IMMUNITY AND POSTVACCINAL REACTION OF TISSUE CULTURE AND CHICKEN EMBRYO ADAPTED LARYNGOTRACHEITIS VACCINES

Kamel I. M. Abou-El-Azm

Dept. of Poultry and Rabbit Dis., Fac. Vet. Med., Mansoura Univ.

ABSTRACT

Two-tissue-culture (Vinland and Schering vaccines) and two chicken-embryo-adapted (Intervet and TAD) vaccines were used for intraocular vaccination of 25-day old chicks to compare the post-vaccinal reaction and degree of protection afforded by each vaccine. Vaccines were applied intraocularlly using specific droppers supplied by the vaccine-producers. Protection was judged by measuring antibody response 15-days postvaccination and the degree of resisting challenge with a virulent local field isolate.

Challenge virus was isolated from field outbreaks in Dakahlia province and identified against specific antisera using the agar gel precipitation test. The ILT isolate was able to cause 92% morbidity and 46% mortality when applied intratracheally to 25-day-old non-ILT vaccinated birds.

Different Vaccinal droppers differ in their speed of releasing vaccinal-drops and the number of drops released involuntary from droppers when turned upside-down. The Intervet dropper was the fastest and more releasing dropper among the tested ones. The Intervet seemed to drip very fast than the vaccinal crows could apply to chicks intraocularly.

Different vaccines differed in their time and in their degree of producing postvaccinal reactions. The higher percentage of birds showing postvaccinal reaction with the Vinland (50%) , Intervet (38%), TAD (25%), and Schering (16%) vaccines were recognized in day 6, 10, 6 and 4 days post-vaccination, respectively.

Protection afforded 15-days postvaccination with the Intervt, TAD, Schering and Vinland vaccines as judged by protection against appearance of signs were 83%, 83%, 63% and 92% and were 92%, 92%, 79% and 96% against mortality, respectively.

INTRODUCTION

Since introduction of Infectious laryngotracheitis to Egypt in 1983 **(Tantawi et al., 1983)**, prophylactic immunization via intraocular, intranasal, or drinking water has been adopted in flocks of commercial layers and breeders in particular and of broilers in some areas of risk.

Duration of resistance after vaccination varies from 6 weeks to as long as 1 year **(Beaudette, 1949, Shibley et al., 1962; and Raggi and Lee, 1965)**. The refractory period and degree of resistance has been shown by several workers to vary with virus strain, concentration of virus in the vaccine, route of application, and age of vaccination **(Gibbs, 1933; Beaudette, 1949; Hitchiner and Winterfield, 1960; Raggi and Lee, 1965, Samberg et al, 1971, Mahmoud, 1987, and Fulton, et al., 2000)**.

Early protection following vaccination is important especially for those birds under the risk of exposure in enzootic areas and in case of emergency vaccination. Although early protection to ILT-vaccines starts as early as 2-3 days post vaccination and complete protection occurs by day 6-8 postimmunization **(Benton et al, 1958; Gelenezei and Marty, 1965; and Alls et al, 1969)**, yet postvaccinal reactions usually starts by day 3-4 and disappears 5-10 day postvaccination **(Brandly and Bushnell, 1934; Hitchner and White, 1958; Shibley et al., 1963; Alls et al., 1969; and Mahmoud, 1987)**.

Recently, some of vaccinated flocks to ILT in Dakahlia and Dommitta provinces showed severe post-vaccinal reaction that extend in complicated cases for 3 weeks. Our aim of work was to comment on droppers supplied with each vaccine and to evaluate the humoral immune response, protection and post vaccinal reaction of 2 tissue culture adapted ILT vaccines and 2 chicken embryo-adapted ILT-vaccines.

MATERIAL AND METHODS

Chickens, housing and diets:

One hundred and twenty 1-day-old Ross broiler chicks were obtained and randomly distributed in 10-wire pens with continuous lighting. Feed (commercial diet) and water was available ad-libitum.

Laryngotracheitis vaccines:

Two-tissue-culture adapted ILT vaccines and 2 chicken embryo adapted ILT-vaccines were used in this work. Fowl laryngotracheitis modified tissue culture live virus vaccines of Schering-

Plough Animal Health corporation (P10500-12, Serial No.:89288A) and of Vinland Laboratories (97027V, Serial No. 54262) and chicken-embryo-adapted laryngotracheitis vaccines of Lohman Animal Health GmbH & Co. KG (TAD ILT vac, Batch No. 1047311) and of Intervet International B.V. (Batch/lot: 001007C) were resuspended in their specific diluent and dropper for intraocular vaccination.

