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Research paper

Immunological status in broiler chickens vaccinated with newcastle vaccine and treated with cephradine

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Abstract

The objective of this study is to clarify the effect of cephradine on cellular and humeral immune responses in broiler chickens. One hundred one-day-old Hubbard broiler chicks were divided into four equal groups (25 chicks in each). 1st group healthy broiler chickens non-vaccinated non medicated (control group), 2nd healthy broilers vaccinated with Newcastle vaccine only, 3rd group healthy broilers received 20 mg cephradine in drinking water daily for 5 consecutive days and 4th group healthy broilers vaccinated and received 20 mg/kg b.wt cephradine daily for 5 consecutive days. At 1st, 10th and 20th day post administration, blood samples were collected for determination total and differential leucocytic count, phagocytic activity, index, killing percentage and HI titer. Vaccinated broilers by Newcastle disease virus vaccine only, showed insignificant increase in leukocytic count, lymphocyte, heterophils, nitric oxide, lysozyme activity, total protein, total, γ globulin and HI titers at 1st day post vaccination. Beside significant increase at 10th and 20th day post vaccination coupled with insignificant increase in eosinophils, basophils, monocyte, phagocytic activity, phagocytic index, killing %, albumin and α globulin and non-significant decrease in serum β globulin and A/G ratio allover experimental periods post vaccination. Broilers received cephradine and/or vaccinated with Newcastle vaccineeither alone or together, showed insignificant increase in leukocyte, heterophils, lymphocyte, eosinophils, basophils, monocyte, nitric oxide, lysozyme activity, total protein, albumin, total, α, β, γ globulin, A/G ratio throughout experimental period post vaccination. Beside significant decrease in phagocytosis, phagocytic index and killing % at 1st day and insignificant decrease at 10th & 20th day post vaccination coupled with significant decrease in HI titers at 1st day post administration and insignificant decrease at 10th & 20th day post vaccination. It was concluded that vaccination by Newcastle disease virus vaccine induced immune-stimulant but cephradine provoked a remarkable immunosuppressive effect in broiler chickens. Therefore, vaccination not recommended during treatment by cephradine.

Keywords: *Broiler chickens; Cephradine; Immunity; Vaccine.*

1. Introduction

Antibiotics are important tool in reducing avian diseases. Antibiotics are extensively used as productivity enhancers in poultry production to control infectious diseases (Moreno et al., 2007). Immunosuppression properties of some antibiotics are effective in inhibition of both cellular and humoral immune responses to a variety of vaccines (Shalaby, 1989). Several antibiotics suppress the immune response by their ability to interfere with protein or immunoglobulin synthesis (Richard and Merle, 1984).
Cephalosporins are a group of antibiotics derived from mould of cephalosporium spp. and are based on 7-aminocephalosporic acid which corresponds to 6-penicilanic acid in penicillin (El-Hewaity et al., 2014). Cephradine is a beta-lactam, first-generation cephalosporin antibiotic with bactericidal activity and available in both oral and parenteral dosage forms (Wilson and Gisvold, 1982). Cephradine have a good activity against Gr +ve bacteria and relatively moderate activity against some enterobacteria, including strains of E coli, K. pneumonia, Proteus mirabilis, Salmonella and Shegella species (James, 1993). Antibacterial activity due to ability of beta-lactamase ring to bind bacterial enzyme Transpeptidase which important for proper cell wall synthesis (Thomson et al., 1984; El Sayed et al., 2016; Aboubakr and Elbadawy, 2017). The present work was planned to investigate the effect of cephradine inducing immuno-suppressive effect on broilers vaccinated with Newcastle virus vaccine.

2. Materials and methods

2.1. Drugs

Cephradinee (Atocef Forte) ® Water-Soluble Powder Each 100 gm of powder contain 20 gm cephradine base. It is available as package containing 500 gm. It is produced by ATCO Pharma Company, Egypt.

2.2. Experimental broiler chickens

One hundred apparently healthy one day old Hubbard broiler chicks obtained from Cairo Poultry Company (CPC) were used in the present study. Chicks were floor reared under hygienic measures. Chicks were fed on balanced commercial ration free from any medications from Cairo Poultry Comp and water provided *ad-libitum*.

