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Isolation, Antigenic and Molecular Characterization of *Bovine Viral Diarrhoea Virus* Field Strains From Apparently Health Buffaloes in Egypt

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In the present study, we report the isolation of BVDV field strains from apparently health buffaloes. One hundred and thirty two samples including buffy coat and organs were collected from apparently health buffaloes in Cairo, Mansoura and Seuz governorates in Egypt during the year 1999. Screening of samples by AGPT using standard known anti-BVDV antisera revealed three positive samples for the presence of BVDV antigen. In a trail for the isolation of the BVDV, 69 buffy coat samples were inoculated in MDBK cells. Five selected samples that induced a clear cytopatheic effect on inoculated cells were passaged for 7 times and subjected for further antigenic and molecular characterization using virus neutralization test and different formats of PCR assays. The results revealed that the isolated BVDV field strains (3 from Cairo designated as Cairo/10/99; Cairo/12/99 and Cairo/13/99 and 2 from Seuz designated as Seuz/60/99 and Seuz/68/99) were of BVDV type I.

INTRODUCTION

BVD is a common disease in cattle populations worldwide and provoking a considerable economic losses (Dufell and Harkness, 1985), with serum neutralizing antibody present in 50-90% of the cattle world population (Hous, 1999). The virus causing a variety of clinical symptoms, including pyrexia, anorexia, diarrhoea and respiratory distress (Malmquist, 1968), while MD is fatal to almost 100% of the infected animals. BVDV was first described in 1946 in new York dairy cattle (Olafson *et al.*, 1946). MD recorded in Iowa, USA as a first description in 1951 (Ramsey and Chivers, 1953).

BVDV with CSFV and BD viruses constitute the genus *Pestivirus* that belonging to the family Flaviviridae (Wengler, 1991). BVDV and BD can cross-infected cattle, sheep, goats and pigs as well as non-domestic species (Paton, 1995). BVDV strains can be differentiated into cytopathic and non-cytopathic isolates (Thiel *et al.*, 1996).

The two biotypes differed in the production of P80 (NS3) which is only generated by cytopathic BVDV (Donis and Dubovi, 1987). BVDV also classified into two genotypes BVD type I and BVDV type II based on phylogenic analysis of the 5`UTR (Pellerin *et al.*, 1994, Ridpath *et al.*, 1994). Using a panel of MAbs against E2 epitopes, BVDV-type I strains classified antigenically into 5 subgroups while type II classify into only 2 subgroups (Becher *et al.*, 1999).

The characteristic feature of *Pestiviruses* is the ability of the placental transmission of NCP-BVDV to infect the fetus at a particular stage of gestation, resulting in persistently infected state (McClurkin *et al.*, 1984).

The viral genome is a positive stranded RNA molecule of about 12.5 kb containing single large ORF encoding approximately 4000 amino acids, 5` non-coding region of almost 400 nucleotides and 3` non-coding

region of more than 200 nucleotides (Collett *et al.*, 1988). The virion of *Pestiviruses* consist, together with the RNA, of four structural proteins, the nucleocapsid C protein and the envelope glycoproteins E^{rns}, E1 and E2 (Thiel *et al.*, 1992). Currently, 11 pestiviral proteins have been identified as products of polyprotein processing, the proteins are arranged in order N^{Pro}/C/E^{rns}/E1/E2/NS2/NS3/NS4A/NS4B/NS5A/NS5B (Collett *et al.*, 1988 and Thiel *et al.*, 1996).

The first isolation of BVDV was NCP-NY-1 and its antigenic relatedness NCP-BVDV (Baker *et al.*, 1954), while the first isolation of CP-BVDV strain (Oregon C24V) was reported by Gillespie *et al.* (1959).

In Egypt, Iman 7912, CP strain of BVDV is the first isolate during 1970-1971 (Hafez, 1973b). Kenna strain is a second CP strain was isolated by Baz (1982), the specimens collected from sero-negative cattle in Upper Egypt-Kenna Province. A NCP-BVDV was isolated from abattoir specimens was also isolated (Baz *et al.*, 1990).

