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BOVINE VIRUS DIARRHEA IN CAMELS: ROLE OF CAMELS INFECTED WITH BOVINE VIRAL DIARRHEA VIRUS IN TRANSMISSION OF THE DISEASE

(With Two Tables and One Figure)

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مرض الإسهال الفيروسى فى الجمال: دور الجمال المصابة بفيروس الإسهال الفيروسى فى نقل المرض

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لدراسة مرض الإسهال الفيروسى فى الجمال والتعرف على الدور الذى تلعبه الجمال فى نقل الفيروس المسبب لهذا المرض إلى الأبقار تم فحص عدد ٥٠ جمل و ٥٠ بقرة (على احتكاك بهذه الجمال) من الناحية الإكلينيكية والسيرولوجية والفيروسية بالإضافة إلى استخدام التكنولوجيا الحيوية. كل الأبقار المستخدمة كانت خالية من مرض الإسهال الفيروسى عند بداية هذه الدراسة كما ثبت من نتائج الاختبارات المعملية الأولى التى تم إجراءها عند بداية العمل. اختبار الاليزا والعزل الفيروسى والتفاعل التبلمرى المتسلسل تم إجراءهم مرتين متتاليتين يفصل بينهما شهر لكل حيوان. الفحص الإكلينيكي أوضح عدم وجود أى أعراض إكلينيكية على الجمال المختبرة حتى فى الجمال التى ثبت إصابتها بالفيروس باستخدام أحد الاختبارات السابق ذكرها (ماعدا جملين أظهروا بعض الأعراض الشديدة). وفى الأبقار، ١٤ بقرة من ٢٣ بقرة ثبت إصابتها بالفيروس كانت تعانى من أعراض إكلينيكية بينما باقى الأبقار المصابة (٩ بقرات) كانت سليمة ظاهريا. كل الجمال أعطت نتائج سلبية مع اختبار الاليزا الأول والثانى (ما عدا جملين أعطوا نتيجة إيجابية ضعيفة مع اختبار الاليزا الثانى) فى نفس الوقت لم تسجل الأبقار أى نتائج إيجابية مع اختبار الاليزا الأول ولكن ١٦ بقرة كانت إيجابية مع اختبار الاليزا الثانى. تم عزل فيروس الإسهال الفيروسى من ١١ جمل فى العزل الفيروسى الأول ولم تتغير النتيجة فى العزل الفيروسى الثاني بينما كل الأبقار المختبرة كانت سلبية للعزل الفيروسى الأول ومع ذلك تم عزل الفيروس من ١٧ بقرة فى العزل الفيروسى الثاني. فى تقنية التفاعل التبلمرى المتسلسل الأول تم التعرف على الحامض النووى لفيروس الإسهال الفيروسى فى ١٥ جمل ولم يتم التعرف على الحامض النووى للفيروس فى أى من الأبقار التى تم اختبارها بينما التفاعل التبلمرى المتسلسل الثاني أوضح وجود الحامض النووى للفيروس فى ١٥ جمل و٢٣ بقرة. كل الجمال والأبقار التى أعطت

نتائج إيجابية مع اختبار الاليزا والعزل الفيروسى كانت إيجابية مع التفاعل التبلمرى المتسلسل. نتائج هذه الدراسة أثبتت أن الجمال يمكن أن تصاب بفيروس الإسهال الفيروسى ولكنها لا تبدى أعراض إكلينيكية ولهذا تستطيع نقل الفيروس للأبقار بعد احتكاكها بها ولو لفترة قصيرة نسبيا وتظل قادرة على نقل الفيروس لمدة طويلة دون ملاحظة. كما أظهرت نتائج هذا العمل أن تقنية التفاعل التبلمرى المتسلسل أكثر حساسية من العزل الفيروسى ومن اختبار الاليزا فى تشخيص مرض الإسهال الفيروسى كما أن العزل الفيروسى أكثر حساسية من اختبار الاليزا فى تشخيص صورة الإصابة المستمرة. من نتائج هذا العمل نستطيع أن نسجل أن الجمال تستطيع أن تلعب دورا هاما جدا فى استمرار بقاء ونقل فيروس الإسهال الفيروسى للأبقار. لهذا يجب وضع هذه النقطة فى الاعتبار عند عمل أى دراسات وبائية وعند التخطيط للتخلص من فيروس الإسهال الفيروسى. كما يمكن القول أن تقنية التفاعل التبلمرى المتسلسل تبدو شديدة الحساسية ومناسبة لتشخيص الإصابة بفيروس الإسهال الفيروسى فى الأبقار والجمال خاصة عندما تفشل الاختبارات الأخرى فى ذلك ولهذا نوصى باستخدام هذه التقنية فى فحص الجمال (خصوصا تلك المستوردة من السودان) للتأكد من خلوها من مرض الإسهال الفيروسى. هذه أول دراسة تتناول الدور الذى تلعبه الجمال فى نقل فيروس الإسهال الفيروسى للأبقار كما أنها أول دراسة تستخدم تقنية التفاعل التبلمرى المتسلسل فى تشخيص الإصابة بفيروس الإسهال الفيروسى فى الجمال.بالإضافة إلى أنها المرة الأولى التى يتم فيها التعرف على فيروس الإسهال الفيروسى_٢ فى الجمال والأبقار فى مصر.