2.3. Newcastle vaccine

Avipro® Polybanco (Live Vaccine against Newcastle-Bronchitis B1 Type, B1 Strain, Mass. & Conn) Manufactured by Elanco animal health Co. was used as eye drops for vaccination of chicks on the 7th day of age against Newcastle disease.
Avipro® ND-chick (Killed Newcastle disease virus, B1 type, Lasota strain produced by Elanco Co.) given SC. at 7th day old

2.4. Experimental design

One hundred one-day-old Hubbard broiler chicks were divided in to four equal groups (25 chicks in each). 1st group healthy broilers non-vaccinated non medicated served as control, 2nd group healthy broilers vaccinated with Newcastle disease virus vaccine only, 3rd group healthy broilers received 20 mg/kg b.wt cephradine in drinking water daily for five consecutive days and 4th group healthy broilers vaccinated with Newcastle disease virus vaccine and received cephradine in same dose and period. At 1st, 10th and 20th day post injection two blood samples was collected from wing vein. First sample was collected in test tub contain EDTA as anticoagulant for estimation total and differential leucocytic count according to Jain (1986). Phagocytic activity, Phagocytic index and killing % were determined according to Rouse et al., (1980) and Woldehiwet and Rowan (1990), respectively. Second sample was collected in test tub for obtain clear serum for estimation of serum total protein according to Doumas et al., (1981), protein fractions were performed by electrophoresis test (Henry et al., 1974). Nitric oxide (Rajarman et al., 1998; Ramadan and Attia, 2003) and lysozyme activity (Schltz, 1987).
Tissue specimens from the internal organs (liver, kidneys, Bursa of fabricious & spleen) of all groups were collected and immediately fixed in 10% neutral buffered formalin for pathological study according to Lillie and Fulman (1976).

2.5. Statistical analysis

Obtained data was analyzed according to Petrie and Watson (1999).

3. Results

Vaccinated broilers with Newcastle vaccine only showed insignificant increase leukocytic count, lymphocyte, heterophils, nitric oxide, lysozyme activity in total protein, total, γ globulin and HI at 1st day post vaccination beside significant increase at 10th and 20th day post vaccination. Coupled with insignificant increase in eosinophils, basophils, monocyte, phagocytic activity, phagocytic index, killing % , albumin and α globulin and non-significant decrease inβ globulin and A/G ratio allover experimental periods post vaccination.
Chickens received Cephradine and/or vaccinated received cephradine, showed insignificant increase in leukocyte, heterophils, lymphocyte, eosinophils, basophils, monocyte, nitric oxide and lysozyme activity, total protein, albumin, total, α, β, γ globulin, A/G ratio allover experimental period post vaccination beside significant decrease in phagocytic activity, phagocytic index and killing % at 1st day coupled with insignificant decrease at 10th and 20th day post vaccination coupled with significant decrease in HI titers at 1st day post administration and insignificant decrease at 10th and 20th day post administration.
Plate (1) Liver (fig 1, 2 & 3) of broiler chickens vaccinated with Newcastle vaccine showed moderate degenerative changes in hepatocytes, mostly hydropic, moderate infiltration of the portal area with heterophiles and lymphocytes beside disorganization of the hepatic cells. Kidney (fig 4) sections revealed hypo-cellularity and atrophy of the glomeruli and degenerative changes in moderate no. of the renal tubules. Bursa of fabricious (fig 5) showed mild depletion of follicular lymphocytes. Spleen (fig 6) showing congestion of splenic sinusoids and depletion of some follicular lymphocytic.
Plate (2) Liver (1 & 2) of broiler vaccinated and received cephradine showed normal parenchyma, however. Some sections showed portal aggregations of heterophils and lymphocytes. Kidney (3 & 4) showed edema within renal papillae beside lymphocytic infiltration, some sections revealed hydropic degeneration in both glomerular and renal tubular epithelium. Bursa (fig 5 and 6) showed normal mucosa and bursal follicles. Some examined sections showed atrophy of some lymphocytic follicles beside edema with mild cystic changes in both the follicles and the mucosal lining. Some sections of spleen (fig 7 & 8) showed apparently normal splenic structures, others showed mild to moderate lymphocytic depletion of both white and red pulp. Mild infiltration of heterophiles was also seen in the splenic sinusoids.

