Laboratory Evaluation of Live Recombinant HVT-IBD Vaccine

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Abstract: Quality control tests were applied to prove the efficacy of the Vaxxitek HVT+IBD vaccine by Identity, sterility, titration, immunosuppression, safety and potency tests. Vaxxitek HVT+IBD vaccine proved to be safe when inoculated in 1-day old chicks free from maternal derived antibodies, broiler chicks and Swiss mice with 10-field dose of the vaccine. The Vaxxitek HVT+IBD vaccine did not interfere with MDA and could be inoculated in 1-day broiler chicks safely and resulted in protective antibody response. Vaxxitek HVT+IBD vaccine was inoculated in 18-day old specific-pathogen-free embryonating chicken eggs (SPF- ECE) (3-days before hatching) without adverse effect on hatchability% and achieved protective response when challenged with vvIBDV compared with CEVA transmune vaccine which gave similar results. Vaxxitek HVT+IBD vaccine was stable after 10 blind passages on CEF cells that showed the same DNA band using specific primer for VP2 gene. Due to intra cellular nature of the vector vaccine so it gave long life protection as the immunity lasts till 9 month in our experiment after inoculation of the vaccine by one field dose to 1-day old broiler chicks then serum sample collected for serological tests (ELISA-SNT) and challenged with vvIBDV.
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Introduction:

Infectious bursal disease virus (IBDV) has a great concern in poultry industry for a long time but particularly for the past decade, The disease was the first reported by **Cosgrove (1962)** as a clinical entry in Gumboro area, southern Delaware, USA and was designed as avian nephrosis due to sever kidney lesions seen on the post mortem examination; Later, it was termed as Infectious bursal disease virus (IBDV) referring to the specific lesions caused by the disease in the bursa of Fabricious **(Hitchner, 1970)**, Immunization of chickens is the principle method used for the control of IBDV in chickens. The vaccine must be safe, pure and efficient **Tsukamoto et al., (1995)**.

In the past years, progresses have been made on new vaccination strategies against IBD, Many attempts have been made to express the structural proteins of IBDV as subunit vaccine in different heterologus systems with biotechnology hold great promises for the future of veterinary vaccines (**Wang et al., 2007)** , The cell-associated IBD vector vaccine $vHVT_{13}$ (currently registered under the name VAXXITEK® HVT+IBD), generated by inserting an IBDV VP_2 gene into the HVT genome used as vector **(Bublot et al., 1999),** The Vaxxitek HVT+IBD vaccine appeared to show no interference with antibodies derived from the mother bird and can be safely administered to one day- old chicks without apparent safety problem or inducing an immunosuppressive effect as reported with **Bublot et al., (2007)** and **Wang et al., (2010)**.

2. Material and Methods:

1. Vaccines:

1.1. Vaxxitek HVT+IBD vaccine: (Bursal Disease - Marek's Disease vaccine serotype 3, live MD vector): It was obtained from MERIAL SELECT, INC. Gainesville, GA 30503 USA U.S.VET. License No. 279, Serial No. R1011 and Exp. date 23 Aug. 2011.

1.2. Live complex vaccine: CEVA TRANSMUNE (winterfield 2512 G-61 strain of infectious bursal disease live virus in complex with IBD antibodies in freeze -dried form), It was obtained from CEVA-PHYLAXIA Veterinary Biologicals Co. Ltd. 1107 Budapest. Hungary, with batch number 0101u4DNB and the expiry date 11-2012.

1.3. Marek's disease vaccine: this vaccine obtained from Intervet international B.V., Box Meer, Holland, batch number A304B with expiry date 11-2012 and a titer of 3200 PFU/dose.

1.4. Classical D78 vaccine: obtained from Intervet International B.V. Boxmeer,Holland, with batch number 9R2Y-2 M.G.,expiry date 10.2011 with titer $10^{5.2}$ EID_{50}/dose.

1.5. Hitchner B1 vaccinal strain: obtained from Intervet international B.V., Boxmeer, Holland with batch number 08811EJ01 Nobilis ND Hitchner, expiry date 11-2012.

2. Challenge virus:

2.1. Challenge IBD virus: vvIBDV used in challenge, It was kindly provided by Dr. Ahmed M. Helal (CLEVB).

2.2. Challenge Marek's disease virus: Oncogenic Marek's disease virus strain, the virus used in challenge was kindly provided by Dr. A. M. Ali (CLEVB).