SUMMARY

To study BVD in camels and to investigate the role played by camels in transmission of BVDV to cattle, 50 camels and 50 cattle in contact with these camels were examined clinically, serologically, virologically and biotechnologically. All used cattle were free from BVD at the beginning of the study as proved by first laboratory investigations. Clinical examination, an indirect ELISA, viral isolation and reverse transcription polymerize chain reaction (RT-PCR) were performed to each examined animal. An indirect ELISA, virus isolation and RT-PCR were carried out two times one month apart. Clinical examination exhibited no clinical signs in camels even in animals proved to be positive for presence of BVDV by one or more of previously mentioned tests (except two camels showed severe signs). In cattle, 14 out of 23 infected animal were suffered from clinical signs after three weeks from starting of the study while the rest of animals were apparently healthy. All examined camels were negative with first and second ELISA except 2 camels gave weak positive result with second ELISA, at the same time, no cattle were positive with first ELISA but 16 cattle were positive with second ELISA. BVDV was isolated from 11camels in the first and second isolation while all examined cattle were negative for first virus isolation,

however BVDV was isolated from 17 cattle in the second virus isolation. In the first RT-PCR, BVD viral nucleic acid (RNA) was detected in 15 camels and no BVD viral nucleic acid was detected in all cattle examined with first RT-PCR while second RT-PCR detected viral nucleic acid of BVD in 15 camels and 23 cattle. Camels and cattle that gave positive result with ELISA and virus isolation were positive with RT-PCR. Results of this study proved that camel could be infected with BVDV but showed no apparent clinical signs so, it can transmit the virus to cattle after contact with it even for a relatively short time and remain infective for a long time without observation. RT-PCR technique seemed to be more sensitive than ELISA and virus isolation while virus isolation was more sensitive than ELISA in diagnosis of persistent form of BVD. From results of the present work we can report that camels could play a very important role in persistent and transmission of BVDV infection among cattle. Therefore, any epidemiological studies or planning of control program to BVDV should put this point of view in consideration. At the same time, RT-PCR technique seemed to be very sensitive and very suitable for diagnosis of BVDV infection in cattle and camels especially when other tests failed to detect the infection. Therefore, we recommend this technique to be used in screening of camels (especially those imported from Sudan) for their freedom from BVDV. This is the first study investigated role of camels in transmission of BVDV to cattle and the first to use RT-PCR assay in diagnosis of BVDV in camels, in addition to the first recognition of BVDV genotype-II in Egypt.

Keywords: *Bovine virus diarrhea (BVD), bovine virus diarrhea virus (BVDV), virus isolation, indirect ELISA, RT-PCR, molecular biology, camel , cattle, epidemiology, Egypt.*

INTRODUCTION

Bovine virus diarrhea virus (BVDV), a positive strand RNA virus is one of the most insidious and ubiquitous pathogen throughout the world (Liebler-Tenorio *et al.*, 2000; Lorenz, 2000; VanLeeuwen *et al.*, 2001). This small *enveloped* virus belongs to the genus pestivirus within the family flaviviridae (Schelp *et al.*, 2000; Schweizer and Peterhans, 2001). Based on its behavior and effect in tissue culture, two biotypes of BVDV are distinguished, a non-cytopathogenic (NCP) and cytopathogenic (CP) biotype (Kummerer and Meyers, 2000; Li and McNally, 2001).

