Natural exposure of Dromedary camels in Sudan to infectious bovine rhinotracheitis virus (bovine herpes virus-1)

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Abstract:

The occurrence of bovine herpes virus-1 (BHV-1) in camels as studied. A total of 186 pneumonic camel lungs were collected from slaughter houses at four different areas in Sudan during 2000–2006. Using sandwich ELISA 1.6% of 186 tested lungs were found positive for BHV-1 antigen, all were from Tambool at Central Sudan. Direct fluorescent antibody test (FAT) was used to confirm the BHV-1 ELISA positives, all ELISA positives were also positive. PCR was used to detect BHV-1 genome with three positive results. BHV-1 was isolated from two camel lungs in MDBK cells. Isolates were identified using ELISA and FAT. Indirect ELISA was used to detect antibodies to BHV-1 in 260 camel sera; 76.9% were found positive. Highest prevalence was observed in sera from Kordofan (84%) then Blue Nile (80%) and Tambool (76.3%). This is the first report for the detection of BHV-1 antigen, genome using PCR, isolation in cell culture and antibodies in camels in Sudan.

1. Introduction:

Infectious bovine rhinotracheitis (IBR), caused by bovine herpes virus-1 (BHV-1) of subfamily Alphaherpesvirinae within the Herpesviridae family, is one of the most economically important diseases of farm animals despite lowmortality rate. BHV-1 genome consists of a single linear molecule of double

stranded DNA. The virus is distributed worldwide and the disease is characterized by clinical signs of upper respiratory tract, nasal discharges, conjunctivitis, fever, depression, loss of appetite and reduced milk yield (Murphy et al., 1999). An outbreak of IBR in dairy cattle in Saudi Arabia characterized by fever, lacrimation, salivation and nasal discharge was reported (Abu Elzein et al., 2008). In Sudan IBR virus was detected in a calf suffering from acute respiratory disease, it was showing pyrexia, profuse bilateral watery nasal discharge and respiratory distress (Eisa, 1983). Prevalence of BHV-1 in camels is poorly studied; detection of antibodies to IBR in camels was reported in Tunisia (Burgemeister et al., 1975) and in Egypt (Eisa, 1998). Nawal et al. (2003) were the first to report the detection and isolation of BHV-1 from camels In Egypt. The widespread of BHV-1 infections in New World Camelidae (NWC) was documented. BHV-1 was isolated from llamas with bronchopneumonia (Williams et al., 1991;Mattson, 1994). Antibodies to BHV-1were 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 the Sudan, the isolation of BHV-1 from cattle has been previously reported (Eisa, 1983; Hassan and E1-Tom, 1985). Seroprevalence of BHV-1 indicating the widespread of the virus in cattle in the Sudan was reported previously (Hassan and Karrar, 1988). In a recent study, a 38% seroprevalence of BHV-1 in Sudanese cattle was reported (El Hussein et al., 2005). This study provides evidences on the association of herpes virus (BHV-1) with respiratory infection in camels.

2. Materials and methods:

2.1. Sample collection:

Pneumonic lung specimens (*n*=186) were collected from slaughter houses at four different localities in Sudan, Central (Tambool and Abudlaiq areas), Northern (Atbara area), Eastern (Gedarif and Kassala areas), and Western (El Obeid and Nyala

areas), Fig. 1 shows these areas. Specimens were transported on ice to the Central Veterinary Research Laboratory at Khartoum and kept at−20 ^{0}C till used. Camel sera (*n*=250)were collected at the same areas where lung specimens were collected. In addition to 10 serum samples were collected from South Central Sudan (Blue Nile area). Serawere kept at −20^{0}C till used.

