Egyptian J. Virol. 1, 489-506 (2004)

Detection of BVDV associated with mortalities in camels

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BVDV was detected in tissues collected from dead camel cases using antigen capture ELISA and fluorescent antibody assay. Histopathology examination of different organs revealed changes similar to those reported with BVDV infection. The existence of BVDV in sera collected from camel population, in the area (Al-Ain city, Western area of Abu-Dhabi- Emirate, UAE) where camel were found, was confirmed. The antigen was confirmed using antigen capture ELISA and FA assay. Testing 356 camel sera by serum neutralization test revealed negative for the presence of serum neutralizing antibodies to BVDV. Using RT-PCR assay based on primers located at the non translated region of BVDV genome to detect BVDV RNA in some buffy coat samples, collected from the co-housed camels in the area where dead camels were found (BVDV-positive), revealed positive results. However, genotyping of such positive samples using RT-PCR genotyping-based assay revealed negative for the presence of BVDV type I, II and border disease virus. After 3 passages of the detected positive buffy coats on MDBK cells, no CPE was detected suggesting that these viruses may be of non-cytopathic type of BVDV. The present study confirm the existence of BVDV among camel population and raise the possibility of the presence of persisting infected camels by BVDV which may cause extensive shedding of the virus among camel population resulting in severe outbreaks. Also, the study demonstrates the continuous genetic diversity of BVDV and exalts the possibility that BVDV in camel may have a distinct group varied from those reported in bovine and ovine populations.

INTRODUCTION

Bovine viral diarrhea virus (BVDV) is an economically important pathogen of cattle throughout the world (Meyling et al., 1990). It was described as transmissible disease of cattle caused by a ruminant pestivirus and characterized by low mortaliy, high morbidity, fever, and diarrhea. Although the virus is a normal pathogen of cattle, BVDV has been isolated from goats, camels, pigs and captive and free living ruminants (Loken, 1996; Yousif et al., 1998 and Belknap et al., 2000). The wide spectrum of signs associated with BVDV infection includes immunosuppression, repeat breeding problems, abortion and mummification, congenital defects, immunotolerance, persistent infection, acute and chronic mucosal disease (Baker, 1987). The first to report the occurrence of natural outbreak of BVD in the dromedary camel and the first isolation of BVDV in Egypt was done and reported by Abo El-Lail (1997) who reported that BVDV infection in camel calves is associated with multiple disease condition, while BVDV is not common in new world camelids. However, the virus was contributed in cases of abortion, diarrhea, ill thrift or weight loss (Belknap et al., 2000). In addition, BVDV was successfully isolated from tissue samples of Lamas (Haines et al., 1992; Belknap et al., 2000).

In Egypt, the prevalence of neutralizing antibodies to BVDV in sera of 59 camels has been proved at a

percentage of 52.5% (Zaghawa., 1998). The sera samples were collected from different localities in Behera and Kafr El-Sheikh provinces of Egypt. Ahmed (2002) detected the BVDV in blood samples and antibody to BVDV among camel sera with high ratio compared to the previous authors who diagnosed a clinical case suffered from BVDV infection (Provost *et al.*, 1967 and Plowright, 1969). The occurrence of similar results of BVDV infection in camels was confirmed and concluded that the presence of neutralizing antibodies to BVDV in camels denotes their susceptibility to the virus and their role in the transmission of the virus (Hedger *et al.*, 1980, Wassel *et al.*, 1996 and Zaghawa, 1998).

In the present study, clinical and dead cases where BVDV is accused were used to detect, isolate BVDV and study its antigenic and molecular characteristics. In the meantime, gather information on the evolution BVDV in persistent infected cases among camel population in different localities of Al-Ain (the eastern region of Abu-Dhabi, UAE) with especial reference to pregnant cases and their newlyborn

MATERIALS AND METHODS

Area of study

Al-Ain region located at about 170 Km to the east of Abu-Dhabi Emirate, United Arab Emirates. It has extensive international borders with Sultanate of Oman towards the east and Saudi Arabia at the south. Al-Ain region accommodates approximately 191,000-125,000 head of camels. The camel's herds distributed at the area of study as small discrete flocks with a flock size vary from 20 to 70 camels per flock.

Collection of tissue samples from dead cases submitted to laboratory examination

Spleen, lymph node, small intestine and lung were collected from nine fresh dead camels, submitted to the diagnostic veterinary laboratory (Al-Quattara Vet. Lab) at Al-Ain city were used in the study during the period of January to April, 2003. Data concerning locality, date of death and age of camels, are presented in table (1).

Collection of blood and buffy coat samples from the area of study

356 serum and 2 buffy coat samples were collected from camels located in 9 different area of Al-Ain region. The detailed data of samples were showed in table (1). The camels were apparently health and not vaccinated against BVDV. There were pregnant and non-pregnant camels. For lymphocyte separation blood samples were collected from camels using sterile vacuitianer with EDTA as anticoagulant. Lymphocytes were separated as mentioned by **Aaron *et al.*, (2002)**. Also, serum samples were collected from apparently healthy animal of those in contact dead cases. The serum samples were categorized according to the locality, age and state of pregnancy.

Tissue samples preparation

A portion of 1 to 2 gram of selected tissue were chopped into small pieces (2 - 5 mm in size). The chopped tissues suspended in 5 ml of PBS (pH 7.4) or the diluent buffer of the applied ELISA kit. The suspension was grind using tissue grinder and sonicated using Soniprep 150 (MSE) for 1 minutes and finally centrifuged at 1500 x g for 10 minutes at + 4 °C.

Working solution of gentamicin was added at a ratio of 0.1 ml / ml of the prepared tissue suspension and incubated for one hour at 37 °C in case of using the suspension for virus propagation on tissue culture. The suspension kept at - 20 °C until used. For PCR analysis; 0.1 ml of tissue suspension was added to 1.5 ml trizol and kept until used.

Laboratory processing of dead tissues for bacteriological examination and exclusion of RP and PPR

Bacteriological examination:

Direct smears from different necropsy tissues were subjected to staining by Gram's stain routinely. Different swabs and portions obtained from different tissues were collected and inoculated into 5 ml. of trypticase broth (BBL) and incubated for 24 hours at 37°C aerobically. The inoculated broth cultures were streaked onto 5% sheep blood agar. MacConkey agar. Hektoen enteric agar and eosin methylene blue agar plates. The inoculated plates were incubated at 37° C for 24 hours. Culture characters of growing organisms including pattern of growth, colonial morphology and haemolysis onto blood agar were recorded. The suspected growing colonies were picked up. Gram stained and checked for cytochrome oxidase. The target colonies were purified by further subculturing. Pure cultures in trypticase broth matching 0.5 McFarland standard were expopsed to bacteria identification using API System (Biomeriux). The biochemical reactions were monitored after 24 hours incubtion at 37° C.

Exclusion of Clostridium perfringens or it's toxins from being one of the probable cause of death:

BIO-X Enterotoxaemia ELISA Kit was applied as manufacturer's recommendation on the collected intestinal contents of the young dead cases. The optical densities (OD) were measured at 450 nm wave length using Multiskan plus MK II (BDSL).