The identification of the biotype (whether it is NCP or CP is based also on known differences between the viral RNAs of the two biotypes (Schweizer and Peterhans, 2001), when compared to NCP BVDV some CP BVDV contain additional sequences in genomic region (Vilcek *et al.*, 2000).Recent serological analyses and sequence comparison studies showed that CP BVDV arise from NCP BVDV by mutation (Kummerer *et al.*, 2000). At the same time, experimental study proved that CP biotype could be changed into NCP biotype (Baroth *et al.*, 2000).

Recently, genetic typing of BVDV is performed and two genotypes of BVDV have recorded, BVDV type I and BVDV type II (Vilcek *et al.*, 1999; Falcone *et al.*, 2001; Luzzago *et al.*, 2001) .Most BVDV isolates were classified as BVDV type 1 (Tajima *et al.*, 2001).

This virus is an economically important pathogen with a worldwide distribution (Schweizer and Peterhans, 2001) causing multiple diseases and clinical syndromes including embryonic mortalities, abortion, fetal mummification, stillbirths, congenital deformities, respiratory disease (Bjorkman *et al.*, 2000; Flores *et al.*, 2002) and haemorrhagic syndrome (Walz *et al.*, 1999). Most of severe signs caused by BVDV type 2 (Tessaro *et al.*, 1999; Stoffregen *et al.*, 2000).

Infection of camels with BVDV has been reported in many countries (Wernery and Wernery, 1990; Belknap *et al.*, 2000)

Animal immunotolerant and persistently infected (PI) with BVDV constitute the mechanism by which BVDV persists and spreads among herds. Detection and elimination of PI animals are necessary for control of BVD (Saliki *et al.*, 2000; Alban *et al.*, 2001). Currently virus isolation and ELISA are used for BVDV PI screening (Saliki *et al.*,2000). The most important control regulation have been blood testing before movements to other herds (Bitsch *et al.*, 2000)

Diarrhea, erosions and ulcers of the oral mucosa, with conjunctival and nasal discharge with other respiratory signs were observed in cattle infected with BVDV. The clinical picture was accompanied by biphasic body temperature elevation (Polak and Zmudzinski, 2000). These signs usually caused by type 2 BVDV (Flores *et al.*, 2000). Infected animals developed respiratory symptoms and seroconversion to BVDV positively after contact with PI animal. Respiratory signs resulted from tissue damage caused by virus (Baule *et al.*, 2001)

The RT-PCR is an in vitro technique that is increasingly being used in diagnosis of viral animal pathogens. Due to its high sensitivity it

is considered as an alternative to current standard methods for detecting BVDV (Scheibner *et al.*, 2000)

In Egypt, no attention has been paid to screening camels (especially those imported from Sudan) for freedom from BVDV infection and there is no data about role of camels in transmission of BVDV to cattle. At the same time, there are only a few publications in the literature that discuss bovine virus diarrhea in camels and their role in epidemiology of this important disease. Therefore, the present study aimed to study BVD in camels and look at the role of camels in transmission and persistence of BVDV among cattle through attempts to detect BVDV, BVD viral antibodies or BVD viral nucleic acid in both camels and contact cattle.

MATERIAL and METHODS

Animals:

This study comprised 50 camels and 50 cattle. Camels were imported from Sudan and found in Daraw, Aswan province, Upper Egypt. Cattle investigated in this work were in contact with examined camels and proved to be free from BVDV at the beginning of the study.

Blood:

5 ml blood was collected from each examined cattle and camel two times one month apart for virus isolation and for extraction of BVDV RNA from purified leukocytes.

Serum:

5 ml blood sample was collected from each examined animal by jugular vein puncture using sterile evacuated tube (Becton-Dickinson, Meylen cedex, FRANCE). Blood without anticoagulant was centrifuged 600g/min for 10 minutes and sera obtained and stored at –20 until the analysis for detection of BVDV antibodies. Two serum samples from each animal were collected one month apart.

Clinical examination:

All camels and cattle used in the present study examined clinically. Animals proved to be infected with BVDV were examined once daily for one month. We used an evaluation system modified from that of Traven *et al.*, (1991) and all specific clinical signs were reported and evaluated.

