Research Article

Isolation and Genetic Characterization of Hobi- Like Pestivirus Circulating in Egyptian Cattle during 2019-2020

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Abstract | HoBi-like Pestivirus is accused in similar clinical manifestations to those of bovine viral diarrhea virus infections. Although, HoBi-like Pestivirus infection has been recorded in multiple countries as in South America, Europe, and Asia, no clinical cases were recorded in Egypt even now. Here, for the first time, we reported natural infection of HoBi-like Pestivirus in Egyptian cattle. Serum and ovarian samples were collected from cattle during the year of 2019. RT-PCR, viral isolation, sequencing, and phylogeny revealed that the primary causative agent was HoBi-like Pestivirus. Our HoBi-like Pestivirus strain, Egypt-020-1ncp, BVDV-Egypt-020-3ncp and BVDV-Egypt-020-2ncp, shared 99.6% homology with BVD3- B1-AU, 99.2% with BVD3- B5-3-MX and BVD3-G2-BR, 98% with BVD3- Hobi-like virus SA-2016-04 and 95% with BVD3- Italy-68-13ncp isolated in Australia, Mexico, Brazil, South America, and Italy. Multiple area of mutations a long 5′ UTR amplicon, were observed by alignment of BVDV- Egypt-020-1ncp BVDV-Egypt-020-3ncp and BVDV-Egypt-020-2ncp.While Npro based detection was not succeeded to detect our HoBi-like Pestivirus in original samples or even in viral isolate despite their ability to amplify the BVDV RNA of reference strain (NDAL). Precisely, this study provides the first evidence of HoBi-like pestivirus infection in Egypt, raising prospective threat to Egyptian cattle industry.

Keywords | HoBi-like Pestivirus, Cattle, Phylogenetic analysis, 5`UTR

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INTRODUCTION

Bovine pestiviruses infection might result in various clinical manifestation extending from mild to severe clinical signs, as reproductive, gastroenteritis and immune disorder and respiratory disease incriminated grave economic concerns in cattle industry. Currently joined, HoBi-like Pestivirus that known as BVDV-3 or atypical bovine Pestivirus. HoBi-like Pestivirus (HoBiPeV) causes similar clinical signs to the other BVDV in cattle (Giammarioli et al., 2015).

Bovine Pestiviruses belong to family *Flaviviridae,* genus *Pestivirus*. The official classification has been comprised Pestivirus A, Pestivirus B and Pestivirus H, that include bovine viral diarrhea virus 1 (BVDV-1),bovine viral diarrhea virus 2 (BVDV-2) and HoBi-like Pestivirus (HoBiPeV) (Smith et al., 2017). Pestivirus is a small, enveloped single-stranded positive sense RNA virus. The viral genome is approximately 12.3 kb with single open reading frame (ORF) flanked by 5′UTR and 3′UTR (Simmonds et al., 2017). ORF encodes a polyprotein (NH2-Npro-C-Erns-E1-E2-p7-NS2-3-NS4A-NS4B-NS5A-NS5B-COOH)

that is cleaved by viral and cellular proteases, forming viral structural and nonstructural proteins (Lindenbach et al., 2007). The conserved region among Pestiviruses is 5′UTR, that commonly used in classification of viral strains and in phylogenetic analyses, the N-terminal protease (Npro) often used in phylogeny (Haider et al., 2014; Liu et al., 2009).

