Prevalence of antibodies against Bovine herpes virus-1 (BHV-1) in camel sera and rapid detection of the virus antigen in camel lungs

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Abstract

Bovine herpes virus-1 (BHV-1) is an important worldwide pathogen that primarily infects cattle and other ruminants and is associated with serious economic impact to the animal production sector. Camels are indispensable domesticated animals for the people of desert areas and share the same environment and pasture with cattle, goat, sheep and other ruminants which increase the likelihood of exposure to these pathogens. The investigation presented in this paper was conducted in Basateen,Cairo and Embaba abattoirs, Giza and designed to detect BHV-1 in 18 lung tissues by immunoperoxidase test (IP) after tissue culture direct inoculation on MDBK cells. Two samples out of the 18 samples were clearly stained by the immunoperoxidase as brown granules in the nucleus of the MDBK cells. The samples were propagated onto MBDK cell culture and the CPE appeared in the two samples 1-2 days post inoculation in the form of rounding of cells and grapes formation. On the other hand serum samples from 384 camels collected from the two abattoirs were tested by indirect ELISA for the detection of antibodies to BHV-1. 98 serum samples (35.3%) out of 278 serum samples from camels in Basateen abattoir and 22 serum samples (20.8%) out of 106 serum samples from Embaba abattoir were positive to BHV-1. Finally the conclusion is that the BHV-1 infection was common between the camels slaughtered in abattoirs and further epidemiological studies is recommended to find out the possible virus pathogenesis and its role in camel bronchopneumonia and economic importance.

Key words: BHV-1, MDBK cells, ELISA , bronchopneumonia.

Introduction

Single humped camels, Camelus dromedarius, together with the two humped camels Camelus bactrianus, inhabit wide areas throughout the world, and have an important impact on people's life as essential sources of human food, entertainment and recent racing sport which is important for the rapidly growing tourism industry in Arab countries. Camels have a unique adaptation to harsh environmental conditions. The adoption of modern husbandry and management methods and availability of efficient veterinary services has steadily increased the number of camels as an alternative source of milk and meat **(Kappeler 1998)**.

Bovine Herpes Virus type 1 (BHV-1) belongs to the Herpesviridae family, subfamily alpha herpesvirinae. BHV-1 is the etiology of clinical diseases including infectious bovine rhinotracheitis, (IBR), infectious pustular vulvovaginitis (IPV) and balanoposthitis (IBP), as well as a systemic generalized form accompanied by encephalitis .It is one of the most economically important diseases of farm animals despite low mortality rate. IBR acts also as an immunosuppressive agent predisposing the host to a several secondary bacterial invasions **(Straub, 2001).** The virus causes loss of appetite and reduced milk yield. It is distributed worldwide and characterized by respiratory syndromes manifested by coughing, rhinitis, fever, and broncho-

pulmonary complications. The virus can remain latent after the primary infection, and might be later provoked after a viral reactivating event **(Murphy *et al*., 1999).**

Evidence of BHV-1 occurrence in Old World Camelid (OWC) is scarce. However, (**Bildfell *et al*., 1996 and Schwyzer & Ackermann, 1996**), describe encephalitis and blindness in camels caused by Equine herpesvirus-1 EHV-1, which has a close genetic relation to BHV-1. Higher incidences of BHV-1 associated diseases are reported in New World Camelid (NWC) than in OWC counter parts: bronchopneumonia, encephalitis, and progressive cough are evident in llamas and alpacas **(Rivera, *et al*.,1987; Rebhunn *et al*., 1988; Williams *et al*., 1991; Picton, 1993; Mattson, 1994)**. Higher prevalence has been recorded when grazing pastures are shared with other ruminants.

Antibody titres (1:5) are reported in 5.8% of dromedary samples screened in Tunisia **(Wernery and Kaaden, 1995)**. Antibodies to BHV-1 `were detected in Peruvian llamas and alpacas **(Rosadio *et al*., 1993)**. Seroprevalence of BHV-1 in alpacas in Peru was found to be 5% **(Rivera *et al*., 1987)**, while **Picton (1993)** reported the detection of BHV-1 antibodies in only 0.7% of 270 llamas in Oregon.