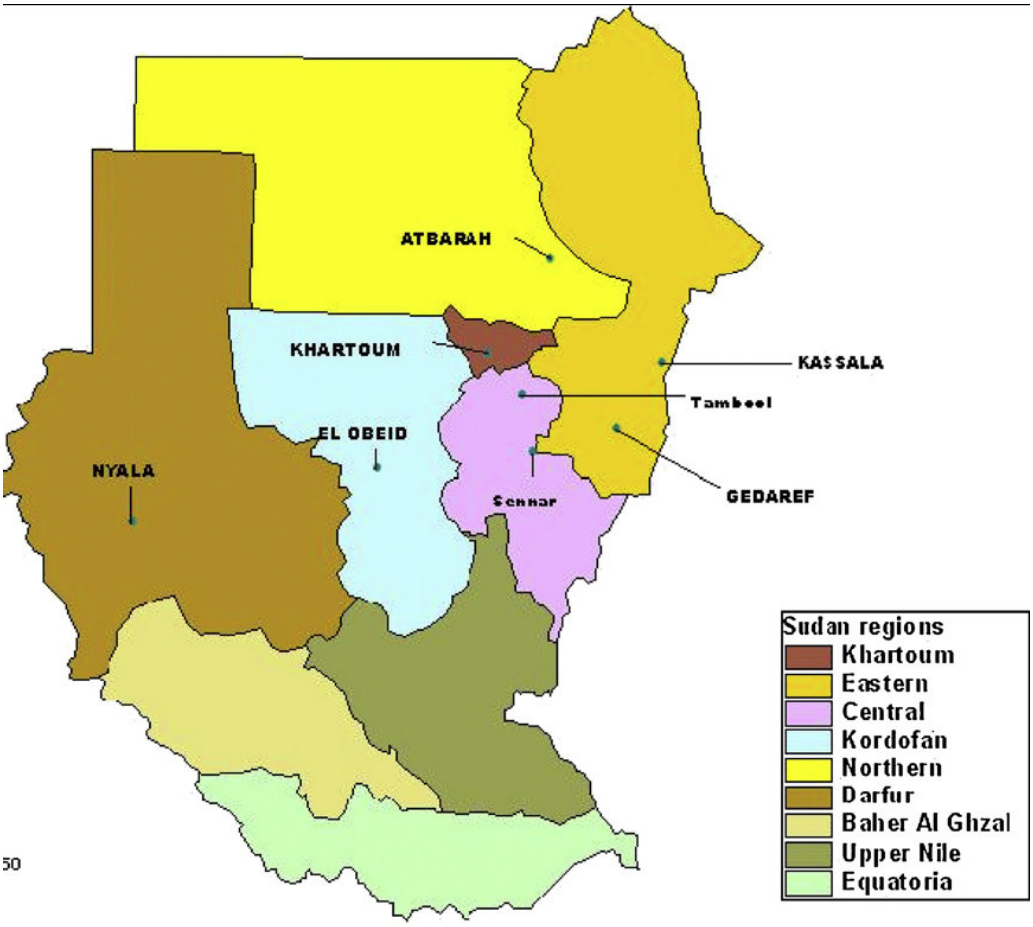

Fig. 1. The locations for collection of camel lung specimens and sera in Sudan: Eastern Sudan (Gedarif and Kassala), Central Sudan (Tambool), Central to South Sudan (Blue
Nile and Sinnar), Northern Sudan (Atbara), andWestern Sudan (El Obaid and Nyala).

2.2. Sandwich ELISA for BHV-1 antigen detection:

Sandwich ELISA kit for detection of BHV-1 antigenwas obtained from Bio-X Diagnostics, Jemelle, Belgium. The sample preparations and test were performed according to the instructions of the manufacturer.

2.3. Direct fluorescent antibody technique (FAT):

Direct immunofluorescence technique was used to confirm the BHV-1 antigen positive ELISA. The conjugate used was obtained from Bio-X Diagnostics Company, Jemelle, Belgium. The test was conducted according to the instructions of the manufacturer.

2.4. Polymerase chain reaction (PCR):

2.4.1. BHV-1 DNA extraction and purification:

The BHV-1 DNA extraction and purification method from test specimens and from control positive BHV-1 strain were described by Galik et al. (1990). Briefly, 500_l of each prepared sample suspension was incubated at 50 ◦C overnight with 1% of SDS and 0.3 mg/ml of proteinase K (Boehringer,Mannheim, Germany). After incubation, a mixture of phenol:chloroform–isoamyl alcohol 1:1 was added and the mixturewas mixed and centrifuged at 5000rpm for 5 min. DNA was precipitated from the supernatants by adding 0.3Msodium acetate and two volumes of absolute ethanol and then DNA was pelletted by centrifugation at 12,000rpm for 30min. The pelletwas purified dried and dissolved in TE buffer (10mMTris (pH 7.5) 1mM EDTA).

