infected groups with DVE for Passive Heamagglutination Assay

From all infected groups with DVE (3,5,6 and 8) liver, spleen and kidney tissue have been collected and prepared to inoculate into 10-12 days old embryonated ducks eggs and chicken eggs by chorioallantoic membrane (CAM) route 0.2 ml/embryo using standard techniques of embryo inoculation. Each of the inoculated embryos was monitored for embryopathy daily for six days. The allantoic fluid and CAM were harvested separately from embryos that died during the period of observation. The harvested

CAM, livers of duck and chicken embryos were made 20% suspension *(26)*.

Passive heamagglutination Assay (PHA)test

This test was carried out with the micro titer technique according to the method described by *(27,28)*. Preparation of duck plague virus antigen according to *(26)*. 20% suspension of the CAM and liver tissues of the duck eggs infected with vaccinal strain used as positive control for test. Virus sensitization of tanned sheep erythrocytes 2.5 % was used for coating the antigen. Anti-sera prepared in rabbits against the vaccinal strain were used as the positive serum. Serial two fold dilution of the 20% CAM and liver tissues of the duck and chicken embryos eggs previously infected with mixture of homogenized liver, spleen and kidney tissues which were collected through experiment (dead or slaughtered). The end point was determined by observing the highest dilution at which cell agglutinated the sensitized sheep RBCs.

Immunohistochemical localization of DVE virus and *S. enteritidis* antigen

Small pieces of tissues were collected and fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned at a thickness of 5µm. The sections were deparafinization then stained for DVE and *S. enteritidis* antigen localization within different samples by using the avidin-biotin –peroxidse complex *(29)*.

Pathological examination

After the appearing of the clinical symptoms of DVE or *S. enteritidis* necropsy was performed and all macroscopic abnormalities were recorded. Specimens were collected from liver, intestine, esophagus, heart, kidneys, spleen and bursa of Fabricius and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100), cleared in xylene, and embedded in paraffin. Five micron thick paraffin section were prepared and routinely stained with hematoxylin and eosin (H&E) and Phloxine Tartrazine dyes according to(*30)* and then examined microscopically.

RESULTS

Clinical Signs

The clinical signs of DVE were appeared in group (3) after 3 days post infection. Ducks in groups (3,4 and5) showed dullness, severe depression, loss of appetite, watery yellowish to greenish diarrhea and ruffling feathers. The clinical signs were very mild in groups (6, 7 and 8).

Inoculated eggs with organs collected from infected groups with DVE

All embryonated ducks eggs and chicken eggs inoculated with collected tissues from infected groups dead within 4-6 days of inoculation exhibiting characteristic pathological lesions of duck plague in embryonic tissue. Irregular patches of congested and petechial haemorrhage throughout the body particularly in the head, neck, legs, abdomen and beak region of the embryo inoculated. The lesions of the chorioallantoic memberane included irregular patches of congestion.

Passive Heamagglutination Assay (PHA) test to infected groups with DVE

This test was adapted to evaluate viral titer. The end point was determined by observing the highest dilution at which cells agglutinated. Agglutination was indicated by a flat or deposition of a diffuse thin layer of clumping RBC at the bottom of well. A compact mass of cells forming a smooth edge button with clear zone was considered as evidence of a negative. The results of the test that calculated on the basis of the highest dilution of infected groups with DVE causing agglutination of sensitized sheep RBC, was considered as titer of infected groups. We found that the high titer was in group4 which infected with filed isolate (6 Log_2 and 100LD50) and *S. enteritidis* (4 $\times 10^5$ CFU/bird orally). The groups 6and 8 gave low titer 4&5 Log_2 in respectively. The group 3 which infected with DEV alone gave 6 Log_2.

Table 1. Results of geometric mean titers (Log_2) of Passive Haemagglutination Assay to infected group by DVE

Group	DVE (GP3)	DVE+*S.enteritidis* (GP5)	DVE+prebiotic (GP6)	DVE+*S. enteritidis*+prebiotic (GP8)
GM	6	8	4	5

GM= geometric mean DVE= Duck Virus Enteritis
S. enteritidis= Salmonella enteritidis

Pathological Findings

Immunohistochemical localization of immunoperoxidase , revealed high levels of expression of the DVE antigen and *S.enteritidis* antigen(high positive signal) in the liver cells and in the epithelial lining of the intestinal villi, bursa of Fabricius and the renal tubules (Fig.A:1-6). These signals were very low or negative in the prebiotic received groups (6, 7 and 8). (Fig. A: 7-12).

Pathological examination of Groups (1 and 2): control and received the prebiotic, respectively. The examined organs (Liver, intestine, heart, esophagus, kidneys and lymphoid organs) were normal and without any gross or microscopic abnormalities.

Group (3): (infected with DVE). Macroscopically, petechial or ecchymotic hemorrhages on the heart (paint-brush like), endocardium and free blood in the body cavity were seen in the experimental ducks. Hemorrhagic bands in the intestinal tract with bloody content, necrosis and hemorrhage on the cloacal surface, grayish-white necrotic foci in the liver (1-2 mm in diameter), and hemorrhages or raised plaque-like areas in the esophagus and rarely on the cloaca were visualized. Many of these esophageal lesions were coalesced, giving the appearance of a diphtheritic membrane. The spleen and bursa were mottled and congested. The kidneys were apparently normal. Microscopically, the liver revealed focal areas of coagulative necrosis (Fig. B: 1). Eosinophilic and basophilic intranuclear inclusion bodies were seen in some degenerating hepatocytes (Fig 2), these inclusions takes bright red color with Phloxine Tartrazine stain (Fig 3). The portal areas showed congested blood vessels, edema and round cells infiltrations (Fig 4). Severe vacuolation and hydropic degeneration were observed in the periportal hepatocytes. Focal interstitial aggregations of lymphocytes and hemorrhage were detected. The intestine revealed variable degrees of catarrhal or necrotic enteritis with intense aggregations of round cells in the submucosa (Fig 5). Basophilic intranuclear inclusions were detected in the intestinal epithelium and stained red with Phloxine Tartrazine stain (Fig 6) . The mucosa of the esophagus was necrotic and focally replaced with caseated material (pseudomembrane) and aggregation of round cells in the mucosa, submucosa and periglandular. Hydropic and ballooning degenerations besides eosinophilic or basophilic intranuclear inclusions were noticed. The spleen and bursa of Fabricius revealed depletion and necrosis in the lymphocytes of white pulp and the follicles. The red pulp was congested and hemorrhagic. The heart showed hemorrhages on the pericardium, among the cardiac muscles and endocardium. Perivascular and interstitial edema and hemorrhage were seen and widely separated the muscle fibers. Zenker's degeneration and necrosis were focally seen. Few round cells aggregations were also noticed. Focal vacuolation and cardiac myolysis were observed. The kidneys showed subcapsular and interstitial hemorrhages besides cloudy swelling in the convoluted tubular epithelia.

