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Efficacy of some Infectious Bursal Disease (IBD) Vaccines Against Recently Isolated IBD Virus

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With the continuity of great losses in poultry farms due to the emergence of very virulent strains of IBDV in recent years combined with the failure of some conventional vaccines to provide full protection, the study of field vaccines efficacy is a mandatory. Therefore, this study was conducted to investigate the protective efficacy of two conventional hot intermediate live IBD vaccines (Xtreme IBD and Bursin plus) those have different sequence analysis in experimental trial challenged with recently well- identified field isolate. One hundred and fifty of one day old broiler chicks were allotted into 3 equal groups; 50 each. The first and the second groups vaccinated with IBD Xtreme vaccine, and Bursin plus" vaccine; respectively, and the third group left without vaccination as a control group. Each group received one dose per bird via oral route (oral drops) at the11 day old (age according to optimal date of vaccination whereas antibodies titer was very low Titer 636). All chicks received New Castle Disease (ND) vaccine at one day old and boostered at 14 days of age. Challenge test were applied on 10 birds from each group on 21 day old and another 10 birds from each group at 28 day old with recently isolated vvIBD. IBD Xtreme – vaccinated group showed significant higher mean IBDV antibody titer till 5 weeks of age (3 weeks post-vaccination) than Bursin plus-vaccinated group. However, at 4 week post- vaccination Bursin plus-vaccinated group showed significant increase the mean titer than that of IBD Xtreme -vaccinated group. RT-PCR results revealed that the vaccinated groups showed a good protection and less virus shedding than control birds.In addition, the virus persistence in the bursa of challenged birds of IBD Xtreme – vaccinated group was less than in Bursin-plus vaccinated one. The size of Bursae of control birds showed normal size with normal structure all over the experimental period. Bursin plus induced significant decrease in bursa/body weight ratio at 3 and 10 days post-vaccination. In the same time Xtreme vaccine has no negative significant effect on the bursa post-vaccination unless slight follicular changes. Challenged virus induced atrophy of the bursa from 3 day post-challenge to 7 day post-challenge in control group with severe macro and micro pathologic changes beside significant difference in B/Body weight ratio and index between the control vaccinated groups regardless the time of challenge either at 21 or at 28 day old. No significant difference between effect of Bursin plus and IBD Xtreme vaccines on bursa and or B/Bw ratio till 7 days post challenge, however, Bursin plus vaccinated group showed sever macro and microscopic lesions and atrophy especially in birds challenged at 21 day old. ND antibody titer was declined post-IBD-vaccination than control non-vaccinated one from first week post-vaccination. At second week post-IBD vaccination the mean ND titer was significant decrease in Xtreme -vaccinated group than Bursin plus –vaccinated group. It could be concluded that both vaccine provided 100 % ptotrction aginst the recently isolated vvIBDV, however, the IBD- Xtreme vaccine was more protective, potent and safer than Bursin plus vaccine.

Keywords: vvIBD. IBD Xtreme, vaccinated, Infectious Bursal Disease Virus, complex evolution

INTRODUCTION

Infectious Bursal Disease Virus (IBDV) or "Gumboro disease belongs to the *Birnaviridae* Family of the genus *Avibirnavirus*,

is one of the most important immunosuppressive agents in modern poultry production (Eterradossi et al.,2013). IBDV continues to be a major threat to commercial poultry all over the world since the first discovery of classical IBDV strains in the USA, and complex evolution of the virus has taken place (Cosgrove An apparently new disease of chickens: avian nephrosis. *Avian Dis*. 1962). Infection with IBDV may induce a temporary or permanent destruction of the bursa cloacalis and other lymphoid tissues depending on the virulence of the IBDV strain, the age at the time of infection, the presence of IBDV antibodies and the genetic background of the infected chicken (Kim et al., 1999).

Two serotypes of the virus have been described until now. Serotype 1 IBDVs cause clinical signs and they are classified as mild, intermediate, and intermediate plus, classical virulent and very virulent strains **(**Van den Berg, 2000**).**The Virus is highly resistant to physical and chemical inactivation (Benton et al., 1967). Therefore, despite strict hygienic measures, vaccination is mandatory to protect chickens against infection by using inactivated and live vaccines. Usually, the breeder layers are vaccinated with a killed-virus vaccine and chickens after hatching are immunized with a live vaccine (Müller et al., 2012). Compared with the inactivated vaccines, live vaccines have many advantages, including being lower cost, easier to administer, and able to be mass administered by drinking water, aerosol spray, and so on (Marangon et al.,2007).

Despite vector vaccines was used since last years, but many honors can't use these types of vaccines due many reasons as their costs and others. So, most commercially available conventional live IBDV vaccines is still used in most chicken flocks. Those classified as "mild" vaccines exhibit only poor efficacy in the presence of certain levels of maternally derived antibodies and against vvIBDV. "Intermediate" and "intermediate plus" or "hot" vaccines have a much better efficacy and may break through higher levels of maternally derived antibodies, but they can induce moderate to severe bursal lesions and, thus, cause corresponding levels of immunosuppression(Mazariegos et al.,1990,Tsukamoto et al.,1995,Kumar et al.,2000 and Rautenschlein et al.,2005). It has been shown that the timing of IBD vaccine administration in broiler progeny is pivotal (Ather, 1993). The optimal vaccination time depends upon the maternally derived antibody (MDA) level of the broiler chicks, the vaccine strain to be used, its breakthrough titre, and the field pressure (de Wit, J.J. 1998.,de Wit , J.J . 2001 and Alam et al.,2002).The optimal timing is often predicted based on serological data following detection of IBDV MDA by an enzyme-linked immunosorbent assay (ELISA) during the first week post hatch (Kouwenhoven et al.,1992 and Kouwenhoven and van den Bos ,1994)**.**Vaccinated chickens may not fully protect chickens against infection by the vvIBDV strains or by antigenic variants(Pitcovski et al.,2003).