4. Discussion

Our results revealed, vaccinated broilers with Newcastle vaccine only showed insignificant leukocytosis, lymphocytosis and heterophiliaat 1st day and significant increase at 10th and 20th day post vaccination beside non-significant eosinophilia, basophilia and monocytosis allover experimental period post vaccination. Similar results were obtained by Wang et al., (2015) who reported that insignificant effect on leukocytic count heterophils, lymphocte in chickens post using Newcastle vaccine. Newcastle vaccine induces insignificant leukocytosis, lymphocytosis, heterophilia and monocytosis in broilers (Ismail, 2017; Mehwish et al., 2018).
Healthy broilers received cephradine in tested dose or received cephradine and vaccinated revealed insignificant heteropenia beside insignificant leukocytosis, lymphocytosis, esinophilia, basophilia and monocytosis allover experimental period. Cephalosporin causes destruction of leucocytes (Bang and Kammer, 1983). Cephradine induce insignificant leukocytopenia and heteropenia beside insignificant lymphocytosis, eosinophilia, basophilia and monocytosis (Borowski et al., 1985). Our results were in complete harmony with those reported by Oleas (2006) who found that ceftiofur sodium induced non-significant leukocytopenia and heteropenia after 3rd day, 1st and 2nd week post vaccination. Insignificant leukocytopenia may due to effect of used drug in the granulopoeisis in bone marrow (Elisa, 1998). Broiler received another beta lactam (ampicillin) with Newcastle virus vaccine induced non-insignificant leukocytosis, heterophilia, lymphocytosis, monocytosis, eosinophilia and basophilia (Gamal, 2019).

Healthy broilers vaccinated with Newcastle vaccine showed insignificant increase in phagocytic activity, phagocytic index and killing % all over the experimental periods post vaccination. Same changes in phagocytic activity were reported by Elsayed (1995) in broiler chickens vaccinated with Newcastle vaccine. Elevation in phagocytic activity, phagocytic index and killing % might be due to activation of macrophages by production of lymphokines by T helper cells after their stimulation by Newcastle disease virus vaccine (Tizard, 1996). Vaccinated hens with Newcastle vaccine revealed increase in phagocytic activity and index (El-Sadek et al., 2008; Wang et al., 2015).

Healthy broilers received cephradine for 5 consecutive days and /or vaccinated with Newcastle vaccine either alone or together showed significant decrease in phagocytic activity, phagocytic index or killing% at 1st day beside nonsignificant decrease 10^{th} and 20^{th} day post vaccination. Cephalosporins reduced phagocytic functions of peripheral neutrophil-rich leukocytes and macrophages (Ohnishi et al., 1984). Cephradine induced decrease in phagocytic activity, phagocytic index or killing% in broiler (Grec and Frei, 1984). Our results were reported previously by Vanholder et al., (1988) in broilers received cefquinome alone or with vaccine. Ceftifur sodium with Newcastle vaccine induces insignificant decrease in phagocytic activity, in phagocytic index and killing% (Refaat, 1999). Our results were supported by those recorded by Ahmed (2015) who stated that cephalexin with vaccination induced significant decrease in phagoioytosis in rabbits. Our results were supported by Gamal (2019) mentioned that another beta lactam (ampicillin) with) with Newcastle virus vaccine induced non-significant increase in phagocytic activity and index throughout the experimental period post administration.

Healthy broilers vaccinated with Newcastle vaccine displayed insignificant increase in nitric oxide and lysozyme at 1^{st} day post vaccination and significant increase at 10th and 20^{th} day post vaccination. Our results were observed by Vanholder et al., (1988) who reported that vaccination induced insignificant increase in lysozyme activity and nitric oxide. Same results were reported by El sayed (1995) who stated that broilers vaccinated with Newcastle vaccine revealed increase in lysozyme activity and nitric oxide. Same change in lysozyme activity and nitric oxide was reported by Hassanein, et al., (2001) in chicken vaccinated with Newcastle vaccine. Lysozyme activity and nitric oxide were increased post vaccination with Newcastle vaccines (Sami and Hamed, 2011).

