Isolation and identification of non-cytopathic strain of bovine viral diarrhoea virus from ovaries of Egyptian heifer and cow in 2019

Zeinab R.A. Aboezz 1, Samia A. Elnagar 2*; Rania S.A. El-Mohamady 2, Ayman S. El-Habbaa 1, Ehab M. El-Nahas 1

1. Department of Virology, Faculty of Veterinary Medicine, Benha University, Egypt
2. Animal Reproduction Research Institute (ARRI), Agricultural Research Center (ARC), Giza, Egypt

ARTICLE INFO

Keywords
- non cytopathic BVDV
- Ovaries
- Genotype 1
- Isolation
- RT-PCR

ABSTRACT

Bovine viral diarrhoea virus (BVDV) infection causes severe losses in the cattle population. Viral isolation may have a substantial impact on BVDV prevention and control. This study aimed to isolate and identify the circulating BVDV in Egyptian cows and heifers during 2019. The isolated virus was identified and detected by immune fluorescent assay and immune peroxidase technique followed by RT-PCR that confirmed circulation of non-cytopathic BVDV-1 in Egyptian cows and heifers. Further molecular analysis is recommended to determine the molecular epidemiology of BVD isolate for updating vaccine strain as a successful preventive strategy.

1. INTRODUCTION

Bovine viral diarrhoea virus (BVD) is a major pathogen that affects the entire world’s ruminant population. It is a major source of economic concern (Krametter-Froetscher et al., 2010 and Chang et al., 2021). It is the major cattle pathogen that involved in reproductive concern (Fray et al., 2000). It is an enveloped virus belongs to family Flaviviridae, genus Pestivirus. It has positive-sense, single-stranded RNA and 12.3 kb long. BVDV is divided into two genotypes: BVDV-1 and BVDV-2, while another pestivirus species “HoBi-like” could be considered as BVDV-3 genotype (Gomez-Romero et al., 2017, Bauermann and Ridpath, 2015). The 5’ and 3’ untranslated regions (5’UTR, 3’UTR) flank the BVD genome. It is translated into a single polyprotein which is then cleaved into 11 structural and non-structural proteins (Npro, C, Erns, E1, E2, P7, NS2/3, NS4A, NS4B, NS5A, and NS4B). Based on its potential to produce noticeable effects on cell culture, BVDV can be categorised into two biotypes; cytopathic (CP) and non-cytopathic (NCP). Virus isolation and detection by immune peroxidase technique and immune fluorescent assay is a golden method for BVDV in diagnostic procedures (Gao, et al., 2011, Bedeković et al., 2011). Moreover, RT-PCR is a valuable diagnostic tool that could determine the BVDV subtypes (Weinstock et al., 2001). In Egypt, BVDV infection is still causing great economic losses in cattle and buffaloes population in spite of vaccination programs (El-Bagoury et al., 2012). The continuous circulation of virus in the field and quasi species nature of RNA genome raises new variants of virus (Domingo et al., 2003).

So, our study directed to word determination the circulating BVDV in Egypt in 2019 by viral isolation followed detection and identification that might help for design appropriate preventive measures including effective vaccination program.

2. MATERIAL AND METHODS

2.1. Specimen collection

Five ovaries were collected from freshly slaughtered heifers and cows from abattoirs in Beheira Governorate in the year 2019, with piversary history of reproductive problem as poor conception rate, early embryonic deaths and abortion. Ovarian sample were collected under complete aseptic condition. In addition; five serum samples were collected from the same animals. Serum samples were kept at -20 °C for further isolation and identification.

2.2. Processing of collected samples

Each ovary was homogenized as one gram vortexed in 4 ml of DMEM with penicillin (100 IU/ml), streptomycin (100 mg/ml), and amphotericin-B (2.5 mg/ml). After centrifugation, the homogenate was clarified, and the supernatant was collected and preserved at -70 °C for further isolation and identification.