Group (4): (infected with *S. entiritidis*): Macroscopically, the liver was slightly enlarged and showed hemorrhagic streaks on its surface. The gallbladder was over distended with viscid greenish bile. The intestine showed focal mucosal congestion and mucoid content. The circumference mesentery was congested. The

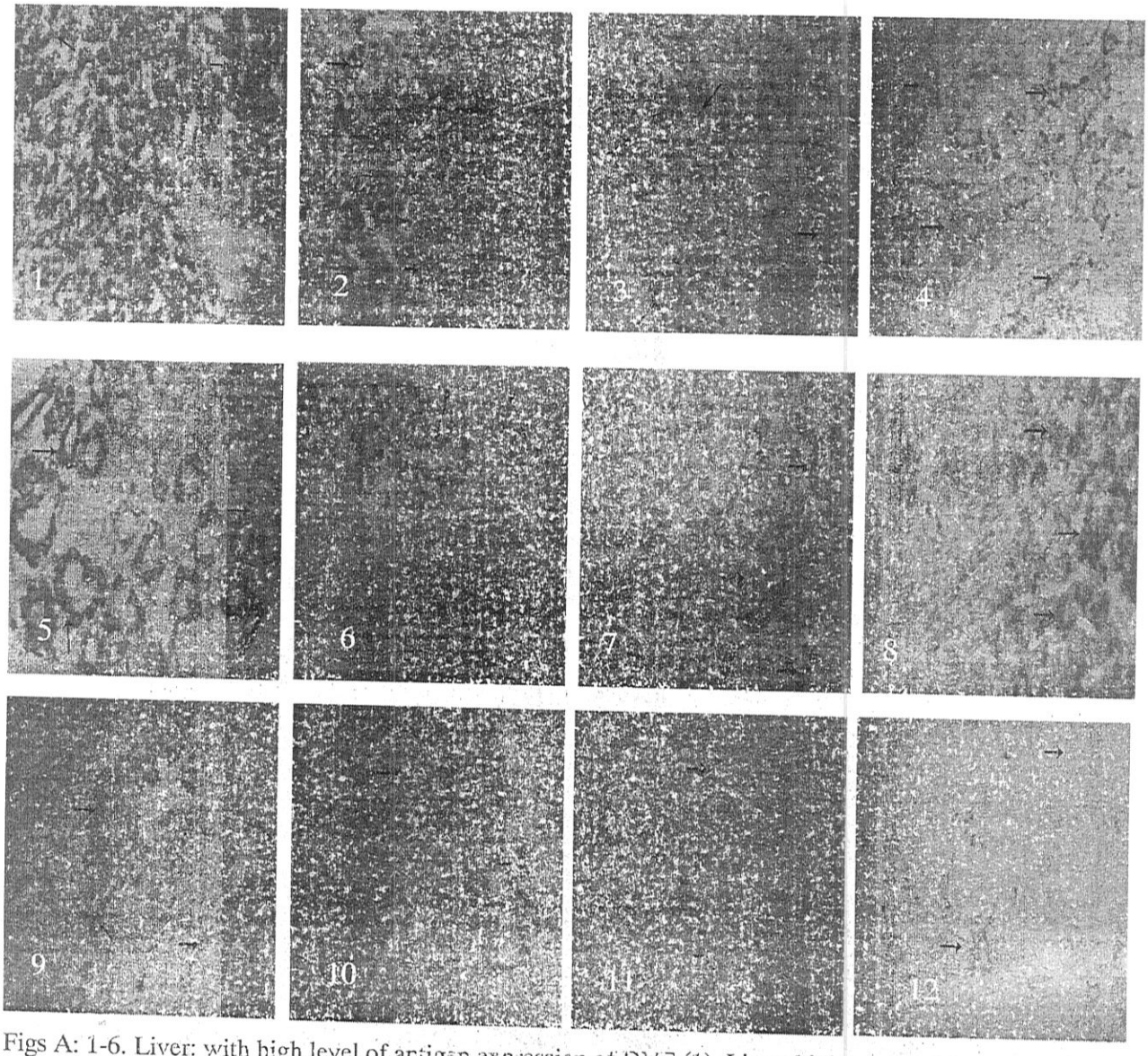
kidneys were swollen and pale in color. The spleen was congested. The heart showed grayish white foci on myocardium. The other organs were apparently normal. Microscopically, the liver showed congested portal blood vessels, fatty change and hydropic degeneration in the hepatocytes (Fig 7). The portal areas were heavily infiltrated with heterophils (Fig 8) and the others showed recently thrombosed portal veins besides few interstitial aggregations of macrophages, lymphocytes, fibroblasts and few heterophils (Fig 9). The gallbladder was chronically inflamed with hyperplasia in the lining epithelium and fibrous connective tissue proliferation. The hepatic artery was thickened and the adjacent hepatocytes were necrotic (Fig 10). The intestine revealed catarrhal enteritis with hyperplasia, desquamation and mucinous degeneration in the lining epithelium besides intense aggregation of macrophages, fibroblasts and few heterophils in the submucosa and lamina propria (Figs 11 and 12). Congested blood vessels and few extravasated erythrocytes were also noticed in the submucosa. The spleen showed mild depletion and necrosis in the lymphocytes of white pulp besides numerous heterophils infiltrations in the red pulp. The heart revealed congestion of the cardiac blood vessels and Zenker's degeneration in the cardiac muscles (Fig 13). The pericardium and adjacent myocardium were infiltrated with macrophages, lymphocytes and few heterophils (Fig 14). The kidneys showed cloudy swelling and hydropic degeneration in the convoluted tubular epithelium. Few round cells and extravasated erythrocytes were visualized among the renal tubules.

Group (5): (infected with both DVE and *S. entiritidis*): The lesions of such group were more severe than those described in groups (3 and 4). Macroscopically, the liver was pale or yellow in color and showed irregular hemorrhagic spots throughout the hepatic surface. The gallbladder was distended with bile. The intestine was severely congested with bloody or watery content. The esophagus and cloaca showed petechations on the mucosal surface. The heart showed petechial hemorrhage on the coronary fat and grayish white nodules on the myocardium. The spleen and bursa were slightly enlarged and congested. Microscopically, the liver revealed micro vesicular and macro vesicular steatosis and vacuolation of the hepatic cells besides hypertrophied kupffer cells (Fig 15). Multifocal areas of coagulative necroses were visualized besides several eosinophilic intranuclear inclusions (Fig 16). The portal areas revealed mononuclear cells infiltration of mostly macrophages and hyperplasia in the biliary epithelium. Focal interstitial aggregations of round cells were also seen. The intestine showed extensive necrosis in the mucosa and intense aggregations of macrophages, lymphocytes and fibroblasts in the submucosa (Figs 17 and 18). In some cases, the submucosa and lamina propria were obliterated with macrophages of abundant eosinophilic cytoplasm (Fig 19). Catarrhal and hemorrhagic enteritis was also observed as in DVE infected group. The heart revealed hemorrhage on the pericardium and Zenker's necrosis in the cardiac muscle fibers. The myocardium was heavly infiltrated with macrophages and lymphocytes particularly subendocardium. The lesions in other organs were similar to those described in groups (3 and 4).

