COMPARISON BETWEEN DUCK EMBRYO FIBROBLAST AND CHICKEN EMBRYO LIVER CELL FOR PROPAGATION OF EGG-DROP-SYNDROME-1976 VIRUS

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ABSTRACT

Propagation of Egg-Drop-syndrome-1976 (EDS-76) virus was compared in Duck Embryo Fibroblast (DEF) and Chicken Embryo Liver (CEL) cell cultures by hemagglutination (HA) test. In fluid and cell associated samples from DEF and CEL cell culture, HA activity appeared on second or third day after inoculation. In CEL, peak of HA titer in fluid samples were observed reached on 5th day after inoculation, and their geometric mean titer (GMT) was 1 : 512. That in cell associated samples were observed reached on 4th day after inoculation and their GMT was 1 : 304. In contrast, peak of virus titer in fluid samples and cell associated samples from DEF were observed reached on 6th day after inoculation, their GMT were 1 : 724 and 1 : 1783, respectively. The result of this study suggest that DEF was better than CEL cell culture for propagation of EDS-76 virus.

INTRODUCTION

The Egg-Drop-Syndrome-1976 (EDS-76) in laying hen is characterised by abberant egg production, usually associated with adverse effects on egg shell quality and colour (Mc Ferran et al., 1977; 1978). The causal agent agglutinates fowl erythrocytes, which permits a hemagglutination-inhibition (HI) test to be used for detecting specific antibodies in sera (Mc Ferran et al., 1977). The EDS-76 virus grows in duck kidney, duck embryo liver, duck fibroblast and chicken embryo liver cells (Adair et al., 1979; Mc Ferran, 1984).

The purpose of this study is to compare the propagation of EDS-76 virus in DEF and in CEL cells by HA test.

MATERIALS AND METHODS

Virus

EDS-76 virus, strain KE-80 which had been propagated in duck embryos, was supplied by PT. Vaksindo Satwa Nusantara, Bogor Indonesia. The virus titer was $10^{7.4}$ TCID 50/0.025 ml and had a HA titer of 1 : 128/0.025 ml.

Cell culture

Cell culture were prepared according to H. Rubin (1973) with a little modification as follows;

DEF Cells : The commercial duck was used as source of eggs for preparation of DEF cultures. Thirteen-day-old embryoes were dispersed in 0.25% trypsin (1 : 250 Difco). The cells were seeded at a concentration of 1.0 x 10^6 cells/ml in growth medium consisting of Eagle's Minimum Essential Medium (Gibco Ltd.) containing 5% fetal calf serum into 45 mm diameter glass petridishes (3.5 ml). Eagle's Minimum Essential Medium with 1% fetal calf serum was used as maintenance medium. Cells were used after 24 hours of inoculation when fully confluent.

CEL cells : The specific phatogen free (SPF) chicken was used as source of eggs for preparation of CEL cell cultures. Livers were obtained from 14-day-old embryoes, were dispersed in 0.25% trypsin (1 : 250 Difco). The cells were seeded at a concentration 1.0 x 10^6 cells /ml in growth medium consisting of Eagle's Minimum Essential Medium containing 5% fetal calf serum (Flow laboratoy Ltd.) into 45 mm diameter petri dishes (3.5 ml). Eagle's Minimum Essential Medium with 1% fetal calf serum was used as maintenance medium. Cells were used after 72 hours of incubation when fully confluent.

Propagation of EDS-76 Virus

Thirty petri dishes of DEF and CEL cell cultures were infected with 0.5 ml containing $10^{3.0}$ $TCID_{50}$

of EDS-76 virus and incubated at 37°C for six days. Viruses were harvested from 5 petri dishes daily. After each culture fluid were transfered to tubes, remaining culture cells were harvested with

and then subjected to freezing and thawing three times. These fluids were centrifuged at 3,000 rpm for 10 minutes. Resulting supernatants were submitted to HA test.

Hemagglutination (HA) Test

The HI test were performed with microtiter method. Two fold serial dilutionof EDS-76 virus was made in phosphat buffered saline. To each dilution, one drop (0.025 ml) of phosphat buffered saline was added, and two drops (0.050 ml) of 0.5% chicken erythrocytes was added and mixed by microplate-mixer. The mixture was allowed to stand at room temperature for 60 minutes. The highest dilution showing complate HA pattern was taken as the HA titer.

RESULTS

As shown in Fig. 1, in fluid and cell associated samples from DEF and CEL cell cultures, HA activity appeared on 2nd or 3rd day after inoculation. Peak of HA titer in fluid samples from CEL were observed reached on 5th day after inoculation and their Geometric Mean Titer (GMT) was 1 : 512. Peak of HA titer in cell associated samples from CEL were observed reached on 4th day after inoculation and their GMT was 1 : 304. Peak of HA titer in fluid samples and cell associated samples from DEF were observed reached on 6th day after inoculation and their GMT were 1 : 724 and 1 : 1783, respectively.

