Concomitant Effect of Bovine IgG rich-fraction and Probiotics in Controlling Chicken Colisepticaemia

BY

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Summary

Usage of the probiotic *Pediococcus acidilactici (P. acidilactici)* and/or bovine IgG-rich fraction in either prophylaxis or therapy of chicken colisepticaemia revealed significant reduction in both clinical and pathological pictures provided that results of prophylactic study were much better than therapeutic one. Bovine IgG rich-fraction potentates the protective effect of *P. acidilactici* under the conditions of the present investigation.

Probiotics and bovine IgG-rich fraction represent a safe and non-pharmaceutical means of combating microbial pathogens that requiring no withdrawal period, substituting antibiotics and can make a valuable contribution to flock health and safety of poultry products.

Introduction

Hence the widespread use of antibiotics as therapeutic agents and growth promoters resulted in the development of resistant population of bacteria which made their subsequent use for therapy difficult and result in occurrence of antibiotic residues in the poultry products **(DuPont and Steele, 1987)**; the direction towards the use of environmentally friendly preparations in the prophylaxis and therapy of poultry pathogens is highly recommended by World Health Organization (WHO). Consequently; the usage of competitive exclusion (probiotics) as natural control method was emerged based on ensuring the bird has an adequate gut micro flora to counter pathogenic bacterial colonization in the digestive tract **(Awaad et al., 2003a).** Probiotics are live microbial feed supplement which beneficially affects the host animals by improving its intestinal microbial balance **(Fuller, 1989)** and stimulating natural resistance through increasing the number of antibodies and the effectiveness of macrophages **(Goldin and Gorbach, 1984)**. *Pediococcus* species are members of probiotic preparations which had been found normally in the caeca of newly hatched chicks **(Barnes et al., 1980)**. *Pediococcus* species are non pathogenic organisms, a part of normal chicken gastrointestinal tract (GIT) flora and thus capable of colonizing the gut producing antagonistic substances which found to be active against enteric pathogens **(Daeschelm and Klaenhammer, 1985)**. On the other hand; several investigators reported that bovine immunoglobulins not only used in the prophylaxis of colibacillosis in calves but also in controlling morbidity and mortality of such infection in the treated groups when compared with untreated ones **(Walser and Brumer, 1967 and Signorini, 1970)**. They have been used in prevention of experimentally induced colisepticaemia in calves **(Penhale et al., 1971; Logan and Penhale, 1971 and Cadman et al., 1994)** and in chickens as well **(Awaad, 1975 and Youseif et al., 2005).**

The purpose of this study is to investigate the possible effectiveness of concomitant usage of bovine IgG rich fraction and/or the probiotic *Pediococcus acidilactici* in both the prophylaxis and therapy of chicken colisepticaemia.

Material and Methods

Bacterial strain: ***E. coli*** serogroup O1 obtained from Animal Health Research Institute was used as a challenge organism. It was identified after **Cruickshank et al., (1975)** and serotyped as described by **Cloud et al., (1985)**. The organism was inoculated on a slope agar and incubated aerobically at 37°C for 24 hours. Colonies were collected and suspended in saline to a density of McFarland No.4 standard for use. Infection was given S/C in a dose of 4.5 $x10^6$ CFU/ bird at 14 days of age.

Probiotic: "Bactocell"® is a product of Lallmand, France containing stabilized strain *Pediococcus acidilactici* MA18/5M. It was given as a dried fed microbial in poultry ration and used in a full dose (100 g/ton) or a half dose (50 g/ton) for the entire period of the experiment starting from one day of age.

Ration: Commercial starter and finisher broiler chicken ration with or without "Bactocell"® was used. The starter ration contained crude protein-not less than 21.64%, crude fat-not less than 2.7%, crude fibers-not less than 2.7%, metabolizing energy-not less than 2950 Kcal/kg ration and used for the first 4 weeks. The finisher ration contained crude protein-not less than 17.73%, crude fat-not less than 2.7%, metabolizing energy-not less than 3010 Kcal/kg ration and used for the remaining of the experimental period. No antibiotics were added to the ration. Semduramicin was added at a concentration of 25 PPM as coccidiostate.