HoBi-like Pestivirus was firstly identified in Germany in fetal bovine serum (FBS) batch from Brazil (Schirrmeier et al., 2004). Subsequently, the HoBi-like Pestiviruses were excessively reported in commercial FBS batches and cell culture derived from several regions, as South America, Europe and Asia (Mao et al., 2012; Ståhl et al., 2010; Stalder et al., 2005; Xia et al., 2011). Natural infection was recorded firstly in aborted fetuses in Brazil in 2006 (Cortez et al., 2006). Subsequently, HoBi-like Pestiviruses were reported in cattle in various districts as Italy, Brazil, Thailand and Bangladesh (Decaro et al., 2016; Haider et al., 2014; Liu et al., 2009; Weber et al., 2016) with variation of clinical symptoms including respiratory diseases and reproductive diseases. However, natural infection of HoBi-like Pestivirus in cattle has not been identified in Egypt so far. So, the objective of this study was applied for determination the existence of HoBi-like Pestivirus in Egyptian cattle. Here, for the first time, we reported and identified a HoBi-like Pestivirus infection in cattle herd in Egypt.

MATERIALS AND METHODS

Samples collection

Ovaries, and serum samples (n= 10) were collected from ten freshly slaughtered heifers and cows from abattoirs in different Egyptian province in the year 2019, with pervious history of reproductive problem as poor conception rate, early embryonic deaths, and abortion. Samples were collected under complete aseptic condition and were kept at -20˚C for further RNA extraction and viral isolation.

Nucleic acid extraction and reverse transcriptase polymerase chain reaction (RT-PCR)

Viral genome was extracted from ovarian and serum samples by using QIAamp® Viral RNA mini-Kit (Qiagen, USA) (Cat. No. 52904) according to the manufacturer's instructions. The extracted RNA was stored at –80˚C for Reverse transcription-polymerase chain reaction (RT-PCR) using specific primer set for 5 'UTR gene, RT-PCR was performed using One-Step RT-PCR Kit (Qiagen) (Cat. No. 210212). The oligonucleotides primer set sequence was F 5'– ATGCCCWTAGTAG GACTAGCA - 3' (forward primer) R 5'- TCAACTCCATGT GCCATGTAC- 3' (reverse primer) targeting 288bp sequence (Vilček et al., 1994). The reaction conditions were 50˚C for 30 min followed by 94˚C for 7 min; and 35 cycles of 94˚C for 10 min 91 sec, 53˚C for 30 sec, 68 ˚C for 30 sec; and final elongation at 68˚C for 7 min. The PCR products were analyzed using 1% agarose gel. Moreover, using of N^{pro} primer set for RT-PCR application on the clinical samples and viral isolate. The used oligonucleotides Npro primer set sequence was F 5'– TGCTACTAAAAATCTCTGCTGT - 3' (forward primer) R 5'- CCATCTATRCAYACATARATGTGGT- 3' (reverse primer) targeting 441bp (Toplak et al., 2004). The RT-PCR reactions were applied under the following conditions 50˚C for 30 min followed by 94˚C for 7 min; and 35 cycles of 94˚C for 10 min, 53˚C for 30 sec, 68 ˚C for 30 sec; and final elongation at 68˚C for 7 min. then the PCR products were analyzed on 1% agarose gel.

Sequencing and phylogenetic analysis of 5 'UTR amplicon of suspected viral sample and BVD Isolate RT-PCR product

The amplified PCR products were purified using a commercial purification kit (QIAGEN (QIAGEN, Valencia, USA) (Cat. No. 28706X4) according to the manufacturer's protocol. These products were sequenced, using big dye chain terminator V3.1 cycle sequencing kit (Perkin-Elmer, Foster city, CA, USA) (Cat. No. 4337454), and analyzed, and their homology to other BVD viruses was determined based on published sequence information. This analysis was performed by using computer software as MEGA X (Kumar et al., 2018).

Trials for viral isolation

Madin-Darby bovine kidney (MDBK) cells were used for isolation of bovine pesti like virus. and reference NADL strain of BVD was used as a control positive. The RNA was extracted from HoBi-like virus third passage isolates and subjected to RT-PCR and sequencing as described above.

Identification of the BVD isolated virus using indirect IFA

The inoculated cells with ovarian homogenate, and serum from suspected viral samples were detected by IFT proved by immune fluorescence (IFX) staining using BVDV polyclonal antiserum that provided by the Department of virology, Animal Health Research Institute, Dokki, Giza, and Rabbit Antibovine-IgG conjugated with fluorescienisothiocyanate that supplied by Sigma (Cat. No. B8395) (Magar et al., 1988).