Indirect ELISA:

An indirect ELISA was used for detection of antibodies to BVDV in sera of camels and cattle according to Juntti *et al.*, 1987; Saliki

et al., 2000 and Manufacturer directions (IDEXX Scandinavia AB, Sweden) as followings:-

(1)-Coated plate was obtained and the sample position was recorded on a worksheet.(2)-100ul of the sample diluent was added to each well.(3)-25ul of negative control was added into appropriate duplicate wells (A1 and A2).(4)- 25ul of positive control was added into appropriate duplicate wells (A3 and A4). (5)- 25ul of serum was added into remaining wells. (6)-Microtiter plate's shaker mixed the content of the microwells. (7)-The microtiter plate was tightly sealed and incubated for 90 minutes at room temperature (18-25°C). (8)-Liquid contents of all wells were aspirated into waste reservoir. (9)-Each well was washed with approximately 300ul of wash solution five times. Liquid contents of all wells were aspirated after each wash. Following the final aspiration, residual wash fluid was firmly taped from microtiter plate onto absorbent material. (10)-100ul of conjugate was dispensed into each well. (11)- The plate was incubated for 30 minutes at room temperature (18-25°C). (12)-Steps 8 and 9 were repeated. (13)-100ul of TMB substrate solution was added into each well. (14)-The plate was incubated for 10 minutes at room temperature (18-25°C). The time was began after the filling of the first well. (15)-100ul of stop solution was added into each well to stop the reaction. The stop solution was added in the same order as the substrate solution was added. (16)-Spectrophotometer was blanked on air. (17)-The absorbance of the samples and control was measured and recorded at 450nm and the results were calculated.

(18)-Results: For the assay to be valid, the difference (P-N) between the positive control mean (PCX) and the negative control mean (NCX) must be less than or equal to 0.150 optical density (OD). In addition, the negative control mean (NCX) must be less than or equal to 0.250 OD. The presence or absence of BVDV antibodies in the samples was determined by S/P ratio for each sample

19-Calculations

(a)-Calculation of negative control mean (NCX)

$$NCX = \frac{NC\ 1\ A(450) + NC2\ A(450)}{2}$$

(b)-Calculation of positive control mean (PCX)

$$PCX = \frac{PC\ 1\ A(450) + PC2\ A(450)}{2}$$

(c)-Calculation for test samples

$$S/P = \frac{Sample\ A(450) - NCX\ A(450)}{PCX\ A(450) - NCX\ A(450)}$$

20-Interpretation of results

(a) -Samples with S/P values less than 0.200 are classified as negative for BVDV antibodies.

(b) -Samples with S/P values greater than or equal to 0.200 but less than 0.300 are considered doubtful and the animal was retested after three weeks.

(c) -Samples with S/P values greater than or equal to 0.300 are classified as positive.

Virus isolation:

Virus isolation was performed according to Niskanen *et al.*, (1991) and Belknap *et al.*, (2000) by inoculation of 0.5 ml blood on coverslip cultures of embryonic bovine turbinate (BTB) cells. The presence of BVDV was determined by immunoperoxidase staining and immunofluoorescence technique, applying monoclonal antibody to BVDV.

Reverse transcription polymerase chain reaction (RT-PCR):

(a) Synthetic oligonucleotide primer

The primer for BVDV (Pharmacia Biotech.) was designed by comparison of published sequences of BVDV (Scheibner *et al.*, 2000; Giangaspero *et al.*, 2001). More specifications of this primer are listed in Table (1).

Table 1: Sequences and location of the oligonucleotide primers used for PCR amplification of BVDV.

Primer	Sequence 5`- 3`	Expected virus	Position
P80 1	CTGCCAAATGCCTCAACCAAAGCT	BVDV	6322–6345
P80 2	GGACAACCCGGTCACTTGCTTCAG	BVDV	7474–7451

(b) RNA extraction

The acid guanidinium-phenol-chloroform (AGPC) method (Chomczynski and Sacchi, 1987) was used to isolate BVDV RNA from purified leukocytes. The RNA pellet obtained was washed with 75% ethanol, dried and suspended in 10ul of 10mM DTT and 10 units of placental ribonuclease inhibitor (GIBCO-BRL). It was heated at 65°C for 5 min, chilled on ice and used immediately thereafter.