ILT-vaccinal dropper evaluation:

Droppers containing sterile diluent for oculo/nasal vaccination were tested for their speed in releasing the first drop, the number of released drops per 5 seconds, and the number of drops released untill complete cessation of dripping occurred. This was done by turning upside down the droppers while it was hold in a rack to count the speed of releasing the drops or holded in hands upside down to measure the number of drops released within 5 seconds and to measure the time from the first drop to the time of last drop before it stopped complete dripping. These criterions were tested in newly opened droppers and when they were half-filled. 15 trials were performed for each dropper for each test and averages were recorded. Tested droppers were those of Schering-Plough Animal Health corporation (P14706-13, Serial No.:106280, 1000 dose), Vinland Laboratories (97061V, Serial No. 11114, 1000 dose), Lohman Animal Health GmbH & Co. KG (TAD ILT vac, Batch No. 1035700, 1000 dose) and of Intervet International B.V. (Lot.: 10097, 2500 dose).

Challenge virus:

Challenge virus was isolated from Balady-SASO flock in Dakahlia province. The flock was suffering from severe respiratory manifestation with expectoration of blood-tinged exudate during the violent coughing. The flock was 60 days old when first signs appeared and 30% mortality occurred within 10 days post-infection. On necropsy, tracheas had bloody cor. Tracheas and/or larynx had pseudodephteritic membranes. Affected tracheas were collected, homogenized, diluted 1:5 in phosphate buffer saline with addition of penicillin and streptomycin. A dose of 0.1 ml of tracheal homogenate was inoculated on chorioallantoic membrane (CAM) of 10-day-old embryonated chicken eggs. CAM with several pock lesions were collected 5 days postinoculation, homogenized and saved at —20°C. Virus identification was done using agar gel precipitation test against ILT-hyperimmune serum prepared according to **Mahmoud (1987)**. Virus titration was carried on through CAM inoculation of 0.1ml/embryonated egg of 10 fold serially diluted CAM homogenates. The virus-CAM homogenate had $10^{4.3}$ EID50/ml.

Challenge test:

Challenge was performed intratracheally through introduction of small-thin plastic tube (5 cm long and 2.5 mm in diameter) connected to the nozzle of a tuberculin syringe into the trachea. 0.1 ml of virulent ILT-field isolate that contains $10^{4.3}$ EID50/ml, was inoculated in trachea of each bird. Challenged birds were observed daily for signs and/or death for 10 days post-challenge. Protection percent were calculated from the formula:

$$100 - \left(\frac{\text{Number of diseased or dead birds}}{\text{Number of challenged birds}} \times 100 \right)$$

Experimental designs:

Ross broiler chicks were vaccinated intraocularly with Hitchiner B1 vaccine at 16 days of age. On day 25 of chicken age 2-replicates each of 12 chicks were either left as non vaccinated negative ILT control groups or vaccinated intraocularly with one of the tested ILT vaccines(2 replicates, each of 12 chicks/ each tested vaccine). Birds were observed daily for 15 days post-vaccination for any post-vaccinal reaction (Conjunctivitis, lacrimation, swollen eyelids, nasal discharge, sneething, and/or coughing). On day 15 post-ILT vaccination, 7-birds/each replicate were bled from wing vein for serum separation. Serum was stored at -20°C until used. 11-birds/ each replicate/each treatment was challenged according to **Mahmoud (1987)**. Challenged birds were observed daily for 10-day post-challenge. Dead birds were subjected for necropsy. Percentage of protection and postvaccinal reaction were calculated to the nearest solid number (No decimal numbers).

ILT antibody titration:

Serum samples were taken off from freezer and left at room temperature for thawing. In a 96 well plate, 6ul of serum samples as well as controls ((positive and negative sera) were added to 300ul-dilution buffer. 50ul of diluted serum samples were transferred to single ILT-virus coated ELISA plate well where positive and negative controls were run in triplicates. ELISA tests were proceeded with normal KPL-ELISA test procedure. Optical densities and calculation of titers were done using computerized BIO-Tek ELX 800 reader and Synobiotics proFile 2.01 software.

Statistical analysis:

Data were grouped and expressed as means ± S.D. Group means for ELISA antibody titers were subjected to analysis of variance **(Snedecor and Cochran, 1967)** using the general linear models procedure and a software package **(SAS, 1987)**. Significant differences (determined by analysis of variance for treatment groups) were compared using Duncan,s multiple range procedure **(Duncan, 1955)**. All statements of significance were based on the 0.05 level of probability.

RESULTS AND DISCUSSION

Several trials of ILT-virus isolation in Egypt by **Tantawi et al., 1983; El-Kennawy et al., 1985; Sultan and El-Gohary, 1999** and others were successful from different outbreaks in breeders, layers and broilers. In our study, isolation of virulent ILT virus from field outbreaks were successful and the isolated strains were able to cause 92% morbidity (conjunctivitis, lacremation, swollen eye led, Sneething, coughing, expectoration of blood tinged exudate) and 46% mortality (Table 3). On necropsy of dead birds, bloody tinged exudate or dephteritic membranes existed in trachea and/or larynx.

