Serum and Vaccine Research Institute,
Abbassia, Cairo,
Head Prof. Dr. S. Salama.

STUDIES ON INFECTOUS LARYNGOTRACHETIS VIRUS
I- EFFECT OF ROUTES FOR INOCULATION ON VIRUS TITRE

(With 3 Tables and 3 Figures)

By

SUSAN TOLBA; I. REDA; SALWA. EL-ASELY;
ELHAM EL-EBIARY; A. EL-SONOSI and NARGES BARHOUMA

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دراسات على فيروس التهاب الحنجرة والقصبة الهوائية المعدى
١ ـ تأثير طرق الحقن المختلفة على القوة العيارية للفيروس

سوزان طلبه ، اسماعيل رضا، سلوى الإصيلي ، الهام الأبيارى، أحمد السنوسي، نرجس برهومة

في هذه التجربة تم حقن العترة اللقاحية لمرض الحنجرة والقصبة الهوائية في أجنة الدجاج وتم الحقن على الغشاء اللقانقي وفي التجويف الالنتوني وكذلك داخل الصفار . وقد أظهرت النتائج أم مكان تكاثر الفيروس هو الغشاء اللقانقي في حالة الحقن على الغشاء اللقانقي والتجويف الالنتوني، وقد نتج عن الحقن بكل من الطريقتين نفس القوة العيارية الفيروسية تقريبا لكل من معلق الأغشية والسائل الامينو النتوني بعد خمسة أيام من الحقن، بينما في حالة الحقن في المخ يصل الفيروس الى الفراغ الالنتوني في اليوم الثالث بعد الحقن ويعطي قوة عيارية منخفضة عند اليوم الخامس.

SUMMARY

In this study, the vaccinal strain of ILT-virus was inoculated in embryonated chicken eggs via chorio-allantoic membrane, allantoic sac and yolk sac routes. Results showed that the site of virus multipliction is CAM where the highet titres were obtained. In both CAM and A.S. routes, the titre reached on the 5th day post inoculation in AAF and CAM suspensions for both routes were almost the same. Therefore for vaccine production the allantoic sac route may be chosen, the best day for virus harvest would be the 5th day P.I. In yolk sach route the virus reached the allantoic sac starting on the 3rd day P.I. and gave the lowest titer in the 5th days P.I.

INTRODUCTION

Respiratory disease of poultry constitute one of the major problems facing the rapidly exanding poultry industry in Egypt. Infectious laryngotrachietis virus (ILT) virus has only been recently introduced to Egypt. TNATAWI et al. (1983), have isoalted for the first time the ILT, during late 1982. Repeated virus isolation and serosurvey studies have proved the widespread existance of the clinical and subclinical forms of the disease among laying and broiler flocks (AMER, 1954 and ABD-EL-SALAM, 1986). These findings pointed to the necessity of producing a live modified virus vaccine, for TLT.

The present study, aimed to investigate the effect of different routes for inoculation of embryonated chicken eggs to choose the best route for vaccine production.

MATERIALS and METHODS

Embryonated chicken eggs: 7-12 days old embryonated chicken eggs were obtained from General Poultry Company. Used for inoculation to three different routes, the yolk sac, the allantoic sac and CAM. It also used for virus titration (HITCHNER et al., 1958).

Vaccinal strain of ILT virus :

A modified egg adapted live and lyophilized virus vaccine produced in specific pathogen free eggs obtained by [TAD-pharmazentisches WERK GMBH]. Each ampoul (100 doses) contained $10^{6.4}$ EID_{50} of the vaccinal strain.

The dried vaccine was preserved at +4°C for 2 hours and CAMs were collected and examined for the presence of pock lesions, and determination of their size and morphology. Embryos were examined concerning there size and weight. Three other eggs from each group were saccrified daily. Pooled amino-allantoic fluids, pooled chorio-allantoic membranes and pooled yolk material and embryos were collected.

The pooled membranes and fluids were harvested at -70°C till their titration. Pooled membrans and fluids from daily collections were titrated for thevirus content by inoculation on the CAM of embryonated chicken eggs. Virus titres were calculated after REED and MEUNCH (1938).