In Egypt BHV-1 was firstly detected and isolated by **Nawal *et al.* (2003)** from camels in Egypt. A prevalence of 13.5% is reported in 171 dromedaries serum samples **(Moussa *et al*., 1990, Eisa, 1998 and Hassanein *et al*, 2008).**

For virus isolation, various cell cultures of bovine origin are used as secondary lung or kidney cells or the Madin–Darby bovine kidney cell line (MDBK).The virus neutralization test and various enzyme-linked immunosorbent assays (ELISA; indirect or blocking) are most widely used for antibody detection **(OIE 2010)**.

Although, the viral pathogen does not directly threaten human health, it has economic impact to animal production and international trade. This work attempted to identify the role of camel population in transmission and spread of BHV-1 in Egypt and study the occurrence of the BHV-1 in camels from Basateen and Embaba abattoirs, Egypt by:

- Detection of Ag by immunoperoxidase test and isolation of the virus on MDBK cell culture.
- Introduce ELISA for the detection of antibodies against BHV-1 in camelids and its use in more studies.

Materials and Methods

1. Sample collection

Lung samples: 18 tissue samples from lung of camels showing lesions of pneumonia were collected from Basateen abattoir. 2 gm of the tissue is mixed with PBS containing antibiotics (800 µg streptomycin, 800 IU penicillin and 400 IU Gentamycin/ ml) and grind in sterile mortar then centrifuged at 1,000 xg for 10 min. The supernatant fluids were separated and kept at –70° C until used for BHV-1 isolation.

Serum samples: serum samples of 384 camels were collected form Basateen and Embaba abattoirs during slaughter. All animals were clinically normal at the time of sampling, (May-July 2008). **Table (1).** summarizes the total number of samples. The sera were stored at -20 ºC until use.

Table (1). Information about serum samples collected from camels during period May-July 2008.

Abattior	Month of collection			Total Number
	May	June	July	
Basateen	123	70	85	278
Embaba	60	10	36	106
total	183	80	121	384

2-Tissue culture, virus and media: In this study a local AbuHammad IBR strain was used as BHV-1 .It was propagated in Madin-Darby Bovine kidney (MDBK) American Tissue Culture Collection **(ATCC CCL-22)** cells grown in Earle's minimal essential medium (MEM) **(Invitrogen S.A., Merelbeke, Belgium)** supplemented with 40mg Gentamycin/L and 2% heat-inactivated horse serum. Positive and negative bovine anti BHV-1 sera (kindly supplied by ELISA unit, AHRI) are used as control positive and negative in ELISA test.

3- Detection of BHV-1 by immunoperoxidase test: The test was carried out according to **(Edwards *et al.*,1983).** The technique was used on lung samples. MDBK cells were suspended in media and 200ul/well were added in tissue culture plate wells. The plates were left for 6-8 hours in Co2 incubator then inoculated with samples 50 ul/well. After 12 hour the plates were washed with sterile PBS three times and then fixed with ethanol, methanol and acetone mixture then in 0.5% H2O2 in methanol for 30 minutes then washed. Bovine Hyper immune serum against BHV-1 (detecting antibody) is added and incubated at 37 ◦C for 1 hr then washed 3 times by PBS and left for air drying. Anti bovine peroxidase conjugate was diluted 1/100 , added and incubated at 37 ◦C for 30 min. The cells then washed and add Tris buffer for 10 min. Colored reaction was obtained by addition of freshly prepared diaminobenzidine solution for 10-15 min at room temp. The stained cells were read by inverted microscope.

4. Virus isolation in cell culture

Lung samples were successfully propagated on MDBK cell line. 200ul of the supernatant of homogenate was inoculated onto the MDBK cells. The cells were examined daily under inverted microscope to observe the cytopathic effect (CPE) appearance. (**Brunner *et al.*, 1988 and Xingnian and Kirkland2008**)

5- Preparation of BHV-1 antigen: According to **Edwards *et al.* (1986**) roux cultures of MDBK cells are inoculated with BHV-1 local isolate AbuHammad, overlaid with medium containing 2% horse serum and incubated for 24 hours at 37°C. The cells are scraped off and pelleted. The supernatant medium is discarded. The pellet is treated with two volumes of 0.5% Nonidet P40 in PBS for 60 minutes at 4°C, and centrifuged at 20.000 rpm for 20 minutes to remove the cell debris. The supernatant antigen is stored in small aliquots at -70°C; Non infected cells are processed in parallel to make a control antigen.