2.3. Challenge ND virus: virulent strain (velogenic-viscerotropic strain) of Newcastle disease field isolate, was obtained from the Newcastle disease Department, Veterinary Serum and Vaccine Research Institute. (VSVRI), and its infectivity titer was 10^6 EID_{50}/ml.

2.4. Newcastle disease Haemagglutinating antigen: Lasota strain has been propagated in embryonating chicken eggs for preparation of ND antigen.

2.5. CEF adapted IBD virus: The virus used in Serum neutralization test and obtained from (CLEVB).

3 .Experimental hosts:

3.1. One day old broiler chicks: were obtained from El-Wady Poultry Co., Giza, Egypt.

3. 2. One day old SPF chicks: chicks free from MDA against IBDV obtained from SPF poultry farm at Koum Osheim El-Fayoum, Egypt.

3.3. SPF embryonating chicken eggs (ECE): were obtained from the SPF production farm, Koum Osheim, El-Fayoum, Egypt.

3.4. Experimental mice: fourty (40) Swiss male mice were obtained from (CLEVB).

4. Material used for Tissue cultures and cell culture media, reagents and solutions:

4. 1) Chicken Embryo Fibroblast cells (CEF), it was obtained from CLEVB.

4. 2)Tissue culture media, reagent and solutions:

4.2. A) Tissue culture media: Minimum Essential Medium Eagle (MEM) was prepared according to the manufacture's instructions.

4.2. B) Trypsin –Versin solution: It was prepared according to **Lennette (1964)**.

4.2. C)Hank's balanced salt solution (HBSS)**:** was prepared according to **Hank and Wallance (1949)** .

4.2. D) Bovine serum: New calf serum, Mycoplasma free and virus screened "Gibco Limited, Scotland, and UK".

4.2. E) sodium bicarbonate solution**:** 4 .4 % sodium bicarbonate "Analar, BDH Chemical, LTD, Pole, England" in ionized distilled water was used for adjustment the required pH value of the cell culture media.

5. Enzyme Linked Immuno -Sorbent Assay (ELISA):

5.1. IBD ELISA Kit**:** ELISA Kit was obtained from SYNBIOTICS Corporation 11011VIA Frontera San Diego, CA 92127, U.S. VET LIC NO.312, Item No. 96-6500 and the Exp. date 9.2011, serial number FS 5155.

5.2. ELISA Reader: Micro plate reader USA, VERSAmax , the serial Number was B02274.

6.Polymerase chain reaction (PCR):

6.1. Materials used in DNA extraction:QIAamp DNA Minikit (50), it was supplied by Qiagen, Cat. Number 51304.

6.1.1. Reagents used for PCR:

6.1.2. d NTP (mixture) 4Mm: it was supplied by Sib Enzyme Ltd. Cat. # N027.

6.1.3. PCR system kit: it was supplied by Biobasic Co., Canada Cat. # D0088.

-10X PCR buffers-Enzyme mixture.

6.1.4. Specific Primer: primer pair (forward and reverse) for VP2 of IBDV.

Using primer specific to VP2 gene it was supplied by MERIAL SELECT, INC. Gainesville.

Forward 5' -CCG TAG AAC GCA GAG CTC CTC-3' (21 mer)

Reverse 5'-CAC CTC CCC CTG AAC CTG AAA C -3' (22mer)

3. Results:

Studying the safety and efficacy of Vaxxitek HVT+IBD vaccine:

The vaccine underwent the quality control tests including Identity, Sterility, Titration, Safety and Potency tests.

1. Identity Test: Polymerase Chain Reaction (PCR) by using Primer specific to infectious bursal disease virus (VP2 gene) vHVT013-69-FW and vHVT013-69-Rev.Visualization of the PCR products on a 1 % agarose gel, DNA band was seen at 1600 bp as shown in photo (1).

2. Vaccine sterility: the Vaxxitek HVT+IBD vaccine was free from bacteria (aerobic and anaerobic), Fungal, Mycoplasmal contamination.

3. Vaccine titration: the vaccine titer was 2600 PFU/dose.

4. Safety: The safety of Vaxxitek HVT+IBD vaccine was performed into 2-parts:

Part -1: the chicks were divided into groups as shown in table (1).

The Vaxxitek HVT+IBD vaccine proved to be safe after S/C vaccination of one-day old susceptible chicks free from maternal antibodies against IBDV and broiler chicks with a 10 X dose.

Part -2: Safety in mammalian species (Ecotoxicity test): fourty Swiss mice were divided into four groups (10/ group) as follow:

Group (1): vaccinated group receive the Vaxxitek HVT+IBD vaccine with 10 x dose intra peritoneal , Group (2): inoculated with parental non-Recombinant strain (Marek's disease vaccine) , Group (3): inoculated with D78 IBD vaccine and Group (4): inoculated with the HVT diluent.

