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OPTIMUM HARVESTING TIME OF S.P.F. EMBRYONATED CHICKEN EGGS FOR PREPARATION OF INFECTIOUS BRONCHITIS VACCINE.

Rahimi,K., Massodi,S., Sadryi,R.

ABSTRACT. **After inoculation of seed viruses of infectious bronchitis in allantoic cavity of embryonating chicken eggs and incubation at 37°C , the virus starts to propagate. The results obtaining indicate that 24hr post-inoculation is the best time for the highest titer of IBV in allantoic fluid of inoculated eggs.**

Key words: INOCULATION/EGGS/ANIMAL DISEASES/VIRUSES/VACCINES

INTRODUCTION

Avian infectious bronchitis is a highly contagious disease of chickens,and it is usually manifested as a respiratory condition specially in young chickens.Layers show less severe respiratory signs than the young chickens but there is a significant drop in egg production and poor quality of eggs. Several types of IBV have been recognized as the cause of infection. All types of such viruses have

been grown in the Amnio-allantoic fluids of embryonated chicken eggs.

Several egg adapted viruses have been used for vaccine production with very satisfactory results.

Massachusetts type egg adapted virus (H_{120}) is also accepted as a vaccine strain, and extensive work has been done on this strain particularly for vaccine production. The purpose of this study was to determine the best harvesting time for infectious bronchitis virus vaccine.

MATERIAL AND METHODS

S.P.F. eggs were received from commercial LOHMAN Company and used for vaccine production. 10 to 11-day-old embryonating eggs were inoculated via the allantoic cavity with vaccine virus strain. Massacusetts type, egg adapted vaccine virus (H_{120}) was selected from a vial of working seed.

The experiment was carried out 5 times, each time 500 S.P.F. embryonated chicken eggs were inoculated with seed virus; subsequently they were divided into five groups of 100 eggs, and were harvested at different post-inoculation times. All groups of embryonated eggs were inoculated via allantoic cavity with 0.1ml of 10^{-3} dilution of working seed which contained originally $10^{6.7}$ EID50/ml.

The eggs were reincubated at 37°C. The embryonated eggs whose embryos died within the first 24hr post-inoculation were discarded.

Twenty four hour following inoculation the first group of 100 inoculated embryonating eggs was harvested.

All harvested embryonated eggs were alive; while in the secone, third, and forth groups , there was a mortality rate of 18.52,37 and 45.86 percent respectively.Dead and alive embryonated eggs were harvested toghether at the end of each experiment. Amniotic-allanto fluid of each group (100 eggs) was collected as vaccine sample at 24, 34, 48 and 56 hours post-inoculation.

Virus titration:

Virus titration was made by inoculation of 0.1ml of serial ten-fold dilutions into allantoic cavity of five 9-day old embryonated chicken eggs.

The fifty percent end point of the embryo infective dose (EID50) was calculated by the Kärber method . The results of virus titration of each experiment is summarized in table No.1.

Table No.1: Results of Titration in different times

State of embryos	Titer of A.F. at 24hr post alive	Titer of A.F. at 34hr post alive-dead	Titer of A.F. at 48hr psot alive-dead	Titer of A.F. at 56hr post alive-dead
Experiment No.1	7.5	7.3	7.1	6.3
" " 2	6.7	5.9	6.5	3.3
" " 3	6.5	6.1	5.5	6.3
4	7.1	6.5	6.7	6.5
5	6.9	6.3	6.1	6.5
	6.94	6.42	6.38	5.78

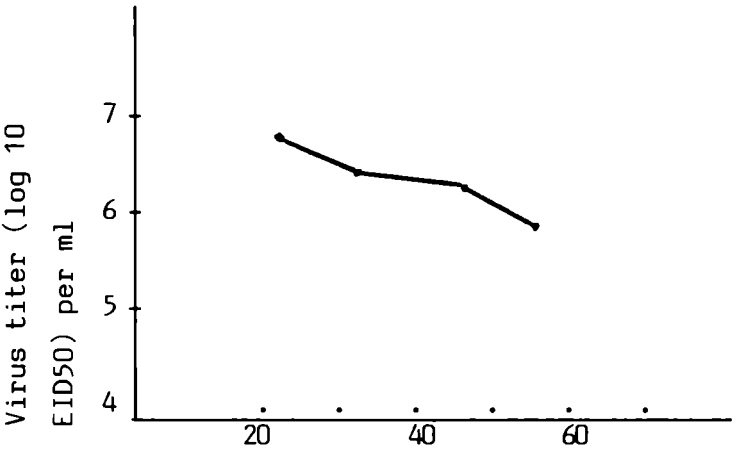

Fig No.1:Embryo-infectivity curve of Massachusestts type of IBV(H-120)

RESULTS AND DISCUSSION

Results of titration of allantoic fluid pools harvested at different times are shown in table & figs. 1.

Our result is in agreement with the results obtained by others (Hitchner and White 1955,Group 1949)indicating that IBV is rapidly propagated in embryonating eggs,reaching a maximum concentration in 24hr after allantoic route inoculation. Our results show that if inoculated eggs were left in the incubator more than 24hr , the embryos begin to die and the mortality rate increases with time. Therefore there is a decline in titer if the eggs are held in the incubator after the demise of the embryos (Hofstad 1984). It has been stated that there is a substance in the allantoic fluid of such eggs which can interfer with the growth of IBV in the chicken embryo if infected eggs were not removed immedieately or in less than

2hr after the demise of the embryo(Group 1949). We conclud that the growth of virus decreaes with the length of incubation period.

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