The neutralizing antibodies to BVD -MD detected in cattle and buffalo sera collected from different localities in lower Egypt and from Cairo slaughter House. BVD -MD recorded in 34% of cattle and 24% of buffaloes (Hafez and Frey, 1973).

Pneumoenteritis was investigated with regard to BVDV in 58.5% of bovine while in 9.3% of buffalo calves (Baz, 1975). Several outbreaks of MD was recorded in cattle and buffalo during 1982 (Baz *et al.*, 1982), while BVDV seroprevalence recorded in 56% of bovine calve and in 10% of buffalo calves collected samples (Baz *et al.*, 1986). The immunosuppressive effect of BVDV in buffaloes which represented almost half of animal population in Egypt was studied by Shalaby *et al.* (1992) and in sheep by Abd El-Ghany (1993).

PCR assay was applied as rapid and sensitive technique for detection of BVDV by using primers designed against P125 gene code (Abd El-Hafez, 1997). Hussein (2001) applied primers designed from 5'UTR and gp48 for characterization and genotyping of Iman strain of BVDV. The results revealed that Iman strain was of CP-BVDV biotype and type I genotype (Hussein, 2001).

On the other hand, several antigenic techniques were used for diagnosis of BVDV as IP, SNT, FAT, AGPT and dot ELISA (Baz *et al.*, 1982, El-Bagoury, 1990, Abd El-Rahim *et al.*, 1988).

Recently, BVDV circulating strains among cattle and in milk were isolated and characterized through the work of our research group (Hussein *et al.*, 2003; Hassanein *et al.*, 2003 and Abdel Hafeiz *et al.*, 2003).

The aims of the present study was to isolate and characterize BVDV circulating in buffalo population using different serological and genomic assays.

MATERIAL AND METHODS

Field Samples

A total of 132 field samples from buffaloes including blood with anticoagulant and different internal organs (spleen, mesenteric lymph nodes and lung) were collected from governorates Cairo, Seuz and El-Mansoura in the period between September 1998 to May 1999. Separation of buffy coat from blood samples (n=69) was carried out as described by Lucy (1974). Preparation of internal organs for processing in the laboratory assays was done according to Burleson *et al.*, (1994).

Agar gel precipitation test (AGPT) for detection of BVDV

Specific polyclonal bovine antiserum to BVDV (CVL-Weybridge, UK) was used in the AGPT to detect BVDV in the field samples. The agar (Defco) was used in a concentration of 1% in phosphate buffered saline and the reaction was incubated for 24-48 hours at 37 oC in CO2 incubator then examined for the presence of the precipitin line.

Trial for isolation, propagation and titration of BVDV from collected samples by inoculation on MDBK cell culture

MDBK cells, which were tested by PCR and found free from latent infection with NCP-BVDV, were used in the isolation and propagation of BVDV from the field samples. The cells were kindly provided from the National Veterinary Disease Laboratory, Ames Iowa, USA. Buffy coat prepared from the samples were inoculated in pre-prepared 96-wells tissue culture microtiter plate containing 50,000 MDBK cells/well. The samples were inoculated and propagated on monlayer cells as described by Marcus and Moll (1968). The BVDV local isolates were propagated in tissue culture flasks that were prepared as 80-90% confluent. All viruses were inoculated on monolayer with adsorption time of 2 hours then serum free media was added and the flasks were incubated at 37 °C with 5% CO2 for 7 days with daily observation of CPE. Virus titration assay was carried out according to Frey and Liess (1971). The virus titer of each isolate was calculated as described by Reed and Munch, (1938).

Antigenic characterization of local isolates of BVDV by microneutralization test

A polyclonal anti-BVDV antiserum, kindly provided from Prof. Dr. El-Azhary Laboratory, University of Montreal, Canada, was used in the neutralization assay to characterize the isolates buffaloes strains of BVDV. The assay was carried out as previously described (Rossitet *et al.*, 1988).