Vaxxitek HVT+IBD vaccine proved to safe for mammalian species as there was no mortality occurred in any group, nor general or local reaction. Growth was identical in the four groups during the period of observation. There was no evidence for abdominal toxicity under the trial condition of the recombinant vaccine and of the parental HVT strain.

5. **Immunosuppression Test**: Sixty one day old chicks free from MDA to IBDV were used for monitoring the immunosuppression of Vaxxitek HVT+IBD vaccine. The experimental chicks of each group were maintained separately in closed cages, divided into four groups as tabulated in table (2).
6. **Potency test**: This part was to assess the potency of Vaxxitek HVT+IBD vaccine when inoculate one field dose of the vaccine subcutaneously to one day old chicks (chicks free from MDA to IBDV and broiler chicks) and the chicks were divided as tabulated in table (3).Vaxxitek HVT+IBD vaccine was potent if vaccinated in both chicks free from MDA and broiler chicks as shown in table (4).

Study the immune response of Vaxxitek HVT+IBD vaccine by in-ovo vaccination compared with live CEVAC-Transmune vaccine.

This experiment consists of two parts:

Part (1): **Safety test:** to determine the safety of Vaxxitek HVT+IBD vaccine by

In-Ovo vaccination in compared with live vaccine CEVAC-Transmune vaccine.

After 21 days post hatching the chicks were necropised and examined for any pathological lesions attributed to IBDV or result from vaccine administration.

No sick or dead birds were recorded and during Post-mortem examination on day 21 did not reveal any macroscopic lesions on the bursa of Fabricious. No significant lesions related to IBDV in both vaccinated groups so both vaccines proved to be safe when administered by in-Ovo route of vaccination and the vaccine has no negative effect on hatchability.

Part (2): **Potency test**: This experiment was designed to compare the effect of in ovo-vaccination of Vaxxitek HVT+IBD vaccine and CEVA-Transmune vaccine on the hatchability then to follow up the immune response of hatched chicks by seroconversion tests. At three weeks old chicks, blood samples were collected for serology to detect the level of antibody titer by using ELISA and SNT, and the result tabulated in table (5) and (6).

Investigation of VP2 gene stability in the Vaxxitek HVT+IBD vaccine:

The vaccine was propagated on CEF cells for 10 successive blind passages and the stability of VP2 gene inserted in the Vaxxitek HVT+IBD vaccine has been studied by Polymerase chain reaction (PCR) and result in visualization of PCR products on a 1 % agarose gel, DNA band was seen at 1600 bp in all 10 passages of Vaxxitek HVT+IBD vaccine as shown in photo (2).

Determination of the duration of immunity of the Vaxxitek HVT+IBD vaccine:

Serum samples were collected for estimation of the duration of immunity of the Vaxxitek HVT+IBD vaccine after 3weeks post vaccination from vaccinated chicks for serology to determine the antibody titers then blood collected weekly until 12 weeks post vaccination and then every month until 9 months post vaccination as shown in table (7).

The highest ELISA titer was obtained at 4^{th} week post vaccination and titer decrease gradually till the end of experiment but still within the protective level, The highest SNT titer was obtained at 3^{th} week post vaccination and remained in the peak till 9WPV then the titer decease gradually till the end of experiment, The protection% after challenge with 10^4 vvIBDV I/O to vaccinated and non vaccinated chicks, the chicks was kept under observation daily for 15 days post challenge to calculate the Protection%, the protection% was 90% till 4MPV then decreased to 88% from 5MPV till the end of the experiment, as shown in table (7).

Following PCR protocol, it was demonstrated as shown in Photo (2) that by Specific amplification of a DNA fragment of the VP2 gene of IBDV inserted into Vaxxitek HVT+IBD vaccine showing DNA band at 1600bp after migration for 30 min. at 100V (1 % agarose gel) in all 10passages of the Vaxxitek HVT+IBD vaccine on CEF cells that approve the stability of the inserted IBD VP2 gene into HVT genome (Vector) even by passages.

