The Journal of Animal & Plant Sciences, 27(4): 2017, Page: 1183-1189
ISSN: 1018-7081

PATHOGENICITY AND IMMUNOSUPPRESSIVE EFFECT OF DIFFERENT VACCINES OF INFECTIOUS BURSAL DISEASE VIRUS

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ABSTRACT

The current experiment was conducted to compare the efficacy of different of Infectious Bursal Disease (IBD) vaccines having different IBD virus strains against field virus. A total of two hundred, one-day old broiler chickens were divided into four groups (50 birds each group). Group A was subcutaneously vaccinated with immune complex vaccine at 1 day of age. The groups B and C were vaccinated with live IBD vaccines D78 and 228E at 20th and 16th day of age according to Deventer formula. The blood samples were taken randomly from five birds of each group at day 7, 14, 21, 28 and 35 to evaluate anti-IBDV antibody titer by ELISA. Five birds from each group were killed on days 14, 21, 28 and 35 to check bursa to body weight ratio, bursometery, gross and histopathological lesions scoring. Ten birds from each treatment group were challenged with virulent field IBDV on 28 day. On day 35 significantly ($P<0.05$) higher ELISA antibody titer was observed in group A vaccinated with immune complex vaccine. Bursa to body weight ratio of group A was significantly higher ($P<0.05$) as compared to other vaccinated groups. Mild to moderate histopathological lesions such as lymphocytic depletion, epithelial necrosis and mononuclear cells infiltration, fibrous tissue proliferation and edematous fluid were observed in vaccinated groups. The live and immune complex IBD vaccine induced adequate protection after challenged. The high mortality and morbidity observed in control group D1 in one week of challenge. The morbidity rate in group A1 and Group C1 was 90% and in group B1 was 70%. It concluded that conventional live IBD vaccines cause more severe damage in bursal follicles as compared to immune complex vaccine. Immune complex vaccine can be safer for day-old chick regardless of maternal derived antibody titers.

Key words: Infectious Bursal Disease, Vaccines, ELISA, Bursa to body weight ratio

INTRODUCTION

Infectious bursal disease (IBD) is an acute, highly contagious infection of young chickens (*Gallus gallus domesticus*), caused by infectious bursal disease Virus (IBDV), which primarily targets the lymphoid tissues of bursa of Fabricius (Kaufer and Weiss, 1980). The IBDV infection may cause immunosuppression and intensity of infection depends on strain of virus (Lukert and Saif, 2003). Most of young chickens are affected by this viral disease and it is important to poultry industry due to immunosuppression (Allan *et al.,* 1972) and increased condemnation rate at processing as a result of muscular haemorrhages. The IBDV actively replicates in B-lymphocytes that found in medullary region of bursa of Fabricius (BF) which its target. Viral replication is initially found in gut-associated lymphoid cells while BF is the site for secondary replication that is mainly responsible for high titers of virus and mortality in young chickens. The B-lymphocytes and other immune cell destruction through IBDV lead to immunosuppression (Khatri *et al.,* 2005).

The IBDV is very stable in the environment and difficult to be destroyed by the standard methods of sanitation and disinfection. Therefore, prevention of infection in chicken is based mainly on vaccination (Saif, 2004). There are two types of IBD vaccines; inactivated and live ones. Inactivated vaccine is used for vaccination of adult hens to protect the progeny at the first three weeks of age (Naqi *et al.,* 1983), while live vaccines are essentially intended for prevention of IBDV infection in young chickens. Live vaccinal strains of IBDV vary in virulence from mild, intermediate, intermediate-plus and hot and they use according to level of maternal derived antibodies (Tsukamoto *et al.,* 1995).

Both mild and intermediate live vaccinal strains of IBDV are neutralized by maternal antibodies (Winterfield *et al.,* 1972), but the intermediate strain vaccines are superior to mild vaccines as these confer better immunity in the presence of maternal antibodies (Mazariegos *et al.,* 1990). Timing of IBD vaccination is most important in broiler farming (van den Berg *et al.,* 2000) as it depends upon the level of maternally derived antibodies (MDA), the strain of vaccine and breakthrough titer (de Wit *et al.,* 1998).

Immune complexes (Icx) play important role in formation of memory B-lymphocytes. The immune complex vaccine developed by mixing live IBD virus with hyper-immune IBD serum (Jeurissen *et al.,* 1998).