Preparation of bovine IgG-rich fraction: This was carried out after **Awaad (1975) and Shepherd and Dean (2000)** as follows; calf serum was taken from calf slaughter house and concentrated using 40% ammonium sulphate to precipitate IgG. The volume of serum to be fractionated was placed in a beaker to which slowly added equal volume of 80 % ammonium sulphate solution while gently stirred. The mixture was left for 4 hrs at room temperature. The precipitated globulin was collected by centrifugation at 6000 r. p. m. for 30 minutes in cooling centrifuge. The supernatant fluid was decanted. The precipitate was resuspended in distilled water to the original volume of serum. The precipitation was repeated 3 times as outlined before. The third precipitated globulin was resuspended in half the original volume of serum. The globulin solution was placed in a dialysis bag of visking tubing (27/ 32 size) and ammonium sulphate was removed by dialyzing at 4°C against frequent changes of 0.85 % Na CI pH 8.0, until barium chloride test gave negative result. The globulin sol. was removed from the dialysis bag and sterilized by filtration (0.45 urn swirex filter).

Experimental chicks: Two hundred and sixty; day-old meat type chicks (Hubbard breed) were obtained from a commercial hatchery. Ten birds were randomly selected, sacrificed and subjected to bacteriological examination to prove that they were free from *E. coli* infection. The birds were floor reared and were given feed and water *ad libitum* consumption. The birds were vaccinated against ND and Gumboro diseases using HB1, D78 and Lasota vaccine at 7, 11, and 18 days of age, respectively using eye drop method.

Experimental design: The experiment was designed according to the schedule illustrated in table 1.

Table 1. Schedule of the experiment.

	Gr. No.	Chick No.	Treatment	Bovine IgG administration time	*E. coli* infection (at 14th Day-old)
Prophylactic study	1	25	Bovine IgG + Probiotic full dose	13 days old	+
	2	25	Bovine IgG + Probiotic half dose		
	3	25	Bovine IgG		
Therapeutic study	4	25	Bovine IgG + Probiotic full dose	16 days old	+
	5	25	Bovine IgG + Probiotic half dose		
	6	25	Bovine IgG		
Probiotic full dose Ctrl	7	25	Probiotic full dose	-	-
Probiotic half dose Ctrl	8	25	Probiotic half dose	-	-
Blank Ctrl	9	25	-	-	-
Untreated Infected Ctrl	10	25	-	-	+

Ctrl= Control

All the groups were kept under observation after *E. coli* infection for 21 days, recording clinical signs, mortality and post mortem lesions.

Lesion scores were recorded in dead as well as sacrificed survived birds at the end of the observation period.

Histopathlogical examination:

Specimens from different organs (liver, heart, airsacs and spleen) were collected, fixed in 10% formol saline, processed using the conventional paraffin embedding technique, sectioned and stained with H & E for routine histopathological examination **(Lilli and Fulmen, 1976)**

Results and Discussion

E. coli is associated with a variety of extra intestinal poultry diseases, including colisepticemia **(Roland et al., 2004)**. Development of resistant population of bacteria might be a sequel for using antibiotics in birds and animals. So; subsequent use for therapy is difficult **(Ghadban, 1999)**. Antibiotics have negative effect on intestinal lactic acid bacteria **(Bougon et al., 1987)** and affect on indigenous gut flora causing intestinal upsets that persist even after their cessation **(Watkins and Kratzer, 1984).** Accordingly; WHO is urging the meat producing countries around the world to use "environmentally friendly" alternative methods for controlling infectious diseases.

In the prophylactic study from *E. coli* infection; a significant increase in the body weight was obtained in groups that received *P. acidilactici* (full dose) plus bovine IgG rich fraction (group 1) followed by *P. acidilactici* (half dose) plus bovine IgG rich fraction (group 2) and finally those received bovine IgG rich fraction alone (group 3) over the infected untreated control group (group 10) at 14, 21, 28 and 35

days of age respectively. Similar results was also obtained in the therapeutic study except at 35 days of age where only significant improving in body weight was obtained in chicken group received *P. acidilactici* (full dose) plus bovine IgG rich fraction (group 4) over infected untreated control group (group 10) (Table 2).

