BENHA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

BENHA VETERINARY MEDICAL JOURNAL

ISOLATION AND GENOTYPING OF BOVINE VIRAL DIARRHEA VIRUS: FIELD ISOLATES FROM INFECTED CATTLE IN KALUOBIA DURING 2011.

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A B S T R A C T

In the present study, we report the isolation of bovine viral diarrhea virus (BVDV) field strains from clinically infected cattle. Sixty samples including 54 buffy coat and 6 nasal swabs were collected from clinically infected cattle in Kaluobia during the year 2011. Screening of samples by Antigen capture ELISA kites revealed ten positive samples for the presence of BVDV antigen. In a trail for isolation of BVDV, 8 buffy coat and 2 nasal swabs were inoculated in MDBK cells. Eight selected samples that induced a clear cytopatheic effect on inoculated cells and 2 non-cytopathic were subjected for further identification and genotyping using nested PCR assay. The results revealed that the isolated BVDV field strains from Kaluobia 2011 were of BVDV type 1.

KEY WORDS: BVDV, ELISA, MDBK, Nested PCR Assay

(BVMJ 23(1): 6-10, 2012**)**

1. I N T R O D U C T I O N

Bovine viral diarrhea virus (BVDV) is an economically important pathogen of cattle with a worldwide distribution. The BVDV can infect cattle of all ages including fetus, and has multiple target organs [3]. Cattle infected with BVDV may display variable clinical signs from subclinical or mild to severe clinical disease [7].Two distinct genotypes were existing, BVDV-I and BVDV-II, together with border disease virus (BDV), classical swine fever virus (CSFV) and Giraffe, constitute the genus Pestivirus of the family Flaviviridae [5]. However, according to cell culture behavior, BVDV occurs in two biotypes, non-cytopathic (ncp) and cytopathic (cp) [17]. BVDVs are genetically variable, containing a single positive-stranded RNA of approximately 12.5 kilo base (kb) in length. The viral genome contains a single large open reading frame (ORF) flanked by 5' and 3' untranslated regions (UTR) [10].

In Egypt, despite control procedures such as vaccination, BVDV is still a problem in the cattle population at different governorates including, Behera [1] El-Sharquia [4], Cairo, Mansoura and Suez governorates [12]. The target of this study was to isolate a new virus from clinically diseased cattle in a private farm at kaluobia governorate during 2011 and its biological and molecular characterization.

2. MATERIAL AND METHODS

Animal:

total number of 54 cattle over 6 months of age suspected for BVDV infection showing signs of respiratory disorders, mucosal ulcers in mouth and diarrhea in a private farm at Kaluobia Governorate were investigated during November 2011.

Preparation of suspected samples: A total number of 60 representative samples (54 buffy coat and 6 Nasal swabs) were used for this work. The samples were prepared according to [6].

Reference BVDV strain:
The Egyptian cytopathic BVDV (Iman) strain was obtained from rinder pest like disease department, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt and was propagated in MDBK cell line and used as positive control in nested RT-PCR.

Antigen-detecting Enzyme Linked Immunosorbent assay (ELISA) based on monoclonal antibodies (MAbs) against the nonstructural BVD virus protein p125/p80: Fifty four buffy coat samples and 6 nasal swabs were investigated using antigen capture ELISA of commercial ELISA BVD/MD antigen mix screening kit from INSTITUTE POURQUIER, France, according to manufacturer description.

Virus Isolation: Madin-Darby bovine kidney (MDBK) cells were used for isolation of BVDV from prepared buffy coat and nasal swabs according to [11].