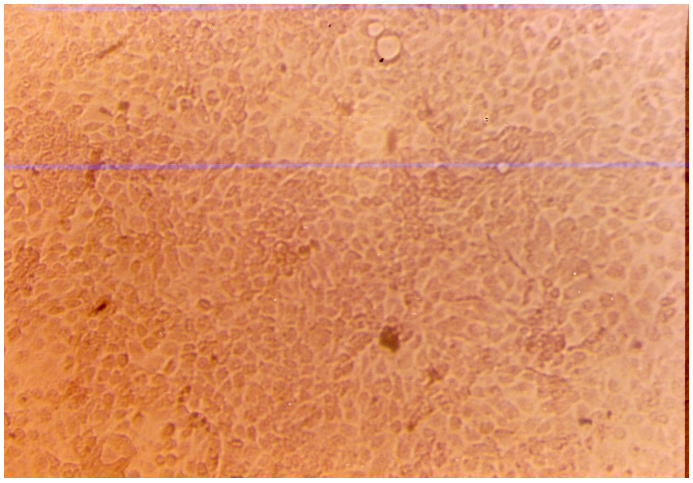
6-Titration of the prepared BHV-1 antigen: Using check board titration against reference positive and negative BHV-1 serum according to **Cho and Bohac (1984).**

7- Indirect ELISA for detection of BHV-1 antibodies in camels sera: according to the method described by **Vollar *et al.* (1979)**: An optimum dilution of the antigen (predetermined by titration) was diluted in carbonate bicarbonate buffer PH 9.6 (the best dilution used 1: 200). 50 ul / well was added to all 96 well flat bottom polystyrene microtiter plate (Nunc Imunolon – I). Then covered and incubated overnight in refrigerator at 4^0C. washing 3 times with washing buffer, and then drying . 50 ul / well of blocking buffer was distributed the plate and incubated at 37^0C for 30 minutes, then washed and dried. 95 ul/well of blocking buffer was distributed, then 5 ul of Tested serum samples were added starting with column 3 in duplicate, the final concentration was 1: 20. The positive and negative control sera were added with the same concentration. The plate was covered with a lid and incubated at 37^0C for one hour on a shaker then washed and dried. 50 ul/well of protein A horse radish peroxidase conjugate diluted 1: 1000 in blocking buffer were added and the plate was incubated at 37^0C for one hour on a shaker, then washed and dried. 50 ul / well of freshly pre-

pared substrate solution was added, incubate the plate for 10 – 20 minutes .The reaction was stopped by adding stopping solution and the plate was read at 492 nm. The cut off point between positive and negative serum values was determined by taking twice the means of the optical density values of negative control serum.

Results

1- Detection of BHV-1 by immunoperoxidase test: The samples were examined for the presence of virus. The virus was clearly stained by the immunoperoxidase as brown granules in the nucleus of the MDBK as shown in **Photo (1a&b).**



Photo(1a). Non infected MDBK cells stained by immunoperoxidase (400X)

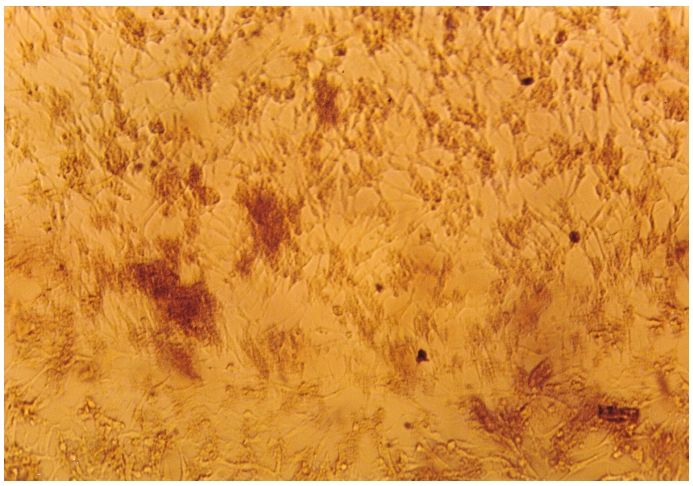


Photo (1b). Bovine herpes virus-1 stained by immunoperoxidase as brown granules in the nucleus of the MDBK cells (400X)

2. Virus isolation in cell culture: The MDBK cell cultures are observed daily for CPE, which usually appears within 1-2 days after inoculation. Two samples showed grape like clusters of rounded cells gathered around a hole in the monolayer; sometimes giant cells with several nuclei may be observed.