Exclusion of RP and PPR from being one of the probable cause of death

Immunocapture ELISA Kit for (RP/PPR) was applied as manufacturer's recommendation on the fresh necropsy tissues obtained from spleen and intestinal lymph nodes of the dead cases showed hemorrhageic gastro-enteritis (Case No 1, 7 and 9).

Different assays applied for BVDV diagnosis: FA staining (direct method) of selected tissue obtained from dead cases

Spleen, mesentric lymph node and lung were stained using the adopted staining procedure followed the manufacturer's recommendation using FITC conjugated BVD antiserum. The slides were mounted with buffered glycerol and viewed by Leitz fluorescence microscope.

Bovine Viral Diarrhoea Virus (BVDV) ELISA Antigen test Kit /Leukocytes (IDEXX, Sweden)

The test was applied to detect BVDV in tissue samples collected from dead animals and selected buffy coat as manufacture's recommendations.

Bovine Viral Diarrhoea Virus (BVDV) Antigen test Kit /Serum (IDEXX)

The test was applied as manufacture's recommendations. The test was applied to detect BVDV in serum samples collected from the contact animals in the area where dead animals were found.

FA staining direct method of selected buffy coat samples obtained from live positive cases

The harvested lymphocytes were collected and washed twice in PBS pH 7.2. Final cell suspension made to contain 400,0000 cells / ml. Using eight well multitest with frosted black mask glass slide (Flow Lab). 0.25 ul of the cell suspension was placed into each well. The slides were air dried at room temperature and fixed with 100 % acetone for 10 minutes. The slides were rinsed for seconds with deionized water. The prepared slides were stained immediately as described earlier or stored at - 20°C until used.

Trials for isolation of BVDV from the positive samples

Two bottles (25 cm^2) with confluent sheet of MDBK cells were used per each sample. The growth medium decanted and the MDBK cell sheet rinsed twice with maintenance MEM. The bottles were inoculated with 0.2ml of each prepared sample. The inoculum allowed to be adsorbed for one hour at 37°C. The bottles were rinsed with 5 ml maintenance MEM followed by addition of 5 ml maintenance MEM. The bottles were incubated at 37°C for 5 days with daily observation for presence of CPE then stored at – 70°C till used. Negative bottles inoculated with maintenance MEM run as cell control. In case of absence of CPE, two more passages were followed using 6 well TC plates with confluent layer of MDBK. On the 4th day, the medium was removed, and allowed to be dried and fixed with 80% acetone for 10 minutes and exposed to FITC conjugated BVD antiserum as described earlier.

Detection of BVDV genome by RT-PCR using UTRI and UTR2 primers:

The technique was applied on the inoculated MDBK cells with the selected samples (FA- and ELISA-positive), the original buffy coat and control non inoculated cells, serum and control positive BVDV. The adapted methods was applied as previously described (Hussein, 2001).

Genotyping of BVDV–positive samples using nested PCR:

The PCR assay was carried out according to Sullivan and Akkina (1995) with modification applied by Hussein, (2001). Amplification of RT products of samples that gave positive for the presence of BVDV using PCR assay in the UTR region was employed. The assay was consisted of 2 round of PCR utilizing 5 primers:

First round PCR Primers (PI=5'AACAAACATGGTTGGTGCA ACTGGT3P2=5'CTTACACAGAATA TTGCCTAGGTTCCA3') and Second round PCR Primers (TS1= 5' TAT ATTATTTGGAGACAGTGAATAG3. TS2 = 5'TGGTTAGGGAAGCAATTA GG3', TS3 = 5'GGGGGTCACTTGTC GGAGG3'). These primers cocktail were designed according to the published sequence of the gp_{48} (E_0) region of BVDV viral genome. P1 and P2 primer pair shared of the maximum homology with all ruminant *Pestiviruses* (BVDV type I and II and BDV) and utilized in the first PCR round. TS primers were type specific primers. TS1 was specific for BDV. TS2 was specific for BVDV type-II and TS3 was specific for BVDV type-I.

Micro serum neutralization test for detection of antibodies to BVDV in camel sera:

A microtitre VN test was been used as previously described by Hawards, (1990).

Pathology and histo-pathology studies:

Dead animals were exposed to necropsy and case No. 3 and 4 were excluded in histopathological examination due to post-mortum autolysis. Specimens from different parts of small intestine, intestinal lymph nodes, spleen and abomasum were immediately fixed in 10 % formalin. The specimens were processed and paraffin sections of 4 - 6 μ were prepared as described elsewhere and stained by Harris Hematoxyline and Eosin.

RESULTS

During the period from January to April 2003, a number of 9 camel cases were found dead for unknown reasons. There were categorized according to their age into 3 groups (Table 1).

Results of monitoring the cases for different bacteria and RP-PPR screening are presented in Table (2). Infection due to clostriduim spp., salmonella spp., pathogenic E.coli 99 was excluded from all cases.

Table (1): The age and locality of the recorded BVDV-positive camel cases

Group No.	Case No.	Date of Recording	Age	Locality
I	1	Jan. 03	45 days	90 km South of Al Ain city
	2	Feb. 03	03 days	80 km West of Al Ain city
	3	Feb. 03	04 days	80 km West of Al Ain city
	4	Feb. 03	04 days	20 km North of Al Ain city
	5	Apr. 03	30 days	100 km North of Al Ain city
	6	Apr. 03	30 days	30 km North of Al Ain city
. II	7	Mar. 03	1 Year Old	20 km North of Al Ain city
III	8	Apr. 03	7-8 Years Old	90 km North/West of Al Ain city
	9	Apr. 03	7-8 Years Old	45 km North of Al Ain city

Table (2): Results of different microbiological assays applied on the dead cases

Animal Group	Case No.	Isolated Bacteria	Anaerobic Cultural	Results of RP-PPR screening
I	1	E. coli	Negative for Clostridum Spp.	Negative
	2	E. coli	Negative for Clostridum Spp.	Negative
	3	Klepsiella Spp.	Negative for Clostridum Spp.	Negative
	4	Klepsiella Spp.	Negative for Clostridum Spp.	Negative
	5	E. coli	Negative for Clostridum Spp.	Negative
	6	E. coli + Candida albicans	-	Negative
II	7	Klepsiella Spp.	-	Negative
III	8	Negative	-	-
	9	Sreptococcus Group C	Negative for Clostridum Spp.	Negative

The necropsy finding showed that 3 cases only possessed typical gastro-intestinal hemorrhage with few focal erupted areas while other cases revealed none of the characteristic signs of BVDV infection. (Table 3).