(A) Effect of route of inoculation on virus titre:

Table (1) shows the titre of daily harvested CAM and AAF of embryos inoculated by different routes.

(B) Effect of route of inoculation on pock morphology:

Table (2) and Photo 1,2,3 show the different on morphology of the pock lesions on CAM of ECE inoculated by different routes.

(C) Effect on embryo size:

Embryos inoculated with ILT virus by the 3 different routes CAMs, As and Ys were generally of a smaller size than controls are shown in photo 4,5 and 6 for CAM, As and Ys routes respectively.

DISCUSSION

The field and laboratory data point clearly that ILT virus infection among Egyptian poultry farms, is established as an evolving disease which require well designed, scientifically based vaccination and control programs.

This study is thefirst step in trials to produce ILT vaccine locally. Experiment I was designed to show the effect of the route of inoculation on virus output. For

this study the most common 3 routes of egg inoculation were tested namely the CAM, As and Ys rotes.

The results as shown in table (1) showed clearly that the route of inoculation of ILT virus influence clearly its development in the chicken embryo. Thus when the virus was inoculated by the CAM route higher virus titres were seen in the CAM, from second day post inoculation till the end of the experiment.

Similar findings were reported by HITCHNER and WHITE (1958) and JORDAN (1964). By inoculating the embryos via the As, it seem that a lage peroid of 24 hours, where no detectable virus titres can be seen in AAF or in CAM, elapses which is than followed by appearance of virus in the AFF at first.

This multiplication phase extends for 48-72 hours, after which the virus reaches a plateau. A similar multiplication pattern was shown by SHIBLEY et al. (1964), while CAM started to show detectable virus titres after 48 hors. This difference may be attributed to residual unabsorbed virus. Seen thereafter and by the third day, the virus titres in CAM took over and exceded that of AAF. The titres reached on the 5th day post day post inoculation in the AAF and CAM suspensions for embryos inoculated by both routes were almost thesame. These findings agree with those of GENTRY (1963), CHURCHILL (1965) and MEULEMANSS and HELAN (1978 b). On the other hand when the virus was inoculated in the yolk sac, the primary site of virus multiplication seems to be the yolk sac and the embryo then the virus reaches the allantoic sac at a later stage, starting on the 3rd day postinouclation when it began to multiply slowly to give a hiher titre on the seventh day post inoculation.

The above mentioned results show that for vaccine production it may be sutiable to inoculate the virus on the CAM, where the highest titres were obtained. These results are in harmoney with those obtained by HITCHNER and WHITE (1958) and JORDAN (1964 & 1966). However, due to thesmall difference between titres reached by the allantoic sac route and chrio-alantoic membrane (CAM) route, the later route may be chosen. Similar conclusions were reached by GENTRY (1963), GHURCHILL (1965), MEULEMANS & HALEN (1978 b) and SAMBERG (1982).

As shown in table (2) and photo 1,2 and 3 embryos inoculated by the Ys route the pock lesions began to appear on the 5th or 6th day, ill defined and then remained as small pin headed whitish pocks. These findings contradict those obtained by BRANDLY (1934) where he failed to dew on strate pock lesions on CAM after inoculation of ILT virus in yolk sac. While in case of inoculation by CAM and AS routes the pock lesions began to appear from the 3rd day which was well defined, large 3-5 mm and yellowish grayish in colour. Similar finding were obtained by MEULEMANS and HALEN (1948 b), in contrast to those results obtained by MORIMAS et al. (1981). It seems that embryos development was affected by ILT virus especially by the As roate, where a real stunting of growth was noticed and the embryos had a body weight which corresponded to less than 1/3 of the control embryos figures 4,5 and

6-A reduction in size of embryos inoculated by the CAM roate was also noticed by EL-ZEIN et al. (1979) and TRIPATHY and HANSON (1980).

REFERENCES

Abd-Salam, M. (1986): Studies on the epidemiology of ILT in poultry in Egypt. M.V.Sc. Thesis, Fac. Vet. Med. Cairo Univ.