(c) Reverse transcription and cDNA synthesis

Reverse transcription was performed in a 20µl reaction mixture containing 10mM Tris.Hcl pH 8.4, 50mM KCl, 2.5mM MgCl2, 1mM of each of the four dNTPs (Sigma), 2.5uM of the reverse primer and 10 units of M-MLV reverse transcriptase (GIBCO-BRL) with incubation at 37°C for 30 min.

(d) PCR amplification

The PCR amplification was performed in a reaction volume of 100ul containing 10mM Tris.HCl pH8.4, 50mM KCl, 2.5 mM MgCl2. 0.2 mM of each four dNTPs, 0.5 uM of each two primers and 0.8 unit of Taq DNA polymerase (CENBIOT), which was overlaid with 100 ul of mineral oil (Sigma). Amplification and reverse transcription were performed for 35 cycles on a gene Amp CR system 9600 (Perkin Elmer) using program as follow: (1) preheating at 94°C for 3min, (2) denaturation at 94°C for I min, (3) primer annealing at 65°C for 1 min and (4) amplification at 72°C for 1 min.

(e) Amplification product analysis

The amplification products was analyzed by electrophoresis of 15ul on a 2% agarose gel stained with ethidium bromide, and examined under ultraviolet light. DNA molecular weight marker type 100bP ladder (GibcoBRL) was applied to identify the size of the PCR products. Negative control for RT-PCR were performed by adding of distilled water instead of the primers.

RT-PCR was performed as described by Scheibner *et al.*, 2000.

RESULTS

1- Clinical examination

(a) Camels

All infected camels were apparently healthy and no clinical signs recorded in all examined camels except two camels showed severe signs in the form of respiratory signs (cough and nasal discharge), biphasic rise of body temperature, and severe pronounced bloody diarrhea. One of these two camels was died after one week from starting of the signs.

(b) Cattle

Great variation in clinical signs among infected cattle was recorded. The signs varied from slight rise of body temperature up to fever, severe respiratory disorders, erosions of oral mucosa and watery diarrhea.

2- Indirect ELISA

(a) First examination: 2 camels were positive for BVDV antibodies while no positive results recorded in examined cattle.

(b) Second examination: 2 camels and 16 cattle were positive for BVDV antibodies.

3- Virus isolation

(a) First isolation: BVDV was isolated from 11 camel (nine camels were harbor NCP biotype and two camels were carry both NCP and CP biotypes) while all examined cattle were negative for BVDV isolation.

(b) Second isolation: BVDV was isolated from 11camels and 17 cattle (twelve NCP biotype isolates and five CP biotype isolates).

4- RT-PCR

(a) First examination: BVD viral nucleic acid was detected in 15 camel and no nucleic acid detected in examined cattle.

(b) Second examination: BVD viral nucleic acid was detected in 15 camel and 23 cattle.

BVDV detected by RT-PCR in both camels and cattle was belong to 2 genotypes, type 1 and type 2 (figure 1).

Results of laboratory investigation of camels and cattle are summarized in table (2).

Table 2: Results of laboratory investigation.

	ELISA		VIRUS ISOLATION				RT-PCR			
	1st exam.	2nd exam.	1st exam.		2nd exam.		1st exam.		2nd exam.	
			CP	NCP	CP	NCP	Type1	Type2	Type1	Type2
CAMELS	0	2	2	9	2	9	13	2	13	2
CATTLE	0	16	0	0	5	12	0	0	18	5

CP = cytopathic biotype of BVDV. NCP = non-cytopathic biotype of BVDV.
Type 1 = BVDV genotype-I. Type 2 = BVDV genotype-II.

DISCUSSION

BVDV was known to cause multiple problems in cattle long time ago but recently, it caused many problems to camels and should be considered as a cause of death in camels (Belknap *et al.*, 2000).

No clinical signs in the examined camels was observed except two camels were suffered from severe signs .All infected camels (except two) were persistently infected and this can explain the cause of absence of any clinical signs among infected camels. Persistently infected animal usually appear healthy (Nagai *et al.*,1998). The same result was recorded by Tessaro *et al.*, (1999) who studied the possibility of infection of elk with BVDV and reported that none of elk inoculated with BVDV developed clinical signs but all elk considered infected as demonstrated by viremia and/or seroconversion.