The main histo-pathological lesions seen were severe congestion in the blood capillaries coincided with edema in the abomasal mucosa in case No.1, 7 and 9. The intestinal section obtained from cases No.1, 7 and 9; revealed presence of congestion, edema, and leukocyte infiltration in the lamina propria of the intestinal villi with desquamation of epithelia lining and villus atrophy. Hyperplasia of lymphoid elements of the white pulpe is the main observation recorded in the spleen of all cases (Plate 1). Spleen of case No.1 possessed severs congestion, hemorrhage and edema in the white pulpe. The intestinal lymph node of all cases showed hyperplasia in the cortical lymphatic follicles. Whereas; case No.1 showed edema in the germinal center coincided with focal area of hemorrhage. The distributions of positive BVDV dead cases among the different field localities of Al Ain city were recorded in (Tables 1 and 5). Specimens prepared from different tissues of the dead cases were subjected to direct FA for further confirmation. All specimens (9 cases) gave positive results for the presence of BVD.

Table (3): Main Pathological and Histopathological Findings Recorded From Dead Cases

Group No.	Case No.	Main Necropsy Findings	Main Histopathological Findings
I	1	Severe hemorrhagic abomasamitis and focal intestinal erosions.	Severe venous congestion, oedema & hemorrhage in the abomasal & intestinal mucosa & hyperlasia of the cortical lymphatic follicies.
	2	No significant lesions.	Hyperplasia of the lymphoid element of the white pulps and the cortical tissues of lymphatic follicles.
	3	ND.	ND.
	4	ND.	ND.
	5	No significant lesions.	Hyperplasia of the lymphoid element of the white pulps and the cortical tissues of lymphatic follicles.
	6	No significant lesions.	Hyperplasia of the lymphoid element of the white pulps and the cortical tissues of lymphatic follicles.
II	7	Severe hemorrhagic abomasamitis & focal intestinal erosions.	Severe venous congestion, oedema & hemorrhage in the abomasal & intestinal mucosa & hyperlasia of the cortical lymphatic follicies.
III	8	No significant lesions.	Hyperplasia of the lymphoid element of the white pulps and the cortical tissues of lymphatic follicles.
	9	Severe hemorrhagic abomasamitis & focal intestinal erosions.	Severe venous congestion, oedema & hemorrhage in the abomasal & intestinal mucosa & hyperlasia of the cortical lymphatic follicies.

Typical severe hemorrhagic abomasumitis was recorded in cases No. 1 and 9. While case No. 7 showed few focal erroted area in the abomasal mucosa. The intestinal mucosa of all the forementioned three cases showed severe congestion and focal hemorrhage.

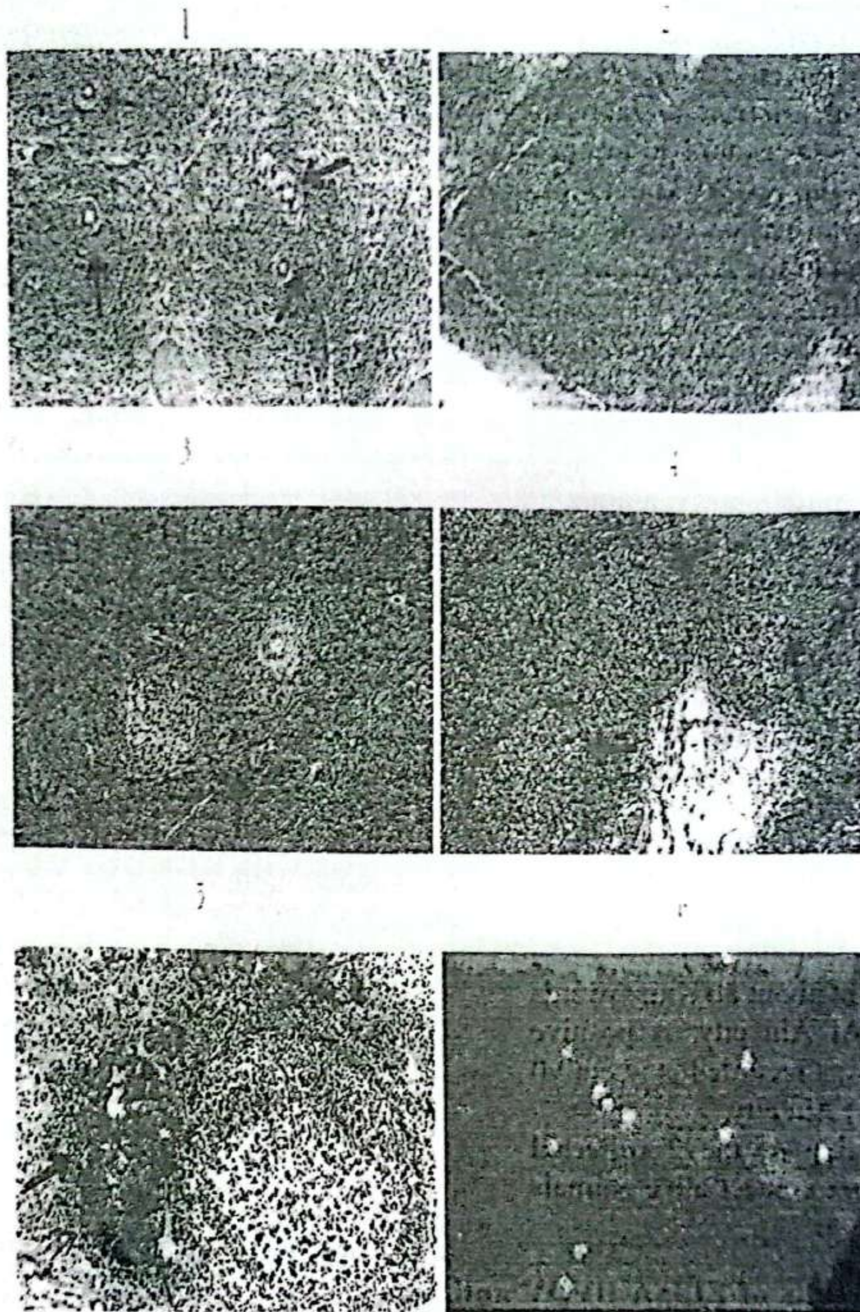

Fig.(1). Camel spleen possessed hyperplasia of lymphoid elements of the white pulpe. 2. Severe congestion and haemorrhage in splenic tissues. 3. Spleen of cases No. 1 revealed severe congestion and hemorrhage. 4. Camel lymph node showed degeneration of lymphocytes in the center of lymphoid follicles while the periphery showed hyperplastic changes. 5. Camel lymph nodes. Notice the center of lymphoid follicle with lymphocytic depletion while the periphery showed reactive hyperplasia. Areas of haemorrhage surrounding the affected follicle. 6. Demonstrates the FA positive buffy coat cells. Intracytoplasmic fluorescence of BVDV infected cells are shown.

Application of BVD antigen ELISA showed that BVDV antigen was detected in the spleen and small intestine of all cases and in the intestinal lymph node in 5 out of 7 cases assayed. The BVDV antigen was also detected in the lung tissues in 4 out of 6 cases assayed (Table 4). Selected 2 buffy coat samples collected from alive animals co-housed in the same area of dead-positive cases were assayed in ELISA and revealed positivity for the presence of BVDV antigen.

