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PROPAGATION OF CHICKEN ANEMIA VIRUS IN DIFFERENT CELL CULTURES

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A B S T R A C T

The present study deals with propagation of chicken anemia virus (CAV) in primary chicken embryo fibroblast (CEF) as well as the continuous cell lines African green monkey kidney (VERO) and baby hamster kidney (BHK) for 10 successive passages. The high virus titers were 7.6 log10TCID50 /ml in VERO and 7.5 log10TCID50 /ml in CEF while BHK yielded virus titer of 6 log10TCID50 /ml. The cytopathic effect (CPE) was characterized by cell detachment and subsequent vacculation of the infected monolayers started by 5th to 7th day post infection (DPI) then began to appear more early by the successive passage to reach the 2nd DPI within all cell cultures. VERO cells yielding the highest virus titer were that one of choice to study the growth kinetics of CAV showing that the highest total virus yield could be obtained 72 hours post cell infection. Direct fluorescent antibody technique and electron microscopy were carried out to ensure the presence of CAV in different used cell cultures. These findings indicate the possibility of the use either CEF or VERO or BHK for CAV propagation instead of the unavailable Marek's disease cell culture (MDCC).

Key Words: CAV, CEF, BHK, VERO

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1. INTRODUCTION

CAV is a unique small virus (CAV) circular DNA belongs to family Circoviridae primarily of young chickens but it also infects the chickens of all age groups. It causes immunosuppressive disease, lower viability and production performance by making birds more susceptible to secondary infection [1]. CAV can be isolated and propagated via the yolk sac at 6 days of age, chick embryos could develop normally into chicks. All the chicks hatched suffered from anemia and died at 10 to 15 days of age with bone marrow aplasia [2]. CAV causes cytopathological effects in chicken thymocytes and cultured transformed mononuclear cells by process of apoptosis. In vitro, expression of VP3 induced apoptosis in chicken lymphoblastoid T cells and myeloid cells which are susceptible to CIAV infection [3].CAV-infected MDCC-MSB1 cells showed the apoptosis-specific pattern of nucleosomal laddering which was absent from mock infected cells. The findings suggested that virus interference with programmed cell death plays a significant role in the pathogenesis of CIAV infection [4]. CAV can be propagated in an established cell line derived from Marek's disease (MD) lymphoma (MDCC-MSB1), chicken embryo fibroblast and also the avian lymphoid leukosis (LL) cell line showing characteristic apoptosis pattern of CAV [5]. The number of cells positive for viral antigen measured susceptibility of MDCC

to CAV in immunofluorescence (IF) tests at 3-10 days post infection using direct florescent antibody technique [6]. In Egypt, a local isolate of CAV propagated successfully in VERO and CEF where the CPE was characterized by cell detachment and subsequent vacuolation of the infected monolayers while no obvious CPE was detected in BHK and MDBK cell cultures [7]. He added that CAV-DNA was detected in the infected cell culture fluids by PCR.
Due to the limited ability of CAV to be propagated in different cell cultures, the present work was planned as a trail for propagation of this virus in different available primary cell cultures (CEF) and some cell lines as Vero and BHK-21in aiming to provide a suitable available cell system for further studies on such virus.

2. MATERIALS AND METHODS

2.1. Virus strain:
Commercial Chicken anemia virus vaccine adapted and propagated on MDCC cell line were kindly supplied by Inter Vet. Company.

2.2. Embryonated chicken eggs (ECE):

Specific pathogen free (SPF) embryonated chicken eggs (ECE) 9-11 days old were used for preparation of chicken embryo fibroblast. It was kindly supplied by VACESRA

2.3. Cell culture:

2.3.1. Primary cell culture:

2.3.1.1. -Cell lines:

African green monkey kidney cell line (Vero) and baby hamster kidney cell (BHK-21) were kindly supplied by Veterinary Serum and Vaccine Research Institute (VSVRI), Abassia, Cairo. The cells were used for CAV propagation according to [5].