Molecular characterization of BVDV local isolates by RT-PCR assays

***RT-PCR for identification of* BVDV *genome in the inoculated MDBK cells with the local strains of* BVDV.**

The assay was carried out as previously described (Hussein, 2001). Extracted RNA from the 5 local Egyptian isolates (from harvested cell culture of the 7th passage), reference strains of BVDV, calf serum and normal cells were used in the RT-reaction. Random hexamers were used as primer sets during RT-reaction at 42°C/45 minutes incubation period, followed by three minutes heating at 94°C to inactivate RT enzyme. The PCR primer pair (UTR1 and UTR2) were designed according to BVDV -NADL published sequence of its viral RNA genome (Collett *et al.*, 1988). The primers amplify a specific stretch of 5`-UTR region of 288 bp, where the sense primer UTR1 located between 108-128 nt-sequence position and antisense primer UTR2 located in between 395-375 nt-sequence position of NADL viral genome PCR cycling profile composed of 35 cycles of 94°C for 1 minute denaturation, 56°C for 1 minute annealing, and 72°C for 1 minute polymerization Pre-PCR heating at 94°C for 1 minute, and final extension step at 72°C for 7 minutes. The products were analyzed on 2% agarose gels stained with ethiduim bromide and photographed with Polaroid camera.

Also, another RT-PCR assay was applied on the same extracted RNA using other sets of primers (P1, P2 and P3) according to Paton *et al.*, (1994).

Genotyping of the isolated strains of* BVDV *by Multiplex RT-PCR

Genotyping of local Egyptian BVDV isolates by multiplex PCR assay was carried out to genotypic these isolates to BVDV type I, BVDV type II, or BDV and to locate these viruses within the phylogenetic map of BVDVs. The genotyping PCR assay applied in the present study originally developed by Sullivan and Akkina, (1995) and used in our previous studies (Hussein, 2001, Hassanin *et al.*, 2003, Hussein *et al.*, 2003 and Abel Hafiez *et al.*, 2003). It depends on the use of pestvirus type specific primer cocktail during second round of the PCR assay. The primers designed according to published sequences of *Pestiviruses*, the first primer set depend on common sequence homology in all three ruminant pestviruses at E^{ms} coding region of the viral genomes (BVDV type I, BVDV type II and BDV). The second PCR depend on type specific primers, which anneal specifically to each of their corresponded genome (Sullivan and Akkina, 1995). The pesti-consensus fragment was 826 bp length amplified by gp 1 and gp 2 primer pair. The BVDV -type I fragment was 223 bp amplified by gp 2 and TS3 primer pair. The BVDV type II fragment was 448 bp hybridized by gp 2 and TS2 primer pair, while BDV fragment was synthesized of 566 nt nucleotide length and amplified by gp 2 and TS1 primer pair.

RESULTS

Results of AGPT of the collected samples

In the present study, 69 field samples (buffy coat), collected from apparently healthy buffaloes located at three different governorates (Cairo, El-Mansoura and Suez), as well as 63 samples (L.N., Spleen and Lung) from the same animals in Cairo after slaughter at Cairo abattoir were included. All samples were prepared and subjected to the AGPT using standard anti-BVDV antiserum. Only, three buffy coat samples revealed positive for BVDV. Table (1) summarized the location and number of the samples used in the study.

All samples were collected from apparently healthy buffaloes. The samples of the animals (buffy coat and internal organs) in Cairo governorate were collected at El-Basateen abattoir.

Table (1): The number and origin of the samples positive by AGPT

Governorates	No. of samples	Status of animals	Age	No. of +ve samples	Code No.
Cairo	97	Apparently healthy	Below 2 years	3	10 12 13
Mansoura	18	Apparently healthy	Below 2 years	-	-
Suez	17	Apparently healthy	Below 2 years	-	-

Trial for BVDV isolation from collected samples by propagation on MDBK cell line

All buffy coat samples (n: 69) collected from apparently healthy buffaloes were further inoculated in BVD -negative MDBK cell culture for virus isolation.

