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Isolation of some respiratory viruses from camels

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The presence of three of the main respiratory viruses found in camels, that is, peste des petits ruminants virus (PPRV), bovine para-influenza virus 3 (PIV3) and bovine herpesvirus-1 (BHV-1), was investigated and isolation of these viruses in different cell cultures was carried out. A total of 100 pneumonic camel lungs collected from slaughterhouses were screened for the presence of PPR, PIV3 and BHV-1 viral antigens using ELISA. Forty five were found to be positive for PPRV, 15 for PIV3 and 4 for BHV-1 virus antigen. Of these samples, 15 PPR, 12 PIV3 and 3 BHV-1 positives were inoculated in MDBK cell line, primary and secondary lamb and bovine kidney cells. Each of the three viruses were successfully isolated in the different cultures used; CPE was seen at day 14 for PPR, day 3 for PIV3 and day 2 for BHV-1 after infection; CPE was observed, characterized by cell rounding, elongation with some syncytia formation for both PPR and PIV3, and BHV-1 samples showed cell rounding, edematous cells and cell sheet detachment. This is the first report of the isolation of PPR virus from camels.

Key words: Camels, cell cultures, respiratory viruses, Sudan.

INTRODUCTION

Respiratory infections are considered one of the main factors decreasing animal production. In Sudan, Abbas et al. (1993) reported that respiratory diseases are complained by 28.6% of pastoralists questioned in eastern Sudan while 1.6% of them considered it as the first constraint to camel production. Viruses encountered in respiratory infections in camels are parainfluenza virus 3, influenza virus A and B, adenovirus, respiratory syncytial virus (RSV) and infectious bovine rhinotracheitis (IBR) (Dioli and Stimmelmayr, 1992). Khalafalla et al. (2010) detected a new emerging morbillivirus in camels causing respiratory outbreak in Eastern Sudan, the virus was proved to be peste des petits ruminants (PPR). Virus isolation in tissue culture is one of the standard techniques in virological work although it is time consuming for some viruses. Anderson (1999) stated that even when diagnosis of PPRV has been carried out by rapid technique like ELISA or AGID, for further studies the virus should always be isolated from field samples in tissue culture. Taylor (1979) reported that PPRV may be isolated in primary lamb kidney cells or VERO cell tissue cultures. CPE of PPRV was described by many authors (El Hag and Taylor, 1984; Abu El Zain et al., 1990). Anderson et al. (1996) noticed that in coverslip cultures CPE might develop earlier than day five. Murphy et al (1999) reported the presence of intracytoplasmic and intranuclear inclusions. Anderson et al (1996) advised that after 5 to 6 days of inoculation, blind passage should always be carried out as CPE may take time to appear. Van der Maaten (1969) examined the replication of the SF-4 strain of bovine para-influenza virus 3 (PIV3) in different cell cultures Madin-Darby Bovine Kidney (MDBK),

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secondary bovine kidney (BK), embryonic bovine trachea (EBTr). He found that the virus replicated equally well in MDBK and secondary BK cell cultures but less successfully in EBTr cultures. Williams et al. (1991) isolated IBR virus from lung of llamas; Nawal et al. (2003) reported the first detection and isolation of this virus in camels in Egypt.

This study intended to isolate PPRV, PIV 3 and BHV-1 from camels in different cell cultures.

MATERIALS AND METHODS

Detection of some respiratory viruses in camel lungs

A total of 100 tissue samples collected from camel lungs showing pneumonia in slaughterhouses were screened for the detection of PPR antigen using IcELISA obtained from CIRAD, France, PIV 3 and BHV-1 antigens using ELISA obtained from Bio X Diagnostics, Belgium. ELISAs were used as instructed by the manufacturers.

Preparation of camel lung tissue

The camel lung tissue specimens were put in pestle and mortar, prepared by cutting with scissor and forceps into small pieces then ground with little amount of sterile PBS. The tissue was then suspended to be 20% in PBS with antibiotics then centrifuged at 2000 rpm for 10 min and the supernatant was separated in sterile tube and stored at -20°C till used. Inoculated specimens were 15 IcELISA positive specimens for PPR antigen, 12 ELISA positive specimens for PIV3 and 3 BHV-1 positive specimens.