The geographical localities of BVD positive cases were recorded in

Table (1). The obtained data showed that positive infected cases were geographically distributed around Al-Ain city from as far as 90 Km towards the north west up to 90 km. to the south and extended up to 80 Km towards the west of the AL-Ain city.

Generally, the camel distributed at the area of study as small flocks with animal size vary from 20 to 70 camels per flocks. Based on FA and ELISA testing, the recorded results showed that the disease was existed allover the area of the study among different camel flocks.

In North direction of Al Ain city, 3 cases were recorded at distance about 20 to 30 Km. and another case was recorded at about 45 Km North of Al Ain city. Another case was found dead as far as 100 Km. towards the North of Al Ain city (Table 5). In addition, one case found dead at about 90 Kim. On the direction of North / West of Al Ain city, and two cases were recorded at about 80 Km. towards the West of Al Ain city. A positive infected case was recorded at about 90 Km south of Al Ain city.

FA testing of the 2 collected buffy coats from contact alive animals revealed positive for the presence of specific intracytoplasmic fluorescence in the buffy coat cells (Plate 1).

Analysis of 356 camel serum samples by ELISA for detection of gp-48 antigen of BVDV revealed that 4 serum samples were positive for BVD-antigen in a percentage of 1.01 (Table 5).

The results showed that the prevalence of BVDV-positive cases vary from locality to another ranged between 1.8% to 4.4% with an average prevalence of 1.01% in all studied localities (Table 5).

Sorting of the obtained results according to the age range of screened animals, the prevalence of positive cases among older animals was higher than that recorded in younger animals (Table 6).

6.45% of the sera representing the samples collected from pregnant animals (n=62) found to be positive (n=4). Whereas, those tested from non pregnant was lower 2% (n=3). Table (7) presents the obtained results.

Table (4): Results of ELISA BVDV antigen assayed on different organs from some dead cases

Camel Groups	Case No.	Results of ELISA on the different tissues			
		Spleen	Small Intestine	Intestinal LN	Lung
I	1	+Ve	+Ve	+Ve	+Ve
	2	+Ve	+Ve	ND	+Ve
	3	+Ve	+Ve	+Ve	ND
	4	+Ve	+Ve	ND	+Ve
	5	+Ve	+Ve	-Ve	ND
	6	+Ve	+Ve	+Ve	-Ve
II	7	+Ve	+Ve	+Ve	+Ve
III	8	+Ve	+Ve	+Ve	ND
	9	+Ve	+Ve	-Ve	-Ve

+ve: Positive -ve: Negative cases. ND: Not Done.

Table (5): Detection of BVD positive cases at the area of study

Geographic Locality	No. of Positive Dead Cases	Surveillance Results As Obtained By Antigen Capture ELISA		
		No. of Serum Samples Assayed	No. Of Positive Samples	Percentage Of Positive Cases
Approximately 20 Km, North of Al-Ain City	**2**	**6**	**0**	**0**
Approximately 25 Km, North of Al-Ain City	**1**	**53**	**1**	**1.8**
Approximately 45 Km, North of Al-Ain City	**1**	**39**	**-**	**0**
Approximately 60 Km, North of Al-Ain City	**0**	**50**	**-**	**0**
Approximately 80 Km, North of Al-Ain City	**2**	**32**	**-**	**0**
Approximately 90 Km, North of Al-Ain City	**2**	**67**	**3**	**4.4**
Approximately 100 Km, North of Al-Ain City	**1**	**109**		**0**
Overall Percentage (%)		**356**	**4**	**1.01**

Table (6): Detection of BVD positive cases in relation to the age of tested camels

Locatities	Total No. of samples	ELISA (+) samples			FA (+) samples		
		Below 1 year	1-2 years	Over 5 years	Below 1 year	1-2 years	Over 5 years
20	**6**	**0/3**	**-**	**0/3**	**0/3**	**-**	**0/3**
25	**53**	**0/9**	**1/35**	**0/9**	**0/9**	**1/35**	**0/9**
45	**39**	**-**	**-**	**0/39**	**-**	**-**	**2/39**
66	**50**	**-**	**0/50**	**-**	**-**	**0/50**	**-**
80	**32**	**0/11**	**-**	**0/21**	**0/11**	**-**	**0/2**
90	**67**	**0/27**	**0/1**	**3/39**	**0/27**	**0/1**	**3/39**
100	**109**	**0/16**	**-**	**0/93**	**0/16**	**-**	**1/93**
Total (%)	**356**	**0/66 (0%)**	**1/86 (1.1%)**	**3/204 (1.4%)**	**0/66 (0%)**	**1/86 (1.1%)**	**6/204 (2.9%)**

Using UTR-1 and UTR-2 primers in RT-PCR assay to amplify 288bp fragment of the un-translated region of BVDV genome in the RNA extracted from 2 buffy coat samples collected from the contact camels in the area where dead camels (highly positive for BVDV in ELISA and FA) were found, specific fragments were amplified and revealed correct size when electrophoresed along with the molecular weight marker. The obtained PCR results confirmed the presence of BVDV in such camel farms.

Utilizing set primers to genotype the detected BVDV genome in the 2 buffy coat in RT-PCR assays, the results were negative for BVD-type I, BVD-type II and BDV. Repeating the RT-PCR genotyping assay in different format with the same set of primers, the results were also negative.

Testing the 356 serum samples for the presence of antibodies to BVDV in a neutralization assay revealed that all sera were negative. BVDV (NADL) strain used in the assay was of type I.

Inoculation of the two buffy coat samples (that were BVDV positive in ELISA and FA test) on MDBK cells has been carried out. The utilized MDBK cells were proved negative for BVDV infection by PCR. Three blind passages were applied in cell culture. Examining of the infected cells daily for 5 days post inoculation, no CPE was observed in the cells during the three passages. BVDV positive and negative control cells were incubated with the inoculums to judge the lab condition.

DISCUSSION

In camels population, several research have been carried out to investigate the existence of BVDV in such species and all the published work have documented the occurrence of BVDV replication in camels (Doyle and Heuschele, 1983; Hamblin and Hedger, 1979; Bornstein and Musa, 1987; Burgemeister *et al.*, 1975; Hedgar *et al.*, 1980; Bohrmann *et al.*, 1988 and Werney and Wernery, 1990). In the last 15 years, BVDV has been studied in the new world camelids and the virus or BVDV antibodies have been detected in camels co-housed with cattle sheep and goat (Belknap *et al.*, 2000; Haines *et al.*, 1992). Belknap (2000) was able to isolate BVDV from 3 different cases in new world camelids. In contrast, Wernery and Wernery (1990) were unsuccessful in isolating BVDV from camels and only they demonstrated the low prevalence of antibodies to BVDV in examined sera (3.6-9.2%).