2.3. Reference BVDV strain and antisera

NADL strain is a cytopathic BVDV strain. It was propagated in MDBK cell line with a titre 10^6.5 TCID50/ml and used as positive control in vial isolation, detection and identification by RT-PCR. BVDV polyclonal antisera was used in viral detection by immune peroxidase technique (IPT) and immune fluorescent test (IFT). Rabbit Antibovine-IgG conjugated with fluorescienisothiocyanate were used in viral detection by (IFT) and goat anti bovine
2.5. BVDV isolation on MDBK cells
Prepared ovarian homogenate and serum were inoculated onto MDBK monolayer cultures. The MDBK cultures were examined for CPE for 6 days. The cultures were frozen at -70°C, then thawed and the supernatants were collected after centrifugation and stored at -70°C. Inoculated cultures were included as negative controls and inoculated NADL as positive control.

2.6. Identification of BVDV by IFT
The inoculated cells with ovarian homogenate were detected by IFT proved by immune fluorescence (IFX) staining (Roberts et al., 1991)

2.7. Extraction of BVDV RNA
QIAamp® Viral RNA mini Kit (Qiagen, USA) was used to extract total RNA from the collected samples, according to the manufacturer’s instructions. The extracted RNA was stored at -80°C for further use.

2.8. Molecular identification of BVDV by RT-PCR
One-Step RT-PCR Kit (Qiagen), was used to perform reverse transcription-polymerase chain reaction (RT-PCR) using specific primer set for 5 UTR gene. The sequence of oligonucleotides is: F 5'- ATGCCWTAGTAG GACTAGCA - 3' (forward primer) R 5'- TCAACTCCATGT GCCATGTAC - 3' (reverse primer) targeting 288bp sequence (Vilcek 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 analysis were performed on 1% agarose gel.

3. RESULTS
There was no cytopathic effect observed in the inoculated MDBK cells for 7 days in comparing with control positive as in fig (1). but, There were 2 of 5 ovarian homogenate are positive by IFT fig (2). This result was parallel to the result of serum samples as two of them were positive by IPT as positive serum showed red-brown cytoplasmic staining, as a positive result suggesting the presence of a non-cytopathic BVD biotype as in fig (3). The positively infected MDBK cell by IPT and IFT showed positive RT-PCR results, and the PCR products were at the predicted size, 288bp for 5 UTR of BVD-1, were observed as clear electrophoretic band. It was similar to 1 was similar to reference NADL strain as fig (4).

4. DISCUSSION
Bovine viral diarrhoea virus is responsible for several problems in cattle population as many reproductive concerns in cattle. The virus could be transmitted from cow to calf, resulting in abortion and the birth of deformed calves and/or persistently infected (PI) calf (Hause et al., 2021). Being an RNA virus, BVDV has a high mutation rate with unique pathogenesis and variability which make BVDV control is challenging (Chang et al., 2021). The standard method for detecting BVDV-infected cattle is virus isolation. BVDV-infected animals, particularly persistent infection (PI) animals, can release high levels of BVDV in their serum (Chang et al., 2021). So in this study serum samples and ovarian homogenate were used for viral isolation. MDBK cell line was suitable for BVDV isolation (Fulton et al., 2006). The presence or absence of observable CPE in infected cells divides BVDV into two biotypes (Ammari et al., 2010). Our BVDV isolate was non-cytopathic biotype as it was not able to produce any cytopathogenic effect on MDBK cell in comparing with NADL strain that produce cell rounding, aggregation,
5. CONCLUSION

It is obvious from the obtained result that immune peroxidase technique and immune fluorocent assay is highly sensitive and specific tool for detection of cytopathic and non-cytopathic BVDV. More over RT-PCR is a highly sensitive tool for genotyping of BVDV. Further molecular analysis is recommended to confirm these finding and determine the molecular epidemiology of BVD-1 isolate and update the nature of the future vaccine for successful preventive strategies.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

6. REFERENCES