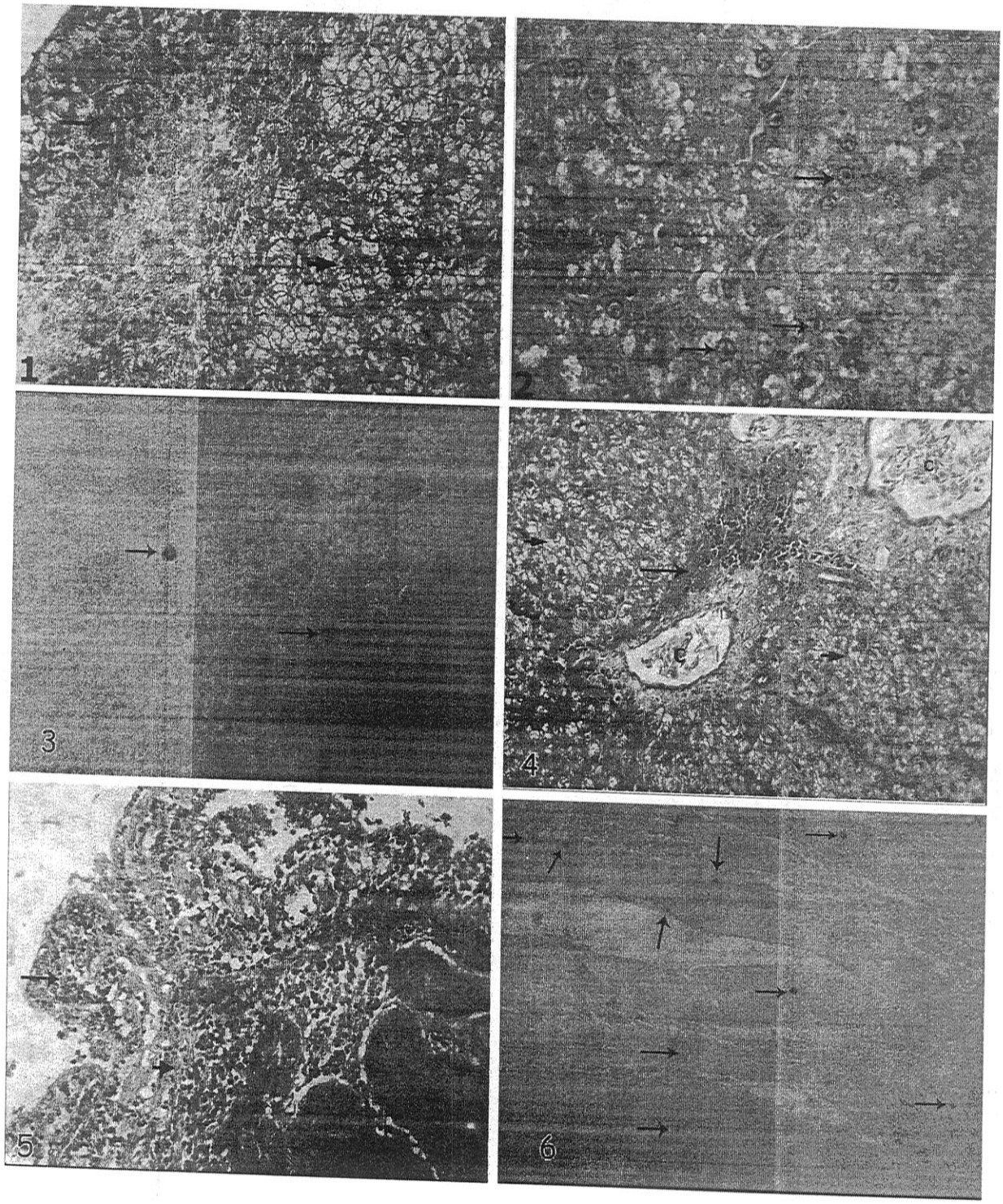
Group (6): (received prebiotic and infected with DVE). The lesions of this group were alleviated than those described in the group (3), where the lesions of digestive tract were absent from esophagus and cloaca and only mild catarrhal enteritis was focally reported. The hemorrhage and edema in almost all examined organs were absent except on the coronary fat and pericardium (Fig 20). The liver showed moderate degenerative changes of hydropic type with no evidence of intranuclear inclusions (Fig 21) and few portal and interstitial aggregations of lymphocytes. The hyperplasia in the lining epithelium of bile ducts were mild or individually absent. The intestine showed mild aggregation of round cells. The kidneys showed mild cloudy swelling and regenerative attempts in the renal tubules (Fig 22). The spleen and bursa were normal or with slightly activation of lymphocytes in the white pulp.

Group (7): (received prebiotic and infected with *S. Entiritidis*): Catarrhal enteritis and aggregation of round cell in the intestine and among the degenerated muscle fibers of the myocardium (Fig 23) were recorded in all experimental ducks. Individually, the liver showed slight congestion in the portal blood vessels and hydropic degeneration in the hepatocytes (Fig 24). The other organs were normal.