Due to the emergence of very virulent strains of IBDV in recent years, some conventional vaccines have been reported less effective (Rautenschlein et al., 2005). It is, therefore, urgent to study the efficacy of currently available vaccines against IBD. Safety and efficacy of these types of vaccines still remain a major concern due to many genetic differences between the vaccines used and circulating vvIBD field virus. To be able to evaluate vaccine efficacy in commercial broilers, parameters such as intrabursal IBDV-antigen load should also be considered in conjunction with bursa lesion scores (TamiruNegashAlkie and SilkeRautenschlein.2016).This study is conducted to evaluate the protective efficacy of two different intermediate plus IBD vaccines; Avipro® IBD Xtreme (V 217 – Winterfield 2512 strain) and Intermediate Plus vaccine against recently field isolated vvIBDV.

MATERIALS AND METHODS

Local variant isolate of Infectious Bursal Disease Virus (IBDV):

Local isolate was in form of infectious allantoic fluid; isolated from field broiler cases in Giza government Egypt (2016) and identified by PCR and sequence analysis (Boot et al.,1999 and Islam, et al.,2001). This virus was propagated in SPF-chicks for several serial passages. Its infectivity titer in SPF ECE was calculated to $10^{3.5}$ EID_{50} /ml for challenge test and the titer of the virus were calculated according to (Reed,L.JandMuench,H. 1937).

Investigational products:

Avipro® IBD Xtreme: (Batch no. G012611, Exp. Date 24.01.18) given to chicks via eye drop at 11day of age with the recommended dose of the manufacture.

Bursin plus: (Batch no. 152781, Exp. Date 16NOV18) given to chicks via eye drop at 11days

of age with the recommended dose of the manufacture.

Polybanco: (Batch no. IB/ND30191. Exp. Date 30.3.18) given to chicks via eye drop at 1 and 14 days of age with the recommended dose of the manufacture.

Experimental design:

One hundred and fifty of one day old broiler chicks were housed in separated rooms provided with commercial balanced feed. Chicks were divided into 3 groups (50 chicks /each group) as following**:**

Group 1; chicks were left as blank control without vaccination, group2; chicks were vaccinated with "Intermediate Bursin Plus"vaccine and group3; chicks were vaccinated with Avipro® IBD Xtreme vaccine. Each group received one dose per bird via oral route (oral drops) at the11 day old (age according to optimal date of vaccination by BioChekwhereas antibodies titer at DOC – Breakthrough Titer 636). All birds were vaccinated against ND and IB using Polybanco at 1 and 14 day old via eye drop.

Challenge test were applied on 20 birds from each group;10 birds were challenged at 21 days old and the other 10 birds were challenged at 28 day old with vvIBDV.

Bursa/Body Weight Ratio and Bursal Lesion Scoring

Clinical signs, BB ratio, bursal size by caliber and physical bursal lesion score (visual) were done at 3,7,10 days on 3 birds from each group.
Blood sampling for serum analysis was taken (10 birds from each group) weekly post-vaccination and / or post-challenge, for estimation of IBD specific antibodies with the BioChek ELISA Test. The blood samples were used to estimate ND titer using HI test to measure immune state.

Bursa of Fabricius sampling/examination:At 3, 7, and10 days post vaccination and challenge 3 birds per group will be euthanized and the bursaeare collected. The bursae were placed in a plastic container (two containers with 3 bursas per group). One container includes part of these bursae for RT-PCR examination and the other container include the other part of these bursae immersed in 10% buffered formalin solution for histopathological examination.

Examination of histopathological lesions of the bursal tissue (bursal scoring) will be carried out according to European Pharmacopoeia monograph (bursal scoring from 0 to 5) in a local laboratory (2 room x 3 bursas per room).

BB ratio = [bursa weight (g)/body weight (g)] × 1000

Bursa-body (BB) index was calculated according to the following formula: BB index = BB ratio of infected (or vaccinated) birds/ BB ratio of the controls **(**Raji et al., 2017)

Detection of IBDV by RT-PCR and sequencing of the VP2-region

RNAs were extracted from pools of bursae using ***QIAamp Viral RNA Mini Kit(Qiagen, Valencia, Calif., USA).*** Cat. No.52904. the procedure was performed according to the company's instruction.A set of primers were used:

Forward primer: [AUS GU: 50-TCA CCG TCC TCA GCTTAC CCA CAT C-30].Reverse primer: [AUS GL: 50-GGA TTT GGG ATC AGCTCG AAG TTG C-30](Boot et al.,1999 and Islam, et al.,2001).

Serology

IBDV antibodies were detected in collected serum samples using a commercially available BioChek ELISA Kits and titres were calculated as described by the manufacturer.

NDV Antibody Titers Measure

To determine the extent of interference from ND antibody production by IBDV vaccine types, all birds were vaccinated against ND and IB using Polybanco at 1 and 14 day old via eye drop. Serum samples were collected at one, 11, 18, and 25 days of age. Sera were examined for hemagglutination (HA)-specific antibodies by HA inhibition (HI) test using NDV antigen.

Production Performance

Weight gain, growth rate, and feed conversion ratio (FCR) were calculated in two flocks to determine the production performance. FCR is a measure of a chicken's efficiency in converting feed mass into increased muscle(24).

Statistical analysis:

Data were collected, summarized and then analyzed by using SPSS, 16.0. One way analysis of variance (ANOVA) test was used to test difference between treated group and vaccinated groups, multiple comparisons test between different studied groups were done using LSD test.

1 tacaccataactgcagccgatgattaccaattctcatcacagtaccaatcaggtggggta
61 acaatcacactgttctcagccaacattgatgccatcacaagcctcagcgttgggggaggg
121 ctcgtgttccaaacaagcgtccaagaccttgtactgggcgccaccatctaccttataggc
181 tttgatgggactacggtgacaaccagagctgtggctgcaaacaatgggctgacggccggc
241 accgacaatcctattccattcaatcttgtgtttccgaccaacgagataacccagccaatc
301 acatccattaaactggagatagtgacctccaaaagtggtggtcaggctggggatcagatg
361 tcatggtcggcaagtgggagcctagcagtgacaatccatggtggcaactatccaggggcc
421 ctccgtcccgtcacacta

Figure 3a:Partial sequence of VP2 of bursinpluse vaccine.