3-Virus passage in cell cultures:

CAV was passage ten successive times in each cell culture where the onset of CPE; time of harvest and virus titration of each virus passage were carried out. On each virus passage, the virus was inoculated on confluent cell sheet seeded 3 days before virus infection and allowed for virus adsorption for one hour at 37^{o}C. The non-adsorbed virus was washed and the infected cell culture was maintained with maintenance MEM supplied with 2% new born calf serum. Non-infected cell control was concluded with each virus passage.

2.4.Virus titration:

Titration for the propagated CAV in different used cell cultures was carried out using the micro titer technique according to [8] and the virus titer was calculated as $TCID_{50}$/ml according to [9].

2.5. Growth kinetics of CAV in cell culture:

The used cell cultures were seeded in Lightens tube containing cover slips then infected with the highest virus passage where the cell free; cell associated and total virus yield were determined on regular intervals post cell infection till harvesting. In addition, infected cells on cover slips were stained with hematoxilin and eosin according to [10] to demonstrate the induced CPE.

2.6. CA hyper immune serum conjugated with fluorescent isothiocyanate:

CA hyper immune serum was supplied kindly by VSVRI and used in the direct FAT to confirm the presence of CAV in the used infected cell cultures.

2.7.Indirect fluorescent antibody technique (IFAT):

Direct FAT was carried out on infected cell cultures according previous method [8].

2.8. Electon microscopy:

Negative contrast electron microscopy was carried out on infected cell cultures to investigate and confirm the incidence of CAV in the infected cell cultures according to the method applied previously [11].

3. RESULTS

Table (1): CAV passage in CEF cell culture

virus passage	Onset of CPE (DPI*)	Time of harvesting (DPI)	Virus titer (log10/mi)
1	6	9	1
2	5	8	2
3	4	7	2.5
4	5	7	3
5	4	6	3.8
6	3	5	4.5
7	3	5	6
8	2	3	7
9	2	3	7.2
10	2	3	7.5

*DPI= days post infection

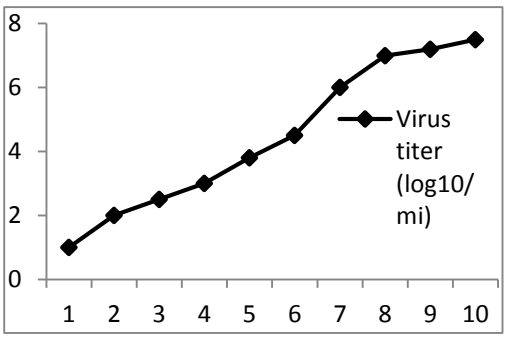

Fig (1): Propagation of CAV in CEF cell culture

Table (2): CAV passage in Vero cell culture

virus passage	Onset of CPE (DPI*)	Time of harvesting (DPI)	Virus titer (log10/mi)
1	7	10	0.5
2	7	9	1
3	6	8	1.5
4	5	7	2
5	4	7	3
6	3	5	4.5
7	2	4	6
8	1	3	7
9	2	3	7.6
10	2	3	7.7

*DPI= days post infection

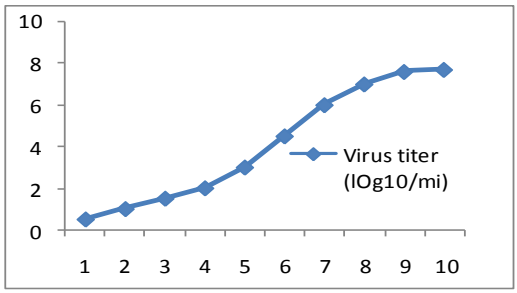

Fig (2): Propagation of CAV in VERO cell culture

Table (3): CAV passage in BHK cell culture

virus passage	Onset of CPE (DPI*)	Time of harvesting (DPI)	Virus titer (log10/mi)
1	5	6	0.6
2	5	7	1
3	5	6	1
4	4	7	1.5
5	3	5	2
6	4	6	3.5
7	3	5	5
8	3	5	5.5
9	2	4	6
10	2	4	6