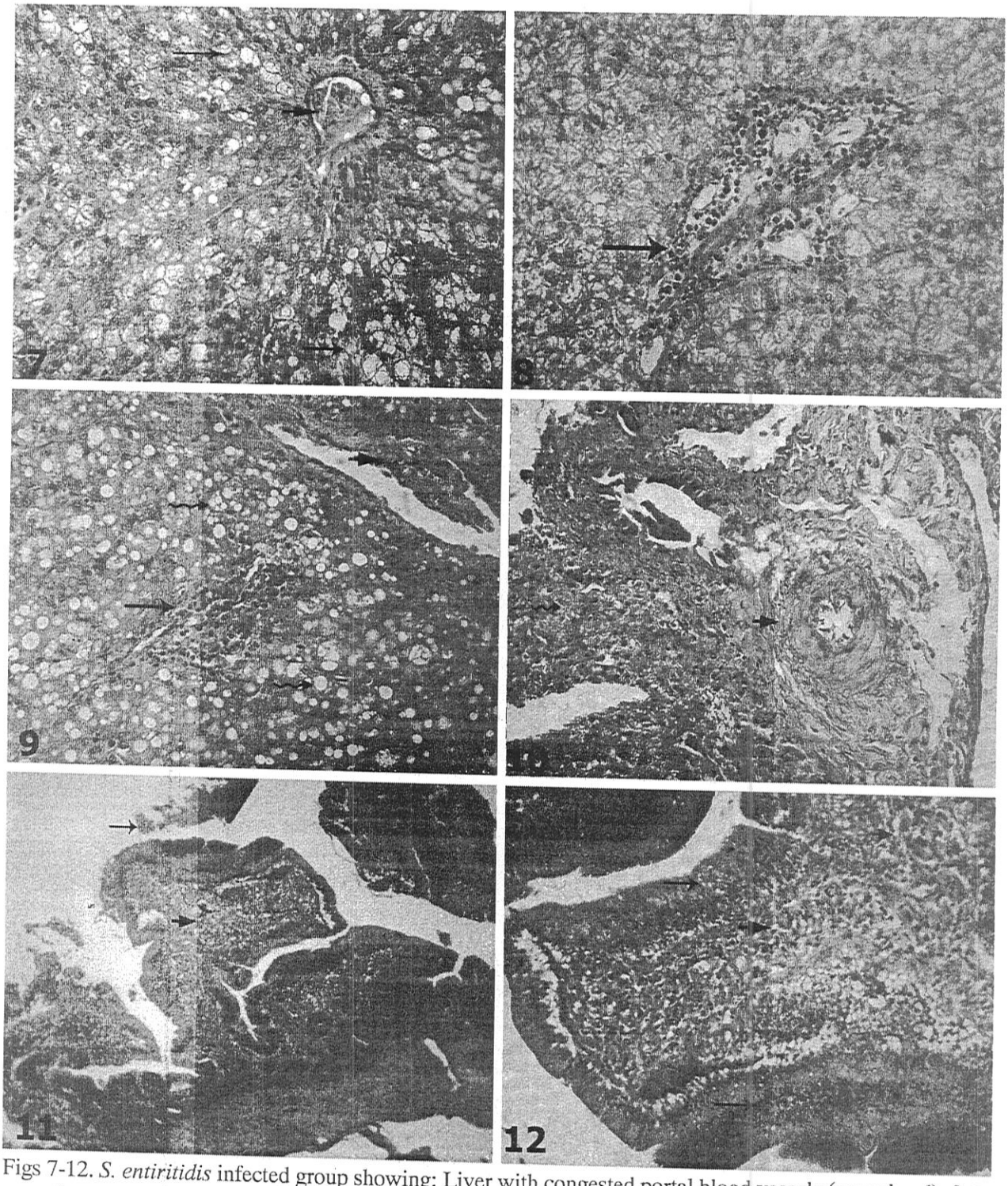
Group (8):(received prebiotic, and infected with both DVE and *S. entiritidis*): The lesions of this group (8) were lowered than those described in group (5) with persistence of some hepatic and cardiac lesions. The portal areas were edematous and showed congested blood vessels, mild hyperplasia in the biliary epithelium and hyalinization in the wall of hepatic arterioles (Fig 25). The heart showed edema, extravasated erythrocytes and lymphocytes among the cardiac muscle fibers. The latter were focally degenerated or necrotic. The kidneys of some cases revealed vacuolation in the renal tubular epithelia and aggregation of macrophages and few heterophils among the renal tubules (Fig 26). Congestion of some renal blood vessels and capillaries was noticed.



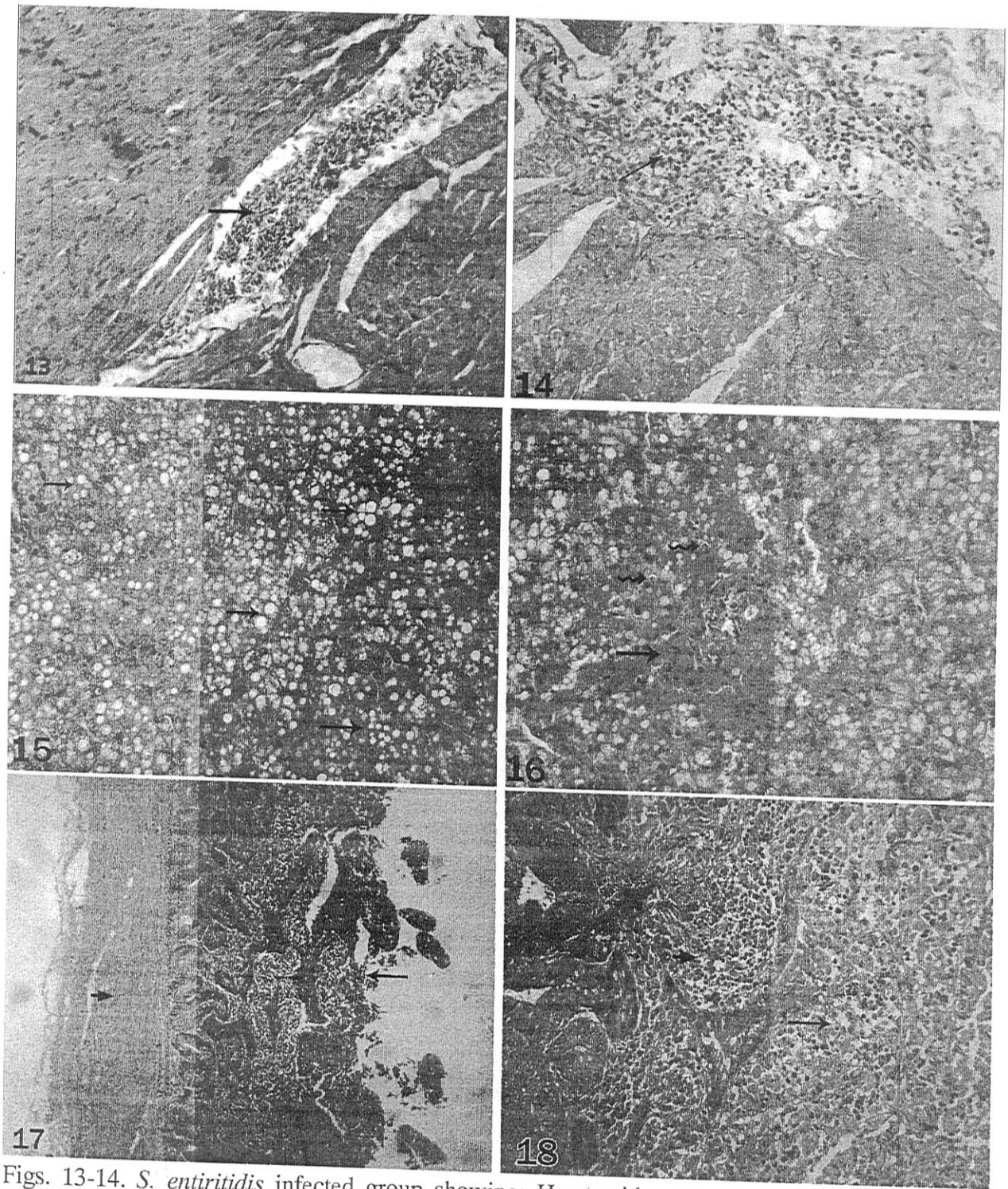
Figs A: 1-6. Liver: with high level of antigen expression of DVE (1), Liver: high level antigen expression of *S.enteritidis* (2), Intestine: high level of DVE antigen expression(3), Intestine: high level antigen expression of *S.enteritidis*(4), Kidneys: with high level of antigen expression of DVE(5), Kidneys: positive *S.enteritidis* antigen at the periphery and negative expression in the central(6), Groups treated by prebiotic and then infected(7-12):Liver: nearly low level of antigen expression of DVE (7), Liver: low level of *S.enteritidis* antigen(8), Intestine: moderate level of antigen expression of DVE(9), Intestine: very low level of *S.enteritidis* antigen expression (10), Kidneys: very low level of antigen expression of DVE(11), Kidneys: very low or nearly absent of *S.enteritidis* antigen expression(12).X.400,Peroxidse stain.