At 1 day of age administered IBDV-BDA complex vaccine can induce active immunity and protection against a standard IBDV challenge of variable levels of maternal IBDV immunity (Haddad *et al.*, 1997). The present experiment evaluated the effect of various vaccinal strains of IBDV in terms of antibody titer, pathogenic effect on immune organs and protective efficacy against field isolate of IBDV in broilers.

MATERIALS AND METHODS

Experimental Design: The one day-old broiler chicks (n=200), obtained from Big Bird, Lahore, Pakistan, were reared on separate disinfected rooms. The birds were distributed in 4 groups with 50 birds in each. Three groups A, B and C were vaccinated with IBD vaccine while group D served as unvaccinated control. Group A was vaccinated with Bursaplex at one day of age, while group B and C were vaccinated with conventional IBD vaccines D78 and 228E at 20^{th} and 16^{th} day of age according to Deventer formula. The optimal vaccination time was estimated by the Deventer formula (de Wit *et al.*, 1998) in which titer of the bird (at sampling) represents a certain percentage of the flock (in this study, 75%).

Vaccination age={(log$_2$ titer bird%-log$_2$ breakthrough) x t½}+age at sampling+correction 0–4

On day 7, 14, 21, 28 and 35, five birds from each group were killed to collect blood and BF. The blood samples were collected from birds and allowed to clot at room temperature for separation of serum. The collected serum samples were then piped out and stored at -20^{o}c till further processing.

Relative Weight of the Bursa of Fabricius and Bursometry: Five birds from each treatment groups were randomly weighted and slaughtered on 7, 14, 21, 28 and 35 day of age. Bursa of Fabricius (BF) was immediately weighed after being removed. BF relative weight was calculated: Bursa of Fabricius weight divided by total bird body weight (BBW) multiplied by 1000 as described by (Debnath *et al*. 2005).

The diameter qualitative measurement of BF was carried out using a Bursometer (Fort Dodge Animal Health) that contains holes with different diameters to each score (Moraes *et al.*, 2004).

Determination of Antibody Titer: The serum samples were thawed and antibody levels against IBD virus were determined using a commercial ELISA kit (FlockChek IBD, IDEXX Laboratories) as described earlier (De Herdt *et al.*, 2005).

Histopathology: Five chickens, randomly selected from each group, were sacrificed on day14, 21, 28 and 35. Bursa of Fabricius tissues were processed, sectioned and stained with Hematoxylin and Eosin in Histopathology lab of Department of Pathology, University of Veterinary and Animal Sciences, Lahore, Pakistan. The bursal lesions were scored as described earlier (Muskett *et al.*, 1979).

Challenge Trial: On day 28, ten birds divided in (Group A1, Group B1, Group C1 and Group D1) were randomly separated from each group and challenged with a field isolate of IBD virus with dose rate of 0.1 ml (virus titer of $10^{5.5}$/100ulEID_{50}) through Eye drops (Abdel-Alim and Saif, 2001). Post challenged mortality and morbidity were recorded for up to one week.

Statistical Analysis: Data on ELISA antibody titers against IBD was analyzed statistically through analysis of variance and geometric mean titers and bursa to body weight ratio were compared through Duncan's Multiple Range test by using SAS (Statistical Analysis Software).

RESULTS

Mean weight gain of different treatment groups of broiler chicken are presented in Table-1. In different treatment groups, no significant difference was observed on day 7. On day 14, birds in the group C (Conventional vaccine 228E) showed significantly higher weight gain compared to other treated groups. On day 35, birds in the control group gained more weight compared to vaccinated groups A, B and C (Table 1). However, birds of the group C (Conventional vaccine 228E) showed low weight gain (p<0-05) compared to the groups A and B on days 28 and 35.