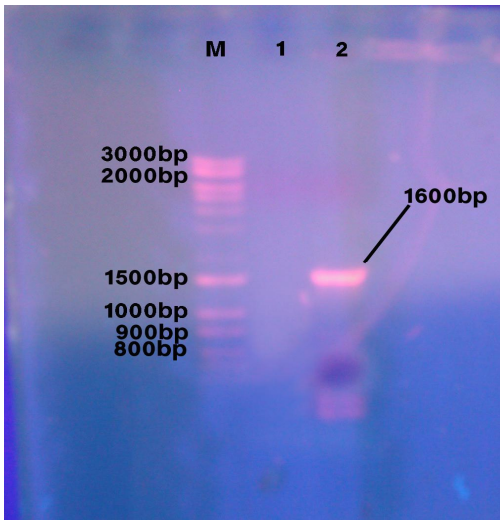

Photo (1): Agarose gel electrophoresis of PCR product of Vaxxitek HVT+IBD vaccine.

Following PCR protocol, it was demonstrated as shown in Photo (1) that Specific amplification of a DNA fragment of the VP2 gene of IBDV inserted into Vaxxitek HVT+IBD vaccine showing DNA band at 1600bp after migration for 30 min. at 100V (1 % agarose gel).

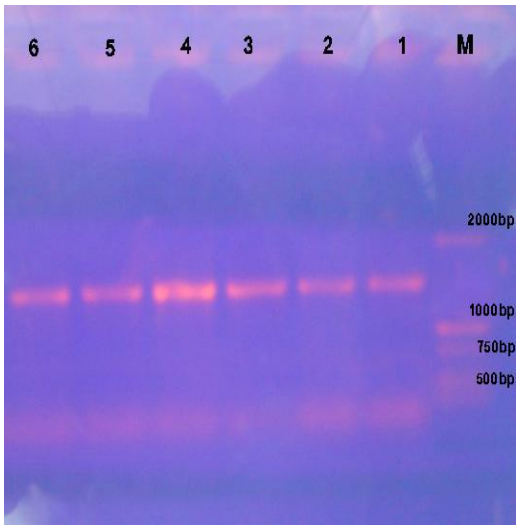

Photo (2): Agarose gel electrophoresis of PCR product of 10 serial passages of Vaxxitek HVT+IBD vaccine on CEF cells.
Lane (1):1 st passage. Lane (2):3 th passage. Lane (3):5 rd passage. Lane (4):7 th passage. Lane (5):9 th passage. Lane (6):10th passage.

Table (1): Safety of the Vaxxitek HVT+IBD vaccine in one day old Chicks free from MDA and One-day old broiler chicks:

Group	Age	Treatment	Dose	Period of observation
1 A	One-day old chicks free from MDA	Vaxxitek HVT+IBD inoculated (1 ampule dispensed into 20 ml of HVT diluent).	10x 0.2 ml	120 days
1 B	One-day old broiler chicks			
2 A	One-day old chicks free from MDA		10x 0.2 ml	21 days
2 B	One-day old broiler chicks			
3 A	One-day old chicks free from MDA	Un-vaccinated challenged group with oncogenic strain of MD (I/P).	0.2 ml	120 days
3 B	One-day old broiler chicks			
4 A	one - day old chicks free from MDA	Un-vaccinated challenged group with vvIBDV (I/O).	0.2 ml	21 day
4 B	one- day old broiler chicks			
5 A	One-day old chicks free from MDA	Un-vaccinated UN-challenged control	-	Until the end of the experiment
5 B	One-day old broiler chicks			

*I/P: intra-peritoneal. * I/O: intra-ocular. *MDA: maternal derived antibody.
**Number of birds in each subgroup: 20 one day old chicks.

Table (1) explain the groups used in safety test: as group A represent One-day old chicks free from MDA and group (B) One-day old broiler chicks and both were divided into 5 subgroups as follow: subgroup (1) refers to vaccinate group with Vaxxitek HVT+IBDV vaccine that examined after 120 days for Marek's disease lesions, subgroup (2) refers to vaccinated group with Vaxxitek HVT+IBDV vaccine that examined after 21day for IBD lesions, subgroup (3) control positive inoculated with oncogenic strain of Marek disease, subgroup (4) control positive inoculated with vvIBDV and subgroup (5) unvaccinated –unchallenged group (control negative).

Table (2): Monitoring of Immunosuppression test by Vaxxitek HVT+IBD vaccine.

Groups	Total number of birds	Number of dead birds	Number of challenged birds	Protection percentage	Mean HI log 2 titer
Group(1) Vaccinated group	20	2	18	90%	7.2
Group (2) Indicatorgroup	20	1	19	95%	7.5
Group (3) Control positive group	15	-	15	0%	1.9
Group (4) Control negative group	5	-	-	100%	-

As shown in table (2) there was no significance difference between HI titer in the vaccinated group with Vaxxitek HVT+IBD vaccine and indicator group (vaccinated with NDV) so the Vaxxitek HVT +IBD vaccine considered as a non immunosuppressive vaccine.