*DPI= days post infection

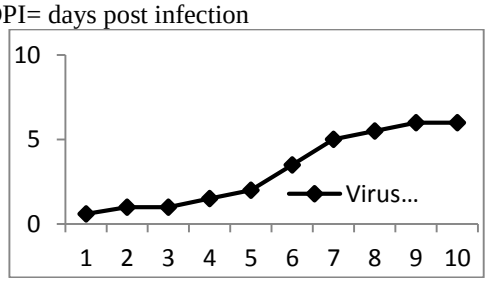

Fig (3): Propagation of CAV in BHK cell culture

Table (4): Growth kinetics of CAV in Vero cell culture

Hours post cell infection	Virus titer (log10 TCID50/ml)		
	Cell free virus	Cell associated virus	Total Virus yield
1	0	≤ 0.5	≤ 0.5
2	0	0.5	0.6
3	≤ 0.5	1.0	1.0
4	0.5	2.0	2.0
5	0.5	3.0	2.0
6	1.0	4.0	3.7
12	1.5	3.0	4.0
36	5.0	2.0	6.5
48	5.7	1.5	6.5
60	6.5	1.0	7.0
72	7.0	0.5	7.6

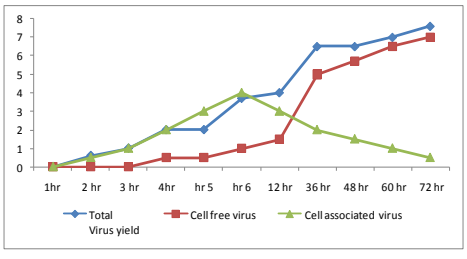

Fig (4): Growth kinetics of CAV in VERO cell culture

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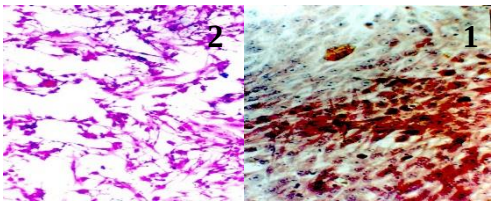

Photo (1): Normal CEF cell culture (H&E, 100 xs).Photo (2): CAV infected CEF (H&E, 100 xs) showing cell rounding and cell lysis.

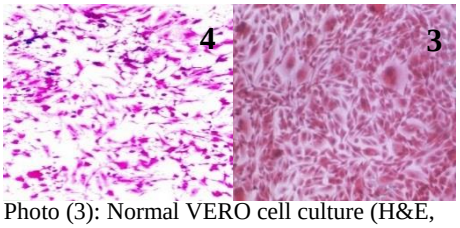

Photo (3): Normal VERO cell culture (H&E, 100 xs).Photo (4): CAV infected VERO cell culture (H&E, 100 xs).

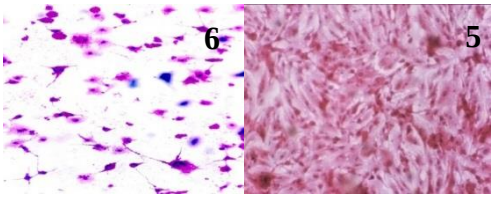

Photo (5): Normal BHK cell culture (H&E, 100 xs), Photo (6): CAV infected BHK cell culture (H&E, 100 xs).

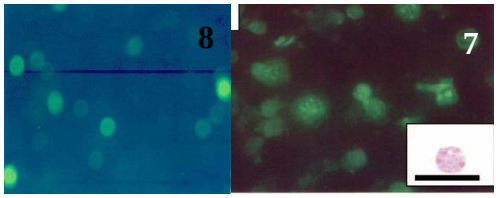

Photo (7), (8): Positive direct FAT on infected VERO cell culture (100 xs) showing intra-nuclear apple green reaction.

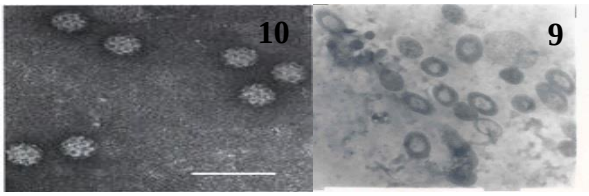

Photo (9): Electron microscopy of CAV in infected VERO Cell, Photo (10) Reference electron microscopy of CAV (International Committee on Taxonomy of Viruses, 2002) Virus Taxonomy Online: Negative contrast electron microscopy of CAV particles stained with uranyl acetate. (Courtesy of M.S. McNulty.) The represents 50 nm (2002).