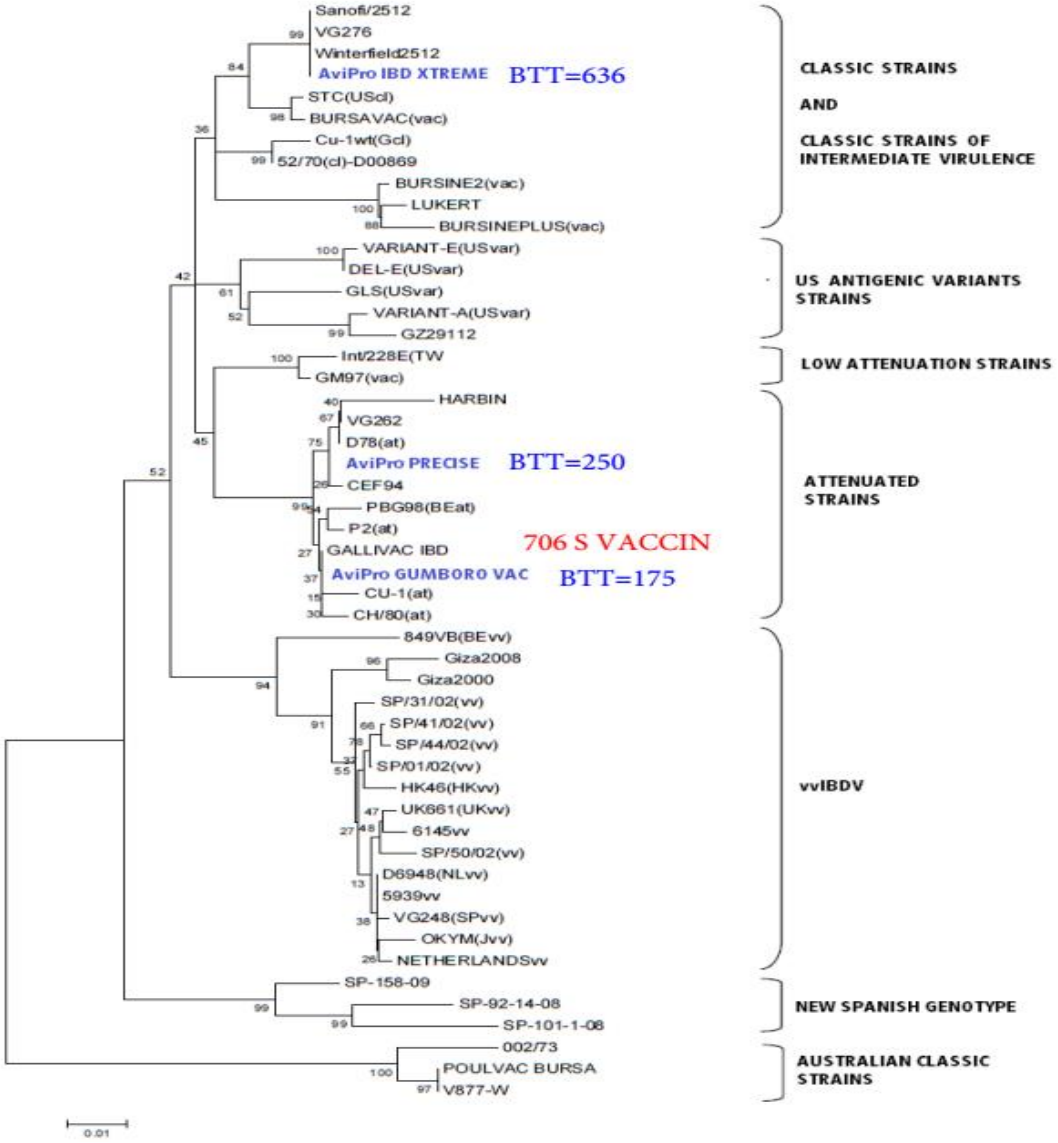
ACAGGCCCAGAGTCTACACCATAACTGCAGCCGATGATTACCAATTCTTATCACAGTACCAACCAG
GTGGGGTAACAATCACACTGTTCTCAGCCAACATTGATGCTATCACAAGCCTCAGCGTTGGGGGA
GAGCTCGTGTTTCAAACAAGCGTTCAAGGCCTTGTACTGGGCGCCACCATCTACCTTATAGGCTTT
GATGGGACTACGGTAATCACCAGGGCTGTGGCCGCAGACAATGGGCTGACGGCCGGCACCGACA
ATCTTATGCCATTCAATATTGTGATTCCAACCAACGAGATAACCCAGCCAATTACATCCATCAAACT
GGAGATAGTGACCTCCAAAAGTGGTGGCCAGGCAGGGGACCAGATGTCATGGTCGGCAAGTGGG
AGCCTAGCAGTGACGATCCATGGTGGCAACTATCCAGGGGCCCTCCGTCCCGTCACACTAGTAGC
CTACGAAAGAGTGGCAACAGGAT

Figure 3b :Partial sequence of VP2 of AVIPRO IBD Xetrem vaccine

GenBank: KX827588.1

1 tcacgctataagggttgacccaatattggtagccacttgtgacagcagtgacaggaccga
61 aatctacaccataactgcagccgatgattaccacttcttatcacagtaccaaccaggtgg
121 ggtaacaatcacactgttctcagccaacattgatgctatcacaagcctcagcgttggggg
181 agagctcgtgtttcaaacaagcgttcaaggccttgtactgggcgccaccatctaccttat
241 aggctttgatgggactacggtaatcaccagggctgtggccgcagacaatgggctgacggc
301 cggcaccgacaatcttatgccattcaatattgtgattccaaccaacgagataacccagcc
361 aattacatccatcaaactggagatagtgacctccaaaagtggtggccaggcaggggacca
421 gatgtcatggtcggcaagtgggagcctagcagtgacgatccatggtggcaactatccagg
481 ggccctccgtcccgtcacactagtagcctacgaaagagtggcaacaggatccgtcgttac
541 ggtcgctggggtgagcaacatcgagctgatcccagatcc