In Egypt, Abo-Ellail (1997) was the first to report the occurrence of a natural outbreak of BVD in dromedary camel and also the first to isolate BVDV from camel in Egypt. By the year 1998, Zaghawa (1998) reported the prevalence of BVDV antibodies when examined 59 camel sera from 2 governorates. Yousif *et al.*, (1998) report in the Annual Meeting hold in the US, the isolation and sequence analysis of type II BVDV in a samples collected by Abo Ellail, (1997) from calf camel with congenital abnormalities Yousif, (2002) reported

Table (7): Detection of BVD in pregnant and non-pregnant camels as detected by antigen capture ELISA on serm samples

	No. of serum samples	No. of BVD positive samples by FA and ELISA	No. of BVD positive by ELISA	No. of BVD positive by FA
Pregnant Animals	**62**	**2**	**2**	**3**
Non-Pregnant Animals	**142**	**2**	**2**	**4**
Total (%)	**204**	**4 (1.9%)**	**4 (1.9%)**	**7 (3.4%)**

the typing of the RNA extracted from 2 BVD isolates from Giza and the results revealed that both isolates were typed as BVDV-type I and BVDV-type II. More recently, Ahmed, (2002) conducted virological studies on BVDV in camels. 14 BVDV isolates were successfully propagated on MDBK cells. Biotyping of such isolates revealed 11 of cytopatheic and 3 non-cytopatheic types (Ahmed, 2002). The samples were collected from 2 different localities (El-Sharkia and Sina governorates). On the other side, the existence of BVDV among camel population has been previously reported in UAE (Wernery and Wernery, 1990).

Natural BVDV outbreaks in camels have been previously reported by Abo Ellail (1997) who reported the histopathological changes associated with BVDV infection in camels. In the present study histopathological finding in the 9 dead cases confirmed the presence of such changes with BVDV infection. Most of the finding was noticed in the spleen, LN and enteric system including abomasums and intestine (Table 4). Macroscopically, hemorrhagic abomasamitis and focal intestinal erosion were seen. However, histopathological examination revealed different changes have observed previously by others (Abo Ellail, 1997; Hegazy, 1995a; Rebhun *et al.*, 1989).

No doughty that the obtained FA positive results confirm the occurrence of BVDV infection in the cases. Moreover, FA results of the 2 examined buffy coat samples add special evidence that BVDV is existing in the population where the problem occur as these 2 samples collected from co-housed camels with the dead ones.

The development of monoclonal antibodies (MAbs) to BVDV and its application in the production of antigen capture ELISA to detect BVDV has add great potential to study BVDV in the field (Sandvik, 1999; Donis, 1995; OIE, 2000 and Cavirani *et al.*, 2000). MAbs directed to the non-structural protein of BVDV NS-2-3 or against P80 have shown remarkable sensitivity and specificity to detect and type BVDV. The application of such ELISA would be grateful when used for typing of BVDV strains. Meanwhile, several MAbs have been developed and used in the prediction of ELISA kits to be applied in the field. ELISA depends on MAbs directed against the structural glycoprotein 44-48 has been successfully used to detect all BVDV strains in the field. The kits have been used by several laboratories worldwide. The ELISA kit can detect BVDV in both persistent infected and clinical infected animals. Also, the test was useful in monitoring the BVDV free herd status. We used such ELISA to examine tissues, buffy coat samples and sera collected. Third, it is possible that camel BVDV is arise from different evolutionary lineage and subsequently may have a distinct structure specially in the area that the MAbs used as the base of the utilized ELISA which differ in other BVDV strains. Fourth, the utilized ELISA was not able to detect BVDV in transiently infected animals in which the virus titre (specially NCP strains) is low as 10^{9} $TCID_{50}$ (Grieser-Wilke *et al.*, 1992 and Graham *et al.*, 1998a). On the other hand, the 4 camel sera revealed BVDV positive by ELISA (Table 5) might represent the real of persistent infected. Camels in such farms as the virus titre in such animals ranged between $10^{8.2}$ to 10^{6} $TCID_{50}$ as reported by others (Bolin *et al.*, 1985b; Bolin and Ridpalh, 1992).

One serum sample revealed BVDV positive in ELISA and was from camel of 1-2 years of age where the other 3 samples were from camels

over 5 years of age (Table 6). Interestingly, the detected BVDV in camel serum of 1-2 years of age might be a case of persistent infection. Certainly, the four detected BVDV infected animals confirm the case of persistence infection by BVDV in the 2 farms of the area of study.

Results obtained by ELISA represent the concrete evidence on the existence of BVDV among camels in the area of study. BVDV antigen detected first in the tissues of dead cases (Table 3), second in the 2 collected buffy coat samples and to some extent in the camel sera (Table 5).

Not surprising is the low percentage of BVDV prevalence in the 356 sera collected from apparently healthy camels in the area of study. Such percentage might represent the real situation of BVDV in camels in the area of study. The cause of such lowest may be due to first samples collected from normal camels, second ELISA used in the current study may be not able to detect all BVDV strains circulating in camel population and this might be due to the nature of the structural proteins of camel BVDV which might differ from the cattle BVDV specially the region at which the MAbs directed (gp 44-48). Such region is known to be useful in genomic classification of *Pestiviruses* (Sullvan and Akkina, 1995). In addition, ELISA may fall in the suspicious range of positively as reported by others (Horner *et al.*, 1995). Also, ELISA depends on the use of MAbs to BVDV has been reported to be not able to detect atypical BVDV viruses (Paton *et al.*, 1995).

In vitro infection by BVDV has been well documented and occurs prior to the development of the fetal immune system result in the birth of persistently infected animals that are continuous shed the virus to the herd mates (Baker, 1995). Detection of BVDV in 2 of the 3 camel sera (over 5 years of age) by ELISA emphasize not only the existence of BVDV but also the possibility of inducing severe outbreaks due to extensive shedding of the virus to the offspring and cause different form of the clinical disease specially in calve camel which might be resemble to those reported in cattle population (Ridpath *et al.*, 1994; Tijissen *et al.*, 1996 and Pellerin *et al.*, 1994).

In the field of pestivirology, RT-PCR assays to amplify the BVDV genome have been extensively used with great potentiality and concrete success (Ward and Mirsa, 1991; Brock *et al.*, 1991; Vilcek *et al.*, 1994, 1999a, 2001; Tajima *et al.*, 1995; Sullivan and Akkina, 1995).

RT-PCR employed in the present study was depending on the use of primer pair located at the untranslated region of BVDV genome (UTR). The used RT-PCR assay has proved its efficacy in term of its sensitivity and specificity in other studies local and international (Deng and Brock, 1992; Harpin *et al.*, 1997; Radwan *et al.*, 1995; Hussein, 2001, 2003; Mohamed, 2004; Hassanein *et al.*, 2003; Hussein *et al.*, 2003 and Abdel-Hafiez *et al.*, 2003).

The success of RT-PCR assay is largely depends on the formulation of the primer, RNA stability and PCR condition. We extracted RNA from 2 buffy coat samples and apply the RT-PCR assay with UTR primers. BVDV genome was detected in both samples. The obtained results were coinciding with the FA and ELISA results confirming the occurrence of BVDV infection such camels.