Broilers received cephradine and /or vaccinated either alone or together displayed non-significant increase in lysozyme activity and nitric oxide allover experimental period. Our results were observed previously by Vanholder et al., (1988) who stated that non-significant increase in lysozyme activity and nitric oxide in broiler received cefquinome alone or with vaccine. Same changes in lysozyme activity and nitric oxide post using cephodizime (Auteri et al., 1990). Cefodizime stimulates nitric oxide production (Shin et al., 1996). Chemotherapeutic agent resulted in an increase in lysozyme activity andnitric oxide (Chin et al., 2000). Same changes in lysozyme activity and nitric oxide were observed previously by Gamal (2019) in broilers received ampicillinwith Newcastle virus vaccine.

Vaccinated broilers with Newcastle disease virus vaccine showed insignificant increase in total protein, total globulin, γ globulin and HI at 1^{st} day post vaccination beside significant increase at 10^{th} and 20^{th} day post vaccination coupled with insignificant increase albumin, α globulin and non-significant decrease in serum β globulin and A/G ratio allover experimental periods post vaccination. Vaccination induced insignificant increase in α globulin beside non-significant decrease in β globulin as well as significant increase in γ globulin (Ahmed, 1991). The γ globulin synthesis immunoglobulins (antibodies) which involved in defense mechanism and immunity of organism by opsonization and neutralization of antigens (Padlan, 1994). Similar results were reported by Elsayed (1995) who stated that broilers vaccinated with Newcastle vaccine revealed increase total protein, γ globulin and decrease in serum β globulin. Same changes in globulin fractions post vaccination were reported by Wang et al., (2015).

Healthy chickens received cephradine and /or vaccinated either alone or together showed insignificant increase in total protein, albumin, globulin, α, β and γglobulin and A/G ratio allover experimental period post vaccination. Broiler chickens received cephradine in therapeutic dose exerts insignificant increase in total protein, albumin, globulin, α, β and γ globulin, A/G ratioat 3^{rd} day, 1^{st} and 2^{nd} week post administration (Borowski et al., 1985). These results parallel with those obtained by Ahmed and Ismail(1998) who found that healthy broilers received ceftiofur sodium increase in total protein, albumin, total, α, β and γ globulin. Our results was previously recorded by Refaat (1999) who stated that ceftifur sodium induced insignificant decrease in total and γ globulin levels in broilers. Another beta lactam (ampicillin) with Newcastle virus vaccine induced insignificant decrease in total and γ globulin levels in broilers (Gamal, 2019).

In the present study, it has been noticed that, healthy broilers vaccinated with Newcastle vaccine resulted insignificant increase in HI titers at 1^{st} day post vaccination and significant increase at 10^{th} and 20^{th} days post vaccination. Similar results were reported by Elsayed (1995) who stated that broilers vaccinated with Newcastle vaccine revealed increase HI titer within 6-10 days and peaks at 3-4 weeks post infection (Alexander, 1997). Same result was reported by Nasser (1998) and Abdu et al., (2012) who stated that Newcastle vaccine induced significant increase in HI titers after 2nd week of vaccination and reach peak at day 21^{st} day post vaccination. Different doses of Newcastle vaccine induced significant increase in the HI titers (Wang, et al., 2015). Our results coincide with Abera (2018) who reported that HI titers increased at 10^{th} day post vaccination and remained up to 35 day post vaccination.

Healthy broiler chickens received tested dose of cephradine and /or vaccinated either alone or together showed significant decrease in HI titers at 1^{st} day post administration and insignificant decrease at 10^{th} and 20^{th} day post administration when compared with control broilers. Our results were observed previously by Vanholder et al., (1988) who reported that insignificant decrease in HI titers production in broiler received cefquinome alone or with another vaccine (Covexin). Same changes in HI titers were reported by Gamal (2019) in broiler received another beta lactam (ampicillin) with) with Newcastle vaccine.