Nucleotide accession numbers

The nucleotide sequences described in this study have been submitted to GenBank and assigned the following nucleotide sequence accession numbers: MZ873104, MZ873105 and MZ873106.

RESULTS AND DISCUSSION

Detection and identification of BVDV in ovary, and serum samples in cattle

In the following study 10 suspected ovarian, and serum samples were examined for existence of BVDV by one step RT-PCR assay for BVDV 5' UTR and resulted in a positive PCR amplicon with size of 288 bp on 1% agarose gel in 7 samples out of 10 samples. While the RT-PCR assay failed to get any PCR product with clinical samples using Npro primer set in spite their efficient work with the control BVDV strain (NDAL) with product size 441bp.

Virus isolation

BVDV was isolated from RT-PCR positive clinical samples, and they were identified by immunofluorescence, RT-PCR, and sequence analysis. As the indirect immunofluorescence assay revealed presence of a non-cytopathogenic strain of BVDV isolates. The sequence of viral isolate was designated as BVDV-Egypt-020-2ncp and deposited in gene bank under the following nucleotide sequence accession number MZ873106.

Sequence analysis of BVDV 5' UTR amplicon

A blast search revealed that the sequence of the PCR amplified 5' UTR fragments were related to BVDV-3. The sequences of original sample from serum and ovary were designated as BVDV-Egypt-020-1ncp and BVDV-Egypt-020-3ncp and deposited in gene bank under the following accession numbers MZ873104, MZ873105 respectively. The nucleotide sequences showed that BVDV-Egypt-020-1ncp (Accession. no, MZ873104) and BVDV-Egypt- 020-3ncp (Accession. no, MZ873105) shared identities 96.2% with each other; BVDV-Egypt-020-1ncp shared identities 99.6% with BVD3- B1-AU (Accession. no, JN967707), 99.2% with BVD3- B5-3-MX and BVD3-G2-BR (Accession. no, JN967747, JN967729); 98% with BVD3- Hobi-like virus SA-2016-04 (Accession. no, KY091655) and 95% with BVD3-Italy-68-13ncp (Accession. no, KJ627179). Meanwhile BVDV-Egypt-020-3ncp (Accession. no, MZ873105) shared nucleotide identities 99.6% with BVD3- B1-AU, 99.2% with BVD3-B5- 3-MX and BVD3-G2-BR; 98% with BVD3- Hobi-like virus SA-2016-04 (Accession. no, KY091655) and 96.2% with BVD3- Italy-68-13ncp.

The dendrogram was generated to determine the phylogenetic position of BVDV- Egypt-020-1ncp and BVDV-Egypt-020-3ncp among BVDV-1, BVDV-2 and BVDV- 3 strains (Figure 1). BVDV-Egypt-020-1ncp and BVDV-Egypt-020-3ncp isolate was related to BVDV-3 strains of Australian, North and South America and Italian strains, respectively.