Amer, M.M. (1984): Evaluation of emergency vaccination in case of out-breaks of Newcastle disease. Ph.D. Thesis, Fac. Vet. Med. Cairo Univ.

Brandly, C.A. (1934): A preliminary report. Effect of ILT on fertility and hatchability. J. Am. Vet. Med. Assoc., 84, 588: 595.

Churchill, A.E. (1965): The development of a live attenuated ILT vaccine Vet. Res., 77: 1227-1233.

El-Zein, A.; El-Awar, F. and Faris, R. (1979): Isolation and identification of avian infectious lanyngotrachietis virus in Lebanon. Avian Dis. 32(4): 1060-1065.

Gently, R.E. (1963): Cultivation of ILT virus in embryonated chicken eggs by various routes of infection. Avian Diseases, 7: 31-37.

Hitchner, S.B. and Philip, G. White (1958): A comparison of embryo and bird infectivity using five strains of ILT virus. Poult. Sci. 37: 684-688.

Jordan, F.T.W. (1964): Diagnosis of ILT by chick embryo inoculation. J. Comp. Pathol. 74: 119-128.

Jordan, F.T.W. (1966): A review of the literature on ILT. Avian Dis., 10 (1): 1-26.

Meulemans, G. and Halen (1978): A comparison of three methods of diagnosis of ILT. Avian Pathol. 7: 433-436.

Morimaso, Y. Kazuhisa, H.; Satoshi, T.; Masayuki, A.; Osamu, U.V. and Vorimosa, S. (1981): Infectivity for chicken embryos of tissue culture modified ILT-virus. Avian Dis. 26, 2: 295-304.

Reed, L.J. and Muench, H. (1938): A simple method of estimating fifty percent and point. Am. J. hyg. 27: 493-497.

Shibley, G.P.; Luginbuhl, R.E. and Helmboldt, G.F. (1963): I- Study of ILT strains. II- The duration and degree of immunity induced by conjunctival vaccination. Avian Dis. 7: 184-191.

Tantawi, H.H. El-Batrawi, A.M., Bastami, M.A.; Youssef, Y.I. and Fawzia, M.M. (1983): Avian ILT in Egypt. I-Epidemiology, virus isolation and identification. Vet. Res. Comm. 6(4): 281-287.

Tripathy, D.N. and Hanson, L.E. (1980): Isolation and identification of avian pathogens 2nd Ed., edited by stephen, B. Hitchner, Ghariman, H.P. and James, E.W. Am. of avian Pathol. College. Station, TX.

ILT Titre

Table (1) The titre of daily harvested CAA and AAF collected from embryos inoculated by the CAM, Ys and As.

Roule / Day PI	CAM route		As route		Ys route	
	AAF	CAM	AAF	CAM	AAF	CAM
1 st	0	0	0	0	0	0
2 nd	2.6	3.4	3.5	0	0	0
3 rd	2.2	4.8	3.4	3.75	2.8	0
4 th	2.5	3.8	3.5	4.00	2.5	0
5 th	4.4	5.8	3.8	4.5	2.3	0
6 th	4.3	5.3	3.8	5.5	3.6	0
7 th	4.5	5.3	5.0	4.7	4.5	

Table (3): The effect of the inoculation of vaccinal strain of ILT virus by different routes on the developing chicken embryos.

Route	Virus dose	Age of embryo	Body weight in grams	Virus content of yolk	" Varius content of embryos
CAM	10^3	12	20.5	0	0
As	10^3	10	8.8	0	0
Ys	10^3	7	16.8	$10^{4.3}$	$10^{4.5}$
Un-ino.	-	10	26.0	0	0

* on the 6 th day.

Table (2) Description of the morphology of pock lesions produced by the vaccinal strain of H.T inoculated in ECE by different routes.