2.4.2. DNA amplification and detection of PCR product:

PCR was performed according to the method described by Von-Beroldingon et al. (1990) in 50_l of a reaction mixture containing a final concentration of 10mM Tris (pH 9.0), 50mM KCl, 0.01% gelatin, 1.9mM MgCl2, 5% (w/v) glycerol, 0.2mM deoxynucleoside triphosphates (Pharmacia, Piscatacia, N.J.), 0.1M primer 1 (5_-CTG CTG TTC GTA GCC CAC AAC G-3_),

0.1M primer 2 (5_-TGT GAC TTG GTG CCC ATG TCG-3_), 10U of Taq polymerase (Boehringer) per ml and 5_l of extracted BHV-1 DNA. The PCR mixtures were subjected to 38 repeated cycles of amplification in a DNA thermal cycler-PerkinElmer/Cetus Research, USA. The cycling conditions were as follows: denaturation (1 min at 95 ◦C), primer annealing (1min at 60◦C) and extension (1 min at 72 ◦C). Negative and positive control reactionswere used. The expected size of the amplicon is 173 bp. Bands were visualized after electrophoresis of 10_l of the PCR product in a 2% agarose stained with ethidium bromide (0.3 mg/ml).

2.4.3. Virus isolation in cell culture:

Two ELISA and PCR BHV-1 positive specimens were selected for isolation in MDBK cell line. The camel lung tissue specimens were prepared by cutting with scissors and forceps into small pieces then ground with little amount of sterile PBS in pestle and mortar. The tissue was then suspended (20%) in PBS with antibiotics, homogenated then centrifuged at 2000rpm for 10 min and the supernatantwas separated in sterile tube and stored at −20 ◦C till used. 0.3 ml of the supernatant of homogenated lung tissue was inoculated in the MDBK cells flask (T25) without media and the inoculated cellswere incubated at 37 ◦C for 1–1.5 h. Then the inoculum was discarded and the cells were washed three times with sterile PD. One to two cell flasks with uninfected MDBK cells were used as control.Newunconsumedmedia without serumwas added to the inoculated and uninfected cells. The cells were examined daily under inverted microscope (KR-SS, Germany) to observe the cytopathic effect (CPE) appearance. Tissue culture harvestswere identified as BHV-1 using ELISAand FAT described above.

2.4.4. Indirect ELISA for BHV-1 antibody detection:

Indirect ELISA kit for detection of antibodies to BHV-1 was obtained from Bio-X Diagnostics, Jemelle, Belgium. The test was applied on all collected samples (n = 260) according to the instructions of the manufacturer.

Table 1
Detection of BHV-1 antigen in camel lungs in different localities in Sudan using ELISA (2000–2006).

Area	Total tested	Total positive	% Positive	Total negative	% Negative
Eastern Sudan	40	0	0	40	100
Central Sudan	68	3	4.4	65	95.6
Northern Sudan	61	0	0	61	100
Western Sudan	17	0	0	17	100
Total	**186**	**3**	**1.6**	**183**	**98.4**

3. Results:

3.1. BHV-1 antigen detection:

Sandwich ELISA kits for detection of BHV-1 antigenwere applied on 186 camel lung specimens; three were found positive (1.6%). BHV-1 antigenwas detected only in specimens fromCentral Sudan; the details are presented in Table 1.

3.2. Direct fluorescent antibody technique:

Direct fluorescent antibody technique was used to confirm the BHV-1 ELISA positives. All ELISA positives (3) were also found to be positive using FAT (Fig. 2).

3.3. Polymerase chain reaction:

Three specimenswere found to be positive by PCR assay (Fig. 3).

3.4. Bovine herpes virus-1 isolation in cell culture:

Bovine herpes virus-1 was successfully isolated in MDBK cells from two ELISA and PCR positive lung specimens, it was showing congestion and swollen; the virus was identified using ELISA and FAT.

3.5. BHV-1 antibody detection:

Indirect ELISA kits for detection of antibodies to BHV-1 were applied on 260 camel sera with 76.9% positivity. The highest prevalence was detected in Western (84%), South Central (80%) then Central Sudan; 1+ was the highest detected prevalence then 2+. The details are shown in Tables 2 and 3.

