

# Molecular characterization of the Egyptian strain (Iman) of BVDV and its RNA synthesis in cell culture

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## ABSTRACT

*In this study, a nested polymerase chain reaction (PCR) that distinguishes between three pestiviruses, bovine viral diarrhea virus (BVDV) type I, BVDV type II as well as border disease virus (BDV), was applied to identify and characterize the Egyptian Iman strain of BVDV. A 826 bp fragment of the genome located in the gp 48 region of BVD viral genome was reverse transcribed and amplified using consensus oligonucleotide primers in a RT-PCR assay. The amplified product was then subjected to second amplification cycles in the presence of type-specific primers (nested PCR) which revealed products with characteristic sizes for each of three viruses (BVDV type I, BVDV type II and BDV). Utilizing this assay, the Egyptian strain of BVDV (Iman) was identified as BVDV type I. The virus (Iman strain) was propagated for 16 successive passages on MDBK cell line to study its cytopathic behavior as well as its RNA synthesis. RT-PCR assay utilizing other primers that are located in the 5' untranslated (UTR) region of the BVDV genome was applied to follow up the BVD viral RNA synthesis and release in cell free and cell associated harvests. Viral RNA was detected in cell associated harvests starting 4 hours post inoculation of BVDV in MDBK cells and at 8 hour in case of cell free harvests.*

**Keywords:** Pestivirus, Bovine viral diarrhea virus, polymerase chain reaction, genotyping, diagnosis.

## INTRODUCTION

The family Flaviviridae consists of three genera: Pestivirus, Flavivirus and Hepacivirus (Pringle, 1999). The Pestivirus genus is composed of three economically important viruses that infect ruminants, namely bovine viral diarrhea virus (BVDV), border disease virus (BDV) and the classical swine fever virus (CSFV) (Collett, 1992; Moening and Plagemann, 1992, Wengler. *et al.*, 1995). Recently, an additional fourth pestivirus species isolated from cattle and sheep has been reported (Corapi *et al.*,

1989; Bolin and Ridpath, 1992 and 1995; Ridpath *et al.*, 1994; Pellerin *et al.*, 1994; Becher *et al.*, 1995; Tijssen *et al.*, 1996). Pestiviruses have single stranded, positive sense RNA genome that are approximately 12.5 kb in length and contain one large open reading frame (ORF) flanked between 5' and 3' highly conserved untranslated regions (UTRs) (Collett *et al.*, 1988). On the basis of sequence and phylogenetic analysis of common regions of different pestiviruses, at least 4 genotypes have been reported including classical BVDV strains: NADL, Osloss, SD1 and R27/27 (pestivirus type 1); CSFV strains