Figs B: 1-6. DVE infected group showing: Liver with coagulative necrosis and hydropic degeneration (1), intranuclear inclusions (2) these inclusions stained reddish brown by (Phloxine Tartrazine stain x1000) (3) and portal area with congested blood vessels © and round cells infiltration (4). Intestine with necrotic mucosa and round cells infiltrations in the submucosa (5).Reddish brown intranuclear inclusions in the intestinal villi (Phloxine Tartrazine stain (x400) (6). H&E x 400.



Figs 7-12. *S. entiritidis* infected group showing: Liver with congested portal blood vessels (arrowhead), fatty change and hydropic degeneration in the hepatocytes "arrow" (7), portal aggregation of heterophils "arrow" (8), recently thrombosed portal vein (arrowhead), interstitial aggregation of round cells (arrow) and fatty change "irregular arrows" (9), and the hepatic artery was thickened and the adjacent hepatocytes were necrotic "irregular arrow" (10). Intestine with catarrhal enteritis with hyperplasia, desquamation and mucinous degeneration in the lining epithelium (arrows) (11x 100), besides intense aggregation of macrophages, fibroblasts and few heterophils in the submucosa and lamina propria "arrow heads" (12). HE x 400.



Figs. 13-14. *S. entiritidis* infected group showing: Heart with congestion of the cardiac blood vessel "arrow" (13) and aggregation of round cells in the myocardium adjacent the pericardial sac "arrow" (14). Figs (15-18): DVE and *S. enteritidis* infected group showing: Liver with macrovesicular and microvesicular steatosis "arrows" (15) and coagulative necrosis (arrow) and intranuclear inclusions "irregular arrows" (16). Intestine with extensive necrosis in the mucosa (arrows) (17 x 100), and intense aggregations of macrophages, lymphocytes and fibroblasts in the submucosa "arrowheads" (18) HE x 400.

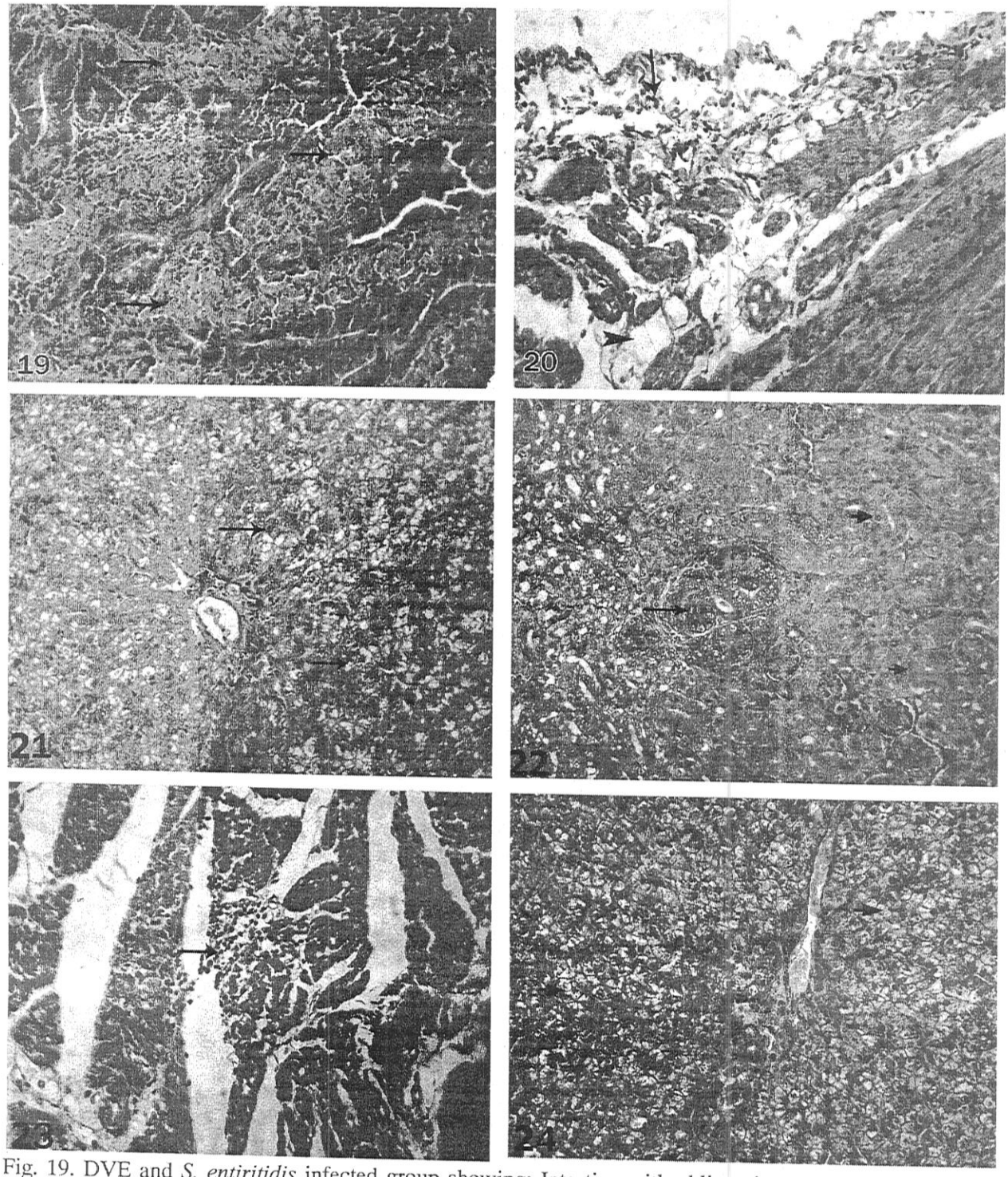
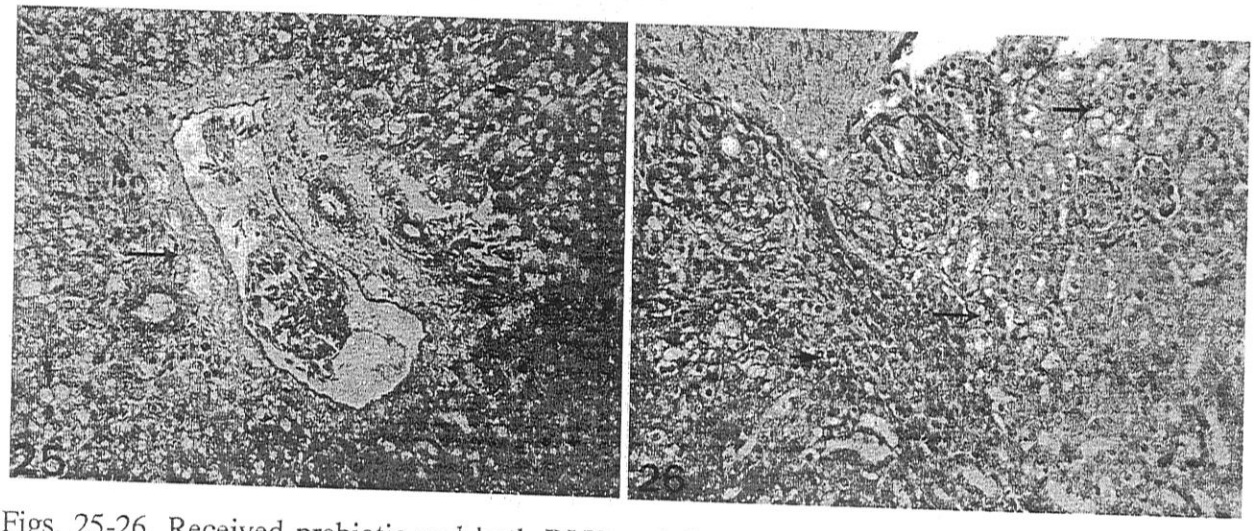