Different ILT vaccines (either chicken-embryo- or tissue-culture-adapted) are claimed to be produced to support birds with maximum protection and minimum postvaccinal reaction. ILT-vaccine caused different degrees of conjunctivitis, lacrimation, swollen eyelids, sneething, coughing and some time increasing mortality rate **(Brandly and Bushnell, 1934; Hitchner and White, 1958; Hitchner and Winterfield, 1960; Shibley et al, 1963; Alls et al., 1969; Mahmoud, 1987; and Cislaghi, 2000)**.

From table 1 it is clear that different droppers differed in their speed of releasing the drops and the number of released drops. Once the dropper was turned upside-down while it was hold in a stable rack, the speed of releasing the first drop were recorded. From table 1, it is clearly obvious that the fastest dropper in releasing the first drop was Intervet-dropper (1 second) while the slowest occurred with Schering dropper (25.6 seconds).

As assumed, the professional vaccinal crows might need 5 seconds for each bird to be intraocularly vaccinated. We tested the number of drops released spontaneously from each dropper in 5 seconds. The 5 seconds started once we turn the dropper upside-down and holding it in almost steady manner by right hand. It is obvious from table 1 that the half-filled droppers dripped more than the filled ones for all tested droppers. It is also obvious that the Intervet-dropper produced in 5 seconds more drops than the other tested ones. Newly opened (full-filled) Schering dropper had the lowest rate of dripping in 5 seconds in comparison to other tested-

droppers while for the half-filled droppers the Vinland-dropper has the lower rate of dripping.

Although the Intervet-droppers had the lower time (9 seconds) of dripping yet it had the higher rate of involuntary dripping and the Vinland-dropper had the lower rate in comparison to the other tested-droppers.

It is obvious from the results of ILT-vaccinal-dropper evaluation that the involuntary dripping from the Intervet-dropper once it is turning upside down is much faster than the vaccinal crows could perfectly apply each drop intraocularly in a separate bird. This means that vaccinal losses and possible contamination of premises may occur.

Following vaccination of chicks at 25 days of age by ILT-vaccines introcularlly, postvaccinal reactions were observed 4 days post vaccination. The reactions comprised lacrimation, conjunctivitis, swollen eyelids and in some cases closure of one or both eyelids and difficult breathing (Table 2). The Vinland-ILT vaccine produced the higher postvaccinal reaction, while the Schering vaccines had the lowest postvaccinal reaction. Although the Vinland vaccines is a tissue culture vaccine, yet it produced the most severe post-vaccinal reaction and this was in contrary to the absence of post-vaccinal reactions with other ILT-tissue culture prepared vaccines **(Glelenezei and Marty, 1964 and Izuchi et al., 1983 and 1984)**.

The peak of postvaccinal reactions appeared in birds vaccinated with Vinland-vaccine (50%) on day 8 post-vaccination, with Intervet-vaccine (38%) on day 10 postvaccination, with TAD-vaccine (25%) on day 6 postvaccination, and with Schering-vaccine (16%) on day 4 postvaccination (Tabl 2).

Protection as judged by antibody titers (measured by ELISA) and resisting challenge to ILT-field isolate (Table 3) indicated that the Vinland-vaccinated chicks were 96% protected for mortalities and 92% protected for morbidity when challenged 15-days postvaccination. Both Intervet and TAD vaccines produced 92% protection as judjed by mortality and 83% protection as judged by morbidity against challenge. Although schering-vaccine had the lowest postvaccinal reactions yet it had the lowest protection rate as judged by antibody titers, percentage of ELISA-negative birds (14%) and resistance to challenge. Antibody titers to ILT did not significantly differ in between vaccinated groups but were significantly different between vaccinated and non-vaccinated groups. Out of the 14 tested serum samples per treatments non of the controls were positive in ELISA test while in vaccinated groups, 1 samples were negative in each of the Vinland- and TAD-vaccinated groups and 2 in Schering-vaccinated ones and non were negative in the Intervet-vaccinated group. Resistance to challenge post-ILT vaccination is not in necessary correlate to antibody titers **(Benton et al., 1958; Cover et al., 1960; and Shibley et al., 1962)** as far as several investigator found that cell mediated immunity is involved in vaccine-induced immunity

to ILT **(Robertson, 1977; Fahey et al., 1983 and 1984; and Mahmoud 1987) .**

We may conclude that the fields complains from the severe post-vaccinal reactions is a matter not only due to the vaccinal strains but also due to the quality of droppers. Losses of vaccines plus contamination of birds and farms might be the normal sequel of excessive fast involuntary dripping that results in post-vaccinal problems. We can also conclude that the ILT isolated virus from Dakahlia province is a pathogenic one that cause 46% mortality and 92% clinical signs in unvaccinated birds and the degree of protection afforded by the different available vaccines in the market differ according to the type of vaccine.