This improvement in weight can be explained by **March (1979)** who stated that intestinal pH may alter both microbial population and nutrient absorption. **Fuller (1997)** attributed this improvement to creating balanced microbial population in the intestinal tract and to the role of probiotic in preventing the harmful bacteria which invade the digestive tract of chickens. On the other hand; the improving in body weight of probiotic treated chicken groups in the present work coincides with results of many investigators **(Ghazalah et al., 1988; Harms and Miles, 1988; Nahashon et al., 1994; Mohan et al., 1995; Abdulrahim et al., 1996; Haddadin et al., 1996; Nahashon et al., 1996; Davis and Qureshi, 1997; Grimes et al., 1997; Yeo and Kim; 1997; Ghazalah and Ibrahim , 1998; Abde El-Samee, 2001; Fairchild et al., 2001; Parks et al., 2001 and Siam et al., 2004)**.

Twenty percent of untreated infected birds (group 10) showed clinical signs of depression, dropping of wings, sleepy appearance and huddling together. Similar clinical signs have been reported by **Awaad (1972) and Khalid (1990)** in *E. coli* infection of chickens. The mean macroscopic lesion scores reached 0.13, 0.33 and 0.53 in prophylaxis study (groups 1-3) and 0.44, 0.56 and 0.81 in therapeutic study (groups 4-6) respectively as compared with 3.5 in infected untreated group (group 10) (Table 3). The milder lesion scores reported in the treated groups accord with those reported by **Samarai and Al-Attar (1995)** who reported on significant lowering in post mortem lesion scores in chickens received lactobacilli.

The protection rates from *E. coli* infection reached 88, 84 and 60% in prophylaxis study in treated groups 1, 2, and 3; whereas in therapeutic study these rates were 72, 64 and 44% in groups 4, 5 and 6 respectively as compared with 36% in infected untreated group (group 10). **Panda et al., (2000)** proved that birds fed probiotic were less susceptible to *E. coli* challenge. **Awaad et al., (2003 b)** reported that the probiotic *P. acidilactici* reduced the mortality to 64% as compared with 72% in untreated chickens infected with *E. coli* O142.

No histopathological changes were found in chickens of the uninfected blank control group (group 9) throughout the experimental period. Histopathological findings of *E. coli* infected groups treated with probiotic and/or bovine IgG rich fraction are shown in Figs. 1-4.

In table 4; the sum microscopic lesion scores reached 5, 13 and 14 in prophylaxis study (groups 1-3) and 8, 12 and 14 in therapeutic study (groups 4-6) respectively as compared with 16 in infected untreated group (group 10). The recoded liver lesions are similar to those observed by **Awaad (1972) and Awaad et al., (2003 b)** who found obvious perihepatitis with moderate degree of hepatitis, portal areas showed some cellular accumulations and dilated sinusoids without marked damage in hepatocytes in *E. coli* infected chickens. Lesions of heart were also similar to those reported by **Awaad (1972)** and **Calnek et al., (1997)**. On the other hand; the recorded milder microscopic lesions in probiotic treated groups support the results of **Awaad et al., (2003 b)** who found that heart of chicken treated group with *P. acidilactici* showed mild myocardial heterophilic cell infiltration with less severe pericarditis with many heterophil seen infiltrating the pericardium and myocardium, whereas untreated infected birds suffered from very severe pericarditis, subepicardial myocarditis and the underlying myocardium intensely infiltrated with mononuclear cells.

The effectiveness of *P. acidilactici* and bovine IgG-rich fraction in the prophylaxis and therapy of chickens significantly reduced *E. coli* infection provided that the prophylactic results were much better than therapeutic one. Moreover; usage of bovine IgG rich-fraction potentate the protective effect of the probiotic *P. acidilactici* under the conditions of the present investigation.

Wafaa (2004) and Zohair (2006) used *P. acidilactici* in poultry to compete experimental infection with different bacterial enteropathogens and pointed out that adding probiotics (both bacterial or yeast), organic acids and symbiotic in broiler chicken ration proved their capability to reduce coliform, *E. coli, Salmonella enteritidis, Campylobacter jujeni and Clostridium perfringens* colonization together with their ability to reduce mortalities, severity of post mortem and pathological lesions due to infection. **Gilliland and Speck (1977)** attributed the antagonistic activity of probiotic (lactobacillus, pediococcus and other lactic acid bacteria) toward pathogens to the production of bactericidal substances and combination of factors (organic acids, hydrogen peroxide and bacteriocins). **Bernet et al., (1994) and Jin et al., (1997)** explained the potential mechanism by which probiotics might exert their protective or therapeutic effect against *E. coli* as competition for nutrients or adhesion receptors (production of inhibitory metabolites as organic acids or antimicrobial agents against pathogens).