Healthy broiler chickens vaccinated with Newcastle disease virus vaccine only revealed many pathological changes in liver as moderate degenerative changes in hepatocytes, mostly hydropic, moderate infiltration of the portal area with heterophiles and lymphocytes beside disorganization of the hepatic cells beside hypocellularity and atrophy of the glomeruli and degenerative changes in moderate number of the renal tubules coupled with changes in immune organ represented by mild deplestion of follicular lymphocytes in Bursa of fabricious. Spleen showing congestion of splenic sinusoids and depletion of some follicular lymphocytic. Same pathological change was reported by Nakamura et al., (2008) in broiler chickens vaccinated with Newcastle disease virus vaccine. Same lesion was observed in healthy broilers vaccinated with Newcastle disease virus vaccine (Kikuyasu et al., 2014). Our findings are nearly agreed with those recorded byAhmed et al., (2017) and Muhammad et al., (2018) who stated that Newcastle disease virus vaccine induced depletion of follicular lymphocytes in Bursa of fabricious and spleen.

Healthy broiler chickens received cephradine in tested dose showed mild round cells infiltration in the portal area and some hepatocytes showed hydropic degeneration beside normal glomeruli and renal tubules meanwhile, bursa of fabricious showed normal glandular epithelium and lymphatic follicles with mild depletion of the central follicular cells but spleen showed normal red and white pulp. Our findings are nearly agreed with those recorded by Tune et al., (1988) who stated that cephalosporin induce renal, cortical, mitochondrial and respiratory toxicity after exposure. Same lesion was observed in healthy broiler chickens received another cephalosporin (cefoperazone) (Shawky, 2007).

It was concluded that vaccination by Newcastle disease virus vaccine induced immuonostimulantbut cephradine provoked a remarkable immunosuppressive effect in broiler chickens. So vaccination during treatment by cephradine is not recommended.