Figure 3c:Partial sequence of VP2 of vvIBD field isolate (2017)



RESULTS AND DISCUSSION

Intermediate" and "intermediate plus" or "hot" IBD vaccines have a much better efficacy and may break through higher levels of maternally derived antibodies, but they can induce moderate to severe bursal lesions and, thus, cause corresponding levels of immunosuppression (Al-Mufarrej . 2014,Mazariegos et al.,1990 and Tsukamoto et al.,1995). They may not fully protect chickens against infection by the vvIBDV strains (Kumar et al.,2000) or by antigenic variants. Safety and efficacy of this type of vaccine still remain a major concern.

Therefore, this study aimed to investigate the protective efficacy of two commonly used hot intermediate live IBD vaccines (Xtreme IBD and Bursin plus) against recently isolated vvIBDV that caused severe losses in our poultry farms.

Detection of IBDV Antibody Titers

Many earlier studies indicated the presence of interference between high maternally derived antibodies (MDA)at a time of vaccination with the vaccine used (Rautenschlein et al.,2005 and Alam et al.,2002),therefore the estimation of optimal time of vaccination is important(Moraes et al.,2005 and Hermann et al.,2010**).**In our study

the MAD levels in one day old chicks were estimated using ELISA kit and according to the level of the MAD, the chicks were vaccinated at 11 days of age by oral routs based on the breakthrough Titer 636for Xtreme vaccine and breakthrough Titer 500 for Bursine plus vaccine.

All vaccinated birds show sero convesion from the first week post vaccination. It was noticed that Xtreme vaccinated group showed significant higher mean titer until 3 weeks post-vaccination than Bursin plus-vaccinated group. However, at 4 week post- vaccination Bursin plus-vaccinated group showed significant increase the mean titer than that of Xtreme vaccinated group (Table 1a).

When the birds challenge at 21 days of age or at 28 days of age, a gradual increasing of the mean antibody titer in non-vaccinated control groups was observed. While at one week post- challenge, vaccinated groups showed slight decrease in antibody mean titer if compared with those vaccinated non-challenged groups at the same age. (Tables 1a,1b&1c). These decreases in antibody titre in challenged groups could be due to neutralization of the antibody with the field challenge virus in these vaccinated groups.

Table (1a) ELISA Results of IBD antibody titreresults in broiler chicks vaccinated orally with IBD Xtreme vaccine or Bursin plus vaccine.

	GMT					
Age of birds	**Non-vaccinated control**	**CV%**	**Extreme**	**CV%**	**Bursin plus**	**CV%**
One day old	1728 ±0.53	22	1728 ± 0.53	22	1728 ± 0.53	22
7 day old	1260±0.37	19	1260±0.37	19	1260±0.37	19
11 day old (time of vaccination)	862±0.75	32	862±0.75	32	862±0.75	32
18 day old (One week post-vaccination)	463±0.63	22	1063±0.93	13	1028±0.87	31
25 day old (Two weeks post-vaccination)	209±0.43	29	2616±0.73*[a]	19	2420±0.53*[b]	41
32 day old (Three weeks post- vaccination)	158±0.99	33	6772±0.37*[a]	26	6390±0.61*[b]	25
39 day old (Four weeks post vaccination)	72±0.0	20	11197±0.61*[b]	27	11503±0.75*[a]	28

* Significant difference at p≤0.05.

[a]highly significant. [b]less significant.

Table (1b) ELISA results in broiler chicks vaccinated with IBD Xtreme vaccine or Bursin plus vaccine and challenged orally at 21 days of age with vvIBDV.

	GMT					
Age of birds	**Non-vaccinated control**	**CV%**	**Extreme**	**CV%**	**Bursin plus**	**CV%**
21 day old (day of challenge	387± 0.85	32	1221 ± 0.89*[a]	20	1190 ± 0.53*[b]	30
28 day old (one week post-challenge)	1117±0.91	29	2209±0.77*	24	2206±0.37*	26
35 day old (two weeks post-challenge)	3061±0.00	27	4270±0.51*	21	4389±0.75*	28

* Significant difference at p≤0.05.

[a]highly significant. [b]less significant.

Table (1c) ELISA Results of IBD antibody titreresults in broiler chicks vaccinated with either IBDXtreme vaccine or Bursin plus vaccine and challenged orally at 28 days of age with vvIBDV.

	GMT					
Age of birds	**Non-vaccinated control**	**CV%**	**Extreme**	**CV%**	**Bursin plus**	**CV%**
28 day old (day of challenge	197±0.91	35	4720 ± 0.41	20	1190 ± 0.71	29
35 day old (one week post-challenge)	2100±0.91	27	10666±0.73*[a]	25	7206±0.53*[b]	29
42 day old (two weeks post-challenge)	3361±0.80	19	13011±0.54*[a]	29	10370±0.63*[b]	31

*Significant difference at p≤0.05.

[a]highly significant. [b]less significant.

Protective Efficacy against vvIBDV Challenge

Results of bursal body weight ratio, index and lesion scoring in chicks vaccinated and non-vaccinated group are shown in table 2.