Three blind passages were applied using 24 well T.C. plates. At the end of the third passage 5 samples appear to have a clear CPE, three were the same buffy coat samples which gave BVD positive by AGPT (10, 12, 13), while the other 2 samples were negative by AGPT. The CPE begin in the form of cell rounding, granulation in scattered area of monolayer followed by cellular darkness and cluster formation. Table (2) summarized the code number and location of samples that revealed a clear CPE.

Results of titration of local Egyptian isolates of BVDV on MDBK-BVDV free cells

The propagated local isolates of BVDV on MDBK cells BVDV free were titrated to determine their infectivity rate. The $TCID_{50}$/ml was determined for each isolate.

Table (2): The number and origin of the samples revealed a clear CPE on MDBK cells

Governorates	No. of samples	Status of animals	Age	No. of samples revealed CPE on MDBK cells	Designated Code No.
Cairo	34	Apparently healthy	Below 2 years	3	Cairo 10/99 Cairo 12/99 Cairo 13/99
Mansoura	18	Apparently healthy	Below 2 years	-	-
Suez	17	Apparently healthy	Below 2 years	2	Suez 60/99 Suez 68/99

Table (3): Demonstrated the results of titration of the local isolates of BVDV on MDBK-BVDV free cells after propagation with NCP-BVDV

	Positive CPE / Total No. of inoculated wells				
Virus dilution	Sample No. 10	Sample No. 12	Sample No. 13	Sample No. 60	Sample No. 68
10^{-1}	8/8	[illegible]	8/8	8/8	8/8
10^{-2}	6/8	8/8	8/8	1/8	8/8
10^{-3}	2/8	8/8	8/8	1/8	2/8
10^{-4}	1/8	8/8	2/8	0/8	1/8
10^{-5}	0/8	0/8	1/8	0/8	0/8
10^{-6}	0/8	0/8	0/8	0/8	0/8
10^{-7}	0/8	0/8	0/8	0/8	0/8
10^{-8}	0/8	0/8	[illegible]/8	0/8	[illegible]/8
Titre*	$10^{2.5}$	$10^{[illegible]}$	$10^{[illegible]}$	$10^{1.5}$	$10^{2.5}$

* Titer was calculated by the method of Reed and Muench $TCID_{50}$/ml.

Results of virus neutralization test for the local isolates of BVDV

Beta procedure of the virus neutralization assay was applied on the 7th passage of the five local isolates using standard anti-BVDV (NADL) antiserum.

In Cairo, 10/99 isolate of BVDV, antiserum able to prevent CPE of the virus on MDBK cells at dilution 1/80, for Cairo 12/99 local isolate BVDV was 1/160, for Cairo 13/99 local BVDV isolate was < 1/640, for Suez 60/99 local isolate was 1/320, while for Suez 68/99 local isolate was <1/640.

Results of detection of local Egyptian BVDVs viral genomes using RT-PCR assay utilizing UTR1 and UTR2 primer pairs

The amplified target regions of the BVDV genome revealed strong visible band corresponding to the molecular weight marker specific band, which confirmed primers and fragment specificity as visualized in 2% agar gel electrophoresis stained with ethidium bromide.

Control MDBK cells (BVDV free cells) were negative when utilized by RT-PCR assay in the present study, which indicated their freedom of latent NCP-BVDV contaminants.

Results of RT-PCR assay in a separate RT-step followed by two rounds of PCR assays (Nested PCR) using two primer pairs (P1 and P3) then (P1 and P2) for detection of BVDV

RT-reaction was attempted on extracted viral RNA 5, 6, 7th passage of the five BVDV local Egyptian isolates, using random hexamers as primer sets during RT-reaction. The Nested-PCR assay utilized, composed of two rounds of PCR. The 1st round yield a specific fragment amplified by pan*Pestivirus* primer set (P1 and P3), in accordance to BVDVs conserved sequence within the 5' UTR region. In the second PCR assay (nested PCR) the primer pair (P1 and P2) amplify a specific sequences located within the amplified fragment produced from the first round.