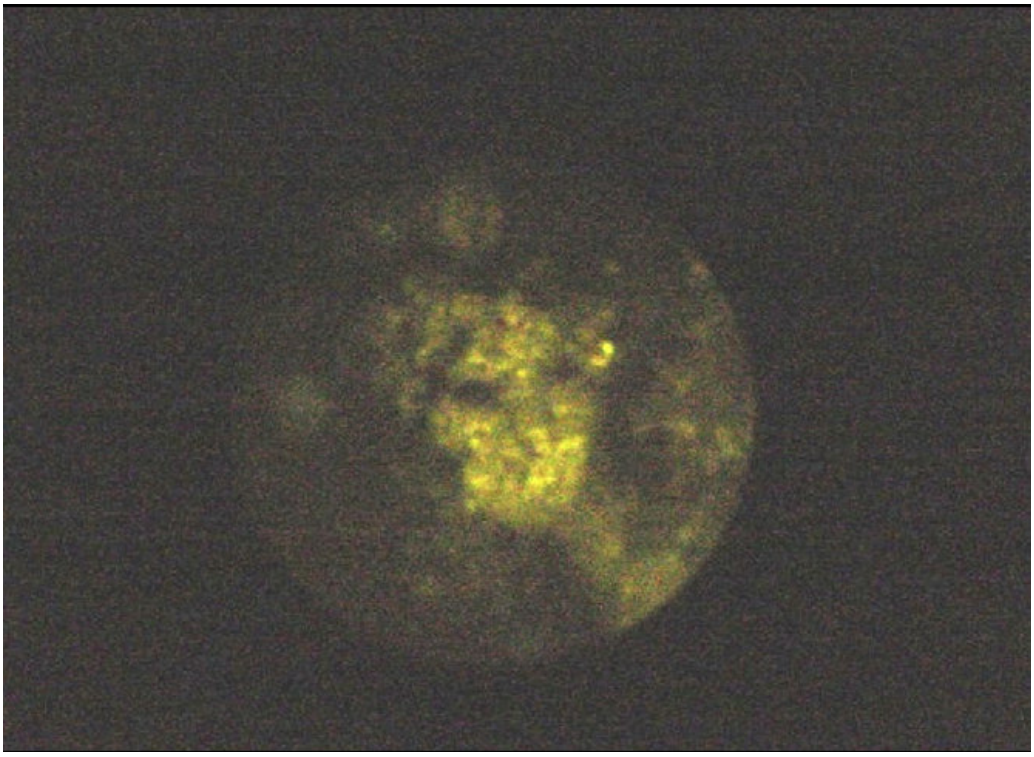

Fig. 2. Positive specimen for bovine herpes virus-1 (BHV-1) antigen detection in camel lung tissues using fluorescent antibody technique (FAT).

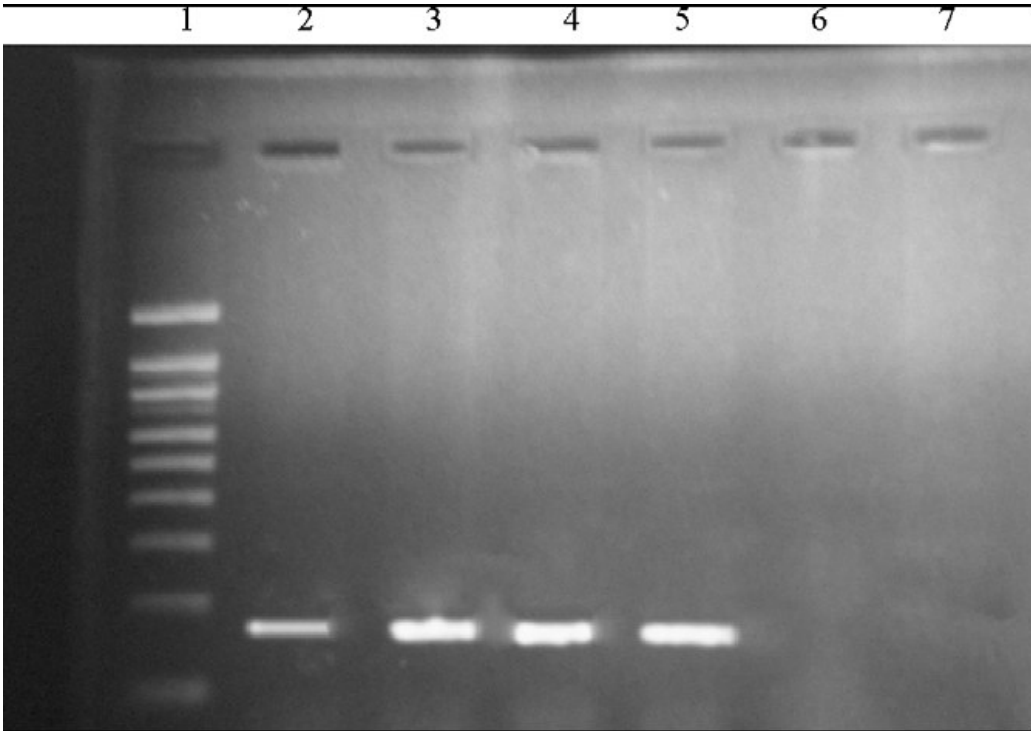

Fig. 3. PCR amplification of BHV-1 (173 bp). Lane 1, 100 bp marker; lane 2, positive control; lane 3–5, positive samples; lane 6, negative sample; lane 7, negative control.