Our study revealed that no changed in the bursa of Fabricuis of control non-vaccinated groups were detected all over the experimental period. In Bursine plus vaccinated group, a decrease in B/bw ratio and index were observed at 3 and 10 days postvaccination, on the other hand no changed in the bursa were observed in Xtreme vaccinated group unless slight follicular changes.In a Previous study on 'intermediate' strain and 'intermediate plus' strain (Boudaoud A, Alloui N. 2008), reported that the two vaccine strains were not of equal pathogenicity and that one was potentially immunosuppressive. The 'intermediate plus' strain proved to be more virulent than the 'intermediate' strain, as it caused greater atrophy and more severe lesions of the bursa of Fabricius. By exerting a more suppressive effect on the serological response of the chicks, the 'intermediate plus' vaccine also proved to be immunosuppressive.

To determine the protective efficacy, group of vaccinated chickens were separated into two subgroups in different rooms, one subgroup was intraocular challenged at 21 days of age and the other subgroup was challenged at 28 days of age. The two different age of challenge were selected based on age of chicken susceptibility to IBDV infectionat 3- 6 weeks (Hebata Allah Mahgoub.2012).

The results of the protective efficacy of both vaccines against challenge with recently field isolate of vvIBDV are shown in table 3.In this study, no mortality were recorded in vaccinated groups wither with Xtreme vaccine or Bursine plus vaccine, while 30% mortality (3/10) were recorded at 7 days postchallenge in non-vaccinated groups that challenged at 21 days of age. In addition, 20% (2/10) and 12.5% mortality were recorded at 7 and 10 days respectively when the birds challenged at 28 days of age (Table 3).

Severe bursa atrophy were observed in control non-vaccinated groups at 3 and 7 days post challenge with severe macro and microscopic lesions in both groups either challenged at 21 days or 28 days of age.

Table 2: Bursal body weight ratio, index and lesion scoring in chicks vaccinated by Bursin plus vaccine or Xterme vaccine by oral route at11 days of age.

Time (day post vacc.)	Group	Size (caliber)	BB ratio	BB index	Lesion score
3 dpv	Group 1	3.75	3.03	-	0
	Group 2	3.5	2.59*	0.86	±
	Group 3	3.5	2.95	0.98	+
7 dpv	Group 1	3.25	2.87	-	0
	Group 2	3.5	2.79	0.98	++(petech. Hg.)
	Group 3	3.75	2.64	0.86	+±
10 dpv	Group 1	4.0	2.72	-	0
	Group 2	4.75	2.18*	0.8	0
	Group 3	4.25	2.81	1.03	0

*

Significant difference at $p \leq 0.05$.

[a]highly significant. [b]less significant.

Group1: Non vaccinated control birds

Group 2: Vaccinated with Bursin plus vaccine orally at 11 days of age.

Group3: Vaccinated with xtreme vaccine orally at 11 days of age.

Table 3 :Bursal body weight ratio, index and lesion scoring in chicks vaccinated by Bursin plus vaccine or Xterme vaccine by oral route at 11 days of age and challenged with vvIBDV at 21 days old or 28 day old.

	Birds challenged at 21 days of age						Birds challenged at 28 days of age				
Time (day post challenge.)	Group	BB ratio	BB index	Size	Lesion score	Mortality	BB ratio	BB index	Size	Lesion score	Mortality
3 dpc	Group 1	1.5	-	5	-	-	1.3	-	5	Bursal edema	-
	Group 2	2.86*	1.9	7*	-	-	1.75*	0.76	6	-	-
	Group 3	2.89*	1.93	6.5*	-	-	1.3	0.56	7	-	-
5 dpc	Group 1	1.05	-	4	Petech. Hg+++	3/10	1.69	-	4	Hg.,gelat. Exudate +++	2/10
	Group 2	3.7*	3.5	6*	+	-	1.456	0.7	5	Hg.,gelat. Exudate +++	-
	Group 3	3.6*	3.43	7*	+	-	1.6	0.8	6	Hg. +*	-
7 dpc	Group 1	1.25	-	4	Hg.+++	-	0.88	-	4	Hg. On ms., Nephrosis Hem. bursa +++	1/8
	Group 2	1.45	1.16	4.5	Edema ++	-	1.6*	1.8	7	Nephrosis Edematous gelat. Bursa +++	-
	Group 3	2.5*	2	7*	Gelatin. Exudate++	-	1.6*	1.68	6.5	++	-

When the birds challenged at 21 days of age, no significant difference observed in the B/bw ratio or index between both vaccinated groups, however, the B/bw ratio was higher in the Xtreme vaccinated group at 10 days post challenge compared with Bursine plus vaccinated group. This results in agreement with (Jung , A. 2006.) who found that broilers that been vaccinated with intermediate strains were protected against mortality, morbidity and the development of severe bursa lesions in compared with non-vaccinated broilers, in which 100% of the birds challenged with very virulent IBDV had developed score lesion ≥ 3.

Histopathological examination of Bursa of Fabricuis:

Microscopically, bursa of fabricuis (BF) of blank control chickens revealed no histopathological changes (Fig.1a). However, BF of chickens vaccinated with Xtreme vaccine (3, 7 and 10 days post vaccination) revealed lymphoblasts activation in both cortex and medulla of bursal lymphoid follicles (Fig. 1b & c). On the other hand, BF of chickens vaccinated with Bursin+ (3 days post vaccinations) revealed normal bursal lymphoid follicles. At 7 and 10 days post vaccination with Bursin+, examined sections revealed modereate lymphoblasts activation in both cortex and medulla of bursal lymphoid follicles (Fig. 1 d). After challenge with IBD virus at 21 days of age, BF of Xtreme vaccinated chichens (3 days PC) revealed no histopathological changes except slight lymphocytic necrosis in some lymphoid follicles (Fig. 1e). At 7 days PC, the examined sections revealed interfollicular oedema associated with inflammatory cells infiltration and fine strands of interfollicular fibrous connective tissue proliferation (Fig. 1f). Moreover, at 10 days PC, most examined sections from this group showed slight lymphocytic necrosis and depletion specially at the medulla associated with interfollicular inflammatory cells infiltration and fine strands of interfollicular fibrous connective tissue proliferation (Fig. 1g). Concerning the group of chickens vaccinated with Busin+, at 3and 7 days Post challenge at 21 days of age, the examined sections showed lymphocytic necrosis and depletion, interfollicular oedema associated with massive inflammatory cells infiltration (Fig. 1h). Additionally, at 10 days PC, examined sections showed small atrophied some follicles with interfollicular oedema, heterophiles infiltration and fine strands of fibroblasts proliferation (Fig. 1i). In contrary, unvaccinated and challenged chickens (at 3 and 5 days post first challenge) showed severe histopathological alterations which decribed as marked lymphocytic necrosis and depletion, marked interfollicular oedema associatd with massive inflammatory cells infiltration specially heterophiles (Fig. 2a). additionally, at 7 days PC, examined sections revealed atrophy of lymphoid follicles associated with marked interfollicular fibrous connective tissue proliferation (Fig. 2b).