P1 sense primer located in the region between 166-186 nt nucleotide sequence, P3 (utilized in the 1st round PCR assay) and P2 (utilized in the second round PCR assay), the antisense primers hybridized the sequences from 404-428 nt and 371-395 nt stretches respectively. The amplified PCR products of second round were analyzed in 2% agar gel electrophoresis stained with ethidium bromide.

Fragments of the five BVDVs local Egyptian isolates (Cairo 10/99, Cairo 12/99, Cairo 13/99, Suez 60/99, Suez 68/99) were visible in specific molecular weight bands parallel to an equal molecular weight marker band, which revealed primers and product specificity. Control MDBK cells (BVD free) were negative when utilized by RT-PCR assay, which indicated their freedom of latent NCP-BVDV contaminants.

Table (4): Shows the results of virus neutralization assay (Beta procedure) of the local isolates of BVDV.

Local isolates	Serum dilution
Cairo / 10	1/80
Cairo / 12	1/60
Cairo / 13	< 1/640
Suez / 60	1/320
Suez / 68	< 1/640

Results of genotyping of local Egyptian BVDVs strains using primers cocktail located at the Erns region viral genome in an multiplex RT-PCR assay:

Genotyping of local Egyptian BVDV isolates by means of multiplex PCR assays were carried out to genotypic these isolates to BVDV type I, BVDV type II, or BDV and to locate these viruses within the phylogenetic map of BVDVs. The genotyping PCR assay utilized in the present study, the amplified PCR products were analyzed in 2% agar gel electrophoresis stained with ethidium bromide, fragments were visible in specific molecular weight band parallel an equal molecular weight marker band, which revealed primers and product specificity.

The obtained results proved that the five Egyptian local BVDV isolates are of genotype I of *Pestiviruses* (BVDV-type I) (Fig. 1). Control MDBK cells were negative when utilized by RT-PCR assay in the present genotyping assay.

DISCUSSION

Different specimens were collected from buffaloes in some governorates in Egypt for isolation of field strains of BVDV. Spleen, lymph nodes, lungs and buffy coat samples were selected for collection (Bolin *et al.*, 1987, Archimbault *et al.*, 2000, Margritte *et al.*, 2000). BVDV has been recovered from blood components, especially circulating lymphocytes and l or whole blood indicating persistent viraemia due to *Pestivirus* infection.

In the present study, we used AGPT as simple and rapid method for BVDV detection in collected samples (buffy coats and internal organs) as previous published studies (Hanel, 1993, Hosny *et al.*, 1996) using standard BVDV antisera.

Only three buffy coat samples were positive. Results of AGPT were considerably low due to many factors which could play a role in such results including, the sensitivity of the test, the nature and time of sample collection with relation of viraemia or clinical disease.