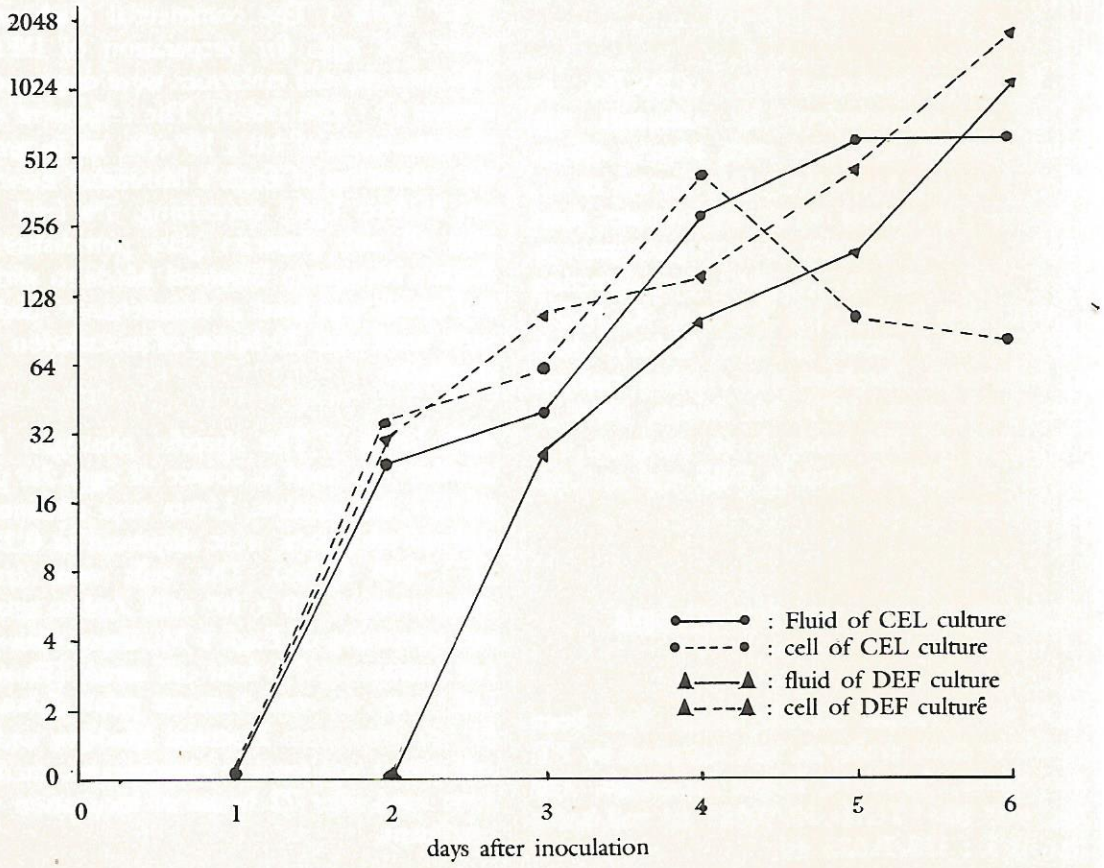

Fig 1. HA titer in DEF and CEL cell culture infected with EDS-76 virus

DISCUSSION

In 1981, Yamaguchi et al. reported that peak of virus titer and peak of intracellular hemagglutination titer were obtained in 48 hours cultivation of CEL and peak extracellular HA titer was obtained in 72 hours of cultivation. But in this experiment, peak of intracellular HA titer and peak of extracellular HA titer were obtained in 96 hours and 120 hours of cultivation, respectively. This different results might be due to the difference of virus strain and the method used.

MC Ferran (1984) described that EDS-76 virus grows to highest titer in duck kidney, duck embryo liver and duck fibroblast cells. It also grows well in chicken embryo liver and duck fibroblast cells. It grows well in chicken embryo liver cells, less well in chicken kidney cells, and rather poorly in chicken embryo fibroblast cells. In our experiment, most high HA titer was obtained from DEF cells, in comparison with CEL cells, suggesting that virus strain used may adapted with duck cells because this strain had been propagated in duck embryo. From the results, we would recommended to use DEF cells for propagation of the EDS-76 virus.

ACKNOWLEDGEMENT

The authour would like to thank Dr. M. Nakamura of National Vaterinary Assay Laboratory, Tokyo, Japan for his technical guidance.

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