4. Discussion:

Bovine herpes virus-1 (BHV-1) is one of the viruses causing espiratory 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 NWC are susceptible; this is due to the detection of BHV-1 in association with different clinical manifestations inNWC (Mattson, 1994).

In this study BHV-1 antigen was detected in 1.6% of 186 pneumonic camel lung specimens using ELISA. Polymerase chain reaction and FAT were used in this study to confirm BHV-

1 detection. All detected specimens were from Central Sudan (Tambool area). The results obtained in this study confirmed the pathogenic effect of BHV-1 in camels reported previously byNawal et al. (2003) and disagree with the conclusion raised by Werner and Kaaden (2002) about the resistance of camels to BHV-1 infections. Field observations in the Sudan pointed to frequent occurrence of abortions in camels (Khalafalla, unpublished data). Accordingly, the role played by BHV-1 in causation of abortion in camels should be investigated.

Additionally, virus isolation provided support to the active role played by herpes virus in camel respiratory infections. In this study BHV-1was isolated fromtwo camel lungs inMDBKcells, 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) isolated IBR from lung of llamas while Nawal et al. (2003) reported the first detection and isolation of this virus in camels in Egypt.

Table 2
Detection of BHV-1 antibodies in camel sera in different localities in Sudan using ELISA during 2000–2006.

Area	Total tested	Number positive	% Positive	Number negative	% Negative
Eastern Sudan	71	49	69	22	31
Central Sudan	38	29	76.3	9	23.7
South Central	10	8	80	2	20
Northern Sudan	35	24	68.6	11	31.4
Western Sudan	106	90	84.9	16	15.1
Total	**260**	**200**	**76.9**	**60**	**23.1**

Table 3
Degree of positivity of BHV-1 antibodies in camel sera in Sudan detected using indirect ELISA.

Area	Total tested	Degree of positivity					
		–	+	2+	3+	4+	5+
Eastern Sudan	71	22	33(46.5%)	15(21.1%)	0	0	1(1.4%)
Central Sudan	38	9	10(26.3%)	12(31.6%)	5(13.2%)	2(5.3%)	0
Central to South	10	2	3(30%)	4(40%)	0	1(10%)	0
Northern Sudan	35	11	16(45.7%)	4(11.4%)	2(5.7%)	1(2.9%)	1(2.9%)
Western Sudan	106	16	50(47.2%)	22(20.8%)	3(2.3%)	0	15(14.1%)
Total	**260**	**60**	**112(43.1%)**	**57(21.5%)**	**10 (3.8%)**	**4(1.5%)**	**17(5.8%)**

IBR seroprevalence in camels in Tunisia was 5.8% Burgemeister et al., 1975), however Wernery and Wernery (1990) did not find antibodies to IBR in camels in Emirates; this is expected as camels in Emirates are mainly kept for racing and milk production in semiclosed farm systems which makes the contact with other animals very rare. Eisa (1998) reported 2.1% IBR seroprevalence in camels in Egypt. This is not unusual 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.

In this study the seroprevalence of BHV-1 in camels was 76.9% of 260 tested camel sera. Highest prevalence was seen in Western Sudan (Kordofan [84%]) then South Central Sudan (Blue Nile and Sinnar [80%]). However, the seroprevalence of BHV-1 in other areas was also high, and the lowest detected percentage (69%) was in Northern Sudan (Atbra). An earlier report on the prevalence of BHV-1 in cattle in the Sudan ranging between 12 and 38% using SNT was reported (Hassan and Karrar, 1988). A serological survey on BHV-1 in Sudanese cattle using passive haemagglutination test revealed the detection of BHV-1 antibodies in 38% of tested sera, highest prevalence (72%) was found in Western Sudan (Kordofan) and 60% in Central Sudan (Khartoum) (El Hussein et al., 2005). Our results are comparable with that reported in cattle sera by El Hussein et al. (2005) about the high seroprevalence in Western Sudan (Kordofan). These reports may raise the possibility that camels might acquire herpes virus from cattle since camels and cattle share pasture and water sources in most of animal rearing areas of the Sudan specially Kordofan which is one of the richest area of animal population and they are usually being in contact. Further molecular characterization

of the isolated virus is expected to reveal the source of infection to camels.