RT-PCR genotyping based assay as a tool to differentiate BVDV strains has been developed and apply with great success in different

laboratories (Sullivan and Akkina, 1995;; Hussein, 2001, Hussein *et al.*, 2003; Hassanein, 2003; Mohamed, 2004; Youssef, 2002). Although similar assays based on primers located in conserved structure within genome of *Pestivirus* as a target for differentiate have been developed and applied (Ridpath *et al.*, 1993; Vilcek, 1993; Radwan *et al.*, 1995). The RT-PCR assay developed by Sullivan and Akkina (1995) proved its ability to differentiate BVD type I, II and BDV ensuring the specificity of the target sequence. Unfortunately, we were not able to demonstrate BVD type I or type II or BDV in the tested 2 buffy coat samples when we applied Sullivan and Akkina, (1995) protocol. There are possible causes of such negativity which could be due to potential genetic diversity of camel BVDV which handicapped the typing primers used in the RT-PCR assay or presence of secondary structure of RNA in extract ran preparation or long preservation of the samples, ice crystal can harmfully affect viral RNA (Boye *et al.*, 1991; Hertig *et al.*, 1991; Radwan *et al.*, 1995).

Sequence analysis of E^{rns} region of BVD genome used to classify bovine and ovine *Pestiviruses*. The emergency of novel BVDV strains from Giraffe-1 and Reindeer-1 based on the sequence of such region constituting new groups raise the question of genetic diversity in such region (Becher *et al.*, 1995, 1997 and 1999a,b). Therefore, it is possible that camel BVDV may constitute a distinct genetic group within BVDV strains isolated from different species. There was only one study allover the world reported the occurrence of BVDV type II in camel (Youssif, 2002). Inspection and analysis of the results of such study indicated nucleotide sequence homology of camel isolate with variable and conflicting with BVD type I and higher homology with Giraffe-1 sequence. However, the isolate was much related to BVDV type II (Youssif, 2002).

Explaining the origin of the detected BVDV type II in camel, (Youssif, 2002) proposed two possible origin of such virus: import or evolution of such virus from unknown African *Pestivirus* or the virus is closely related to BVD type II viruses like those reported in USA. On the contrary, BVDV detected in the current study failed to be typed by the employed RT-PCR assay. We believe based on the current research on the genetic diversity of BVDV that BVDV in camels may constitute a distinct lineage of evolution or most probably if the cross species of BVDV strains occurs between camel and other population in the area of study. the adapted BVDV strains will be originating from ovine or caprine species as cattle were not present in the farms where the samples were collected.

The continuous evolution and diversity of BVDV via mutation and recombination have been previously described (Meyeres *et al.*, 1996 and 1998). Similar regions in *Pestiviruses* within a species or even between two different species facilitate the template switching of RNA dependent RNA polymerase during the replication cycle yet yield a new or direct strain (Becher, 1999a,b).

E^{rns} region could be a region in which such template switch occurs. Therefore, the BVDV in camel is possible to be a distinct type not only type I and type II as previously reported in the study of Youssif, (2002). The template switch phenomena as the cause of mutation was reported to be the process of continuous evolution in CP *Pestiviruses*. Indeed, BVDV detected in the present study in buffy coat of

contact camel might be represent a new strains originating from 2 sources camel or cross species most probably ovine.

Propagation of the BVDV positive buffy coat samples on MDBK cells for three passages has been applied. As expected, there was no CPE detected among the 3 passages which might reflect that the detected strains of NCP types. Further propagation titration and characterization studies needs to be conducted on these 2 samples in further study.

In conclusion, the study raise the possibility of the presence of persistent infected camels by BVDV in area of study and those camels may cause extensive shedding of the virus in camel population resulting in severe outbreaks or may play a primary cause in the recombination and diversity in BVDV strains in camel population in area of study. The prevalence of BVDV in camel population is somewhat low but depends on the diagnostic test used. BVDV was detected in association with dead cases and may be contributed as a cause of such cases. Failure in RT-PCR genotyping assay to type the detected BVDV confirm the continuous genetic diversity of such viruses and exalt the possibility that camel BVDV may have a distinct group varied from those described in cattle and ovine population. BVDV in the area of study is circulating in pregnant and non-pregnant camels. Therefore, great attention should be taken for further disease problems especially to those resemble BVDV infection. The 2 detected BVDV strains may be of NCP type. This result in the current study is of added interest and was expected. Those NCP strains may be the cause of fetal infection which might lead to fetal deaths, leg deformity, abortion observed in the area of the study. The present study; add a piece of knowledge, which might help in the study of the molecular epidemiology and genetic diversity of BVDV strains. Increasing our knowledge on such virus on camel population in further studies will help in understand the mechanism of evaluation of such strain.

REFERENCES

Aaron, F.H.; Lum, Chad, E.G.; Claretis, R.L.; Donald, E.S. and Scott, I.S. (2002). Dynamic regulation of LFA-1 activation and neutrophil arrest on intracellular adhesion molecule 1 (ICAM-1) in shear flow. J. Biol. Chem., Vol. 277 Issue 23, 20660-20670.

Abd El-Hafeiz, Y.G.M.; Hussein, H.A.; Amin, A.S. and Reda, I.M. (2003). Current status of BVDV in Egypt: 3. Isolation, antigenic and genetic characterization of cytopathic and non-cytopathic strains of genotype II from Milk. 7th Sci. Cong. Egypt. Soc. Cattle Dis., 7-9 Dec. 2003, Assiut, Egypt, pp. 207-218.

Abo- Ellail, T.A. (1997). Pathological and virological studies on camel diseases with special reference to bovien viral diarrhea virus infection. Thesis, Ph. D. Fac. Vet. Med., Cairo Univ..

Ahmed, A.H. (1998). Virological and immunological studies on BVDV, Thesis Ph. D. Fac. Of Vet. Med. Zagazig University.

Baker J. C. and Houe, H. (1995). Bovine Viral diarrhea Virus. Vet. Clin. North. America, Food Anim. Prac., 11(3): 393-651.

Baker, J.C. (1987). Bovine viral diarrhea virus: A review. J. A. V. M. A., 190(11), June (1): 1449-1458.

Baker, J.C. (1995). Up date and the clinical manifestations of Bovine

Viral Diarrhoea Virus Veterinary Clinics of North America: food animal practice. 11: 425-445.

Becher, P.; Orlich, M.; Shannon, A.D.; Horner, G.; Konig, M. and Thiel, H.J. (1997). Phylogenetic analysis of Pestiviruses from domestic and wild ruminants. J. Gen. Virology, 78(6): 1357-1366.

Becher, P.; Konig, M.; Paton, D.J. and Thiel, H.J. (1995). Further characterization of border disease virus isolates: evidence for the presence of more than three species within the genus Pestivirus Virology. 10; 209(1): 200-206.

Becher, P.; Orlich, M.; Kosmidou, A.; Konig, M.; Baroth, M. and Thiel, H.J. (1999a). Genetic diversity of Pestiviruses: identification of novel groups and implications for classification. Virology. 15; 262(1): 64-71.