Fig. 19. DVE and *S. entiritidis* infected group showing: Intestine with obliteration of the submucosa and lamina propria with macrophages of abundant eosinophilic cytoplasm "arrows" (19). Figs (20-22): received prebiotic and DVE infected group showing: Heart with edema (arrowhead) and hemorrhage "arrow" on the pericardium (20) and liver with mild hydropic degeneration "arrows" (21).Kidney with cloudy swelling (arrowheads) and regenerated renal tubules "arrows" (22).Figs (23-24): received prebiotic and *S. entiritidis* infected group showing: Heart with few round cells aggregation among the cardiac muscle fibers "arrow" (23) and liver mild hydropic degeneration (arrowheads) (24). HE x 400.



Figs. 25-26. Received prebiotic and both DVE and *S. entiritidis* infected group showing: Liver with edematous portal area and congested blood vessels (arrow) besides hydropic degeneration in the hepatocytes "arrowheads" (25), and kidney with vacuolations of renal tubular epithelia (arrows) and heterophils and macrophages infiltration "arrowhead" (26). HE x 400.

DISCUSSION

Due to the increased prevalence of *S.enteritidis* and its complex pathogenesis, it is important to understand the correlation between the levels of this bacterium in internal organs and the progression of the infection. Generally, little is known about the pathogenesis of *S.enteritidis* infection. Up to day, the mechanisms by which *S.enteritidis* and other serotypes persist within the host and the reasons for absence of immune clearance are not known. Understanding this correlation will help gain further, insight into the pathogenesis of *S.enteritidis* infections *(7, 13,31)*.

The clinical signs in the present study in GP.(3) which infected by DVE showed the typical symptoms of the disease, loss of appetite, retarded neck and watery diarrhea with no mortality. Our findings are reported by *(1,32)*. Our results are accepted with, *(32)*, he explained that, outbreaks on duck farms in DVE infection are usually caused through transmission of the causal agent by wild-fowl flying over the farms, the outbreak may have been caused by the combination of two factors: first, the presence of anon-identified carrier, and, second, the stress suffered by the birds as the result of vaccination and the reproductive season.

The clinical symptoms in Gp.(4) which infected by *S.enteritidis* were loss of appetite, depression, ruffled feather and diarrhea without outbreaks. These results are in accordance with the results described by *(33- 35)*. No deaths occur in this group. This result is explained by *(34)* in turkey poults infected by *S.Gallinarum*, he reported that no mortality in any of the treatment groups, this may be due to the resistance of white turkeys to *S. Gallinarum* infection as well as to low virulence of the agent. The Gp.(5) which infected by both DVE and *S.enteritidis* in the present work showed more severe clinical symptoms of the two disease. Our results are explained by *(32)*, he decided that, the viral disease when complicated by secondary bacterial invaders such as *salmonella* spp. could induce a diphteroid form of enteritis. Gps. (6,7 and 8), showed mild clinical symptoms of the DVE and *S.enteritidis*. This may be due to action of the prebiotic which has a positive influence on

defense mechanisms of the digestive system and neutralization of pathogens; they also activate digestive enzymes and improve nutrients absorption from the diet *(20)*.

In our study we used Passive Haemagglutination Assay to measure DVE titer in infected groups (3, 5, 6 and 8), these are according to *(36)* who described a Reverse Passive Haemagglutination (RPHA) test for detection of DVE, another virus that lacks the Haemagglutination antigen. *(37)* Said that, the modified PHA for viral antigens, unviable viruses and subunit viruses will not able to infect the RBCs. So, PHA for viral antigens may be more specific and yet as sensitive as the reverse PHA test. That test was adapted to evaluated viral titer. We found that the high titer (8 log_2) was in group 5 which had mixed infection with filed isolate of DVE (6 Log_2 and 100LD50) and *S. enteritidis* (4 $\times 10^5$ CFU/bird orally). The groups 6and 8 gave low titer 4 & 5 Log_2 in respectively and this is due to this 2groups which administrated prebiotic in water in recommended dose from one day-old to the end of expirament. The group 3 which infected with DEV alone gave 6 Log_2 these results were agreement with *(26)*.

Our findings of the immunoperoxidase localization showed high levels on DVE antigen expression in parenchymatous organs(liver cells and the epithelial lining of the renal tubules), also epithelial lining the intestine and the bursa. These results are confirmed with *(24)*, he concluded that staining was strong without significant background staining particularly in epithelial tissues and lymphoid tissue. Organs were most frequently and strongly affected were the. liver, spleen and bursa. Tissues that also stained to a lesser extent, included kidneys and intestine. Immunoperoxidase on *S.enteritidis* antigen in the present study are agreement with *(7)*, he showed that high level of expression in the epithelial cells, lymphocytes of jejunum and ileum. Also in the parenchymatous organs as liver tissue more than lungs and kidneys.

In the present study, the gross lesions in Gp. (3), were petechial or ecchymotic hemorrhage on the heart and free blood in the body cavities. Hemorrhagic bands in the intestinal tract with bloody content, necrosis and hemorrhage on the cloacal surface and grayish white necrotic foci on the liver. Similar results are obtained by *(1, 32 , 38)*.