Regarding the challenge with IBD virus 28 days of age, BF from the group of chickens vaccinated with Xtreme vaccine (3days PC) revealed lymphocytic necrosis and depletion in some lymphoid follicles (Fig. 2c). However, at 7 and 10 days PC, examined bursae showed moderate changes confined as moderate lymphocytic necrosis and depletion as well as interfollicular fibroplasia and inflammatory cells infiltration (Fig. 2d). Meanwhile, BF of chickens vaccinated with Bursin+ (3 days PC) showed marked interfollicular oedema and massive inflammatory cells infiltration (Fig. 2e). Moreover, at 7 and 10 days PC, examined sections showed lymphocytic necrosis, depletion, follicular atrophy and interfollucular fibroblasts proliferation (Fig. 2f). Severe histopathological changes were noticed in examined section from control, unvaccinated chickens. At 3 days Pc, the BF showed severe necrosis in both cortex and medulla of lymphoid follicles as well as marked oedema and massive interfollicular inflammatory cells infiltration (Fig. 2g). Moreover, at 7 days PC, marked necrosis and atrophy of lymphoid follicles associated with fibroblasts proliferation and massive interfollicular inflammatory cells infiltration (Fig. 2h) were noticed in all examined sections. Marked lymphoid necrosis and atrophy as well as marked interfollicular fibrous connective tissue proliferation were also recorded in sections at 10 days PC (Fig. 2i).

Detection of IBDV by RT-PCR and sequencing of the VP2-region

In a previous research study (Abdel-Alim and Saif. 2001**)** it was found that, in commercial one day old broiler chicks, the bursa derived virus were detected only at 7 and 14 days post inoculation when inoculated ay a high dose 104 EID50/birds, whereas the virus was detected only at 14 days PI when inoculated at low dose 103 EID50/birds, therefore in our study the bursal samples were collected at 3, 7 and 10 days post challenge for detection of the virus persistence by RT-PCR.