Cells

Madin Dairby bovine kidney cell line (MDBK)

Madin Dairby bovine kidney cell line (MDBK) was kindly provided by Dr. Ibrahim El Sabagh, Virology Department, Faculty of Veterinary Medicine, Cairo University.

Cells were grown in new GMEM media with antibiotics, other supplements, plus 5% feotal bovine serum was added to the media. The cells then distributed to 25 cm² plastic flasks (SIGMA, USA) and incubated at 37°C until confluence sheet formed.

Primary lamb and bovine kidney cells

Primary lamb and bovine kidney cells were kindly supplied by Department of Viral Vaccines of the Veterinary Research Institute, Khartoum, Sudan. Primary cells were inoculated by the suspected specimens; some flasks were used to prepare secondary cell culture.

Inoculation of the suspected specimens in MDBK, lamb and bovine kidney cells

All inoculation procedure was done in sterile condition in laminar air flow chamber (BDSL). The growth media of uninfected confluent MDBK cell line cells was discarded in sterile glassware then 0.3 ml of the supernatant of homogenized lung tissue was inoculated in the MDBK cells flask without media and the inoculated cells were incubated at 37°C for 1 to 1.5 h. Then the inoculum was discarded and the cells were washed 3 times with sterile GMEM media containing antibiotic and antifungal. One to two cell flasks with uninfected MDBK cells were used as control. New media without serum was added to the inoculated and uninfected cells. The same process was used for inoculation into lamb and bovine kidney cells. The cells were examined daily up to 21 days under inverted microscope (KRÜSS, Germany) to observe the appearance of cytopathic effect (CPE). Tissue culture procedure was applied as previously described (OIE, 2013).

Adaptation of the isolated viruses to the MDBK, lamb and bovine kidney cells

After the CPE of the inoculated virus reached more than 80% of the cell sheet, a final harvest of the infected cells was done by keeping the infected cells at -20°C, freezing and thawing was undergone for three times and a second passage of the virus was done by inoculation of 0.3 to 0.5 ml of the first passage onto new uninfected cells by the same method of specimen inoculation mentioned above. The time of CPE appearance was observed. Five blind passages were applied.

Identification of the isolated viruses

Tissue culture harvests were examined for the identification of the isolated viruses; PPR antigen was detected using IcELISA obtained from CIRAD, France and PCR using Qiagen one-step RT/PCR Kit, primers used were Np3 and NP4, expected amplicon size was 350 bp (Kwiatek et al., 2011). PIV3 and BHV-1 antigens were detected in the harvests using FAT; conjugates were obtained from BioX Diagnostics, Jemele, Belgium.

RESULTS

Detection of some respiratory viruses in camel lungs

Using ELISA, out of 100 camel lung specimens tested, 45 were found to be positive for PPR, 15 positive for PIV3 and 4 for BHV-1 antigen.

Isolation and identification of respiratory viruses in tissue culture

Isolation of PPRV in MDBK cell line, lamb and bovine kidney cells

A total of 30 specimens ELISA positive for PPR (15), PIV3 (12) and BHV-1 (3) were inoculated into the 3 types of tissue culture. Fifteen IcELISA positive specimens for PPR virus, of which 6 were positive also for PIV3 and 3 were also positive for BHV-1 were inoculated in MDBK cell line, lamb and bovine kidney cells.