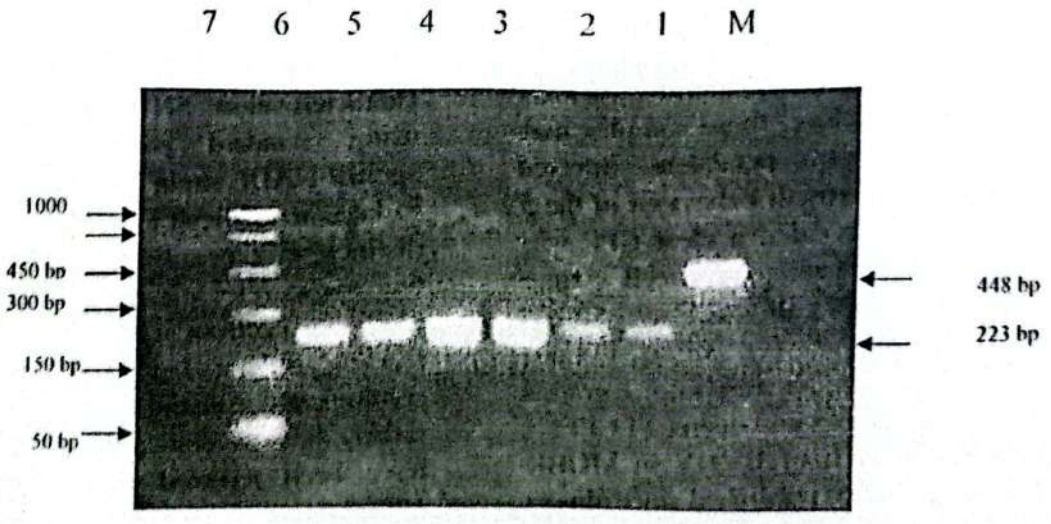

Fig. (1): E^{rns}-based genotyping of the Egyptian buffalo BVDV strains. PCR amplification was done according to Sullivan and Akkina, 1995, products were electrophoresed on 2% agarose gel and stained with ethidium bromide. M: molecular weight marker; Lanes 1 to 5, PCR amplification products of the five local buffalo BVDV strains; Lane 6, the BVDV type 1 positive control; Lane 7, the BVDV type 2 positive control; C, the negative control.

Indeed, AGPT was able to detect three samples which might be of viremic or persistent infected animals. Hanel, 1993 reported that the virus isolation was more sensitive than AGPT. Therefore, different types of cell culture were used in the current study for virus isolation. As previously reported, different cell culture system have been used for BVDV isolation. The cells were of different origin with MDBK cell line is considered the most common cell culture system used for virus isolation and propagation (Dereget *et al*., 1992, Melendez and Celedon, 1995). In the current study, MDBK cells were used for isolation and propagation of BVDV from field samples. Only the buffy coat samples were inoculated on MDBK (BVD free) cells and for three successive blind passages were applied. CPE begin to appear in 5 samples.

Out of 34 samples collected from Cairo governorate 3 sample revealed a clear CPE on the cell. Of 18 samples collected from Mansoura, no CPE was revealed from such samples on MDBk cells, whereas out of 175 samples collected from Suez governorate, 2 samples gave a clear CPE. All samples were originally from buffy coat of apparently healthy animals. Therefore, these samples may be taken from persistent infected animals. The growth behaviour of the 5 isolates that revealed a clear CPE was studied along passages in cell culture (4th-7th passage) the severity of CPE was differed between the 5 isolates classifying these viruses into 3 groups according to onset and degree of CPE.

The CPE of BVDV in MDBK cell line has been published since almost 40 years (Marcus and Moll, 1968). BVDV induced early and marked effect in cell culture characterized by severe vacuolation and granulation of the cytoplasm followed by patchy destruction and detachment of cells. MDBK always started at 24-48 hours and reached its maximum by 7 days post inoculation (Marcus and Moll, 1968). In the current study, the CPE of the 5 isolates although it differed within the isolates, the growth behaviour was typical of BVDV.

The CPE was started to some extent delayed at 3 days post isolation characterized by rounding and granulation of cells, cell clustering and the CPE progressed in behaviour similar to the BVDV adapted strains (Marcus and Moll, 1968, Dereget *et al*., 1992, Melendez and Celedon, 1995).

To characterize the 5 isolates, virus titration was applied after screening of the cells by PCR to confirm its freedom from latent infection with NCP-BVDV. The results of virus titration revealed that the virus titres were ranged from $10^{1.6}$ to $10^{4.9}$ $TCID_{50}$/ml, it was concluded that these viruses needs more passages for adaptation of cell culture to increase titres.

However, we started the characterization of these viruses after 7 passages in cell culture. The characterization studies were focused on the antigenic and genomic relatedness of such viruses to BVD . For antigenic characterization SNT was employed using standard polyclonal antisera against NADL strain of BVDV. BVDV isolates are difficult to be classified into distinct serotypes. Even though within the single serologically related group, there is a spectrum of antigenically cross reacting strains but in many cases serologically distinguishable strains rather than several distinct serotypes have been reported (Howard *et al*., 1987). Strain differences in BVDV can be demonstrated by virus neutralization test (Fernelius *et al*., 1971, Hafez and Liess, 1972a,b). Identification of BVDV strains using panels of MAbs was facilitate the classification of BVDV different strains into distinct types (Wensvoort *et al*., 1988, Cay *et al*.,