Other means of controlling infectious diseases have been employed by **Hu and Linna** (**1976**) who used serotherapy in avian reticuloendotheliosis virus using immune serum; **Ikemori et al., (1992)** who administered egg yolk powder from hens immunized with K99-piliated enterotoxigenic *E. coli* to protect neonatal calves against fatal enteric colibacillosis and **Kariyawasam et al., (2004)** who orally ingested egg yolk immunoglobulin from hens immunized with an enterotoxigenic *E. coli* strain to prevent diarrhea in rabbits challenged with the same strain. Usage of specific and non-specific gamma-globulins in the prophylaxis and therapy of *E. coli* infection in chickens has been studied by **Awaad (1975); Awaad et al., (1992); Youseif et al., (2005) and Hassanien et al., (2007)**.

Chansiripornchai et al., (1995) reported that genes located on plasmids of *E. coli* often encode resistance to antibiotics; these plasmids easily spread through bacterial populations, which lead to the spread of resistance, so rendering the drug ineffective. **Panigrayh et al., (1983)** mentioned that chemotherapy in the presence of infectious drug resistance has been looked as transient and unreliable, besides representing a potential health hazard for human.

Therefore, the present work might be necessary searching for another natural tool to protect the chickens from this emerging food born pathogen.

In conclusion; usage of probiotics and bovine IgG-rich fraction represent a safe and non-pharmaceutical means of combating microbial pathogens that requiring no withdrawal period. They eventually can substitute antibiotics and can make a valuable contribution to flock health and safety of poultry products as food in the domain of prophylaxis and therapy from poultry disease

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Table 2: Body weights of *E. coli* infected chicken groups treated with *Pediococcus acidilactici* and/or bovine IgG-rich fraction

	Gr. No.	Treatment					Body weight (gram / day)				
		P.acidilactici		*E.coli* infection	Bovine IgG		Pre *E.coli* infection		Post *E.coli* infection		
		Half	Full		*Before	**After	7 days	14 days	21 days	28 days	35 days
Prophylacti -c study	1	-	+	+	+	-	114.5±2.1	335.2±8.1	463.3±18.2	795.5±34.7	1074.2±364
	2	+	-	+	+	-	139.1± 3.8	337.2±11.8	406.5±15.1	690.8±26.6	980.0±32.4
	3	-	-	+	+	-	150.2± 3.3	373.8±6.1	355.7±11.1	536.9±25.2	818.3±38.6
Therapeuti -c study	4	-	+	+	-	+	141.1± 3.9	328.4±9.4	388.5±19.3	733.2±46.4	974.5±43.3
	5	+	-	+	-	+	128.9± 2.2	349.7±10.1	427.3±18.2	701.7±34.8	877.0±40.2
	6	-	-	+	-	+	150.2± 3.7	293.3±7.9	348.1±11.9	525.4±24.2	677.7±38.1
Controls	7	-	+	-	-	-	116.6± 3.8	326.5±5.5	472.6±18.2	736.8±24.9	1029.2±716
	8	+	-	-	-	-	117.6± 2.3	310.0±8.8	496.6±14.8	700±20.3	911.1±37.8
	9	-	-	-	-	-	122.5±3.9	288.7±6.9	421.0 ±12.4	628.4±18.0	895.2±33.0
	10	-	-	+	-	-	153.1± 4.9	223.0±7.0	272.8±9.5	420.7±19.4	887.1±23.0
Least Significant differences (LSD)							6.91	23.7	38.22	66.85	99.6

Values represent means ± SEM of 20 broiler chickens each per treatment (n = 20)
Means in a column with no common superscripts differ significantly (P <0.05).
* Bovine IgG-rich fraction administered before *E. coli* infection (prophylactic study).
** Bovine IgG-rich fraction administered after *E. coli* infection (therapeutic study)

Table 3: Results of *E. coli* infection of treated and untreated groups with *Pediococcus acidilactici* and/or bovine IgG-rich fraction

