

## **STUDIES ON THE IMMUNOGENIC PROPERTIES OF A LIVE INFECTIOUS LARYNGOTRACHEITIS VIRUS VACCINE(\*)**

by  
*V. SOHRAB, A. TAVASSOLI, E. GHAZARIANS  
and F. AFLATOŌNI (\*)*

### **INTRODUCTON**

Live virus vaccine against Infectious Laryngotracheitis (ILT) has now been used for over 40 years. The first vaccine consisted of tracheal exudate containing virulent virus and was applied to the mucous membrane of the cloaca by BAUDETTE and HUDSON in 1933 (2). HITCHNER and WINTERFIELD (6) described the use of live virus by the intraocular route as a method of revaccination and found it to be superior to the cloacal route.

SHIBLEY et al. (7) investigated an intraocular vaccine which was prepared with Benton's strain. They found some degrees of reaction five to seven days after vaccination consisting of swollen or closed eyelids. This was followed by the development of high degree of immunity lasting for one year. The attenuation of a virulent strain of ILT by continuous passage in chick embryo kidney and liver tissue cultures was achieved by GELENCZEL and MARTY in 1964.

A live vaccine against ILT for intraocular administration was developed from a naturally mild strain of ILT by CHURCHILL in 1965. This vaccine was non pathogenic for chickens when given intraocularly, intratracheally, intracerebrally and into infraorbital sinus, but was pathogenic for young chickens (two-weeks-old) when given as a high titer aerosol. The purpose of this study was to investigate the immunogenic properties of a live mild Infectious Laryngotracheitis virus vaccine prepared at the Razi Institute.

(\*) Reprinted from: Bull. Off. int. Epiz, 1979, 91 (9-10), 671-678.

## MATERIALS AND METHODS

### Seed virus.

The mild strain of ILT was used as vaccine seed virus after six passages on the chorioallantoic membrane (CAM) of 11 to 12-day-old SPF embryonated eggs. The infected CAM was homogenized and after being freeze-dried was stored at  $-40^{\circ}\text{C}$  prior to use. The titer of seed virus was  $10^{5.7}$  pock forming units (PFU) per ml. Locally isolated ILT virulent virus was used for challenge. The challenge virus had a titer of  $10^5$  PFU per ml.

### Chickens.

Non vaccinated White Leghorn chickens hatched from SPF eggs were used as experimental chickens.

#### *Vaccine preparation.*

SPF embryonated chicken eggs, 11 to 12 day-old, were inoculated on the chorioallantoic membrane with 0.1 ml of  $10^{-2}$  dilution of seed virus. Five days post-inoculation, the chorioallantoic membranes were harvested, pooled, homogenized and after clarification the supernatant was freeze-dried and used as vaccine.

#### *Virus titration*

Titration was made by inoculating 0.1 ml serial ten-fold dilutions onto the chorioallantoic of 11-12-day-old embryo-nated chicken eggs. Titration end points were calculated from the pock counts obtained from the dilution which gave between 3 and 10 pocks per membrane five days post inoculation. The titer of virus vaccine was  $3 \times 10^6$  pock forming units (PFU/ml).

#### *Vaccination procedures.*

150 chickens were divided into three main groups, A, B and C and were used in the following manner:

*Group A:* consisting of 50 four-week-old vaccinated intraocularly with one drop (0.03 ml) of  $10^{4.5}$  PFU/ml of ILT vaccine. These vaccinated chickens with 30 of their contact control chickens were held together in an isolation unit.

*Group B:* Consisting of 50 four-week-old chickens immunized with 0.1 ml of  $10^{4.5}$  PFU/ml of ILT virus vaccine in drinking water (three times greater than intraocular vaccine). The fifty vaccinated chickens with their contact controls (30 chickens) were housed in a separate isolation unit.

*Group C:* Consisting of 30 four-week-old non vaccinated chickens which were held in another isolation unit and used as challenge controls at the end of the experiment. They had not been vaccinated against any disease.

#### *Challenge procedure.*

4 weeks post-vaccination, all of the vaccinated and unvaccinated control chickens were challenged intratracheally with 0.2 ml of  $10^5$  PFU/ml of virulent ILT virus. Reactions and death to challenge were checked by clinical observation from the third to the seventh day post-inoculation.

### Neutralization tests.

Virus neutralization tests were conducted by mixing serial ten-fold dilutions of the viral suspension with equal quantities of undiluted pools of ten antisera. The mixture was incubated for one hour at room temperature prior inoculation. 0.2 ml of the mixture was inoculated onto the chorioallantoic membrane of 11 to 12-day-old embryonated chicken eggs.

An undiluted negative serum was included each test. The neutralizing index (N.I.) was expressed as log difference between the control virus and the titer of virus after incubation with test serum.

## RESULTS

### *Immunological responses of vaccinated chickens.*

The results obtained indicated 950 pock forming units of ocular ILT vaccine and 3, 160 pock forming units of drinking water vaccine were sufficient to immunize 98 and 65% of chickens respectively (Table I).