Route / Days PI	CAM route	As route	Ys route
1 st	No change	No change	No change
2 nd	The membranes were odematous and turbid	" "	" "
3rd	The membrane began to show lesious yellowish greyish in colour with depressed center distirbuted in all the membrane.	Pock lesions began to appear circular in shap 2-3 mm. in and regulary distributed in all the membrane.	" "
4th	Presence of yellowish greyish coalscent with depressed center, of 3-5 mm. in Ø distributed all over the CAM.	Large No. of Pock Lesions appear evently distributed in all the membrane 3-4 Ø yellowish greyish in colour.	The membranes were highly odemateous No Pock lesions were detected
5 th	Presence of coalscent lesions with a depressed center on inoculation site and clear Pocks on the other parts of the membrane.	Pock lesions still appear with large perforation in the site of inoculations in all eggs opened.	Lesions began to appear not well defined whitish in all membrane
6 th	As above	Distribution of pock lesions all over the membrane.	More defined, fewer in number scattered all over the membrane
7th	As above	As above	1-2 Ø whitish in colour scattered all over the membrane.

ILT Titre

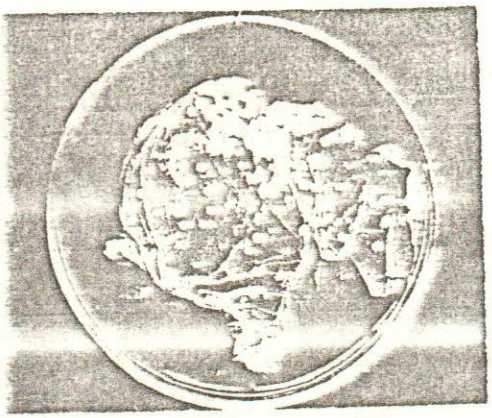

Photo (1): The pock lesions produced by the vaccinal strain of ILT inoculated by CAM route (5th day post inoculation)

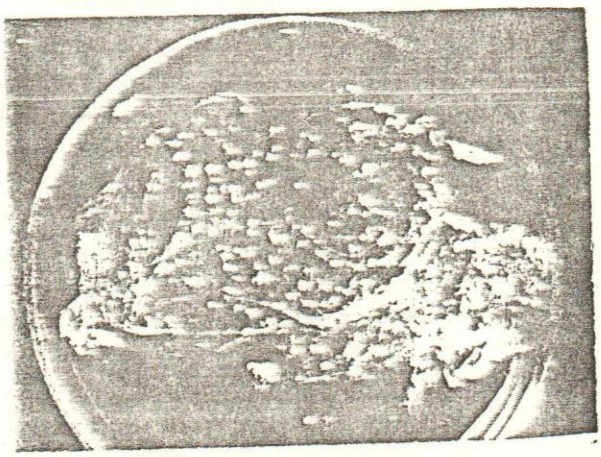

Photo (2): The pock lesions produced by the vaccinal strain of ILT inoculated by the allantoic sac route (6th day post inoculation).

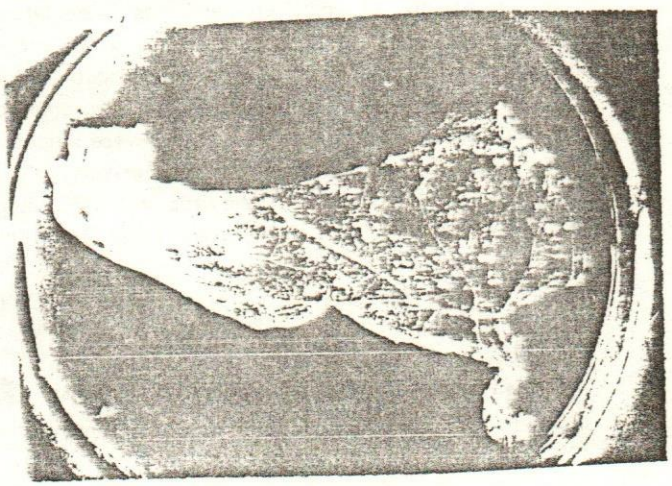

Photo (3): The pock lesions produced by ILT vaccinal strains inoculated by yolk sac route (7th day post inoculation).