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STUDIES ON INFECTIOUS LARYNGOTRACHEITIS VIRUS VACCINE II- THE EFFECT OF INCUBATION TEMPERATURE ON THE PATHOGENICITY OF I.L.T. VIRUS (FOR CHICKEN EMBRYOS INOCULATED BY THE ALLANTOIC SAC ROUTE)

(With 2 Tables)

By

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دراسات على فيروس التهاب الحنجرة والقصبة الهوائية المعدى

٢ ـ تأثير درجة حرارة التحضين على ضراوة فيروس التهاب الحنجرة والقصبة الهوائيـــــة في أجنة البيض المحتونة في التجويف الالنتوني

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في هذه التجربة تم تعيين ضراوة فيروس التهاب الحنجرة والقصبة الهوائية المحقون فـــي الأغشية اللقانقية للاجنـة المحنة عند درجات الحرارة ٣٤م، ٣٧م، ٤٠م، وقد أشارت النتائـــج بوضوح أن هناك تأثيرا لدرجة حرارة التحضين على ضراوة الفيروس في الأجنة المحقونـــة ، فبينما كان التحضن عند درجة حرارة ٣٤ م و ٣٧م حافظا لعترة اللقاح عند المؤشر المميـــز للعترة غير الضارية للفيروس (حيث بقى معامل النفوق أقل من ٠١٦ر٠).

SUMMARY

The incubation of chicken embryos inoculated with original vaccinal strain at different temperature 34°C, 37°C and 40°C had a considerable influence on the pathogenicity of virus inoculated embryos:

1- The mortality index for chicken embryos (MICE) of the original vaccinal strain when the embryos were incuabted at 34°C, 37°C and 40°C were 0.072, 0.028 and 0.46 respectively.

2- One passage of thevirus at 34°C kept thevirus pathogenicity at an indices of 0.05, 0.067 and 0.166 for temperatures of 34°C, 37°C and 40°C respectively.

3- One passage of the virus at 37°C resulted in pathogenicity indices of 0.06, 0.029 and 0.45 for 34°C, 37°C and 40°C and 40°C respectively.

4- One passage of the virus at 40°C resulted in a virus which showed MICE of 0.11 and 0.44 when embryos were incubated at 34°C, 37°C and 40°C respectively.

INTRODUCTION

Infetious laryngotracheitis virus (I.L.T.) is an acute respiratory disease of adult chicken group-A herpes virus (MOTTANTY and DUTTA, 1981). susceptible chickens of all ages may be affected.

ILT was considered to be exotic disease for Egypt since 1982 (TANTAWI et al., 1983). Repeated virus isolation and serosurvy studies have proved the wide spread existance of clinical and subclinical forms of the disease among laying and broiler flocks (AMER, 1984 and ABD-SALAM, 1986).

The production of efficient and safe live modified virus vaccine locally for ILT is of great importance and the present study aimed to investigate the effect of incubation temperature on the pathogenicity of ILT virus for chicken embryos inoculated via allantoic route. The importance of this study lies in the fact that changes in temperature for few degree higher than that commonly 37°C for incubation of infected embryos could have a series effect on the virulence of the virus and consequantly on the quality of produced vaccine.

MATERIAL and METHODS

Materials :

1. Embryonated chicken eggs 7-12th days of age for virus titration and isolation, supplied by poultry organization.
2. Vaccinal strain of ILT virus, a modified adapted lvie and lyophilized virus vaccine produced in specific pathogen free obtained from a well known commercial firm (by TAD-pharmacutisches WERK GMBH). Each ampoul (1000 doses) contain $10^{6.4}$ EID_{50} of vaccinal strain. It was kept at 4°C till it's use.
3. Saline, antibiotics, tincture iodine.

Methods

1. Virus titration: Serial tenfold dilutions starting from 10^{-1} till 10^{-10} were made from the vaccinal strain in sterile PBS. Calculation of 50% embryos infective dose (EID_{50}) was according to REED and MUENCH (1938).
2. Determination of virus pathogenicity: 10^{-3} EID_{50}/ml of virus strains to be tested inoculated in 11 days old chicken embryos via allantoic cavity with 0.1 ml using 15 eggs for each strain incubated at 37°C. (The CMICE) mortality index for chicken embryos was calulated according to (IZUCHI and HASEGAWA, 1982).
3. Virus isolation: It was performed on chorio-allantoic membrane (HITCHNER et al., 1985).

EXPERIMENTAL DISIGN:

(A) Determination of the pathogenicity of vaccinal strains for chicken embryos under different incubation period.

(B) Determination of the pathogenicy of ILT-virus in CAM of embryos which have

been incubated at different temeprature with 0.1 ml of 10^{-3}-10^{-4} EID_{50}/ml and then divided into 3 groups each containing 15 embryos andincubated at the above mentioned temperature.