The geometric mean antibody titer (GMT) of different groups is presented in Table-2. On day 7, no significant difference was observed in GMT of broiler chicken in different treatment group. On day 14, 21, 28 and 35, group A (Immune complex Bursaplex) showed significantly higher GMT compared to vaccinated groups B and C. On day 21, 28 and 35 group D (Control, Unvaccinated) showed significantly low antibody titer compared to other vaccinated groups. Bursometry of different treatment groups of broiler chicken were determined weekly up to 35 days of age is presented in Table-3. On day 7, no significant difference was observed in bursometry of broiler chicken in different treatment groups. On 14, group B (Conventional vaccine D78) showed significantly higher bursal size compared to other treated groups A, C and D. On day 21, 28 and 35, group C (Conventional vaccine 228E) showed significantly lower bursal size as compared to vaccinated groups A (Bursaplex) and Group B (D78). Group A (Bursaplex) showed higher bursal size as compared to other vaccinated group B and C. The relative weights of BF of different treatment groups of broiler chicken were determined weekly up to 35 days of age as presented in Table-4. No ssignificant differences were found in bursa to body weight index of broiler chicken on day 7 in

different treatment groups. The group A (Immune complex Bursaplex) showed significantly lower weight of bursa on 14 day as comparison to others IBDV vaccinated group B and C. On day 21, 28 and 35 group C (Conventional vaccine 228E) showed significantly ($P<0.05$) lower bursal weight ratio in comparison to IBDV vaccinated groups A (Bursaplex) and group B (Conventional vaccine D78).On day 28 and 35 group D (Control, Unvaccinated) showed higher Bursa to Body Weight Ratio as compared to other treated groups A (Bursaplex), B (Conventional vaccine D78) and C (Conventional vaccine 228E).

Gross and histopathological lesions were scored in different treatment groups. The severe lesions were scored as 4, marked bursal lesion scored 3, moderate bursal lesions scored 2, mild bursal lesions scored 1, no bursal lesion scored as 0 (Table-5). On day 14, group A (Bursaplex) showed mild changes in bursa with swollen bursal fold. On day 21, group A showed mild hemorrhages on bursa. Group B showed mild bursal swelling and group C showed moderate changes with hemorrhages and edematous swelling and fluid present. Group D (unvaccinated) showed no changes in BF. On day 28, Group A showed mild changes with presences of bursal fluid. Group B showed moderate type of changes with bursal fold swelling and congestion. Group C showed marked congestion, bursal fold swelling along with blood accumulation and atrophy. Histopathological lesions are summarized in Table-6. At 14th day of age, group A showed mild lymphocytic depletion and epithelial necrosis in bursa (Fig. 1). Lymphocytic depletion, epithelial necrosis and mononuclear cells infiltration, fibrous tissue proliferation and edematous fluid were observed (Fig.1). Group C showed severe necrosis of epithelium and formation of cyst in epithelium. No histological lesions were observed in bursa of group D (control group) (Fig. 1 E).

Post-challenge mortality was observed up to 7 days (Table-7). Group A1 (Immune complex Bursaplex) and Group C1 (Conventional vaccine 228E) showed 10% mortality at the 1 week of post-challenge. Group B1 treated (Conventional vaccine D78) caused 30% mortality. Group D1(Unvaccinated) showed 60% mortality.

Table 1. Showing average weight gain (gm) of broiler chicken in different groups from day 7 to day 35 of age

Group	7th day	14day	21 day	28day	35 day
A (Bursaplex)	160 ±3.72	435^{c} ±3.53	780^{a} ±3.53	1290^{a} ±3.53	1722^{b} ±4.46
B (D78)	156±3.20	440^{c} ±3.53	720^{b} ±5.02	1210^{b} ±4.32	1690^{c} ±3.53
C (228E)	164 ±3.61	472^{a} ±4.46	690^{c} ±4.09	1150^{c} ±3.53	1620^{d} ±3.53
D (Control)	163 ±4.66	457^{b} ±6.15	772^{a} ±3.63	1295^{a} ±4.76	1754^{a} ±2.82

Values showing different superscripts within a column differ significantly ($p<0.05$)
Group A=Immune complex vaccine, Group B=Conventional vaccine (D78), Group C=Conventional vaccine (228E), Group D=Control (unvaccinated)

Table 2. Showing geometric mean ELISA antibody titer of different groups vaccinated with IBDV vaccines from day 7 to day 35 of age.

Groups	7 day	14 day	21 day	28 day	35 day
Group A	2354^{b}± 5.09	1238^{a}± 4.88	1733^{a}±4.66	1958^{a} ±2.98	2976^{a} ±4.24
Group B	2425^{a} ±3.41	953.2^{c}±2.66	1235.8^{c}±4.11	1675^{c} ±5.67	2107^{c}±3.00
Group C	1965.6^{d} ±4.26	981.6^{c}±3.86	1349.2^{b}±3.77	1919.8^{b} ±4.12	2841.8^{b} ±4.04
Group D	2289^{c} ±3.50	1047.4^{b}±4.11	342.4^{d}±3.96	229.2^{d}±2.87	208^{d} ±3.00

Values showing different superscripts within a column differ significantly ($P<0.05$).
Group A=Immune complex vaccine, Group B=Conventional vaccine (D78), Group C=Conventional vaccine (228E), Group D=Control (unvaccinated)

Table 3 Showing geometric mean bursometry in different groups from day 7 to day 35 of age.