The microscopic picture of the liver in Gp. (3) in this work showed focal areas of coagulative necrosis, intranuclear inclusion bodies in some degenerating hepatocytes together with congested blood vessels and interstitial aggregations of lymphocytes. These results are in accordance with that reported by *(1,32 , 38)*. The microscopic finding in the intestine in the present study revealed necrotic enteritis in addition to intranuclear inclusion bodies in the intestinal epithelium with intense aggregation of round cells. The mucosa of the esophagus was necrotic and focally replaced by caseated material (pseudomembrane), in addition to the microscopic finding on other organs (spleen, bursa of Fabricius and heart). Our obtained results are similar to those reported in previous studies *(1, 32, 38, 39 , 40)*; they elucidated that, DVE replicates in the epithelial cells and given its alpha herpes virus nature, produces inclusion bodies composed of large clusters of virions. These features have been observed in our study in the epithelial cell of intestine, kidneys and bile ducts.

The gross picture of organs in Gp.(4) in the present work, were slightly enlarged liver with hemorrhagic streaks on its surface together with over distended gall bladder. The over distention of the gall bladder are discussed by

(35 , 41) on *S. Gallinarium* infection. They reported that, this is due to the fact that *S.Gallinarium* organisms have a predilection for bile canaliculi which causes the stasis of bile in the liver. The intestine and spleen showed congestion. The kidneys were swollen and pale in color. These results are confirmed with *(13 ,42)* in *S.enteritidis* infected ducks. On the same context our results are in accordance with *(34)* on *S.Gallinarium* infection in turkey poults and with *(7)* on pigeon. The microscopic findings in this group were vascular damage, severe congestion or hemorrhage in all examined organs. Fatty and hydropic degeneration of the hepatocytes together with

infiltration of heterophils in addition thrombosed portal veins beside interstitial aggregation of macrophages and lymphocytes. Similar results previously reported by *(7,13,42)* in *S.enteritidis* on ducks. They decided that necrosis and varying degrees of hepatocytes fat degeneration together with severe hyperemia, hemorrhages and heterophils infiltration. On the same line our results are confirmed by *(35,43,44)* in *S.Gallinarium* infection on chicken. The intestine showed catarrhal enteritis with desquamation in the epithelial lining beside aggregation of macrophages, fibroblasts and heterophils in the submucosa and lamina propria. Depletion and necrosis of the lymphocytes in spleen and degeneration in the cardiac muscle were observed. The kidneys showed cloudy swelling and hydropic degeneration in the tubular epithelial. Similar to our findings observed by *(13,42)* on *S.enteritidis* infection in ducks , *(34)* in *S.Gallinarium* infection in turkey and with *(7)* in pigeon, he suggested that, the lymphoid and intestinal organs are the major target organs of *S.enteritidis* replication.

The gross picture and microscopic examination of Gp.(5) which infected with both, DVE and *S.enteritidis* were more severe than those described in Gps.(3,4). Typical lesions of DVE obtained previously by *(1, 3, 32, 38)* were observed in this group and typical lesions of *S.enteritidis* were also observed which confirmed by *(34, 35 ,42)*. Our obtained results on *S.enteritidis* infection were discussed by *(42)*, he reported that the mechanism of colonization by *S.enteritidis* in the gut is not clear and require further studies. *Salmonella* can induce the suppression of cellular responses *(45)*, further, it has been reported that *S.enteritidis* infection induces low-grade inflammation, which favors the colonization of the bacteria in the host. On the other hand *S.typhimurium* infection activates inflammatory molecules and is cleared more rapidly *(31)*. *S.enteritidis* infection leads to an increase in the number of spleenic CD_3, T cells and decrease in the number of B cells: however, it was difficult to associate this increase with *S.enteritidis* clearance due to lack of significant changes in the number of CD_4^+ or CD_8^+ T cell *(46 , 47)*. Therefore, these changes may be the reason for why a significant number of *S.enteritidis* cells can persist for a long time in the spleen, ileum, jejunum and ceccum without causing any apparent symptoms.

In the present study the microscopically picture of Gps.(6,7 and 8) which received prebiotic and infected with DVE, *S.enteritidis* and both respectively, were alleviated than those described in the Gps.(3,4 and 5), the liver showed moderate hydropic degeneration in addition few interstitial aggregations of lymphocytes. The intestine showed mild aggregation of round cells and the kidneys showed mild cloudy swelling and regenerative changes in the renal tubules. The spleen and bursa were normal with slight activation of lymphocytes. The Gp. (7) showed catarrhal enteritis together with aggregation of round cells. Degeneration in the muscle fiber of the myocardium, slight congestion of the portal blood vessels together with hydropic degeneration of the hepatocytes was observed. The Gp.(8) showed edema and congested blood vessels together with mild hyperplasia in the bilary epitheliam, the heart showed edema, extravasated erythrocytes and lymphocytic aggregation among the muscle fiber. The kidneys showed vacuolation of the renal tubular epithelium.

Prebiotic compounds offer on attractive alternative to the use of Antibiotic Growth Promotors (AGP). Growth promotion associated with prebiotics is believed to result from enhanced energy gained by the fermentation of these compounds within the lower gastrointestinal tract (GIT) allowing the host animal to generate muscle mass and effectively producing a desirable marketing weight *(48 ,49)*. Other health benefits such as stimulation of intestinal motility, mineral absorption, elimination of ammonium, direct stimulation of the immune system and the inhibition of toxin binding, are associated with host, prebiotic synergy *(49 ,50)*. However the greatest protection against pathogenic bacterial infections are achieved by stimulation GIT bacteria to produce short chain fatty acids that are inhibitory to some pathogens and increase

in quantity, therefore reducing attachment sites for pathogens on the GIT mucosa *(49 ,51)*. Prebiotic compounds such as galacto-oligosaccharides have been previously shown to increase the composition of beneficial bacteria, such as Bifidobacterium and Lactobacillus, in the colon of humans and mice *(52)*. ***Price et al., (49)***, showed that the inclusion of a commercial dietary supplement, *XPC*, containing nutritional metabolites, mannanoligo-saccharide(MOS)and β-glucans produced during the anaerobic fermentation of *Saccharomyces cerevisiae* when administrated to weaned pigs during salmonella infection results an increased in the number of copies for Bacteriods and Lactobacillus in the faces of pigs compared with control. Increased amounts of β-glucans have been shown to increase digesta retention time in the small intestine, affecting the digestibility of other nutrients, particularly protein and starch *(53)*. Inclusion of mixed- linked β-glucans in the diet of rats *(54)* and pigs *(49)* corresponded with increased populations of *Lactobacillus (55)*. On the same line weanling pigs supplemented with a mixture of prebiotic bacteria and subsequently challenged with salmonella showed reduced incidence and duration of diarrhea and shedding of salmonella *(56 ,49)*.