The titers detected in this study in sera from South Central Sudan (Sinnar) were higher than that reported by El Hussein et al. (2005) which is mostly due to the increase in the disease prevalence beside the increased sensitivity of the used technique (ELISA). Variable titers of BHV-1 antibodies were detected which was expressed as +1 to +5. Most of the highest titers (+5) observed were in Western Sudan (Kordofan) sera (16.7%) of positives. Highest percentage of positive sera (56%) showed only low titer (+). This indicates the high circulation rate of BHV-1 in camels in Sudan. The seroprevalence of BHV-1 in camels detected in this study was higher than most of reviewed reports.

This is the first report on the detection of BHV-1 antigen, genome, antibodies and isolation from camels in Sudan. Detailed molecular characterization of BHV-1 circulating in camels and comparison with that affected cattle in Sudan is highly recommended.

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ملخص البحث

أجريت هذه الدراسة على عدد 186 من رئات الإبل تظهر فيها علامات الالتهاب و قد جمعت من المسالخ في أربعة مناطق مختلفة في السودان في الفترة من 2000-2006. و قد اهتمت وركزت الدراسة بالبحث عن فيروس BHV-1 وهو احد الفيروسات المسببة للالتهابات الرئوية في البقر.

باستخدام اختبار التراص المناعي المرتبط بالإنزيم (Sandwich ELISA) قد وجد أن 1.6% من مجموع 186 من الرئات ايجابي للفيروس مما يؤكد إمكانية إصابة الإبل بهذا الفيروس. و من الملاحظ أن جميع العينات الايجابية كانت من منطقة تمبول وهي منطقة في وسط السودان وفيها سوق للإبل التي تأتي من مختلف أنحاء السودان خاصة كردفان.

اجري اختبار آخر مناعي لتأكيد وجود الفيروس في العينات الموجبة وهو اختبار التألق المناعي المباشر (FAT) وقد كانت كل العينات موجبة بهذا الاختبار.

اجري اختبار تفاعل البلمرة المتسلسل (Polymerase chain reaction PCR) على ثلاثة من العينات الموجبة وقد كانت النتيجة موجبة لكل العينات المفحوصة.

مررت عينتين من العينات الموجبة في الخلايا الحية (MDBK) لاختبار الأثر المرضي في الخلايا (CPE) وقد أظهرت الخلايا الأثر المرضي مطابق لما يفعله هذا الفيروس. تم حصاد الخلايا المصابة بعد أن أظهرت الأثر المرضي في أكثر من 80% من الخلايا خلال ثلاثة أيام من الحقن. أكدت ايجابية الاختبار باستخدام اختباري ال (ELISA & FAT) علي الخلايا المصابة.

تم إجراء مسح مناعي لعدد 260 مصل للتأكد من وجود أجسام مضادة لهذا الفيروس وقد استخدم اختبار التراص المناعي المرتبط بالإنزيم غير المباشر (Indirect ELISA) لهذا الغرض وقد كانت النتيجة وجود أجسام مضادة بنسبة 76.9% من العينات المفحوصة وهذه النسبة تعتبر عالية مما يوحي بمدى انتشار هذا المرض بين قطعان الإبل. أعلى نسبة لوحظ وجودها في أمصال الإبل من كردفان تليها من النيل الأزرق ثم تمبول.

هذا البحث هو الأول لإظهار هذا المرض في الإبل وباستخدام اختبارات المناعة المختلفة والمتطورة في مجال الفيروسات في السودان.

Abstract:

The occurrence of bovine herpes virus-1 (BHV-1) in camels was studied. A total of 186 pneumonic camel lungs were collected from slaughter houses at four different areas in Sudan during 2000–2006. Using sandwich ELISA 1.6% of 186 tested lungs were found positive for BHV-1 antigen, all were from Tambool

at Central Sudan. Direct fluorescent antibody test (FAT) was used to confirm the BHV-1 ELISA positives, all ELISA positives were also positive. PCR was used to detect BHV-1 genome with three positive results. BHV-1 was isolated from two camel lungs in MDBK cells. Isolates were identified using ELISA and FAT.

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This is the first report for the detection of BHV-1 antigen, genome using PCR, isolation in cell culture and antibodies in camels in Sudan.