Table 1: ILT-dropper evaluation

Company	Hanging time	No. of drops/5 seconds		No. of involuntary drops
		Full volume	Half volume	
Intervet	1	5	8.2	11 drops/9 seconds
TAD	7.2	1.6	2.6	3 drops/12.2 seconds
Schering	25.6	0.4	2.0	2.0 drop/15 seconds
Vinland	5.2	1	1.4	0.6 drops/15 seconds

Table 2: Post-vaccinal reaction at different intervals post-vaccination with different ILT vaccines:

Type of vaccine	Type and percentage of birds showing post-vaccinal reaction														
	4-days P.V.			6-days P.V			8-days P.V.			10-days P.V.			12-days P.V.		
	LC	SW	SC	LC	SW	SC	LC	SW	SC	LC	SW	SC	LC	SW	SC
None	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Intervet	13	0.0	8	17	0.0	4	33	17	17	38	13	8	13	0.0	0.0
TAD	21	4	4	25	4	8	17	4	4	17	4	0.0	17	0.0	0.0
Schering	16	0.0	4	13	0.0	8	8	0.0	8	13	8	4	8	0.0	0.0
Vinland	33	17	4	50	17	17	46	17	8	46	46	17	13	0.0	0.0

P.V. Post-vaccination
SW Swollen eye-lids
LC Lacrimation and/or conjuctivitis
SC Sneething, coughing and/or nasal discharge.

Table 3: Antibody titers and protection to challenge with virulent ILT-field isolates at 15-days post-vaccination

Vaccinal type	Antibody titers	Percentage of protection from	
		Morbidity	Mortality
None	196 ± 7.0 b	8	54
Intervet	1654 ± 96 a	83	92
TAD	1402 ± 144 a	83	92
Schering	1461 ± 337 a	63	79
Vinland	1597 ± 166 a	92	96

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الملخص العربى

المناعة وردود الفعل الناجمة من إستخدام لقاحات مرض إلتهاب الحنجرة والقصبة الهوائية المعدى الحضرة فى أجنة البيض أو خلايا الزرع النسيجى

المشتركون فى البحث

كامـل إبراهيـم محمـود أبوالعـزم

قسم أمراض الدواجن والأرانب

كليـة الطـب البيطرى - جامعـة المنصـورة

تم دراسة المناعة و ردود الفعل الناجمة عن تحصين بدارى التسمين بالتقطير العيني فى عمر ٢٥ يوم بلقاحات مرض إلتهاب الحنجرة والقصبة الهوائية المعدى الحضرة فى أجنة البيض (أنترفت وتاد-لوهمان) أو خلايا الزرع النسيجي (شيرنج و فنلاند) .

أستخدم فى اختبار التحدى عترة ضارية من فيروس إلتهاب الحنجرة والقصبة الهوائية المعدى تم عزلها من إحدى المزارع بالدقهلية وتصنيفها ضد مصل مضاد باستخدام اختبار الترسيب في الأجار وكذلك عن طريق قياس مستوى الأجسام المناعية باستخدام اختبار الإليزا . وقد تسببت العترة المعزولة في حدوث أعراض فى ٩٢٪ وكذلك ٤٦٪ وفيات في الطيور الغير محصنة والمحقونة في القصية الهوائية .

تفاوتت سرعة نزول القطرات وكذلك عدد القطرات الغير إرادية من القطارات المختلفة الواردة من الشركات سابقة الذكر. وكانت قطارة إنترفت أسرعهم وأكثرهم عددا في نزول القطرات الغير إرادية عند الاستخدام وكانت سرعة نزول القطرات وعددها يبدو أسرع مما يستطيع فريق التحصين استخدامه .

وقد تباينت اللقاحات المستخدمة فيما بينها في سرعة إحداث ودرجة ظهور ردود الفعل . كانت أعلى نسب ظهور أعراض للقاحات فنلاند (٥٠٪)، إنترفت (٣٨٪)، تاد-لوهمان (٢٥٪)، وشيرنج (١٦٪) فى اليوم ٦ . ١٠ . ٦ . ٤ بعد التحصين على التوالى .

كانت نسب الوقاية فى اختبار التحدى في اليوم الخامس عشر بعد التحصين بلقاحات إنترفت، تاد - لوهمان، شيرنج، فنلاند ٨٣٪، ٨٣٪، ٦٣٪، ٩٢٪ من ظهور أعراض و٩٢٪، ٩٢٪، ٧٩٪، ٩٦٪ من حدوث وفيات على التوالى.