Groups	7 day	14 day	21 day	28 day	35 day
A Group	3	5	6	5	6
B Group	3	6	5	4	4
C Group	3	4	4	3	3
D Group	3	5	6	6	7

Group A=Immune complex vaccine, Group B=Conventional vaccine (D78), Group C=Conventional vaccine (228E), Group D=Control (unvaccinated)

Table 4. Showing mean bursa to body weight ratio in different groups from day 7 to day 35 of age.

Groups	7 day	14 day	21 day	28 day	35 day
Group A	2.17^{a}±0.38	2.02^{a} ±0.50	1.72^{a} ±0.33	2.14^{a} ± 0.07	1.28^{a}± 0.04
Group B	1.99^{a}±0.05	2.57^{a}±0.08	2.16^{a}±0.04	1.01^{b}±0.02	0.67^{b}±0.03
Group C	1.88^{a}±0.03	2.34^{a}±0.04	1.68^{a}±0.03	0.75^{c}±0.04	0.62^{b}±0.04
Group D	1.95^{a}±0.03	1.84^{a}±0.04	2.05^{a}±0.03	2.26^{a}±0.03	1.32^{a}±0.03

Values showing different superscripts within a column differ significantly (P<0.05)
Group A=Immune complex vaccine, Group B=Conventional vaccine (D78), Group C=Conventional vaccine (228E), Group D=Control (unvaccinated)

Table 5. Gross Bursal lesions scores in bursa of Fabricius of various groups.

Days	Groups	Vaccine Strain	Hemorrhages	Hypertrophy	Swollen Bursal Folds	Fluid Presence
14th	A	Immune complex Vaccinev (Bursaplex)	0	0	1	1
day	B	D78	0	0	0	0
	C	228E	0	0	0	0
	D	Control	0	0	0	0
21st	A	Immune complex Vaccine (Bursaplex)	1	1	1	1
day	B	D78	1	1	0	0
	C	228E	3	2	3	3
	D	Control	0	0	0	0
28th	A	Immune Complex Vaccine (Bursaplex)	1	1	1	2
day	B	D78	2	1	2	1
	C	228E	3	1	3	3
	D	Control	0	0	0	0
35th	A	Immune Complex Vaccine	0	0	0	1
day	B	D78	0	0	1	1
	C	228E	2	1	1	2
	D	Control	0	0	0	0

0: no Lesion 1: Mild Lesion 2: Moderate Lesion 3: Marked Lesion 4: Severe Lesion
Group A=Immune complex vaccine (Bursaplex), Group B=Conventional vaccine (D78), Group C=Conventional vaccine (228E), Group D=Control (unvaccinated)

Table 6. Histopathological bursal lesions scores in bursa of Fabricius in various groups

Day	Group	Vaccine	LN	LD	EH	FT	CF	MP	BA	OF
14 day	A	Immune Complex Vaccine (Bursaplex)	0	1	1	0	0	1	0	1
	B	D78	0	0	0	0	0	0	0	0
	C	228E	0	0	0	0	0	0	0	0
	D	Control	0	0	0	0	0	0	0	0
21 day	A	Immune complex Vaccine (Bursaplex)	1	1	1	0	0	1	0	0
	B	D78	1	1	1	1	0	1	0	2
	C	228E	4	3	4	4	4	3	0	3
	D	Control	0	0	0	0	0	0	0	0
28 day	A	Immune complex Vaccine	2	1	1	1	1	2	0	1
	B	D78	2	2	2	2	1	2	0	2
	C	228E	4	3	4	3	4	4	3	0
	D	Control	0	0	0	0	0	0	0	0
35 day	A	Immune Complex Vaccine	1	1	1	0	1	2	1	1
	B	D78	2	2	2	2	1	2	2	2
	C	228E	3	3	3	3	4	4	3	1
	D	Control	0	0	0	0	0	0	0	0

LN=Lymphoid necrosis, LD=Lymphoid depletion, EH=Epithelial hyperplasia, FT, Fibrous tissue presence, CF. Cyst Formation, MP=Mononuclear cellular presence, BA=bursal atrophy, OF=oedematus fluid
Scoring: 0: No Lesion 1: Mild Lesion 2: Moderate Lesion 3: Marked Lesion 4: Severe Lesion
Group A=Immune complex vaccine (Bursaplex), Group B=Conventional vaccine (D78), Group C=Conventional vaccine (228E), Group D=Control (unvaccinated)

Table 7. Challenge protection trials of birds inoculated with different groups.