	Gr. No.	Treatment					Macroscopic lesion scores					
		P.acidilactici		*E.coli* Infection	Bovine IgG		Survived birds / group	Organ lesion score				Total lesion score	Total score / survive-ors
		Half	Full		*Before	**After		1+	2+	3+	4+		
Prophylactic study	1	-	+	+	+	-	22/25	0	0	1	0	3	0.13 (3/22)
	2	+	-	+	+	-	21/25	3	2	0	0	7	0.33 (7/21)
	3	-	-	+	+	-	15/25	1	2	1	0	8	0.53 (8/15)
Therapeutic study	4	-	+	+	-	+	18/25	2	3	0	0	8	0.44 (8/18)
	5	+	-	+	-	+	16/25	4	1	1	0	9	0.56 (9/16)
	6	-	-	+	-	+	11/25	1	1	2	0	9	0.81 (9/11)
Controls	7	-	+	-	-	-	25/25	0	0	0	0	0	0 (0/25)
	8	+	-	-	-	-	25/25	0	0	0	0	0	0 (0/25)
	9	-	-	-	-	-	25/25	0	0	0	0	0	0 (0/25)
	10	-	-	+	-	-	9/25	5	7	3	1	32	3.5 (32/9)

Lesions=Airsacculitis and pericaditis after **Awaad (1975):** +1= Mild +2= Moderate+3= Severe+4= Very severe. *Bovine IgG-rich fraction administered before *E. coli* infection (prophylactic study). **Bovine IgG-rich fraction administered after *E. coli* infection (therapeutic study).

Table 4: Histopathological lesion scores of *E. coli* infected chicken groups treated with *Pediococcus acidilactici* and/or bovine IgG-rich fraction

	Gr. No.	Treatment					Histopathological Severity index				
		P.acidilactici		*E.coli* infection	Bovine IgG		Organ				
		Half	Full		*Before	**After	Liver	Heart	Air sacs	Spleen	Sum
Prophylactic study	1	-	+	+	+	-	1	1	1	2	5
	2	+	-	+	+	-	3	3	4	3	13
	3	-	-	+	+	-	4	3	4	3	14
Therapeutic study	4	-	+	+	-	+	2	2	2	2	8
	5	+	-	+	-	+	3	3	3	3	12
	6	-	-	+	-	+	4	4	3	3	14
Controls	7	-	+	-	-	-	0	0	0	0	0
	8	+	-	-	-	-	0	0	0	0	0
	9	-	-	-	-	-	0	0	0	0	0
	10	-	-	+	-	-	4	4	4	4	16

Bovine IgG-rich fraction administered before *E. coli* infection (prophylactic study). ** Bovine IgG-rich fraction administered after *E. coli* infection (therapeutic study).

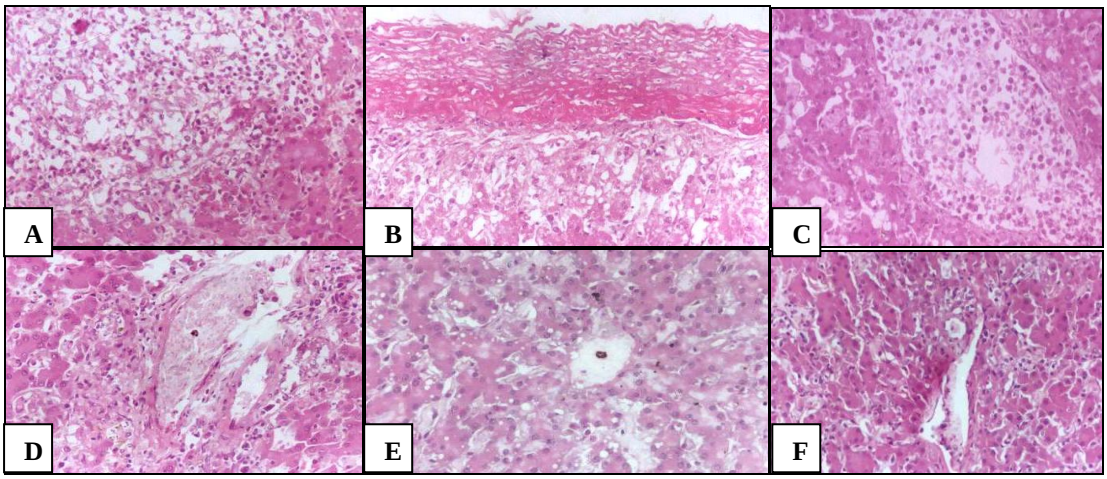

Fig. 1: **Liver A**, Infected group showing focal necrotic area infiltrated with mononuclear cells (H&E x 200). **B**, The same group revealed perihepatitis with subcapsular necrosis of hepatocytes (H&E x 200). **C**, Treated groups with half dose of probiotic and Igs showing necrobiotic changes of hepatocytes and aggregation of hetrophils (H&E x 200). **D**, The previous groups was also showing perivascular hetrophils infiltration and necrosis of hepatocytes (H&E x 200). **E**, Treated group with full dose of probiotic and Igs after infection showing fatty degeneration of hepatocytes with leukocytic infiltration (H&E x 200). **F**, Treated group with full dose of probiotic and Igs before infection showing mild degenerative changes of hepatocytes with leukocytic cells infiltration (H&E x 200).