Becher, P.; Shannon, A.D.; Horner, G.; Konig, M. and Thiel, H.J. (1999b). Phylogenetic analysis of Pestiviruses from domestic and wild ruminants. J. Gen. Virology. 78(6): 1357-1366.

Belknap, E.B.; Collins, J.K.L. and Conrad, K.P. (2000). Bovine Viral Diarrhoea Virus in new world camelids. J Vet. Diagnostic investigation 12:6, 568-570.

Bohrmann, R.; Frey, H.R. and Lifss, B. (1988). Survey on the prevalence of neutraizing antibodies to bovine viral diarrhea (BVD) virus, bovine herpsvirus type I (BHV-I) and parainfluenza virus type 3 (PI3) in ruminants in the Djibouti Republic. Dtsch. TieraerztL. Wschr. 95, 99-102.

Bolin, S.R. and Ridpath, J.F. (1992). Differences in virulence between two NCP-BVDV in calves. Am. J. Vet. Res. 53: 2157-2163.

Bolin, S.R.; McClurkin, A.W.; Cutlip, R.C. and Coria, M.F. (1985). Severe clinical disease induced in cattle persistently infected with noncytopathic Bovine Viral Diarrhoea Virus by Superinfection with cytopathic bovine viral diarrhea virus. Am J Vet Res 46, 573-6.

Bornstein, S. and Musa, B.E. (1987). Prevalence of antibodies to some viral pathogens, Brucella abortus and Toxoplasma godii in serum from camels (Camelus dromedarius) In Sudan. J. of Vet. Med., Series B. 34:5, 364-370.

Boye, M.; Kamstrup, S. and Dalsgaard, K. (1991). Specific sequence amplification of BVDV and HCV and sequencing of BVDV nucleic acid. Vet. Microb., 29 (1): 1-13.

Brock, K.V.; Redman, D.R. and Vickers, M.L. (1991). Quantitation of Bovine Viral Diarrhoea Virus in embryo transfer flush fluids collected from a persistently infected heifer. J. Vet. Diagnostic. Invest 3: 99-100.

Burgemeister, R.; Leyk, W. and Gossler, R. (1975). Studies on the occurrence of parasites, bacterial and viral infections in southern Tunisian dromedaries. Deutsche Tierarztliche Wochenschrift, 82: 9, 352-354.

Cavirani, S.; Foni, E.; Cabassi, S. and Donofrio, G. (2000). Detection of BVDV antigen by capture ELISA and immunofluorescence assay in cell culture. Selezione Veterinaria No. supp., s189-194.

Deng, R. and Brock, K.V. (1992). Molecular cloning and nucleotide sequence of a Pestivirus genome, noncytopathic BVDV strain SD-1. Virology, 191: 867-879.

Donis, R.O. (1995). Molecular biology of Bovine Viral Diarrhoea Virus and its interactions with the host. Vet. Clin. North Am. Food Anim. Pract., 11 (3): 393-423.

Doyle, L.G. and Heuschele, W.P. (1983). BVDV infection in captive

exotic ruminants. J.A.V.M.A., 183 (11): 1257-1259.

Graham, D.A.; Mclaren, I.E. and German, A. (1998). Evaluation of the suitability of a commercial Bovine Viral Diarrhoea Virus antigen capture ELISA for diagnostic testing. Vet J. 156(2): 149-154.

Greiser-Wilke, I.; Dittmar, K.; Liess, B. and Moennig, V. (1992). Heterogenous expression of the non-structural protein P80/P125 in cells infected with different Pestiviruses. J. Gen. Virol., 73: 47-52.

Haines, D.M.; Clark, E.G. and Dubovi, E.J. (1992). Monoclonal antibody-based immunohistochemical detection of BVDV in formalin-fixed, paraffin- embedded tissues. Vet. Pathology, 29(1): 27-32.

Hambiln, C. and Hedger, R.S. (1979). The Prevalence of antibodies to BVD/MD virus in Africal Wildfe. Comparative Immunology, Microbiology, and infectious diseases. Vol 2, 295-303.

Harpin, S.; Talbot, B.; Mbikay, M. and El-Azhary, Y. (1997). Immune response to vaccination with DNA encoding the Bovine Viral Diarrhoea Virus major glycoprotein gp53 (E2). FEMS Microbiol. Lett. 15: 146 (2): 229-234.

Haward, C.J. (1990). Immunological responses to Bovine Virus Diarrhoea Virus infections. Rev Sci Tech 9,95-103.

Hedger, R.S.; Barnett, I.T.R. and Gray D.F. (1980). Some virus diseases of domestic animals in the Sultanate of Oman. Trop. Animal. Hlth. Prod. 12: 107-114.

Hegazy, A.A.; El-Sanousi, A. A.; Lotfy, M.M.; Hamouda, M.A.; Hassan, H.B.; Hussein, A.H.M. and Abo-Ellail, T.A. (1995) Pathological and virological studies on calf mortality B-Mortalities associated with BVDV infection. J. Egypt. Vet. Med. Ass., 1-2: 493-503.

Hertig, C.; Pauli, U.; Zanoni, R. and Peterhans, E. (1991). Detection of bovine viral diarrhoea (BVD) virus using the polymerase chain reaction. Vet. Microbiol., 26 (1-2): 65-76.

Horner, G.W.; Tham, K.M.; Orr, D.; Ralston, J.; Rowe, S. and Houghton, T. (1995). Comparison of an antigen capture enzyme linked assay with reverse transcription polymerase chain reaction and cell culture immunoperoxidase tests for the diagnosis of ruminant Pestvirus infections. Vet. Microbiol., 43 (1): 75-84.

Hassanein, S.A.; Hussein, H.A.; Karim, I.A.; Reda, I.M. and Shalaby, M.A. (2003). Current status of BVDV in Egypt: Isolation, antigenic and genomic characterization of BVDV genotypes I and II from calves in Egypt. J. Egypt. Vet. Med. Assoc. 63: 399-416.

Hussein, A.H. (2001). Molecular characterization of the Egyptian strain (Iman) of BVDV and its RNA synthesis in cell culture. Arab J. Biotech., 4 (1): 23-36.

Hussein, H.A.; Hassanein, S.; Karim, I.A. and Shalaby, M.A. (2003). Molecular diversity of bovine viral diarrhoea virus: Genotype I and II among apparently health calves in Egypt. J. Egypt. Vet. Med. Ass., 63 (3): 87-104.

Hussein, H.A.; Mohamed, N.A.; Mohamed, F.M. and Shalaby, M.A. (2004). Cytopathogenicity of local field strains of Bovine Viral Diarrhoea Virus isolated from apparently health buffaloes in Egypt. Egyptian Journal of Virology. Volume 1 (In press).

Loken, T. (1996). Ruminant Pestivirus infections in animals other than cattle and sheep. Vet. Clin. North Am. Large Pract 11: 597:614.London.