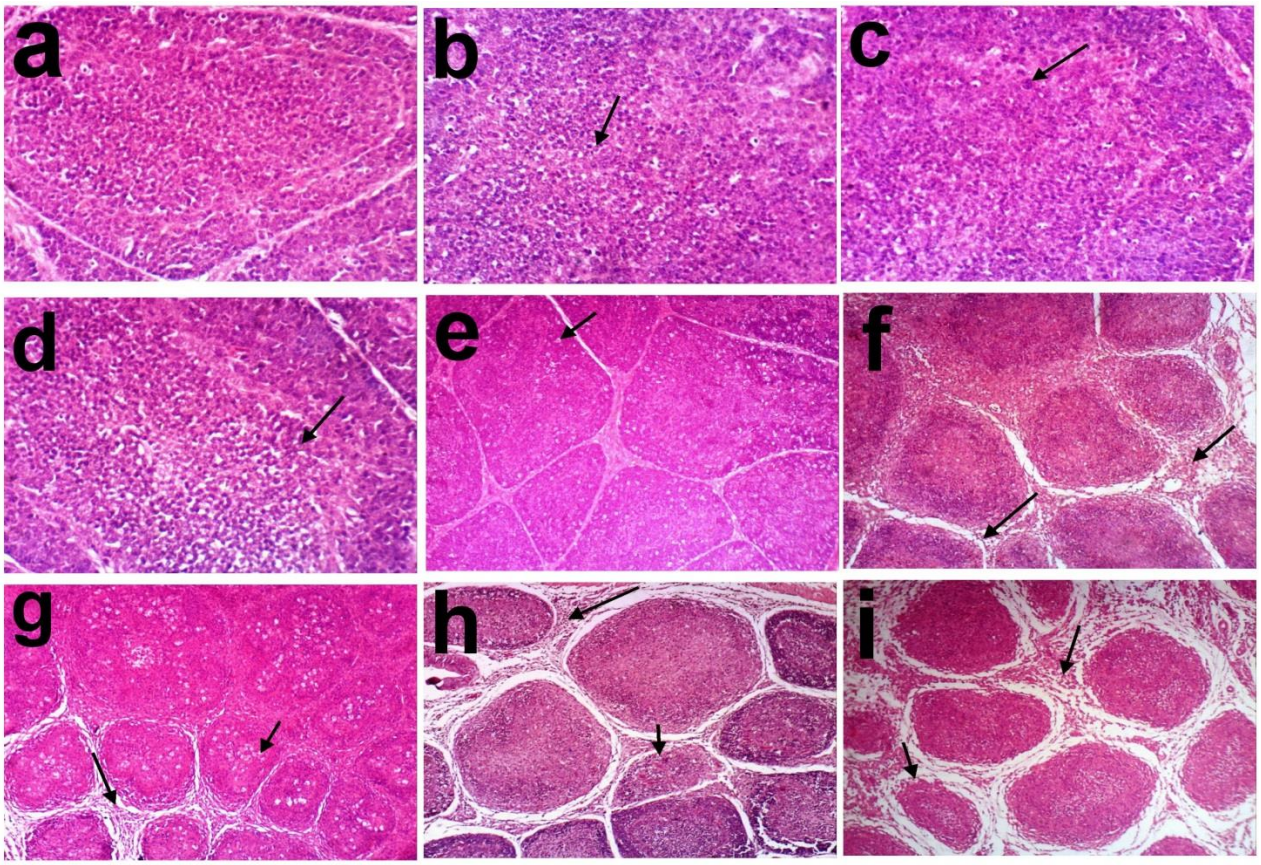


Figure 1: Bursa of fabricius of chicken **a)** Blank control showing no histopathological changes (H & E X 400). **b& c)** vaccinated with xtreme vaccine (3and 7 days post vaccination) showing lymphoblasts activation in both cortex and medulla of bursal lymphoid follicles(H & E X 400). **d)**vaccinated with Bursin+ (7 days post vaccination) showing modereatelymphoblasts activation in both cortex and medulla of bursal lymphoid follicles (H & E X 400). **e)** xtreme vaccinated and challenges at 21 days (3 days PC) showing slight lymphocytic necrosis in some lymphoid follicles (H & E X 100). **f)** xtreme vaccinated and challenges at 21 days (5 days PC) showing interfollicularoedema associated with inflammatory cells infiltration and fine strands of interfollicular fibrous connective tissue proliferation (H & E X 100). **g)** xtreme e vaccinated and challenges at 21 days (7 days PC) showing slight lymphocytic necrosis and depletion with fine strands of interfollicular fibrous connective tissue proliferation (H & E X 100). **h)**Bursin + vaccinated and challenges at 21 days (5 days PC) showing lymphocytic necrosis and depletion, interfollicularoedema associated with massive inflammatory cells infiltration (H & E X 100**). i)**Bursin + vaccinated and challenges at 21 days (7 days PC) showing small atrophied some follicles with interfollicularoedema, heterophiles infiltration and fine strands of fibroblasts proliferation (H & E X 100).