Primers Design: According to [16] had designated the primers P1 5'…..AAC AAA CAT GGT TGG TGC AAC TGGT….3' (1424-1449 nts) and P2 5'… CTT ACA CAG ACA TAT TTG CCT AGG TTC CA….3' (2221-2250 nts) sequences that shared maximum homology with all ruminant pestiviruses. TS1 5'….TAT ATT ATT TGG AGA CAG TGA ATG TAG TAG CT…3' (1684-1716 nts) TS2 5'…. TGG TTA GGG AAG CAA TTA GG….3' (1802-1821 nts) and TS3 5'… GGG GGT CAC TTG TCG GAGG….3' (2027-2045 nts) sequences were type specific for BDV, BVDV genotype II and I respectively with the amplified products (P1 and P2) by using nested reverse transcription-polymerase chain reaction (nRT-PCR) technique

Polymerase Chain Reaction (PCR) reagents: Supplied with the Kit by QIAamp® MinElute® Virus Spin Handbook kit For simultaneous purification of viral RNA from cell-culture supplied by QIAGEN.

Genotyping the BVDV Isolate by n RT-PCR technique: In 35 cycles, the first amplification of the reverse transcript RNA using the primers P1 and P2 was done while the second amplification using the primers TS1,TS2 TS3 and amplified products of the first round (P1 and P2) was carried out in 25 cycles as described by [16].

3. RESULTS

4.1. *Detection of BVDV in buffy coat and nasal swab Samples using antigen capture ELISA*: It was found that 10 samples (8 from buffy coat and 2 from nasal swabs) induced change in colour of substrate by ELISA as reveled in table (1).

Table 1 Number of positive BVDV antigen isolates from Buffy coat and nasal swabs of infected cattle using antigen capture ELISA.

Total No.of samples	Positive samples by antigen capture ELISA		Total positive
	Buffy coat	Nasal swabs	
60	8/54	2/6	10

4.2. *Isolation and biotyping of suspected BVDV from buffy coat and nasal swab Samples on MDBK cell*:
It was found that 8 samples induced cytopathic effect by 3 blind serial passages in MDBK cell culture while other 2 samples one from buffy coat and other from nasal swabs are non cytopathic as

shown in table (2). The CPE of suspected BVDV isolates in infected cell culture was characterized by cell rounding, cell aggregation, vaculation followed by cellular darkness and cluster formation. The CPE was clear between the 3rd and 5th day post inoculation increased gradually till 70-80 % of sheet was completely detached. These findings were demonstrated in photo (2) as compared with normal control monolayer demonstrated in photo (1).

Table 2 Biotype of BVDV isolates from cattle.

	positive samples	BVDV biotypes	
		Cp	Ncp
Buffy coat	8	7	1
Nasal swab	2	1	1
total	10	8	2

Cp. cytopathic strain. Ncp. Non cytopathic strain

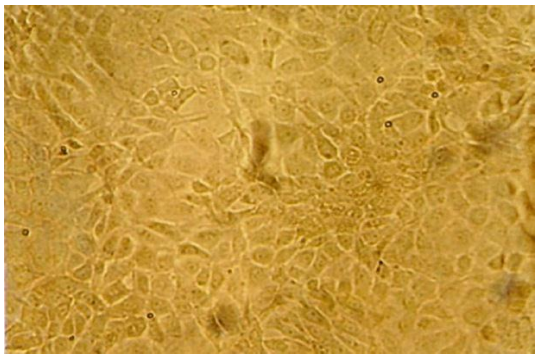

Photo (1) Control non infected complete sheet of MDBK cells

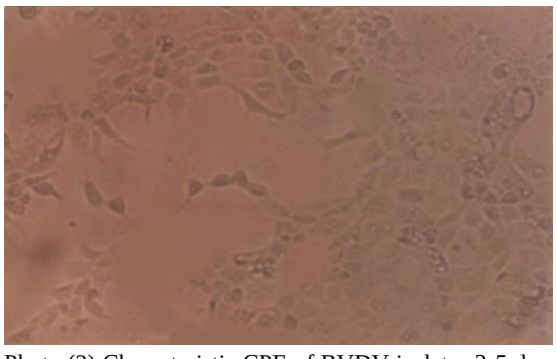

Photo (2) Characteristic CPE of BVDV isolates 3-5 days post inoculation on MDBK cells in the form of cell rounding, cell aggregation, vaculation followed by cellular darkness and cluster formation and cell detachment.