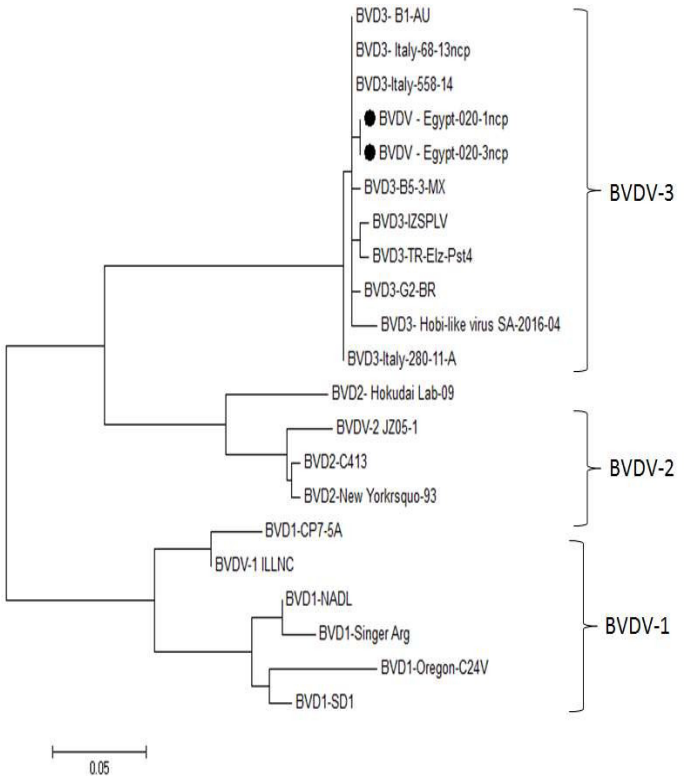

Figure 1: Neighbor-joining Phylogenetic tree for partial 5` UTR nucleotide sequence of BVDV- Egypt-020-1ncp (MZ873104) and BVDV-Egypt-020-3ncp (MZ873105). strains compared with other BVDV-1, BVDV-2 and BVDV-3 reference sequence obtained from GenBank database. The scale bar represents the number of substitutions per nucleotide. Egypt-020-1ncp and BVDV-Egypt-020-3ncp strains from the present study are indicated by black circle.

Rate of 5` UTR sequence diversion after isolation

BVDV-Egypt-020-1ncp and BVDV-Egypt-020-2ncp were 5 `UTR BVDV sequence from serum original sample and the third passage of serum on MDBK cells, respectively. The nucleotide analysis of 5' UTR products of BVDV-Egypt-020-1ncp and BVDV-Egypt-020-2ncp revealed slight nucleotide variations at three sites of 5UTR products including sites 31, 32 and 96 where G nucleotide in BVDV-Egypt- 020-1ncp replaced by C, A, A in BVDV-Egypt-020-2ncp at this site, respectively Figure 2.

Also, nucleotide variations were traced between BVDV-Egypt-020-1ncp (BVDV original serum sample) and BVDV-Egypt-020-3ncp (BVDV original ovary sample). The nucleotide analysis of 5` UTR products revealed nucleotide variations in the sites from 27 to 40 where ATCTGGGCTCGTGT in BVDV-Egypt-020-1ncp replaced by GATGTCAGCTAGCA in BVDV-Egypt-020-3ncp, also there is variation at the site 42 where A in BVDV-Egypt-020-1ncp replaced by T in BVDV-Egypt-020-3ncp Figure 3.

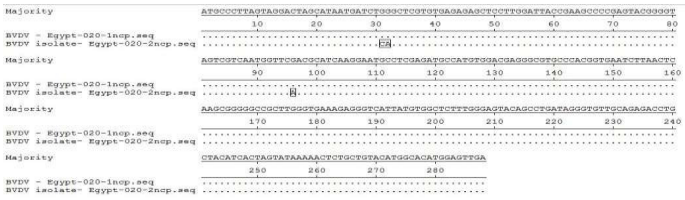

Figure 2: Nucleotide variations in 288bp 5UTR products of BVDV-Egypt-020-1ncp (original serum sample) and BVDV-Egypt-020- 2ncp (third passage on MDBK cells from serum). Dots or hide refer to nucleotide similarities while box refer to nucleotide differences.

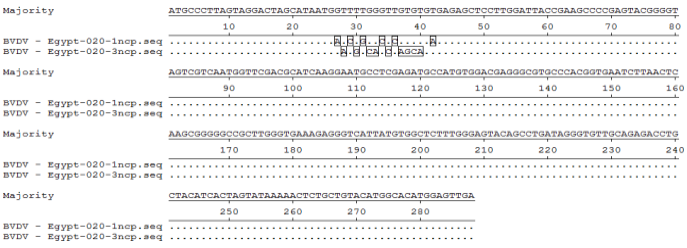

Figure 3: Nucleotide variations in 288bp 5UTR products of BVDV-Egypt-020-1ncp (original serum sample) and BVDV-Egypt-020- 3ncp (original ovary sample). Dots or hide refer to nucleotide similarities while box refer to nucleotide differences.