Table (3): The potency of vaccine in one- day old chicks free from MDA and One- day old broiler chicks:

Groups	**Age**	**Treatment**	**Period of observation**
1A (Vaccinated group)	One-day old chicks free from MDA	Vaxxitek HVT+IBD vaccine was inoculated S/c then challenge with oncogenic strain of MDV I/P at 7 day post vaccination.	70 days post challenge
1B (Vaccinated group)	One-day old broiler chicks		
2A (Vaccinated group)	One-day old chicks free from MDA	Vaxxitek HVT+IBD vaccine was inoculated S/c then challenge with vvIBD I/O at 3 weeks post vaccination.	14 days post challenge
2B (Vaccinated group)	One-day old broiler chicks		
3A (control positive group)	One-day old chicks free from MDA	I/P challenge with oncogenic strain of MDV at 7 day old (un vaccinated-challenged).	70 days post challenge
3A (control positive group)	One-day old broiler chicks		
4A (control positive group)	One-day old chicks free from MDA	I/O challenge vvIBDV at 3 weeks old with dose 10^4 EID_{50} (un vaccinated-challenged).	14 days post challenge
4B (control positive group)	One-day old broiler chicks		
5A (control negative group)	One-day old chicks free from MDA	unvaccinated –unchallenged control	until the end of the experiment
5B (control negative group)	One-day old broiler chicks		

*S/C: subcutaneously. *I/P: intra-peritoneal. I/O: intra-ocular. *Number of birds in each subgroup: 30 one day old chicks.

Table (3) explain the groups used in Potency test: as group A represent One-day old chicks free from MDA and group (B) One-day old broiler chicks and both were divided into 5 subgroups as follow: subgroup (1) refers to vaccinate group with Vaxxitek HVT+IBDV vaccine and challenged with standard oncogenic MDV at 7 days intraperitonial and observed for 70 days post challenge, subgroup (2) refers to vaccinated group with Vaxxitek HVT+IBDV vaccine then blood samples were collected after 3 weeks for serology and challenge with vvIBDV then examined for 2 weeks post challenge, sub group(3)control positive challenged with oncogenic strain of Marek disease, subgroup (4) control positive challenged with vvIBDV and subgroup (5) unvaccinated –unchallenged group (control negative).

Table (4): Potency test (protection % of birds Challenged with vvIBDV and Relative protection score (RPS) birds Challenged with oncogenic Marek's disease virus):

Groups	Antibody mean ELISA Titer	Antibody mean SNT Titer	Bursal /Body weight Ratio	RPS	Protection%
Group (1A) vaccinated group	-	-	-	91.3%	-
Group (1B)vaccinated group	-	-	-	90%	-
Group (2A)vaccinated group	7832	512	1.3	-	90%
Group (2B)vaccinated group	7741	512	1.5	-	90%
Group (3A)control positive MD	-	-	-	0%	-
Group (3A)control positive MD	-	-	-	0%	-
Group (4A)Control positive vvIBDV	467	16	0.9	-	0%
Group (4B)Control positive vvIBDV	760	32	0.9	-	0%
Group (5A) control negative	467	16	0.9	100%	100%
Group (5B) control negative	760	32	0.9	100%	100%

Group (A): One-day old chicks free from MDA. Group (B): One-day old broiler chicks.

The result in table (4) indicate that the RPS of One-day old chicks free from MDA was 91.3% and in One-day old broiler chicks was 90% that indicate the Vaxxitek HVT+IBD vaccine was potent if vaccinated in either chicks free from MDA and broiler chicks and the Protection % of both groups (One-day old chicks free from MDA and One-day old broiler chicks) was 90%, The protective Synbiotics ELISA titer for IBDV was 3000, IBD serum neutralizing antibody titer =the reciprocal of serum dilution which neutralized and inhibit the CPE of 100 TCD_{50} of IBDV, bursal/body weight index was calculated according to the following equatinon:

$$\text{**Bursal weight index} = \frac{\text{B: BW ratio of the inoculated group}}{\text{Mean of B: BW ratio of the non inoculated control group}}$$

And chicks with bursal index lower than 0.7 were considered to have bursal atrophy. so from above mentioned results Vaxxitek HVT+IBD vaccine was considered satisfactory and potent if administered to one-day old chicks free from MDA or broiler chicks. The results indicating that the Vaxxitek HVT +IBD vaccine not affect hatchability % if administered to 18-day old SPF ECE by in-ovo vaccination that there hatchability % was 97% in compared with CEVAC-Transmune vaccine 93% and control group 90%.