4.7. *Identification and Genotyping of BVDV local isolates from cattle using nested reverse transcriptase Polymerase Chain Reaction (RT-PCR)*:

Electrophoresis of the amplified products revealed the presence of specific PCR product at the correct expected size of the BVDV type I (223 bp). The size of the resulted fragments were analyzed by gel documentation system and showed that the BVDV reference strain and the local isolate had the same size of gene fragment 223 bp, without significant differences between the strains as represented in photo. (3**).**

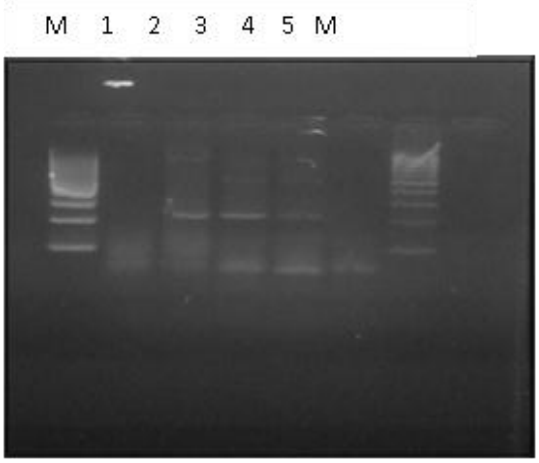

Photo 3 genotyping of BVDV local isolates. Revealed the presence of specific PCR product at the correct expected size of BVDV type I gene (223 bp), in the same pattern with no differences between reference strain and local isolates. Lane M: High molecular weight nucleic acid marker Lane 1: negative control. Lane 2: Reference BVDV strain (Iman) Lane 3, 4: BVDV local isolates either cytopathic or non-cytopathic

Table (3) biotype and genotype of BVDV isolates from cattle at different governorates

	Positive samples	BVDV biotypes		BVDV genotypes	
		Cp	Ncp	BVDV/1	BVDV/2
Buffy coat	8	7	1	8	0
Nasal swab	2	1	1	2	0
total	10	8	2	10	0

Cp. cytopathic strain. Ncp. Non cytopathic strain

4. DISCUSSION

Accurate diagnosis of BVDV infection depends upon isolating the virus from nasal swabs or blood or tissue samples

from affected animals [13] mononuclear cells obtained from whole blood are the ideal sample for BVDV isolation, because neutralizing antibodies to BVDV present in serum may interfere with virus isolation [8].

Antigen-detecting ELISA based on monoclonal antibodies (MAbs) against the nonstructural BVD virus protein p125/p80 was considered to be the test of choice for circulated infection as well as offered sensitivity equal to virus isolation if they used for calves over 6 month of age [2]. BVDV antigen was detected in buffy coat and nasal swabs by Antigen-detecting ELISA table (1) indicating circulated infection and the role of nasal discharge in establishment of infection in the herd.

A Trial for isolation of BVDV on MDBK cell line from positive ELISA samples was similar [2] and our findings in table (2) revealed that cytopathic strain BVDV is the most common biotype in nature. These findings agree with those of [4] and disagree with [9] who reported that the most common biotype in nature is noncytopathic strains

The Egyptian BVDV isolates from Kaluobia governorate are a cytopathogenic biotype of BVDV. The detection of a cp BVDV supports the hypothesis that the origin of cp BVDVs is the result of a random genetic recombination leading to expression of the NS3 protein separate from the NS2-3 expressed by the non-cytopathogenic biotype. Such genetic recombinations are likely to occur independently of the evolutionary history of the virus [13].

Previously, several PCR-based assays for typing BVDV have been reported [15, 16]. In our search to rapid detection and typing of BVDV field isolates, RNA extracted from cytopathic and noncytopathic biotypes were amplified by RT-PCR using a nested set of primers complementary to sequences in the Erns of the pestivirus genome [16]. The nested PCR product was characteristic of a bovine viral diarrhea virus (BVDV) type 1 revealed specific PCR product at the (223 bp) (photo.3).our results comes in agreement with [14] who reported that genotype 1 may exist as cytopathic or non cytopathic.

5. REFERENCES

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