For the 6 specimens negative for the other two viruses, CPE appeared on day 14 post inoculation (PI) which was rounding, floating cells with some syncytia and detachment of cell sheet, 80% CPE was reached at day 21 PI, the harvests were tested positive for PPR using IcELISA and PCR. In the other 9 specimens which were positive also for PIV3 or BHV-1, one of which showed the

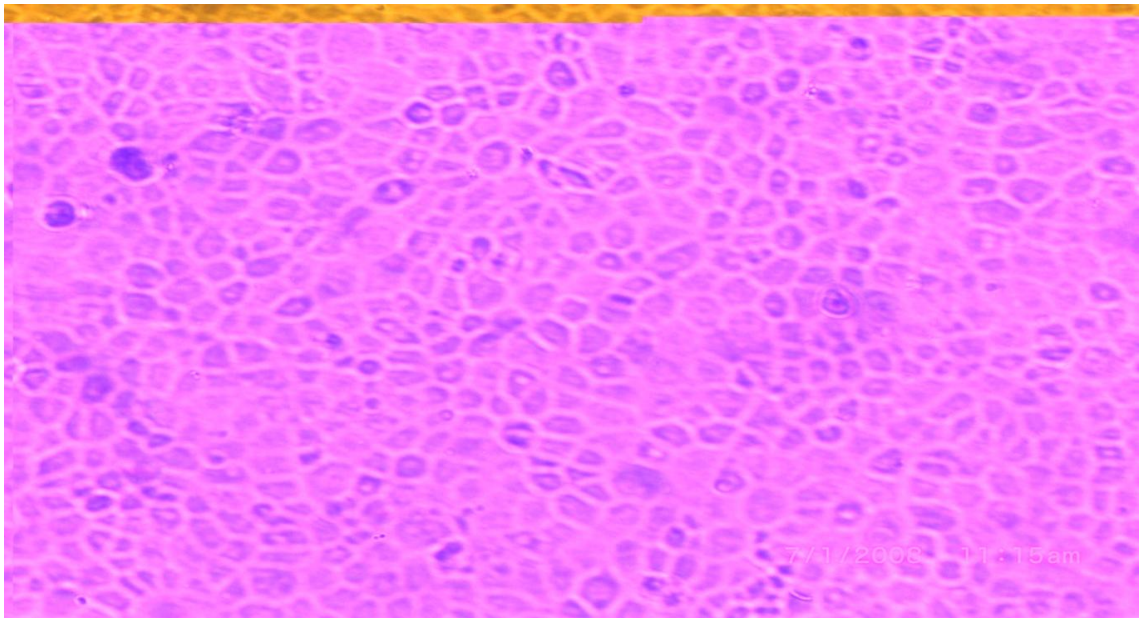

Figure 1. Uninfected MDBK cells used as control.

CPE after 48 h of inoculation and reached more than 80% in the third day post inoculation with rounding and aggregation of infected cells, approximately all cells except few were detached and floated in the media. When applying direct fluorescent antibody technique (FAT) for BHV-1 to the harvest it revealed positive.

In other 4 specimens, the CPE appeared in the third day post inoculation; began with floated rounded cells then two days after, few number of cell aggregations were noticed, there were increase in the floated rounded cells, appearance of some floated syncytia and elongation of cells; the CPE reached more than 80% between 7 and 10 days post inoculation ending by detachment of most of the cell sheet. The resulted harvests were positive for PIV3 when examined by FAT.

Twelve only PIV3 ELISA positive specimens were isolated in MDBK cell line, lamb and bovine kidney cells; the CPE appeared after the second to third day of inoculation as cells aggregation with rounding and floating cells, some multinucleated cells, elongation of the cells, cells sloughing from the intact sheet; the CPE reached more than 80% within 7 to 10 days post inoculation. The final harvests for the 12 specimens were detected by FAT with positive results for PIV3 antigen.

Out of 3 camel lung specimens, BHV-1was isolated from two in MDBK cells, the characteristic cytopathic effect (CPE) appeared 24 to 48 h post-inoculation; it was as previously reported (rounding, edematous cells, sloughing).

It was noticed that there was no difference in the CPE observed in the different cell cultures except for the appearance of the CPE in the primary lamb and bovine kidney cells one day before the other cultures in some specimens. The normal cells and CPE of the isolated viruses are shown in Figures 1 to 3.