Groups/Days	1	2	3	4	5	6	7	Mortality	Morbidity
A1	-	-	-	-	1	-	-	10%	90%
B1	-	-	-	3	-	-	-	30%	70%
C1	-	-	-	1	-	-	-	10%	90%
D1	-	-	2	2	1	1	-	60%	40%

Group A1: Immune complex vaccine, Group B1: Conventional vaccine (D78), Group C1: Conventional vaccine (228E), Group D1: Control (Unvaccinated).

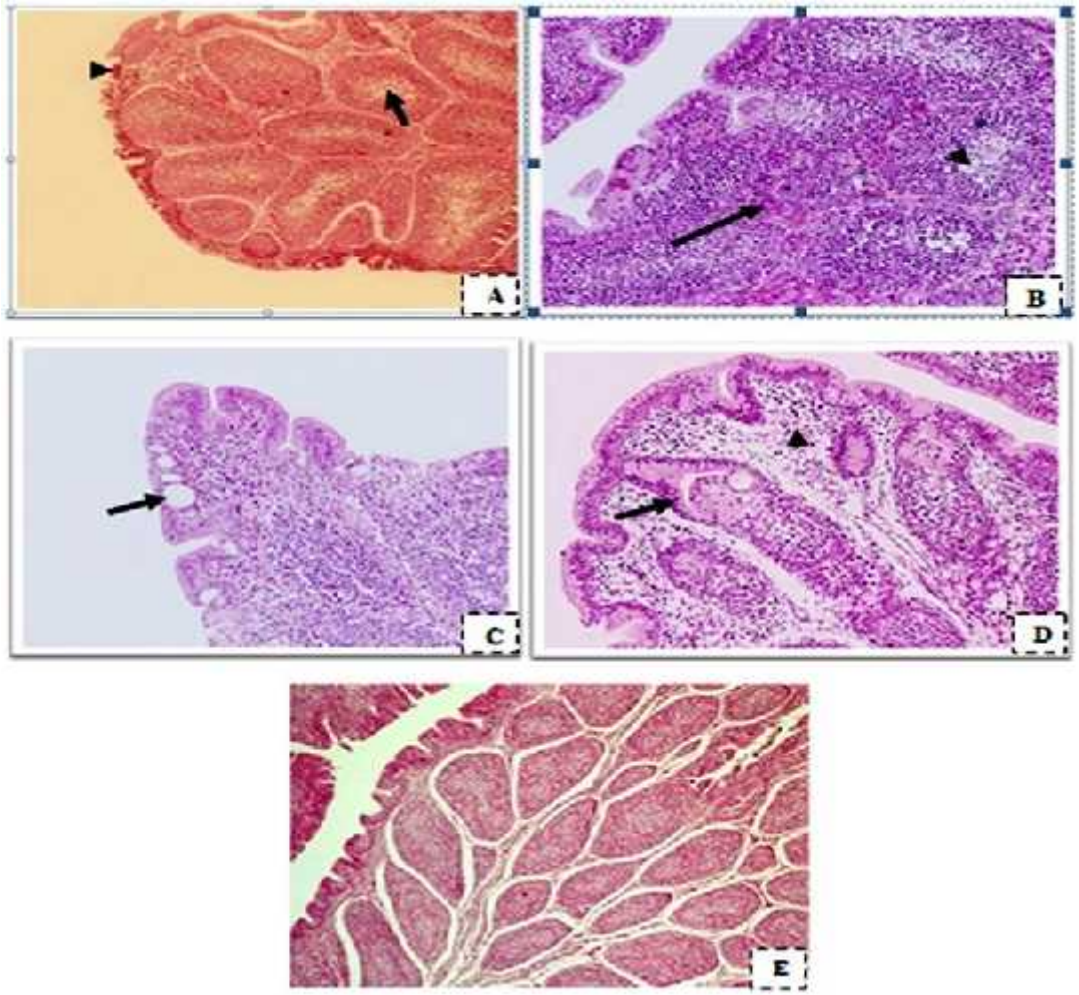

Fig. 1. Histopathology of Bursa of Fabricius showing A) Bursa from group A at 14th day of post-vaccination showing mild lymphocyte depletion (arrow) and epithelial necrosis (arrowhead)