The two camels that suffered from clinical signs were harbor two biotypes of BVDV, cytopathic and noncytopathic while persistently infected camels were carry only noncytopathic BVDV. Therefore we can

consider these two camels acutely infected and so showed severe signs. However, one of these two camels died one week after appearance of clinical signs. Belknap *et al.*, (2000) recorded severe clinical signs and many deaths among camels infected with BVDV.

Severe bloody diarrhea was observed in the camel that died. This can be explained by the results of Walz *et al.*, (1999) which suggested that platelet function may be depressed in animals infected with type II BVDV. Although the mechanism for altered platelet function was not determined, it likely involved an increase the percentage of aged platelets in the circulation. Therefore, a direct virus platelet interaction or an indirect virus-platelet interaction, platelet dysfunction, in addition to thrombocytopenia, may contribute to the hemorrhagic syndrome associated with acute type II BVDV infection .Stoffergen *et al.*, (2000) reported bloody diarrhea in animals infected with noncytopathic BVDV from the type 2 genotype and they reported that the cause may be thrombocytopenia that cause hemorrhagic syndrome.

Great variation in clinical signs was observed in infected cattle. These signs varied from slight rise in body temperature up to biphasic fever, severe diarrhea, erosions of oral mucosa and nasal discharge. These severe signs were observed only in 5 cattle, type 2 BVDV was isolated from all these cattle while type-1 BVDV was isolated from cattle show slight or moderate signs. The same result was recorded by Flores *et al.*, (2000); Polak and Zmudzinski (2000) and Baule *et al.*, (2001) who reported that type-2 BVDV more virulent than type-1 BVDV and most severe signs accompanied to type-2 BVDV. At the same time, Hamers *et al.*, (2001) concluded that BVDV diversity is important for understanding the wide variety of signs caused by BVDV.

ELISA used in this study failed to detect any of PI camels. The same observation was reported by Grooms *et al.*, (2001) who concluded that PI animal has no or undetectable antibodies. Another explanation of failure of ELISA in detection of PI animals in our study was mentioned by Rufenacht *et al*, 2000 who reported that the prevalence of BVD viral antibodies was lowest in young animals and highest in aged animals (5 years or more), all animals used in our study were young (1-2 years old).Therefore, ELISA failed to diagnose BVDV in PI animals. In the other hand ELISA detected BVD viral antibodies in two camels and 17 cattle. These animals were acutely infected and so, ELISA could detect these animals. Beaudeau *et al.*, (2001a) and Beaudeau *et al.*, (2001b) used ELISA in detection of BVDV antibodies in acutely infected animals.

All infected cattle used in this work were negative for first ELISA but seroconverted positively within one month of contact with PI camels. The same result was reported by Grooms *et al.*, (2001) who studied the possibility of transmission of BVDV from PI cow to seronegative steer.

BVDV was isolated from 11 camels, 9 of these isolates were NCP biotype and belong BVDV type-1 while 2 isolates were CP biotype and belong BVDV type-2. BVDV was isolated from 17 cattle, all of these isolates were NCP, 5 isolates were BVDV type-2 and 12 were BVDV type-1. Two biotypes and two genotypes were recorded in camels and cattle (Belknap *et al.*, 2000; Kummerer and Meyers, 2000; Falcone *et al.*, 2001). However, BVDV type-1 was more prevalence than BVDV type-2. Tajima *et al.*, 2001 concluded that most BVDV isolates classified as type 1.

Our result included presence of 15 camels persistently infected with BVDV while BVDV isolated only from 11 of these camels. This result can be explained by the conclusion of Grooms *et al.*, (2001) who mentioned that cattle persistently infected with BVDV. That lack virus detectable in virus isolation can transmit the virus to susceptible animals.

ELISA used in the present work was as sensitive as virus isolation in diagnosis of acute BVDV infection. Saliki *et al.*, (2000) compared between sensitivity of ELISA and virus isolation in diagnosis of BVDV and recorded agreement between the two assay in diagnosis of BVDV.