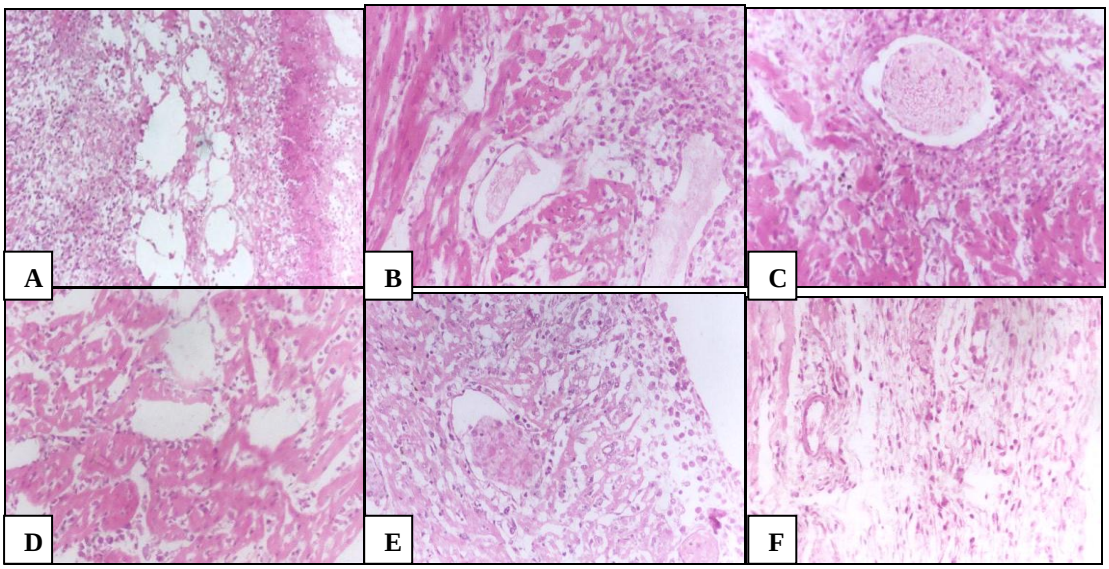

Fig. 2: **Heart. A**, Infected group showing severe pericarditis with early stage of organization (H&E x 200). **B**, The same group revealed myocarditis with perivascular heterophils infiltration (H&E x 200). **C**, Treated groups with half dose of probiotic and Igs showing less severe pericarditis in which serofibrinous exudates with hetrophils infiltration (H&E x 200). **D**, The previous groups was also showing myocarditis with minimal heterophils infiltration (H&E x 200). **E**, Treated group with full dose of probiotic and Igs after infection showing serofibrinous pericarditis and mild degeneration of cardiac muscle (H&E x 200). **F**, Treated group with full dose of probiotic and Igs before infection showing mild serofibrinous pericarditis with few hetrophils infiltration (H&E x 200).