Meyers. G.; Tautz. N.; Becher, P.; Thiel. H.J. and Kummerer. B.M. (1996). Recovery of cytopathogenic and non-cytopathogenic bovine viral diarrhoea viruses from cDNA constructs. J. Virol. 70 (12): 8606-8613.

Meyers, G.; Stoll. D. and Gunn. M. (1998). Insertion of a sequence encoding light chain 3 of microtubule-associated proteins 1A and 1B in a Pestvirus genome: connection with virus cytopathogenicity and induction of lethal disease in cattle. J.Virol. 72. (5): 4139-4149.

Meyling. A.; Houe. H. and Jensen. A.M. (1990). Epidemiology of BVDV. Rev. SCI. Et. Tech. Of Inter.Des. Epiz, 9(1): 75-93.

Mohamed. N.A.; Hussein. H.A.; Mohamed, F.M. and Shalaby. M.A. (2004). Isolation, antigenic and molecular characterization of Bovine Viral Diarrhoea Virus field strains from apparently health buffaloes in Egypt. Egyptian J. of Virology. Volume 1 (In press).

OIE. (2000). Manual of Standards Diagnostic tests and Vaccines. Part 3. Section X. Chapter X 5 Bovine viral diarrhoea.

Paton. D.J.; Sands. J.J.; Lowings. J.P.; Smith. J.E.; Ibata. G. and Edwards. (1995). A proposed division of the Pestivirus genus using monoclonal antibodies. supported by cross-neutralisation assays and genetic sequencing. Vet Res 26: 92-109.

Pellerin. C.; Van den Hurk. L.; Lecomte. J. and Tijssen. P. (1994). Identification of a new group of Bovine Viral Diarrhoea Virus (BVDV) strains associated with ever outbreaks and high mortalities. Virol. 203: 260-268.

Plowright. W. (1969). Other diseases in relation to R.P. Ist Ann mee' Joint campaign against Rinderpest (J.P. 15 program. Mogadisico. December 1969).

Provost. A.; Bogel. K.; Borredon. C. and Maurice. Y. (1967). Epidemiological observation. Rev. Elev. Med. Vet. Pays. Trap.. 20:27-29.

Radwan. G.S.; Brock. K.V.; Hogan. J.S. and Smith. K.L. (1995). Development of a PCR amplification assay as a screening test using bulk milk samples for identifying dairy herds infected with Bovine Viral Diarrhoa Virus. Vet. Microbiol. 44: 77-92.

Rebhun. W.C.; French. T.W.; Perdrizet. J.A.; Dubovi. E.J.; Dill. S.G. and Karcher. L.F. (1989). Thrombocytopenia associated with acute bovine virus diarrhoea infection in cattle. J. Vet. Intern. Med.. 3 (1): 24-26.

Ridpath. J.F.; Bolin. S.R. and Dubovi. E.J. (1994). Segregation of Bovine Viral Diarrhoea Virus into genotypes. Virology. 205(1): 66-74.

Sandvik T. (1999). Laboratory diagnostic investigations for Bovine Viral Diarrhoea Virus infections in cattle. Vet Microbiol. 64(2-3) 123-134.

Sullivan. D.G. and Akkina R.K. (1995). A nested polymerase chain reaction assay to differentiate Pestiviruses. Virus Res. 38(2-3): 231-239.

Tajima. M.; Kirisawa. R.; Taguchi. M.; Iwai. H.; Kawakami. M.; Yagiwara. K.; Ohtsuke. H.; Sentsui. H. and Takahashi. K. (1995). Attempt to discriminate between BVDV strains using PCR. J. Vet. Med.. B. 42 (5): 257-265.

Tijssen, P.; Pellerin, C.; Lecomte, J. and Van den, H.J. (1996). Immunodominant E2 (gp53) sequences of highly virulent bovine viral diarrhea group II viruses indicate a close resemblance to a subgroup of border disease viruses. Virology. 217(1): 356-361.

Vilcek, S. (1993). Amplification in vitro of a genome fragment of Bovine Diarrhoea Virus by the PCR method. Veterinarni Medicina, 38 (5): 257-266.

Vilcek, S. and Belak, S. (1996). Genetic identification of Pestivirus strain Frijters as a border disease virus from pigs. J. Virol. Methods. 60 (1): 103-108.

Vilcek, S.; Alenius, S.; Paton, D.J.; Mittelholzer, C. and Belak S. (1999a). Genetic clustering of bovine viral diarrhoea viruses in cattle farms: genetic identification and analysis of viruses directly from cattle sera. Vet J. 158(1): 33-38.

Vilcek, S.; Drew, T.W.; McGoldrick, A. and Paton D.J. (1999b). Genetic typing of bovine Pestiviruses from England and Wales. Vet Microbiol. 69(4): 227-237.

Vilcek, S.; Herring, A.J.; Herring, J.A.; Nettleton, P.F.; Lowings, J.P. and Paton D.J. (1994). Pestiviruses isolated from pigs, cattle and sheep can be allocated into at least three genogroups using polymerase chain reaction and restriction endonuclease analysis. Arch Virol. 136(3-4): 309-323.

Vilcek, S.; Paton, D.J.; Durkovic, B.; Strojny, L.; Ibata, G.; Moussa, A.; Loitsch, A.; Rossmanith, W.; Vega, S.; Scicluna, M.T. and Paifi, V. (2001). Bovine Viral Diarrhoea Virus genotype 1 can be separated into at least eleven genetic groups. Arch Virol. 146(1): 99-115.

Ward, P. and Misra, V. (1991). Detection of Bovine Viral Diarrhoea Virus, using degenerate oligonucleotide primers and the polymerase chain reaction. Am. J. Vet. Res., 52 (8): 1231-1236.

Wassel, M.S.; Souad, S.A.; Girgis, S.; Hussein, A.Z. and El-Rawy, E.J. (1996). Trial for preparation and evaluation of a combined vaccine for IBRV, BVDV, PI-3 V, P. multocida and P. haemolytica in Egypt. Assiut Vet. Med. J., 35 (70): 130-143.

Wernery, U. and Wernery, R. (1990). Sero-epidemiological investigation in female camels (Camelus dromedarius) for the demonstration of antibodies against brucella, chlamedia, leptospira, BVD/MD, IBR/IPV and enzootic bovine leukosis (EBL) virus. Dtsch. Tierarztl. Wschr. 79:3, 134-135.

Yousif, A.A. (2002). Ruminant Pestiviruses genetic diversity: The key to detection and control. Ph.D. Thesis, Biological Sciences, South Dakota State Univ., South Dakota, USA.

Yousif, A.A.; Braun, L.J. and EL-Leil T.A.A. (1998). Sequence analysis of a type II cytopathic BVDV of BVDV of Dromedary Camels. Proc. Conf. Res. Workers Anim. Dis. 79:113.

Zaghawa, A. (1998). Prevalence of antibodies to Bovine Viral Diarrhoea Virus and/or border disease virus in domestic ruminants. Zentralbl Veterinarmed (B).45(6): 345-351.