B) Bursa from group B at 21day of post-vaccination showing moderate lymphoid depletion (arrowhead) with proliferating fibrous tissue (arrow) C) Bursa from group C at 28th day of post-vaccination showing epithelial cyst formation (arrow) D) Bursa from group C at 28th day of post-vaccination showing moderate to severe lymphoid depletion (arrowhead) with infoldings of epithelium and marked fibroplasias (arrow).E) Bursa from group D (Control unvaccinated) at 21day of age lymphoid follicle with normal epithelium.

DISCUSSION

This study demonstrated the optimal time for IBDV vaccination to obtain the protective immune response in chicken. It has been reported that high maternal derived antibodies (MDA) at the time of IBD vaccination in the field conditions interfere with vaccine efficacy by neutralizing the vaccinal virus (Rautenschlein *et al.*, 2007; van den Berg, 2000). The optimal time of vaccination for conventional vaccines to induce detectable humoral immunity was estimated by the

Deventer formula. On 21, 28 and 35day of post vaccination significantly low body weight gain was observed in group C vaccinated with 228-E. Similar observation was reported by Azhar *et al.,* (2012). The geometric mean titer was significantly higher (p<0-05) in the group A (vaccinated with Bursaplex Immunecomplex IBDV vaccine) compared to other vaccinated groups. The similar findings have also been observed in our previous report (Beenish *et al.,* 2015). The immune complex vaccine used in the present study has been developed by mixing live IBD virus with hyperimmune IBD serum. In our previous study, we used the same vaccine that demonstrated the immunomodulatory effect as IBDV replicated in bursa of Fabricius and damaged the immature B lymphocytes causing immunosuppression (Beenish *et al.,* 2015). The immune organ to body weight ratio and bursal diameter, estimation is also important indication for evaluating the efficiency of disease (Bolis *et al.,* 2003, Bennett *et al.,* 2006). In the present study, mean Bursa to body weight ratio and bursometry was performed weekly up to thirty fifth day of age. The higher bursal diameter was observed in live immune complex vaccinated group compared to other groups vaccinated with intermediate strains of IBD. These findings were matched with previous findings of Ayyub *et al.* (2003). On 7^{th}, 14^{th} and 21^{st} day post-vaccination, no significance difference was observed in different treatment groups. The group C (conventional vaccine 228E) showed significantly lower bursal weight compared to other vaccinated groups. The intermediate plus IBDV vaccines carry hot IBDV strain and adversely damage the immune organs and cause bursal regression as reported by Samanta *et al.* (2011). The present study indicates the immune-suppressive effect of hot strains of live intermediate vaccines in terms of bursal damage which is in agreement with findings of Boudaoud *et al.* (2008).

Gross and histopathological lesions were noticed in different treatment groups on 14, 21, 28 and 35 days of post vaccination. No specific gross lesions were observed before vaccination. Group A showed mild changes in bursa of Fabricius with swollen bursal fold and mild hemorrhages. The moderate changes were observed in group vaccinated with (Conventional IBD vaccine D78). This finding is closely related with the work of Ayyub *et al.* (2003) who reported moderate damaging effect of D78 strain of IBDV. The marked congestion of bursa, bursal folds swelling along with blood accumulation and bursal atrophy were observed in the group vaccinated with intermediate plus 228E strain. The similar findings of damaging and immune suppressive effect of virulent strain of IBD vaccines have been reported by various researchers (Van den Berg *et al.,* 2000; Al-Zubeedy, 2009). The post challenge mortality and morbidity in the control group was 60%. The Bursaplex and group vaccinated with 228E showed mortality of 10%. Similar observations were reported by Azhar *et al.,* (2012).

Conclusion: Conventional vaccines cause more severe damage in bursal follicles as compared to immune complex vaccine. Immune complex vaccine can be safer for day-old chick regardless of maternal derived antibody titers.

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