The results indicating that the highest ELISA titer was obtained at 4th weeks post vaccination and then declined gradually till 9mpv, the highest SNT titer was obtained at 3th wpv then declined gradually till 9mpv, and the protection% was 90% till 4mpv then declined to 88% at 5mpv till 7mpv then decreased to 86% at 8mpv and 9mpv.

Table (5): Antibody response to Vaxxitek HVT+IBD vaccine compared with CEVA-Transmune vaccine as monitored by ELISA, SNT and protection% after in-ovo vaccination

Groups	Type of vaccine used	GMT of ELISA	SNT	Treatment	Protection % when challenged with vvIBDV
Group (A)	Vaxxitek HVT+IBD vaccine	5945	256	In-Ovo vaccination then after 3weeks blood samples were collected for serology and then challenge with vvIBDV.	90%
Group (B)	CEVA-Transmune vaccine	5840	256		90%
Group (C)	Control positive	156	16	Un vaccinated –challenged with vvIBDV.	0%
Group (D)	Control negative	156	16	Un vaccinated -unchallenged	100%

The results shown in table (5) indicate that both vaccines (Vaxxitek HVT+IBD and CEVAC-Transmune vaccine) were potent when inoculated into 18-day old SPF ECE (In-ovo vaccination).

Table (6): Hatchability percentage of SPF eggs inoculated with Vaxxitek HVT+IBD vaccine compared with CEVAC-Transmune vaccine.

Treatment	Number of Embryonating eggs	Number of hatchment	Hatchability %
Group (A)	100	97	97%
Group (B)	100	93	93%
Control (un vaccinated)	50	45	90%

Group A: vaccinated with Vaxxitek HVT +IBD vaccine. Group B: vaccinated with CEVAC-Transmune vaccine.

4. Discussion

In the past years, progresses have been made on new vaccination strategies against IBD, Many attempts have been made to express the structural proteins of IBDV as subunit vaccine in different heterologus systems with biotechnology hold great promises for the future of veterinary vaccines **(Wang et al., 2007)**. This different heterologus system including E.coli (**Azad et al.,(1986)** ,Yeast **(Wang et al., 2003)**,Vaccinia virus (**Shaw and Davison 2000)**, Recombinant NDV vector **(Huang et al.,2004)** ,Recombinant baculovirus **(Lu et al.,2002)** and DNA vaccine **(Li et al.,2004)**; Expressing the VP2 coding sequence of Infectious Bursal Disease Virus (IBDV) for protection of chickens against Gumboro disease and Marek's disease. Vp2 is a major structural protein of IBDV containing antigenic epitopes responsible for the induction of neutralizing and protective antibody **(Fahey et al., 1991 b**; **Müller et al., 1992)**.

Studying the safety and efficacy of the Vaxxitek HVT+IBD vaccine. So the vaccine underwent the quality control tests including Identity, Sterility, Titration, Safety and Potency tests. Polymerase Chain Reaction (PCR) by using Primer specific to IBDV and Specific amplification of a DNA fragment of the VP2 gene of IBDV inserted into Vaxxitek HVT+IBD vaccine showing DNA band at 1600bp after migration for 30 min. at 100V (1 % agarose gel). This result agree with that of the manufacturing company (Merial). The size of vHVT13- expressed VP2 protein was similar to that of the parental Faragher 52/70 IBDV strain (**Bublot et al., 2007)**.

A 1.7 kb PCR segments that covered the partial VP2 gene, 850bp of the US2 region and partial genome of MDV and the whole VP2 gene was obtained from DNA extracted from rMDV- infected cells by different primers (**Wang et al., 2010)**.

Table (7) Duration of immunity induced in broiler chicks after vaccination with Vaxxitek HVT+IBD vaccine.

Time	Mean ELISA titer	Mean SNT Log 2 titer	Protection rate %
3WPV.	7.832	512	90%
4WPV.	7.891	512	
5WPV.	7.716	512	
6WPV.	6.981	512	
7WPV.	6.971	512	
8WPV.	6.782	512	90%
9WPV.	6.753	512	
10WPV.	6.364	256	
11WPV.	6.195	256	
12WPV.	5.951	256	90%
4MPV.	5.617	256	90%
5MPV.	5.512	256	88%
6MPV.	5.219	128	88%
7MPV	5109	128	88%
8MPV	5023	128	86%
9MPV.	5.013	128	86%

*WPV: weeks post vaccination. ** MPV: month post vaccination.