Our study proved that RT-PCR more sensitive than virus isolation and ELISA in detection of both acutely and persistently infected animals. Rufenacht *et al.*, (2000) recorded that detection of persistent infection improved by RT-PCR and their results showed that the prevalence of persistent infection may be underestimated by as much as one-third when virus isolation used alone. Scheibner *et al.*, (2000) and Graham *et al.*, (2001) concluded that RT-PCR is more sensitive than any conventional test used in diagnosis of all forms of BVDV infection.

In this investigation we classified camels that gave positive result with virus isolation and/or RT-PCR two successive times one month apart as persistently infected camel. Animals were verified as persistently infected when proved to be positive with two viral examination 3 weeks later (Grooms *et al.*, 2001)

In the present work, PI camels transmitted BVDV to susceptible cattle. PI animal is the main source of infection with BVDV, animal persistently infected with BVDV may serve as virus reservoir for infecting susceptible cattle (Groomes *et al.*, 2001) and most new

infections were associated with movement of PI animal (Alban *et al.*, 2001). Nineteen herds were found infected with BVDV because of purchase of PI calves (Bitsch *et al.*, 2000) and from our study we can add that new BVDV infections appear due to purchase of PI camels.

From results of this study we can conclude that camels could be infected with BVDV and exhibit two forms of the disease, persistent infection form and acute form. Persistent infection form is more prevalence and very important and dangerous form from epidemiological point of view, as PI camel appear healthy, sheds and transmits virus to susceptible animals especially cattle along its entire life without observation. Therefore, camel should be considered as a source of infection and transmission of BVDV to cattle. Acute form may causes death of infected camel so, it could be encountered as a cause of death in camels. RT-PCR seems to be more sensitive than ELISA and virus isolation in diagnosis of different forms of BVDV infection especially dangerous persistent form. Therefore, we recommend using of this sensitive technique in diagnosis of BVDV in camels and cattle and in screening of imported camels especially those imported from neighbouring countries for freedom from BVDV before introducing of camels into Egypt. We think that this step is one of the most important steps in control of BVDV in Egypt. This is the first work concerning role played by camels in transmission of BVDV to cattle and the first study used RT-PCR in diagnosis of BVDV infection in camels, in addition to the first recognition of BVDV genotype-II in Egypt.

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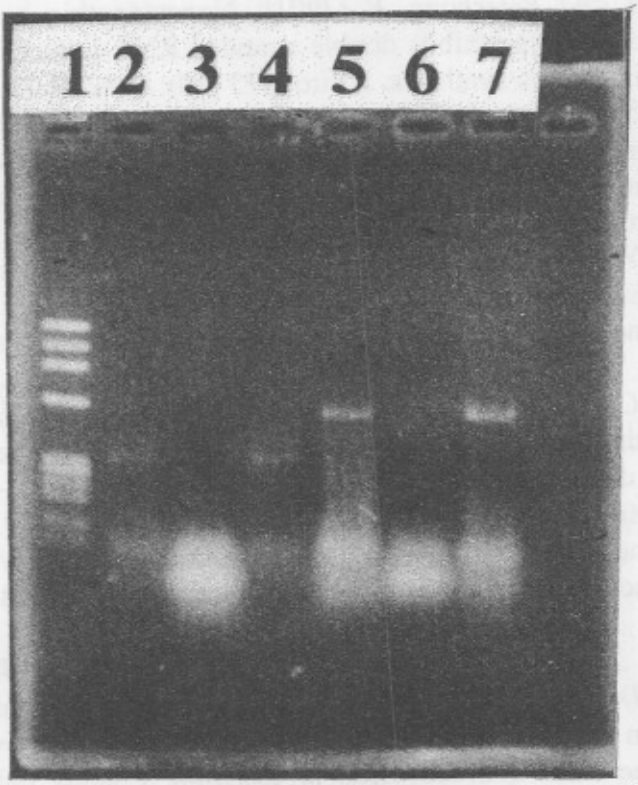

Fig. 1: Agarose gel electrophoresie of PCR products in examined camels and cattle. Lane 1 (DNA ladder), Lane 2 (BVDV type 1 in camels), Lane 3 (negative result in camels), Lane 4 (BVDV type 1 in cattle), lane 5 (BVDV type 2 in camels), lane 6 (negative result in cattle) and lane 7 (BVDV type II in cattle).