3-Results of the prepared BHV-1 antigen titration: The results of titration of the prepared BHV-1 viral antigen against positive and negative standard antiserum are shown in **Fig (2).** It shows that the mean optical density reading of the prepared antigen against positive serum is 0.500, while the mean optical density reading of the prepared antigen against nega-

tive serum is 0.088, and the best working dilution of the prepared antigen for ELISA is 1: 200 for BHV-1 antigen. It could be noticed that the binding ratio (mean optical density of positive control serum / mean optical density of negative control serum) is more than 5.

4- Results of BHV-1 antibodies detection in camel sera: as shown in **table (2)** BHV-1 antibodies were detected in 120 (31.25%) samples out of 384, where (98 (35.3%) and 22 (20.8%)) samples were collected from Basateen and Embaba abattoirs respectively.

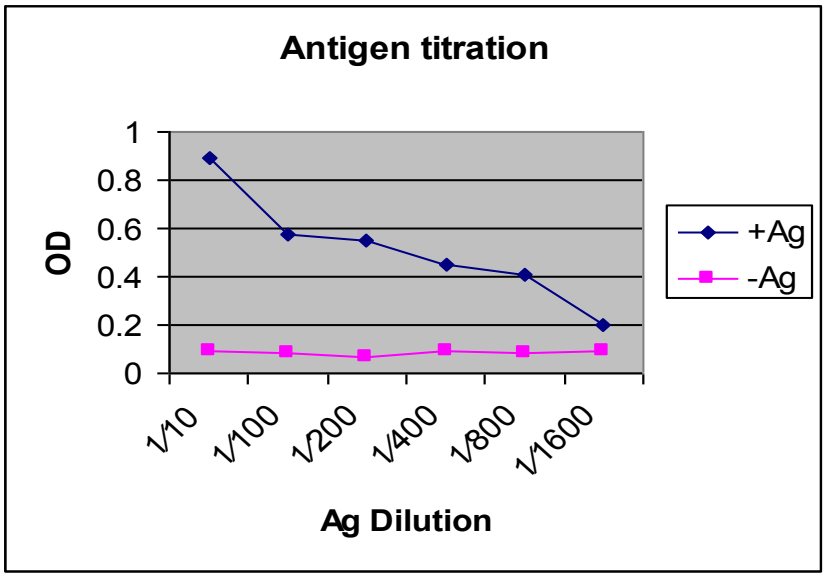


Fig. (2). Titration of the prepared BHV-1 antigen against positive and negative reference sera by check board titration.

Table (2). Results of BHV-1 antibodies detection in camel sera collected from Basateen and Embaba abattoirs.

Abattoir	Total	Positive (%)	Negative (%)
Basateen	278	98 (35.3%)	180 (64.7%)
Embaba	106	22(20.8%)	84 (79.2%)
Total	384	120 (31.25%)	264 (68.75%)

Discussion

Bovine herpes virus-1 (BHV-1) is one of the viruses causing respiratory infections in camels **(Dioli and Stimmelmary, 1992).** In a review on viral diseases of camels, **Werner and Kaaden (2002)** concluded that camels are resistant to BHV-1 infections while New World Camelides (NWC) are susceptible; this is due to the detection of BHV-1 in association with different clinical manifestations in NWC **(Mattson, 1994).**

In this study BHV-1 virus was detected in 2 samples out of 18 pneumonic camel lung specimens using Immuno Peroxidase(IP). The results obtained in this study confirmed the pathogenic effect of BHV-1 in camels which reported previously by **Nawal *et al.* (2003)** and disagree with the conclusion raised by **Werner and Kaaden (2002)** about the resistance of camels to BHV-1 infections.

Additionally, virus isolation provided support to the active role played by herpes virus in camel respiratory infections. In this study BHV -1was isolated from two camel lungs on MDBK cells, the characteristic cytopathic effect (CPE) appeared 24–48 h post-inoculation; it was as previously reported (rounding, edematous cells, sloughing). This is not the first report since **Williams *et al.* (1991)** had isolated IBR from lung of llamas while **Nawal *et al.* (2003)** reported the first detection and isolation of this virus in camels in Egypt.