DISCUSSION

Virus isolation in tissue culture is routinely used as diagnostic tool and for further characterization work. PPRV had been isolated in primary lamb kidney and VERO cells (Taylor, 1979). The CPE produced by PPRV can develop within 5 to 19 days and consists of cell rounding, refractile cells and aggregation culminating in syncytia formation with eventual detachment of cells. The syncytia are characterized by a circular arrangement of nuclei giving a clock face appearance (Hamdy et al., 1976; El Hag Ali and Taylor, 1984; Abu El Zein et al., 1990).

In this study trials for isolation of PPR virus from camel tissues were applied using MDBK cell culture, primary and secondary lamb and bovine kidney cells. PPRV was isolated in the different cell cultures used. CPE was observed at day 14 PI in 6 PPR positive PIV3 and BHV-1 negative specimens, as expected, observed CPE were cell rounding, refractile cells, elongation and aggregation with syncytia formation ended with detachment of cells, it reached 80% at day 21 to 22 PI. The isolation of PPR virus failed in some specimens due to growth interference of other viruses (BHV-1 and PIV3) that were found in association with PPRV, they were more faster in showing CPE as BHV-1 needs 3 days to infect almost all cell sheet, PIV3 needs 7 to 10 days to infect more than 80% of the cell sheet while PPRV needs between 8 and 19 days to show CPE (Abu Zain et al., 1990, Anderson et al.,

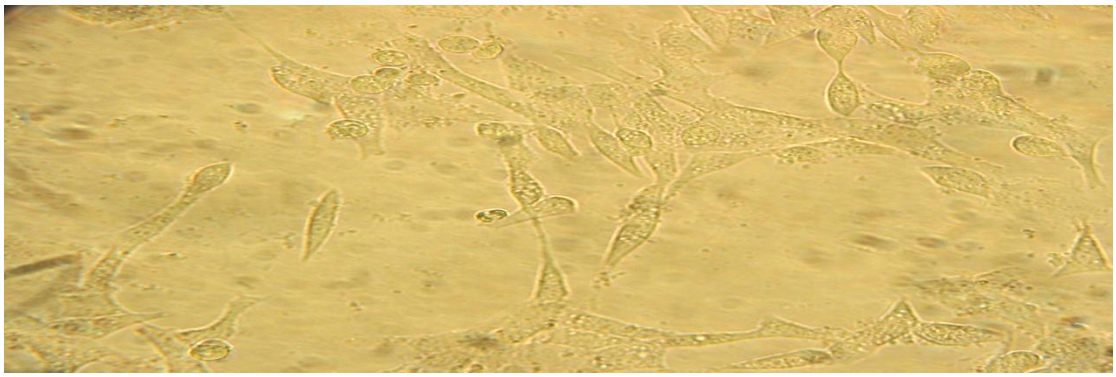

Figure 2. Cytopathic effect induced in MDBK cells by paramyxoviruses (PPRV or PIV3). Note: Elongation, rounding and sloughing of infected cells with paramyxovirus 7 days for PIV3 and 21 days post inoculation for PPR.