The Vaxxitek HVT+IBD vaccine titer was 2600 PFU/dose as our results judged according to the parameters of Code of Federal Regulation USA ‘‘Part 133.331-9 CFR ch. 1, 1-1-97 Ed.’’ which recorded the minimum titration level of Marek’s disease must be not less than 2000PFU/ dose , Sterility test proved that the Vaxxitek HVT+IBD vaccine was free from bacterial (aerobic and anaerobic), fungal and Mycoplasma contamination. These results came in agreement with the parameters of Code of Federal Regulation USA ‘‘Part 133.331-9 CFR ch. 1, 1-1-97 Ed.’’

The safety test revealed that tested vaccine was safe when administered to one day old SPF chicks and broiler chicks, The findings of safety experiment have been found to be in compliance with these published by **(Witter and Lee 1984** and **Heine et al., 1991**, **Ismail and Saif 1991)**. The HVT vaccine is well -known to be safe and poorly sensitive to interference from MDA, That is why it has been proposed as a vector for IBD where **(Darteil et. al., 1995** and **Jacob et al., 2003)** proved that injection of threefold of the dose of the rVP2 vaccine into birds not cause mortality or signs of any disease.

The Vaxxitek HVT+IBD vaccine is non immunosuppressive as shown in table (3). Our results agree with (**Bublot et al., 2007)** who mention that by the experimental studies showed that $vHVT_{13}$ did not interfere with conventional Newcastle disease or Infectious Bronchitis virus uptake. These results indicated that the $vHVT_{13}$ vaccine was safe in SPF chicks and did not induce immunosuppression.

The potency test was applied on the Vaxxitek HVT+IBD vaccine that the vaccine was inoculated in 1-day old chicks free from maternal derived antibody to IBDV and 1-day old broiler chickens s/c by full dose of the tested vaccine. The maternal immunity interfering with the live IBD vaccine replication but had no detectable effect on the vector vaccine take (**Le Gross et al., 2009)** as the $vHVT_{13}$ vaccine administered at a time when maternal antibody was maximal. The cell associated nature of this $vHVT_{13}$ vaccine, the lack of expression of the vp2 on the surface of the infected cells or by the $vHVT_{13}$ virus, and the nature of replication of the HVT vector virus probably all contribute to the ability of this vaccine to avoid interference from MDA (**Bublot et al., 2007)**. Because HVT is propagated through cell-to-cell infection, it is relatively free from the influences of anti-HVT specific antibodies present in the circulating blood. Therefore, the HVT live vaccine has a good character exhibiting its full effect even in the case of the presence of maternal antibody which often attenuates the efficacy of a live vaccine **(Takanori, 2009)**.

The results obtained in this thesis and depicted in table (6) agree with (**Bublot et al., 2007** and **Le Gros et al., 2009)** who used the HVT-IBD vector vaccine in one day old broiler chicks and obtain protection 93% against the very virulent challenge IBDV and with (**Wang et al., 2010)** who reported that the efficacy of the rMDV against virulent IBDV in commercial chickens(87%) is high enough for it to be considered competitive with the attenuated live vaccine (95%), and with (**Tsukamoto et al., 1999)** who mentioned that rMDV can confrere full protection to chickens against vvMDV.

Antibody response evaluated by serological tests, results of ELISA test was tabulated in table(6), That reached to 7741 and 7832 for chicks free from MDA against IBDV and broiler chicks respectively while the mean antibody log 2 titer of SNT gave 512 in vaccinated chicks free from MDA and broiler chicks, the Bursal/body weight ratio for chicks free from MDA against IBDV was 1.3 and in broiler chicks was 1.5, the protection % after challenge with vvIBDV was 90% in both groups of chicks and RPS after challenge with oncogenic strain of MDV was 91.3% in chicks free from MDA and 90% in broiler chicks.

RPS% for SPF chicks free from antibody against IBDV was 91.3% and in broiler chicks was 90% .These results were matched with **Lemiere et al., 2011** who confirmed that MD challenge tests that were superior to 90% in relative score in all the groups vaccinated with both vaccines showed that the mixture of HVT + IBD and Rispens vaccines had no effect on clinical protection against MD, and IBD challenge tests showed that the mixture of HVT + IBD and Rispens vaccines had no effect on clinical protection against IBD, which was equal to 100% protection in all the groups vaccinated with both vaccines.