The virus can be concentrated and purified by density gradient centrifugation. Alternatively, a

potent antigen can be prepared by treatment of infected cell cultures with detergents, such as Nonidet P40, Mega 10, Triton X-100 or 1-octyl -beta-D-glucopyranoside (OGP)**(OIE,2010).**

This study was designed to determine the occurrence of antibodies to BHV-1 in camels in Egypt, as camels are reared together with ruminants (cattle, sheep and goats).

The detection of antibodies against BHV-1 in camelids has difficulties problematic, even in experimentally induced infection, and seroconversion was not always observed **(Wernery & Kaaden, 1995).** Different studies by **Bornstein & Musa (1987)** and **Wernery & Wernery (1990)** revealed that there is no specific antibodies against BHV-1 were detected in Old World Camelides (OWC) contrary to the results observed in OWC , evidence of BHV-1 isolation and seroconversion in New World Camelides (NWC) has been reported by **Rivera (1987), Picton (1993), Rosadio *et al.* (1993)** and **Mattson (1994).**

Different ELISAs are used for antibody detection for BHV-1 in individual or pooled blood sera, and this is useful for determining herd status. In this study antibodies to BHV- were detected in 120 (31.25%) samples out of 384 tested camel sera. This agreed with **Wernery & Kaaden (1995)** who reported a prevalence of 16.2% in Peruvian alpacas, which declined to 5.1% in subsequent years when a change in production systems was introduced and the animals were reared in separate pastures not shared with other ruminants. If these finding are to be generalized, the close contact between camel and cattle herds supports the inter -species transmission of viral infection.

Eisa (1998) reported that 2.1% are positive for IBRV in seroprevalence of camels in Egypt. This is not usual as most of camels in Egypt are imported from Sudan for slaughter with some kept for breeding and used for agricultural purposes especially in Sharkia Governorate. In a recent report 13% of 496 camels were found to be positive for IBR antibodies in different areas in Saudi Arabia **(AL-Afaleq *et al.*, 2007);** this is far lower than our results, however in the eastern region the prevalence rate was 65% of 102 tested sera.

The indirect ELISA has 100% sensitivity when undiluted test serum samples are used. Therefore, the test can detect low levels of specific antibodies undetected by other conventional assays **(Lemaire *et al.*, 1995)** and it is a reliable alternative to the VN test in areas of high prevalence **(Pritchard, 2001)**.

In Conclusion: The scarcity of scientific publications on infectious diseases in camels has urged scientists to focus more attention on research and expand knowledge of this species. Manufacture of specific diagnostic reagents for rapid detection of IBRV infection in camel is recommended.

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مدى انتشار الاجسام المناعية المضادة لفيروس الهربس البقرى-1 فى مصل الجمال والكشف السريع عن انتيجين الفيروس فى الانسجة الرئوية للجمال

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الملخص العربى

يعتبر فيروس التهاب القصبة الهوائية المعدى فى الماشية من الفيروسات التى تصيب الابقار والمجترات الاخرى ولها تأثير اقتصادى على الانتاج الحيوانى. تعتبر الجمال من الحيوانات التى يستخدمها الانسان فى المناطق الصحراوية وتشارك الابقار والاغتام والماعز فى المرعى مما يزيد فى انتشار الفيروس.فى هذه الدراسة تم تجميع عدد 18 عينة رئة 374 عينة دم لفصل السيرم من مجزرى البساتين وامبابة بالقاهرة والجيزة. بفحص عدد 18 عينة رئة باستخدام البيروكسيداز المناعى للتعرف على وجود فيروس التهاب القصبة الهوائية المعدى كانت عينتين ايجابيتين. بتمرير العينات على الخلاياحدثت تغيرات من 1-2 يوم على شكل استدارة فى الخلايا وتكوين عناقيد تشبه عناقيد العنب ووجد عدد 2 من 18 عينة ايجابية .بفحص عدد 384 عينة سيرم باستخدام اختبار الاليزا الغير مباشر 98 عينة سيرم من 278 من مجزر البساتين بنسبة 3503% و 22 عينة سيرم من 106 عينة من مجزر امبابة بنسبة 20.8% كانت ايجابية للاجسام المناعية ضد فيروس التهاب القصبة الهوائية المعدى.