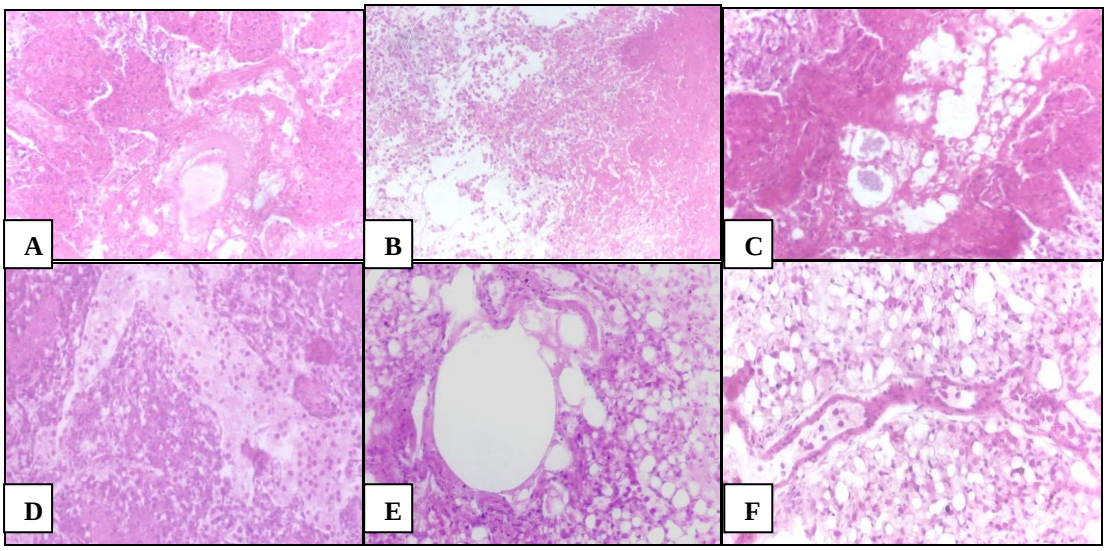

Fig. 3: **Air sacs. A**, Infected group showing very severe mucopurulant airsaculitis (H&E x 200). **B**, The same group revealed massive areas of suppuration with heterophils infiltration (H&E x 200). **C**, Treated groups with half dose of probiotic and Igs showing less severe air saculitis with hetrophils infiltration (H&E x 200). **D**, The previous groups was also showing suppurative airsaculitis with thrombosis of blood vessel (H&E x 200). **E**, Treated group with full dose of probiotic and Igs after infection showing serofibrinous airsaculitis with minimal heterophils infiltration (H&E x 200). **F**, Treated group with full dose of probiotic and Igs before infection showing mild serofibrinous air saculitis (H&E x 200)

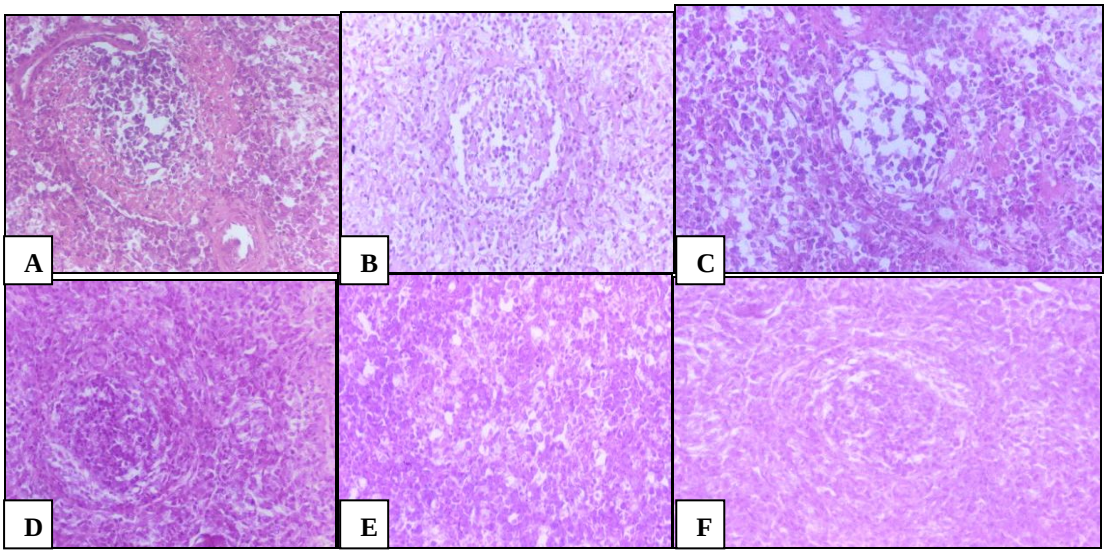

Fig. 4: **Spleen. A**, Infected group showing severe damage of lymphoid follicles and hemorrhages (H&E x 200). **B**, The same group revealed necrosis of lymphoid follicles (H&E x 200). **C**, Treated groups with half dose of probiotic and Igs showing less severe damage of lymphoid follicles (H&E x 200). **D**, The previous groups was also showing depletion of lymphoid follicles with heterophils infiltration (H&E x 200). **E**, Treated group with full dose of probiotic and Igs after infection showing moderate depletion of lymphoid follicles (H&E x 200). **F**, Treated group with full dose of probiotic and Igs before infection showing mild depletion of lymphoid follicles (H&E x 200).