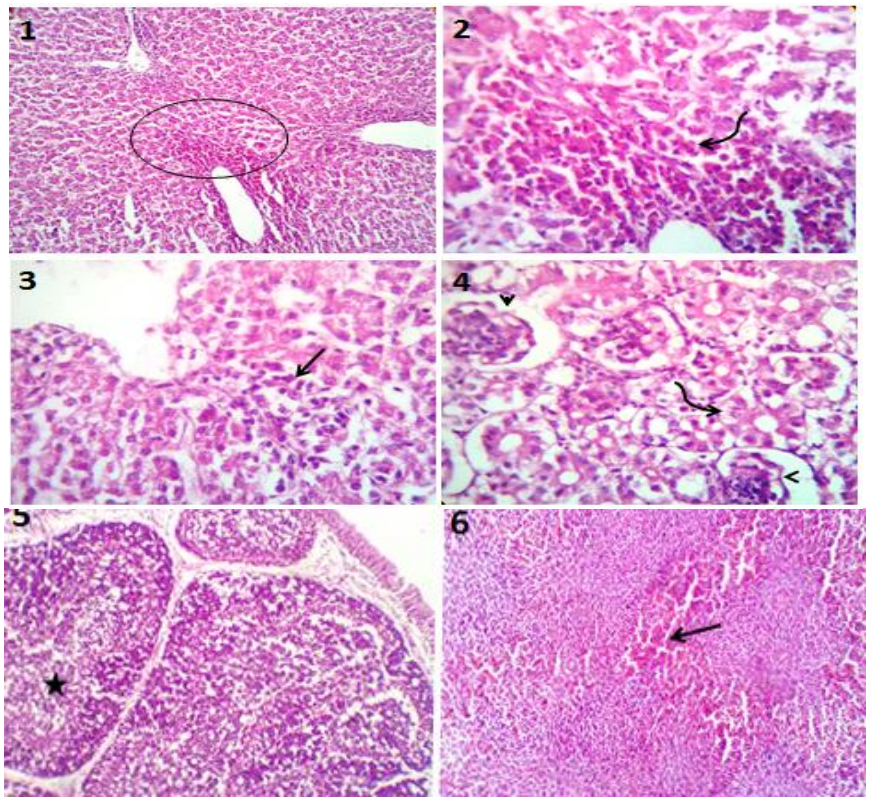

Fig. 1: Photomicrograph of Vaccinated Broiler with Newcastle Vaccine Revealed Liver (Fig 1,2,3) Showing Moderate Degenerative Changes in Hepatocytes, Mostly Hydropic,Moderate Infiltration of the Portal Area with Heterophiles (Curved Arrow) and Lymphocytes (Open Arrow) Beside Disorganization of the Hepatic Cells (Arrow Head). Kidney (Fig 4) Showing Hypocellularity and Atrophy of the Glomeruli (Arrow Heads) and Degenerative Changes in Amoderte No. of the Renal Tubules (Curved Arrow). Bursa (Fig 5) Showing Mild Deplestion of the Follicular Lymphocytes (Star). Spleen (Fig 6) Showing Congestion of Splenic Sinusoids (Open Arrow).

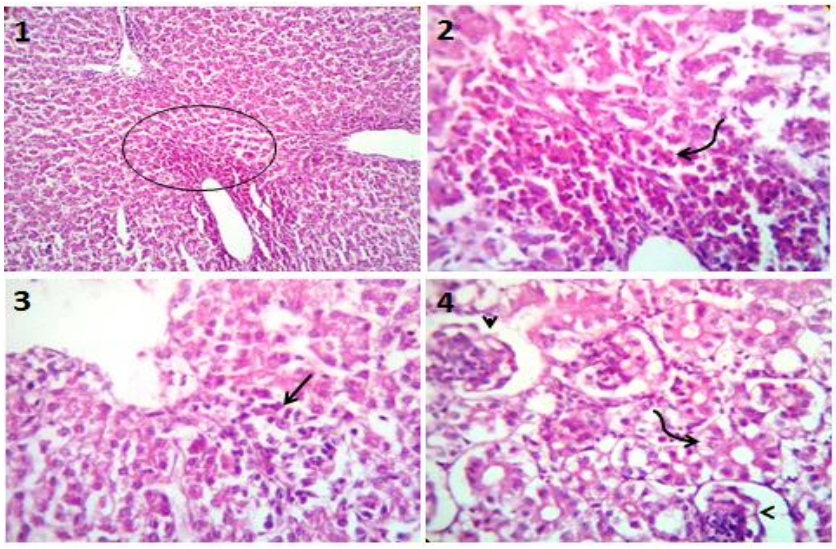

Fig. 2: Photomicrograph of Liver (Fig 1 and 2) Showing Portal Aggregations of Heterophils (Open Arrow) and Lymphocytes (Curved Arrow). Kidney (Fig 3 And 4) Showing Edema Beside Lymphocytic Infiltration Within Renal Papillae (Curved Arrow), Hydropic Degeneration In Glomerular Epithelium (Arrow Head) and Renal Tubular Epithelium (Open Arrow).