Bursal indexes in vaccinated SPF and broiler groups were significantly higher than the challenge controls. The commercial vaccine protected against bursal damage as indicated by significantly lower bursal lesions in vaccinated birds **(Perozo,et al., 2009)**, the bursae from chickens with bursal/body weight index higher than 0.7 found to be histologically normal **(Lucio and Hitchner 1979)**.

Studying safety and efficacy of Vaxxitek HVT+IBD vaccine when administered by in-ovo vaccination and compared with CEVAC Transmune (IBD complex) vaccine. So both vaccines proved to be safe when administered by in ovo-route. The results of ELISA test was tabulated in table (5) which gave 5945 and 5840 in vaccinated birds with Vaxxitek HVT+IBD vaccine and CEVAC Transmune vaccines respectively. While the SNT was 256 in two vaccinated groups, then challenge with vvIBDV and examined for two weeks post challenge to calculate the protection%. No clinical signs or lesions were recorded in all vaccinated groups the Protection % against vvIBDV gave 90 % in vaccinated two groups. Our results were agreed with (**Bublot et al., 2007)**.

DNA band was seen at 1600 bp in all 10 passages of Vaxxitek HVT+IBD vaccine photo(2) . These results agree with (**Liu et al., 2006)** who reported that rMDV is stable after 31 passages and **Bublot et. al., 2007** who reported that the construct of $vHVT_{13}$ was shown to be genetically and phenotypically stable after at least eight in vitro and nine in vivo passages.

Determination of the duration of the immunity with Vaxxitek HVT+IBD vaccine by monitoring the immune status for 9 months post vaccination by ELISA, SNT and protection % was calculated after challenge with vvIBDV as shown in table (7). The highest ELISA titer was detected 4 th week post vaccination with 7891 and gradually decrease until reach 5013 at 9 month post vaccination while of SNT test gave 256 at 5 weeks post vaccination and decline gradually and reach 128 at 9 month age of birds post vaccination. The protection % gave 90% from three weeks till 4 month post vaccination and gradually decrease till reach 85% at 9 month of age post vaccination these findings coinude with (**Tuskamoto et al .,1999**) who reported that antibody level against IBD following the vaccination increased continuously for at least 10 weeks and was agreed with **OIE 2008**. The VP2 Ag accumulate in cytoplasm, Therefore, the Ag can escape from the host immune system and establish a persistent infection in chickens continuous stimulate of host immune system by expressed antigen was demonstrated by finding that immune responses to IBD-VP2 increased for 16 weeks in chickens after vaccination with rHVT-pec VP2**(Tuskamoto et al ., 2002)**.

In SPF birds, the vHVT13 vaccine induced high SN IBD titers that reached a persisting plateau (~4log 10) at about 6 weeks of age. Excellent level of protection% (90-100%) was observed after challenge with the different challenge strains after s/c or in-ovo vaccination. Similar immunogenicity induced by another HVT-vectored IBD vaccine in SPF birds has been reported previously **(Tsukamoto et al., 2002)**. In broiler birds ,vHVT13 induced full protection against challenge performed at 3 and 6 weeks post vaccination, despite the presence of very high concentration (>log10) of anti-IBDV MDA at time of vaccination ,Furthermore ,a clear vHVT13 induced IBD seroconversion could be detected 6 weeks post vaccination ,full protection was observed as soon as 1 week after vaccination **(Bublot et al.,**

2007).

The highest IBD serum neutralizing antibody titer at the 4th week post vaccination then remained unchanged till 12 weeks post vaccination **(Afaf et al.2000)**.From above results indicated that recombinant tested vaccine not affected by maternally derived antibodies as another vaccines, As the interference by maternally derived antibody (MDA) considered a major problem for the vaccination of young chicken against IBDV as reported with (**Bublot et al., 2007**), A significant increase in antibody titers detected in flocks vaccinated with the vector vaccine indicated its ability to induce an immune response in birds with a high level of maternally derived antibodies**(Zorman et al.,2011).**

In conclusion the recombinant vaccines produced with biotechnology hold great promises for the future of veterinary vaccines and have great advantage over traditional vaccines to be accepted to the poultry industry. Because Vaxxitek HVT+IBD vaccine is administered to health chicken embryo at 18 or 19 days of embryonation, or to healthy chicks at one day of age and proved to be safe, as an aid in the prevention of Marek's disease and Infectious bursal disease. This vaccine was an effective and stable vaccine in correlation with the vaccine efficacy against lethal IBDV challenge and can provide a better protective effect that is likely to persist for the life of the chickens, together with cost-effectiveness and easiness of production are critical factors to make a commercially feasible vaccine.

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