There was a significant difference in the antibody responses (SN titers) of birds with the two different methods of vaccine administration (Table I). 98% of those vaccinated by intraocular route and 65% of those given the vaccine in drinking water were resistant when they were challenged four weeks after vaccination with 0.2 ml of  $10^5$  PFU/ml of virulent ILT virus (Tables I and II).

TABLE I.  
*Immunological responses of 4-weeks-old chickens vaccinated with two kinds of ILT vaccine.*

GROUP	NUMBER	Pock forming units (PFU) per dose	N.I. ** three weeks post-vaccination		% Survival after challenge (at nine-weeks age)
GROUP « A »	50 chickens vaccinated by intraocular route	950	1 3 1	1.5 2.5 2	98
	80 chickens contact control	—	3	0	8
GROUP « B »	50 chickens vaccinated by drinking water	3,160	3 2	1.5 2	65
	80 chickens contact control	—	3	0	10
GROUP « C »	30 chickens challenge control	—	5	0	6

\* Number of undiluted pools of 10 sera for S.N. Test.

\*\* Neutralizing Index.

\*Number of undiluted pools of sera for S.N. Test.

\*\*Neutralizing Index.

TABLE II.  
Effect of intratracheal challenge after vaccination\*

GROUP	NUMBER	% of reactors to challenge	% of deaths	% of survival after challenge	Clinical signs
GROUP «A» 80 chickens	50 chickens vaccinated by intraocular route	8	2	98	Cough, rales, watering of eyes.
	30 chickens contact control	95	92	8	Typical gasping, severe haemorrhage, rales.
	50 chickens				
Group «B» 80 chickens	by drinking water	45	35	65	Watering of eyes, rales, cough.
	30 chickens contact control	94	90	10	Severe haemorrhage, typical gasping.
Group «C» 30 chicken	challenge control	98	96	6	Cough, watering of eyes, typical gasping.

\* challenge dose = 0.2 ml,  $10^5$  PFU/ml of virulent virus.

## DISCUSSION

HITCHNER and WINTERFIELD used a live virus vaccine by intraocular route, and found it to be superior to the cloacal route. The results obtained by CHURCHILL in 1965 indicated that 300 pock forming units of ILT virus vaccine was able to immunize the majority of vaccinated birds. The results of the present study indicate that 950 pock forming units of ocular vaccine and 3, 160 PFU of drinking water vaccine are sufficient to immunize 98 and 65% of birds respectively (Table I).

Other investigators such as GELENCZE and MARTY (5), CHURCHIL (3) ALLS et al. (1) and CHANG et al. (4), in laboratory and field trials, showed that ILT vaccine prepared in embryonated eggs and tissue cultures induced significant levels of neutralizing antibody. The vaccine gave good protection and resistance against a virulent strain of ILT virus. Results of neutralizing antibody in our trials shown in Table I are similar to the results obtained by CHURCHILL, GELENCZEI and CHANG.

Serological tests, 4 weeks after vaccination, showed that there was no transmission of the ILT vaccine virus to the unvaccinated control chickens kept in the same unit with the vaccinated chickens (Table I). The time required for the immunity was studied in 4-weeks-old chickens.

The results indicated that a solid immunity was produced three to four weeks post-vaccination (Tables I and II).

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## ACKNOWLEDGEMENTS

The authors thank Dr.M. KAVEH, Deputy Minister of Agriculture and Dr. H. RAMYAR, Director General of the Razi Institute for their supports, encouragements and permission to publish this paper.

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## SUMMARY

A live vaccine against Infectious Laryngotracheitis (ILT) for intraocular and oral administration developed from a mild strain of viruses described. 950 pock forming units of ocular ILT vaccine and 3, 160 pock forming units of oral vaccine were sufficient to immunize 98 and 65% of chickens respectively. No evidence of spreading could be found in contact control chickens five weeks after their exposure to vaccinated ones.

Four-weeks-old chickens were vaccinated by intraocular and oral routes and challenged five weeks later. Reactions and death to challenge were checked by clinical observation from third to seventh day post-inoculation. Both laboratory and field trials with ocular vaccine gave better results than the oral vaccine.

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## RESUME

Un vaccin vivant contre la Laryngotrachéite Infectieuse a été préparé avec une souche naturellement apathogène. Ce vaccin a été administré par les voies intraoculaire et orale.

950 unités formant plage (PFU) du vaccin oculaire et 3, 160 PFU du vaccin oral sont suffisantes pour immuniser respectivement 98 et 65% des poules.

La propagation du virus, après cinq semaines, entre les poulets vaccinés et non vaccinés mis ensemble, a été presque nulle. Les poulets vaccinés, âgés de quatre semaines, ont été éprouvés avec une souche virulente, à l'âge de neuf semaines, en même temps que les poulets témoins.

La réaction et la mort des poulets éprouvés ont été contrôlés cliniquement à partir du troisième jusqu' au septième jour d'épreuve.

Les expériences du laboratoire et du terrain montrent que le vaccin oculaire a donné une meilleure protection que le vaccin oral.

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