On the other hand the microscopical findings of spleen and bursa of Fabricius in the present work showed depletion and necrosis in the lymphocytes of white pulp besides numerous heterophils infiltration in the red pulp in all infected groups (3,4 and 5) and these lesions were alleviated in prebiotic received groups (6,7 and 8). Our findings are discussed by *(57)*, in broiler chicks challenged with *S.enteritidis* and received prebiotic- based on mannan-oligosaccharide and β-glucan, he stated that, the exact mechanisms that mediate the immunomodulatory activities of prebiotics are not clear. However, several in vitro and in vivo studies have shown that salmonella infection stimulates different subsets of immune cells to produce the inflammatory cytokine interleukin 1*(58,59)*. The lower humoral immune response of challenged broilers can be explained by lower lymphocyte count and lower immune organ weights, because of the inflammatory effects of interleukin-1. Inflammatory factors stimulate the hypothalamic production of corticotrophin releasing factor *(60)*. Interleukin-1 stimulates the hypothalamus, leucocytes, or both to produce the corticotrophin-releasing factor, which stimulates the production of adrenocorticotropic hormone by the anterior pituitary, leucocytes or both. Adrenocorticotropic hormone then stimulates corticosterone production from the adrenal gland *(58)*. Corticosterone has been found to be immunosuppressive *(60)* inhibiting the production and actions of antibodies *(61)*, increasing the H/L(heterophils/lymphocytes) ratio and depressing the immune organ growth *(62)* led to conclude that the challenged chicks were in a physiological stress state. Heterophils are parts of natural immunity and cellular defense against microbial infections and lymphocytes are cells that produce antibodies. The increase in H/L ratio in challenged chicks may be attributed to increased corticosterone secretion *(62)* resulted in decrease of the antibody titers. Dietary inclusion of prebiotics had a significant positive effects in salmonella challenged chicks. On the same line *(63, 64)*, they reported that MOS and β-glucan are effective on humeral and cell immunity. Another explanation in our results are supported by *(65 , 57)*, they stated that prebiotic with high mannas levels will bind macrophage reception sites by recognizing specific sugars found in glucoproteins of the epithelial surface, triggering a cascading reaction that would eventually activate macrophages and release cytokines thereby activating the acquired immune response. *(66 ,20)*, concluded that FOS and MOS prebiotics have stimulating effect on lymphocytic tissue of GIT. This is mainly due to lactic acid action which affect the mechanisms of non specific immunity (increased proliferation of macrophages and their phagocytic activity as well as NK cell synthesis) specific (to stimulate macrophage to produce cytokines that activate Tc cells) and humeral(stimulation of B lymphocytes to produce antibacterial antibodies including IgA). IgA activate the digestive system and protect the intestinal epithelium against pathogenic microorganism.

Conclusion:

It could be concluded that *XPC* prebiotic alleviated the immunological and pathological alteration induced from the experimentally infected ducks by Duck Virus Enteritis or *Salmonella enteritides* infection *and both* .

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الملخص العربى

دراسات مناعية وباثولوجية على البط المصاب تجريبيا بالتهاب الامعاء الفيروسى و السالمونيلا انتريتيدس مع دلالة تأثيراكس بى سى بريبيوتك

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استهدف هدا البحث دراسة التغيرات الباثولوجية التى تحدثها العدوى التجريبية لكلا من مرض التهاب الامعاء الفيروسى(طاعون البط) وبكتريا السالمنيلا انتريتيدس كلا على حدة او معا على البط مع دلالة تأثير دور اكس بى سى بريبيوتك فى تحسين التغيرات الباثولوجية التى تحدثها العدوى التجريبية على الاعضاء المختلفة للبط. اجريت هده التجربة على عدد ٨٠ بط مسكوفى تم تقسيمها بالتساوى الى ٨مجموعات . المجموعة الاولى تركت ضابط للتجربة، تم اعطاء المجموعة الثانية ١مل/لتر بريبيوتك اكس بى سى فى ماء الشرب من عمر يوم وحتى نهاية التجربة، كما يتم عدوى المجموعة الثالثة بجرعة ١مل/بطة عن طريق الحقن العضلى بمعلق اعضاء بط مصاب سابقا بالتهاب الامعاء الفيروسى ونافق حديثا مجهز معمليا ودلك عند عمر ١٢ يوم، كما يتم احداث عدوى تجريبية عند نفس العمر للمجموعة الرابعة بعترة السا لمونيلا انتريتيدس عن طريق الفم ، ويتم عدوى المجموعة الخامسة بكلا من معلق اعضاء البط المصاب سابقا بالتهاب الامعاء الفيروسى و عترة السالمنيلا بنفس الجرعات سالفة الدكر وعند نفس العمر كما فى المجموعات (٣ ،٤).يتم اعطاء المجموعات(٦،٧،٨) ١مل/لتر فى ماء الشرب من اكس بى سى بريبيوتك من عمر يوم وحتى نهاية التجربة وعند عمر ١٢ يوم يتم احداث العدوى التجريبية بمعلق اعضاء البط المصاب بالتهاب الامعاء الفيروسى ونافق حديثا ، وعترة السالمونيلا انتريتيدس كلا على حده ومجتمعين مع بعض على التوالى كما فى المجموعات(٣،٤،٥) بنفس الجرعات المدكورة سابقا. وعند ظهور اعراض المرض يتم اجراء الصفة التشريحية وتسجيل التغيرات المختلفة ويتم تجميع عينات من الاعضاء ودلك لعمل الفحص الفيرولوجى. كما يتم اخد عينات من الكبد ، الامعاء، المرئ، القلب، الكلية، الطحال ،و البرسا ويتم وضعها فى فورمالين ١٠% ودلك لعمل الفحص الباثولوجى و اختبار المناعة الكيميائية النسيجية(IHC) .

وقد اظهرت النتائج الاعراض المرضية لالتهاب الامعاء الفيروسى والسالمونيلا على المجموعات المصابة تجريبيا. وقد أظهر الفحص المجهرى لهده المجموعات احتقان فى معظم الاوعية الدموية للمجموعات المصابة تجريبيا كما لوحظ وجود تجلط حديث فى الوريد الكبدى للمجموعة المصابة تجريبيا بالسالمونيلا، كما لوحظ ايضا وجود تنكسات وتنكرز بدرجات مختلفة مع وجود فرط فى الخلايا الالتهابية فى جميع الاعضاء ، وايضا لوحظ وجود اجسام احتوائية فى خلايا الكبد والامعاء فى المجموعة المصابة تجريبيا بمعلق التهاب الامعاء الفيروسى. وقد اظهرت النتائج تحسن ملحوظ للتغيرات الباثولوجية فى المجموعات التى تم اعطائها *XPC* بريبيوتك فى ماء الشرب ثم تم عدواها.

مما سبق نستنتج ان *XPC* بريبيوتك له تأثير فعال فى تحسين التغيرات الباثولوجية التى احدثتها العدوى التجريبية لكلا المرضين على حدة او مع بعضهم البعض.