4. DISCUSSION

The present work trays to provide a cell culture system suitable for propagation of CAV instead of MDCC which considered unavailable host and used for not neglectable time. The virus was propagated in each of CEF; VERO and BHK cell culture for ten successive passages. On the starting of viral passage in such cell cultures, it was noticed that the onset of CPE was retarded to be ranged from 5-7 days post cell infection then began to appear more early to be on the 2nd day post infection with harvesation time ranged from 6-9 days post cell infection during the first viral passage to be 3 days on the 10th passage in most used cell culture. Vero cell yielded the highest virus titer (7.6log$_{10}$ TCID$_{50}$ /ml) followed by CEF yield (7.5 log$_{10}$ TCID$_{50}$ /ml) and BHK yield (6 log$_{10}$ TCID$_{50}$ /ml) as shown in tables (1,2&3) and fig (1, 2&3).

These findings agree with what obtained by [7] who found that Vero cell culture yielded the CAV titer higher than that obtained by CEF but differ from his findings that BHK cell culture was unsuitable for CAV propagation the thing which could be attributed to the virus nature where he used primary isolated field isolate while this work used MDCC adapted virus. The stained infected different cell cultures showed that the noticed CPE was characterized by cell rounding, detachment, apoptosis and vacculation (photo 2, 4&6) in agreement with what recorded by[3]; [4]and [7].

On the other hand direct FAT carried out on infected different cell culture confirmed the presence of CAV showing clear apple green intra-nuclear positive reaction (phot-6) in a parallel manner to the reference

findings (photo-7). In this respect [6] applied direct FAT on infected MDCC and determined the presence of CAV intra-nuclear. In addition electron microscopy applied to different infected cell cultures indicated and confirmed the presence of CAV as icosahedrons (photo 8) as what demonstrated by the reference findings (photo-9) in agreement with [12].

Studying the growth kinetics of CAV in VERO cell culture (it was of choice where it yielded the highest virus titer), it was found that the best time to obtain the highest virus yield is 72 hours post cell infection. There were no available data that discuss the growth kinetics of CAV in cell cultures but (7) found relative intensity value of CAV in VERO cells using PCR. Depending on the obtained results, it could be concluded that VERO, CEF and BHK cell cultures could be used successfully for CAV propagation instead of the unavailable MDCC.

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امرار فيروس أنيميا الطيور فى مزارع نسيجية مختلفة

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الملخص العربي

أجريت هذه الدراسة محاولة لإيجاد مزارع نسيجية بديلة لخلايا مرض ميريك (MDCC) الغير متوفرة لأقلمة فيروس أنيميا الطيور حيث تم استخدام خلايا أجنة الدجاج (CEF) كمزارع أولية وخلايا كلى القرد الأخضر الأفريقي (VERO) وخلايا كلى اليربوع السوري الذهبي (BHK) تم تمرير الفيروس فى كل منها عشر مرات متتالية حيث وجد أن خلايا VERO تعطى أعلى معيار للفيروس تليها خلايا CEF ثم خلايا BHK (7.6 لوج 10 ، 7.5 لوج 10، 6 لوج 10 جرعة نصف معدية للزرع النسيجي/مل على التوالي) وقد تميز تأثير الفيروس المرضى على الخلايا المختلفة باستدارة الخلايا ثم موتها مع ظهور فجوات ثم انفصال الخلايا عن سطح التذريع كما اوضحت ذلك شرائح الخلايا المصبوغة كما تم التأكد من وجود الفيروس فى المزارع النسيجية المختلفة بإجراء اختبار الوميض الفلوريسنتى المناعي المباشر وبالفحص المجهري الإليكتروني. وبدراسة منحنى نمو الفيروس فى خلايا VERO تبين أن أفضل وقت للحصول على أعلى معيار كلى للفيروس هو 72 ساعة بعد عدوى الخلايا.

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