RESULTS

The reults of the influence of the pathogencity of ILT-virus for chicken embryos according to different incubation temperature was shown in table (1). From the table it is cleared that the original vaccine virus had a mortality index for chicken embryos (NICE) of 0.072, 0.028 and 0.46 when embryos were incubted at 34°C, 37°C and 40°C respectively.

Table (2) shows the influence of the pathogenicity of ILT-virus in CAM of embryos which have been indubated at different temperature (34°C-37°C and 40°C) and collected 5 days post inoculation from this table we can notice that one passage of the virus at 34°C kept the virus pathogencity at an indices of 0.05, 0.067 and 0.166 for temperature of 34°C, 37°C and 40°C rspectively. One passage ofthevirus at 37°C resulted in pathogenicity indices of 0.06, 0.029 and 0.45 for 34°C, 37°C and 40°C respectively. One passag of the vacinal strain virus at 40°C resulted in a virus which showed MICE of 0.11, 0.18 and 0.44 when embryos were incubated at 34°C, 37°C and 40°C respectively. Also the rsults show that the inoculated eggs kept at 40°C resulted in higher embryos mortalities if compared to the other temeprture (34°C and 37°C).

DISCUSSION

ILT was considered to be an exotic disease for Egypt since 1982, the country was attacked by a serious outbreak of this disease which spread rapidly to cover several ares in Egypt. Loses were enormous, mortality reached as high as 19.9 and egg production was lowered 35% in some flocks (TANTAWI et al., 1983). Since the ILT-virus infection occurred among Egyptian poultry farms this needed scientifically based vaccination and control programs.

In this study trials were made to determine the effect of temperature of incubation on virus yield and pathogenicity on the keeping quality of harvested virus. The results as shown in table (1) clear that the incubation of the chicken embryos incubated with the original vaccinal strain at different temperature 34°C, 37°C and 40°C had a considerable influence on the pathogenicity of th virus in inoculated embryos. Thus while the incubation at 34°C and 37°C kept the vaccinal strain at indices characteristic for a virulent ILT virus strain (less than 0.16). The (MICE) mortality index for chicken embryos was calculated according to (IZUCHI and HASEGAWA, 1982), results of these authors suggested that strain with MICE more than 0.27 would be highly pathogenic.

The keeping of incubated eggs at 40°C resulted in higher embryo morlatities giving rise to an index of 0.46 which was clearly denoting an increase of virus pathogenicity indices from that determined for the original vaccinal virus, yet passage of

virus once at 40°C had resulted in higher pathogencity indices even when in the second time the virus was kept (for a second time) at 40°C didn't change the MICE and remined by 0.44. The importance of these results lie in the fact that, a change in the temperture for few degrees higher than that commonly used (37°C) for incubation of infected embryos, a matter which may occure accidently, would have a serious effect on the quality of the produced vaccine in the direction of its pathogenicity to chiken embryos. this later may be a reflection to its increased virulence to the chickens.

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Table (1): Studies on the pathogenicity of the original vaccinal strain of ILt virus for chicken embryos incubated at different temperature.

Sample	Status of embryos	No. of embryo on day/PI							Cumulative No.	*MICE
		1	2	3	4	5	6	7		
34°C	dead	-	-	-	1	1	2	4	8	
	a live	15	15	15	14	14	13	11	97	0.073
37°C	dead	-	-	-	-	-	1	2	3	
	a live	15	15	15	15	15	14	13	102	0.028
40°C	dead	-	-	2	4	7	11	14	38	
	a live	15	15	13	11	8	4	1	82	0.46

Table (2) Effect of temperature on the pathogenicity of ILT in CAM of embryos which have been incubated at different temperatures 34°C , 37°C and 40°C as determined by inoculation of embryonated chicken eggs in allantoic sac.

Sample	Status of embryos	No. of embryos on day PI 1	2	3	4	5	6	7	Cumul-alive No. of embryos	MICE
Group 34°C 34°C	Dead	0	0	0	0	0	2	3	5	
	a live	15	15	15	15	15	13	12	100	0.05
37°C	Dead	0	0	1	1	1	1	3	7	
	a live	15	14	13	13	13	13	11	92	0.076
40°C	Dead	0	0	0	0	2	5	8	15	
	a live	15	15	15	15	13	10	7	90	0.166
Group 37°C 34°C	Dead	0	0	0	0	1	2	3	6	
	a live	15	15	15	15	14	13	12	99	0.06
37°C	Dead	0	0	0	0	0	1	2	3	
	a live	15	15	15	15	15	14	13	102	0.029
40°C	Dead	0	0	0	4	6	9	12	33	
	a live	15	15	13	11	9	6	3	72	0.45
Group 40°C 34°C	Dead	0	0	0	0	2	3	5	10	
	a live	15	15	15	15	13	12	10	95	0.11
37°C	Dead	0	0	0	0	2	4	9	15	
	a live	14	14	14	14	12	10	15	85	0.18
40°C	Dead	0	0	0	2	5	9	12	28	
	a live	13	13	13	11	8	4	1	63	0.44