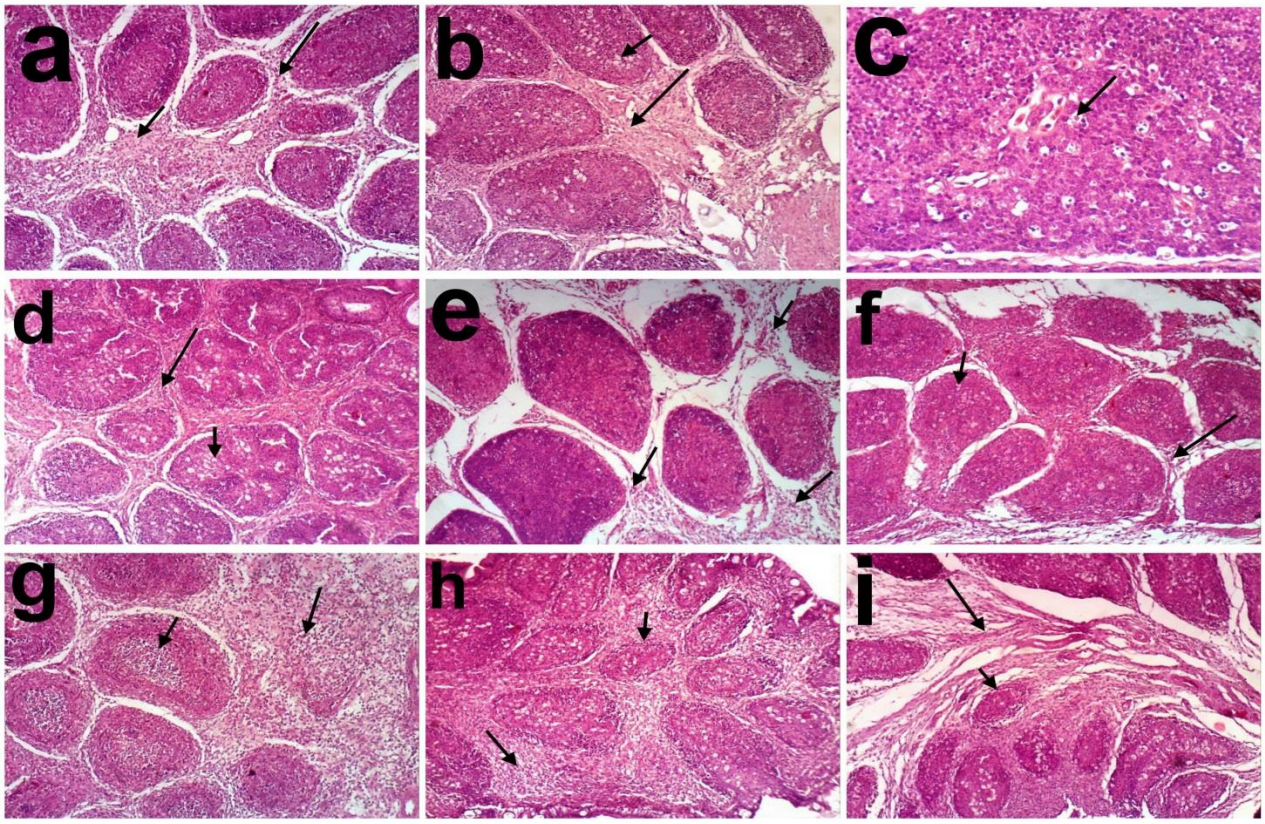


Figure 2: Bursa of fabricius of chicken **a)** unvaccinated and challenged chicken at 21 days (3 days PC) showed marked lymphocytic necrosis and depletion, marked interfollicularoedemaassociatd with massive inflammatory cells infiltration (H & E X 100). **b)**unvaccinated and challenged chicken at 21 days (7 days PC) showed atrophy of lymphoid follicles associated with marked interfollicular fibrous connective tissue proliferation (H & E X 100). **c)** xtreme vaccinated and challenges at 28 days (3 days PC) showing lymphocytic necrosis and depletion in some lymphoid follicles (H & E X 100). **d)** Extreme vaccinated and challenges at 28 days (7 days PC) showing moderate lymphocytic necrosis and depletion as well as interfollicular fibroplasia and inflammatory cells infiltration (H & E X 100). **e)**Bursin+ vaccinated and challenges at 28 days (3 days PC) showing marked interfollicularoedema and massive inflammatory cells infiltration (H & E X 100). **f)**Bursin+ vaccinated and challenges at 28 days (7 days PC) showing lymphocytic necrosis, depletion, follicular atrophy and interfollucular fibroblasts proliferation (H & E X 100). **g)** unvaccinated and challenged at 28 days (3 days PC) showing severe necrosis in both cortex and medulla of lymphoid follicles, marked oedema and massive interfollicular inflammatory cells infiltration (H & E X 100). **h)** unvaccinated and challenged at 28 days (5 days PC) showing marked necrosis and atrophy of lymphoid follicles associated with fibroblasts proliferation and massive interfollicular inflammatory cells infiltration (H & E X 100**). i)**unvaccinated and challenged at 28 days (7 days PC) showing follicular atrophy and marked interfollicular fibrous connective tissue proliferation (H & E X 100).

The result of RT-PCR are shown in table 4.The virus was detected in all control non-vaccianted challenged groups at 3, 7 and days post challenge either challenged at 21 days of age or 28 days of age. In Bursine plus vaccianted groups, the virus was deteced in one out of 3 birds at 3 and 7 days postchallenge in birds challenged at 21 days of age and the virrus was deteced in 2 birds out of 3 at 3 and 7 days postchallenged at 28 days of age. While in Xtreme vaccinated groups the virus was detected in one birds at 3 days post challenge only when challenged at 21 days of age and in 2 birds out of 3 and in one bird out of 3 at 3 and 7 days post challenge at 28 days respectively (Table 4). These finding indicating the persistence of the virus in the bursa of fabricuis in bursine plus vaccianted groups more than in Xtreme than Bursine plus vaccine.The late incidence of bursa lesions after vaccination may be confused with field virus-induced lesions, in which case sequencing may offer a valuable tool for differentiation**. (30).** The Partial sequence of VP2 region of vaccines used and the field isolated vvIBDV are shown on Fig.3 a, 3b and 3c.

ND HI Titer

The effect of the IBDV vaccines on antibody titers of ND vaccines was conducted. Serum antibody titers to the ND vaccine were measured at one, 11, 18 and 25 days of age by HI. The results of HI titre are shown in table 5. The IBDV live vaccine may also interfere with other vaccines **(8).** Consistent with this finding, our results also showed that the NDV antibody titre of non vaccianted groups were higher than vaccinated groups.

The feed convesion ratio (FCR) was better in Xtreme vaccinated groups at 14, 21, and 28 days of age (2.0, 1.8, and 1.7) compared with Bursine plus vaccinated groups (2.3, 1.9, and 1.8) respectively. However, the FCR in Bursine plus vaccianted groups was batter than that in Xtreme vaccianted groups only at 35 days of age.

Table 4 :RT-PCR results in broiler chicks vaccinated with Xtreme vaccine or Bursin plus vaccine and challenged orally either at 21 days of age or at 28 days of age with vvIBDV.

Group	RT-PCR positive Bursa					
	Challenge at 21 days of age			Challenge at 28 days of age		
	3dpc	7dpc	10dpc	3dpc	7dpc	10dpc
1	3/3	3/3	3/3	2/3	3/3	3/3
2	1/3	1/3	0/3	2/3	2/3	0/3
3	1/3	0/3	0/3	2/3	1/3	0/3

Group1: Non vaccinated control birds
Group 2: Vaccinated with Bursin plus vaccine orally at 11 days of age.
Group3: Vaccinated with xtreme vaccine orally at 11 days of age.
Challenge: Birds were challenged orally by vvIBDV.

Table 5: Haemagglutination Inhibition (HI) of Newcastle disease antibodies of chickens vaccinated with IBDV Xtreme vaccine or Bursin plus vaccine.

Age	Group	No. of sera	ND (HI titer log-2)												Mean
			1	2	3	4	5	6	7	8	9	10	11	12	
One day		15						6	6	3					6.8
11 day		11				2	7	2							5
18 day old	Group 1	10				4	3	3							4.9
	Group 2	10				5	5								4.5*[b]
	Group 3	10				5	4	1							4.6*[a]
25 day	Group 1	10				4	3	3							5.9
	Group 2	10	1			2	4	1	2						4.8*[a]
	Group 3	10			2	4	2	2							4.4*[b]

Group1: Non vaccinated control birds
Group 2: Vaccinated with Bursin plus vaccine orally at 11 days of age.
Group3: Vaccinated with xtreme vaccine orally at 11 days of age.

- Birds were vaccinated with NDV vaccine via eye drop at 1 and 14 days of age with the recommended dose of the manufacture.

Table 6: Mean body weight and feed conversion rate in groups vaccinated orally with IBD Xtreme vaccine or Bursin plus vaccine.

Age / Group	7 day old		14 day old		day old21		28 day old		35 day old	
	Avg. BWt. (gm)	FCR	Avg. BWt. (gm)	FCR	Avg.BWt. (gm)	FCR	Avg. BWt. (gm)	FCR	Avg. BWt. (gm)	FCR
Control IBD	115	1.7	225.2	1.8	342.3	1.9	481.1	1.9	1250.6	1.6
Bursin +	-	-	233.6	2.3	471.5	1.9	833.5	1.8	1546.2	1.7
xtrem	-	-	247.3	2	484.7	1.8	868.2	1.7	1500	1.98

N.B. day old average body weight (**Avg. BWt.**)= 47 gram (gm)

CONCLUSION

Based on our findings, it can be cocluded that the both vaccines achived a good protection against vvIBDV, however, Xtreme vaccine seems more efficient, safer than Bursine plus vaccine and resulted in more production performance and feed conversion value.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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