HoBi-like Pestivirus was detected in cell cultures, commercial FBS, small ruminants, and cattle in several countries. It was accused in several clinical manifestations smellier to BVDV in South American, Europe and Asia. Although no available data about existence of HoBi-like Pestivirus infection in Egypt so far. We firstly recorded clinical cases with HoBi-like Pestivirus infection in cattle in Egypt. The 5′ UTR is frequently used for classification of Pestivirus strains. Interestingly, the newly isolated Pestivirus was non cytopathic strain of HoBi-like Pestivirus. It is known that Cytopathic and noncytopathic are equally able to produce severe disease in cattle, moreover, only non-cytopathic stain is produced persistent infection (Ridpath et al., 2006).

This Egyptian HoBi-like pestivirus had a great homology in 5`UTR region with the HoBi-like pestivirus strains from Italy, Australia, Mexico, Brazil, and South America. It is possible that this HoBi-like pestivirus in Egypt was introduced from other regions through cattle imports and trading of contaminated biological products (Bauermann et al., 2013). Further, the 5` UTR slight nucleotide variations between BVDV-3 Egyptian isolate and original BVDV-3 virus as shown in (Figures 2 and 3). These clarify the genetic diversity by the third passage of BVDV-3 Egyptian strain on MDBK cells as a characteristic for Pestiviruses genus (Fulton et al., 2003; Bachofen et al., 2008). Our suggestion is that the nucleotide substitutions in 5` UTR may differentiate between the passaged and un passaged virus as well as the viral tropism. Similarly, like (Topliff and Kelling, 1998) who distinguishing between eight isolates of low and high virulence BVDV-2. A cytosine at position 219 and an uracil at position 278 were present in the low virulence isolates, while the opposite was observed in the high virulence isolates.

Moreover; the failure of Npro primer set to amplify target sequence with our HoBi-like Pestivirus samples and isolate in spite of their work with control NDAL BVDV. This might be attributed to genetic diversity of our HoBi-like Pestivirus that might incorporate with mismatching of Npro primer sequences and viral nucleotide as described in previous studies So, HoBi-like viruses detection and identification required using HoBi-like primers.it is prospective like other pestivirus membres genetically diverse (Decaro et al., 2012; Bauermann et al ., 2014).

In summary, our study described the first clinical cattle case of natural HoBi-like Pestivirus infection in Egypt. It is worthy to note that the epidemiological status and virus variation of the HoBi-like Pestivirus were still largely unclear in most countries (Bauermann et al., 2015). In addition, there is no vaccine available to specifically control the infection of HoBi-like Pestivirus. Thus, the emerging HoBi-like Pestivirus could be a considerable threat to the cattle industry in Egypt and worldwide.

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NOVELTY STATEMENT

The existence of HoBi-like pestivirus was not recorded absolutely as natural infection in Egypt so far; till our study identified and reported HoBi-like pestivirus as natural infection in cattle herd.

AUTHOR'S CONTRIBUTION

Conceptualization of this study were performed by E.M. El-Nahas, A.S. El-Habbaa, and Z.R. Aboezz. Investigation and methodology were performed by S.A. Elnagar, E.M. El-Nahas, and R.S El-Mohamady. Data curation and phylogenetic analysis were performed by A.S. El-Habbaa and Z.R. Aboezz. The first draft of manuscript was written by Z.R. Aboezz and S.A. Elnagar. Reviewed and editing of the manuscript were performed by E.M. El-Nahas, A.S. El-Habbaa and R.S El-Mohamady. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors have declared no conflict of interest.

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