1989). BVDV type I and type II can be serologically distinct using virus strain specific antisera. Difference with at least 4 fold dilution higher against strain from the same group could be used for strain classification (Pellerin *et al.*, 1994) whereas the difference in the virus neutralization titres between the two groups of BVDV found to be ranged between 15-80 fold using non-specific antibodies against E2 protein of BVDV (Tijssen *et al.*, 1996). In the present study, VN test revealed that all isolates of BVDV reacted with the polyclonal antibodies confirming its relatedness to BVDV subgroup I. It seems that the local isolates have various degree of reactivity ranged between 1/80 to < 1/640. Antigenic variation among BVDV by VN have been reported (Hafez *et al.*, 1976, Nettleton, 1987). Moreover, the use of MAbs was useful in grouping and segregation of different strains of BVDV (Peters *et al.*, 1986, Bolin *et al.*, 1988, Magar *et al.*, 1988). Using MAb in characterization of BVDV strains represent a successful approach to segregate BVDV strains however some times, the MAb failed to differentiate between strains in the neutralization test (Peters *et al.*, 1986, Edward *et al.*, 1988). Several epitopes on the viral BVD viral proteins have been reported to be involved in virus neutralization.

In the current study, differences in neutralization titres indicate the heterogenicity of these local isolates. Such heterogenicity may be due to loss changes or even lost of some epitopes leading to lowering its reactivity with the antibodies causing differences in neutralizing activities. The result of the current study are consistent with the previous observation and works of others which indicate that neutralizing antisera was specific for certain BVDV isolates (Xue *et al.*, 1990, Bolin, 1988, Magar *et al.*, 1988). Indeed it still functionally to use the VN test to categorise BVDV strains as reported by others (Xue *et al.*, 1990, Bolin, 1988).

For molecular characterization of the local isolates we utilized different PCR assays based on the adaptation and optimization of the published work on BVDV (Sullivan and Akkina, 1995, Harpin *et al.*, 1995, Fulton *et al.*, 1999, Hussein, 2001). In the current study, RNA isolation in a single step liquid phase separation method was applied. The method has been previously described after comparison with different extract on methods and appeared to be the most reliable method.

In the field pestivirology PCR has been applied in different formate and used in the characterization of different field strains of BVDV.

Single steps nested and multiplex PCR have been widely used by several researches to detach and characterize BVDV genome (Tusboi and Imada, 1998, Radwan *et al.* 1995, Vilcek *et al.*, 1999 a and b). In the current study first step PCR was employed to detect BVDV in cell culture harvest along all passages. The results confirmed the presence of BVDV in inoculated cultures and monitor all passages in cell culture. These single step PCR was depend on the use of a pair of primers that amplify 288 bp located in the 5'UTR of BVDV genome.

Nested PCR assay based on the use of 2 round of PCR after first strand cDNA synthesis of BVDV genome using random hexamer primers was applied. The used of 2 round of PCR found to improve the sensitivity of the assay (Harpin *et al.*, 1995). Multiplex PCR was used for genotyping of the isolated local strains of BVDV. The assay has been originally developed by Sullivan and Akkama (1995) and modified in other studies which carried out in the department of virology, Faculty of Veterinary Medicine (Hussein, 2001, Hassanien *et al.*, 2003,

Hussein *et al.*, 2003, Abdel Hafiez *et al.*, 2003). Application of such PCR genotyping assay was successful in differentiating between type I and type II of BVDV and insured its specificity. The assay was used to type the 5 local isolates in the current study and revealed that these isolates are belonging to BVD type I. BVDV type-I strains have been widely reported world wide and known to be associated with most of BVDV isolated from clinical as well as persistent infected animals. In previous study by Hussein (2001), genotyping of the first report of BVDV in Egypt (Iman strain) was found to be of type I genotype. In the current study, we reported for the first time the isolation and genotyping of BVDV in buffaloes in Egypt. The isolated viruses fit in the same type of BVDV, however, the antigenic characterization study of such viruses indicates their heterogeneity. Indeed in the present study we report the isolation and characterization of these local strain of BVDV isolated from buffaloes population in Egypt.

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