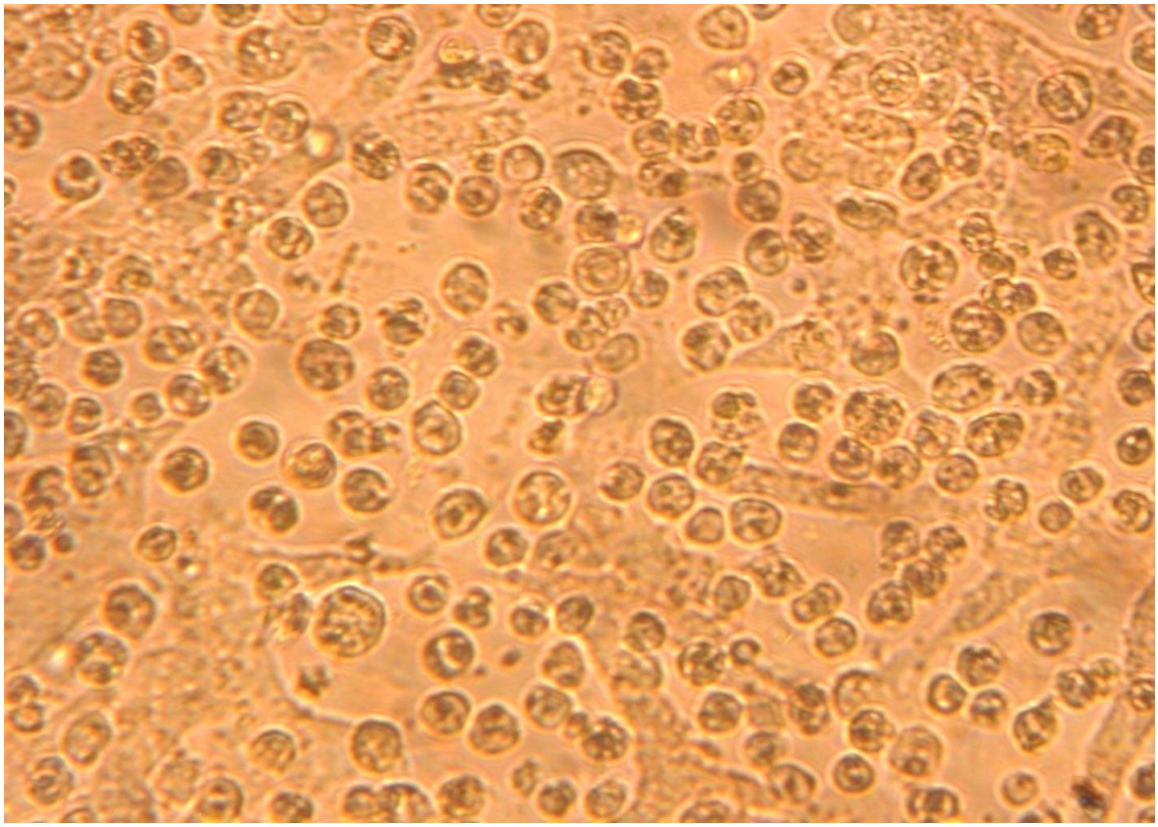

Figure 3. Cytopathic effect induced in MDBK cells by IBRV. Note: Edematous cells, rounding and floating of infected cells and multinucleated cells 3 days post inoculation.

2000, Henrickson, 2003).

Isolation of PIV3 in different cell cultures was documented. Van der Maaten (1969) compared three tissue culture systems for BPI3 isolation, MDBK, secondary bovine kidney were more suitable for virus replication than embryonic bovine trachea. Shaker (2003) reported the isolation of PIV3 from camel lung (1 of 2) and 4 out of 40 nasal swabs in MDBK. In the present study isolation of PIV3 virus from camel lung specimens in MDBK, primary and secondary lamb and bovine kidney cell cultures was reported. Typical PIV3 CPE appeared; rounding refractile cells, cells elongation and sloughing with some syncytia formations, which are similar to that described by Henrickson (2003). The virus was identified

by FAT. The first detection and isolation of PIV3 in camels in Egypt was reported by Nawal et al. (2003) while the first report for the isolation of PIV3 from camels in Sudan was reported by Intisar et al. (2010).

BHV-1 has been isolated from Sudanese cattle by Eisa (1983) and Hassan and El Tom (1985). Williams et al. (1991) isolated IBR from lung of llamas while Nawal et al. (2003) reported the first detection and isolation of this virus in camels in Egypt. In this study BHV-1 was isolated in MDBK, primary and secondary lamb and bovine kidney cells. Observed CPE in different cultures used was like that described previously in MDBK, which were rounding, refractile edematous cells, with complete detachment of cell sheet (Intisar et al., 2009). No big difference was observed between different cell cultures used, however Wellenberg et al. (2002) reported that MDBK and Vero cells were more susceptible for BHV1.

This is the first report for the isolation of PPRV from camels in different cell cultures, as well as the isolation of PIV3 and BHV-1 from camels in lamb and bovine kidney cells.

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