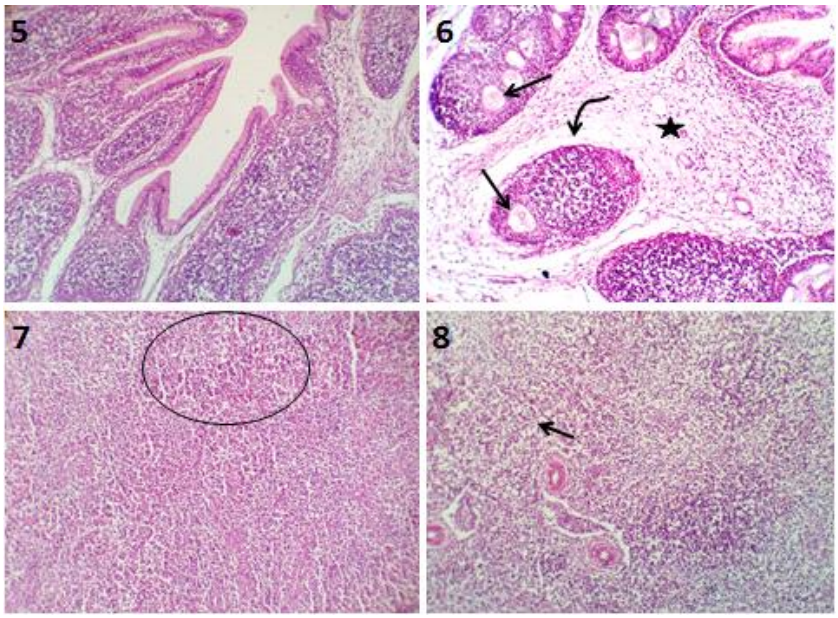

Fig. 3: Bursa (Fig 5) Showing Normal Mucosa and Bursal Follicles. Some Bursal Sections (Fig 6) Showing Atrophy of Some Follicles(Curved Arrow), Edema (Star) between Lymphocytic Follicles with Mild Cystic Changes Within Follicles (Open Arrows). Spleen (Fig 7, 8) Showing Mild Lymphocytic Depletion (Open Arrow) and Mild Infiltration of Hetereophiles in Splenic Sinusoids (Circle).

Table 1: Effect of Vaccination and Cephradine on Total and Differential Leucocytic Count of Broilers (N=5)

Parameter		1st day				10th day				20th day			
		G 1	G 2	G 3	G 4	G 1	G 2	G 3	G 4	G 1	G 2	G 3	G 4
T. leukocytic		11.21±	11.61±	11.35±	11.69±	11.27±	12.62±	11.53±	11.73±	11.26±	12.73±	11.27±	11.59±
10^3/ul		0.15	0.40	0.28	0.35	0.16	0.49*	0.32	0.49	0.18	0.22*	0.25	0.37
Differenial count (10^3/ul)	hetero	2.50±	2.59±	2.55±	2.51±	2.44±	2.90±	2.56±	2.46±	2.43±	2.89±	2.44±	2.45±
		0.18	0.05	0.17	0.16	0.19	0.07*	0.23	0.19	0.18	0.06*	0.24	0.18
	Lymph	4.25±	4.31±	4.30±	4.44±	4.30±	4.85±	4.30±	4.45±	4.35±	4.93±	4.35±	4.43±
		0.11	0.19	0.20	0.40	0.10	0.20*	0.21	0.32	0.11	0.25*	0.24	0.39
	Esino	1.36±	1.48±	1.43±	1.47±	1.39±	1.49±	1.40±	1.46±	1.37±	1.50±	1.40±	1.40±
		0.13	0.21	0.12	0.24	0.11	0.16	0.18	0.22	0.12	0.18	0.21	0.23
	Baso	1.52±	1.53±	1.56±	1.63±	1.51±	1.67±	1.60±	1.64±	1.50±	1.66±	1.60±	1.65±
		0.12	0.14	0.19	0.20	0.14	0.18	0.18	0.19	0.11	0.20	0.19	0.21
	Mono	1.65±	1.66±	1.65±	1.69±	1.68±	1.71±	1.69±	1.72±	1.68±	1.70±	1.70±	1.68±
		0.24	0.23	0.21	0.24	0.26	0.24	0.20	0.21	0.28	0.26	0.24	0.27

*Significant at P < 0.05.

Table 2: Effect of Vaccination and Cephradine on Phagocytic Activity%, Phagocytic Index, Killing% of Broilers (N=5)

Parameter	1st day				10th day				20th day			
	G 1	G 2	G 3	G 4	G 1	G 2	G 3	G 4	G 1	G 2	G 3	G 4
phagocytic	40.13±	40.62±	38.83±	38.94±	40.15±	41.21±	38.58±	38.78±	40.1±	41.4±	38.95±	39.84±
activity%	0.54	0.31	0. 4*	0.48*	0.41	0.48	0.22*	0.43*	0.59	0.85	0.39*	0.55
phagocytic	4.86±	4.93±	3.92±	3.86±	4.86±	4.98±	3.92±	3.79±	4.80±	4.94±	3.92±	4.46±
index	0.46	0.23	0.17*	0.29*	0.39	0.21	0.17*	0.25*	0.38	0.19	0.17*	0.19
killing%	76.39±	76.81±	74.44±	75.89±	76.43±	76.95±	74.65±	75.95±	76.7±	76.9±	75.05±	76.05±
	0.23	0.49	0.61*	0.45	0.29	0.62	0.70*	0.39	0.26	0.23	0.42*	0.42

*Significant at P < 0.05.

Table 3: Effect of Vaccination and Cephradine on Serum Nitric Oxide, Lysozyme Activity and HI Titreof Broilers (N=5)

Parameter	1st day				10th day				20th day			
	G 1	G 2	G 3	G 4	G 1	G 2	G 3	G 4	G 1	G 2	G 3	G 4
Nitric	26.16±	28.03±	26.24±	26.81±	27.11±	29.18±	27.22±	28.42±	27.1±	29.7±	27.19±	27.15±
oxide	0.56	0.41*	0.29	0.23*	0.39	0.37*	0.36	0.36*	0.43	0.4*	0.38	0.38
lysozyme ac-	94.13±	96.20±	94.62±	99.88±	93.89±	99.32±	94.12±	95.12±	93.9±	99. 48 ±	94.78±	95.10±
tivity	1.04	1.35	1.21	1.73*	1.10	1.61*	1.73	1.61*	1.12	1.7*	1.42	1.35
HI titre	3.13±	3.12±	1.98±	2.47±	3.23±	5.60±	2.92±	2.84±	3.26±	5.55±	2.98±	2.95±
	0.14	0.12	0.39*	0.18*	0.32	0.39**	0.23	0.22	0.25	0.9**	0.25	0.23

*Significant at P < 0.05.

Table 4: Effect of Vaccination and Cephradine on Serum Total Protein and Protein Fractions of Broiler Chickens (N=5)

Parameter		1st day				10th day				20th day			
		G 1	G 2	G 3	G 4	G 1	G 2	G 3	G 4	G1	G 2	G 3	G 4
T. protein (gm/dl)		5.57± 0.13	5.63± 0.12	5.59± 0.16	5.68± 0.17	5.54± 0.11	6.14± 0.33*	5.90± 0.19	6.05± 0.16*	5.58± 0.14	6.32± 0.37*	5.81± 0.18	6.08± 0.1*
Albumin (gm/dl)		2.89± 0.21	2.80± 0.14	2.92± 0.17	2.88± 0.16	2.84± 0.22	3.12± 0.18	2.96± 0.15	2.95± 0.16	2.87± 0.26	3.25± 0.14	2.99± 0.17	2.99± 0.18
Globulin (gm/dl)	α	0.63± 0.13	0.69± 0.14	0.64± 0.14	0.65± 0.15	0.62± 0.15	0.68± 0.12	0.66± 0.12	0.74± 0.17	0.60± 0.13	0.66± 0.14	0.65± 0.13	0.69± 0.12
	β	0.85± 0.06	0.83± 0.04	0.84± 0.09	0.84± 0.06	0.86± 0.08	0.84± 0.07	0.85± 0.04	0.85± 0.08	0.85± 0.07	0.84± 0.06	0.84± 0.05	0.84± 0.07
	γ	1.20± 0.04	1.31± 0.09	1.05± 0.07	1.28± 0.07	1.23± 0.05	1.41± 0.04*	1.12± 0.06	1.48± 0.07*	1.23± 0.03	1.38± 0.05*	1.12± 0.09	1.42± 0.1*
	Total	2.68± 0.21	2.83± 0.13	2.67± 0.20	2.80± 0.12	2.69± 0.10	3.02± 0.10*	2.71± 0.10	3.07± 0.11*	2.73± 0.10	3.17± 0.12*	2.72± 0.14	3.09± 0.1*
A/G Ratio		1.08± 0.16	1.06± 0.14	1.08± 0.14	1.08± 0.11	1.05± 0.19	1.02± 0.16	1.08± 0.16	1.09± 0.18	1.06± 0.17	1.06± 0.14	1.06± 0.14	1.07± 0.13

*Significant at $P < 0.05$.

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