DETECTION OF BOVINE VIRAL DIARRHOEA VIRUS IN SWINE FROM MALAYSIA

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ABSTRACT. Bovine viral diarrhoea virus (BVDV) is an economically important disease of cattle. Infection of cattle results in mucosal disease, acute infections of respiratory systems and gastrointestinal tracts and reproductive disorders. Besides cattle as the virus natural host, BVDV infection has been found to extend to swine and other ungulates with the first case observed in swine recorded in 1973. Although BVDV infection seldom causes severe disease in swine, symptoms mimicking those of classical swine fever virus (CSFV) are sometimes observed, thus requiring extra caution during disease investigation and management. Here, we report the detection of BVDV in swine from Sarawak, Malaysia from a pooled swine tonsils received for CSFV surveillance. Detection was carried out using reverse transcriptase polymerase chain reaction (RT-PCR). In the initial screening, a 288 bp amplicon from the 5′ untranslated region (5′ UTR) of the viral genome was successfully amplified using universal pestivirus primers. However, the samples were tested negative for CSFV-specific PCR. Purified amplicon is further sent for sequencing for virus confirmation. Phylogenetic analysis of the sequencing result was done by Neighbor-Joining method and indicated that this virus clustered with BVDV-2. This finding may have important implications for the epidemiology, diagnosis and control of BVDV and CSFV infection in the country.

Keywords: Bovine viral diarrhoea virus (BVDV), swine, Sarawak, reverse transcriptase polymerase chain reaction (RT-PCR), phylogenetic analysis

INTRODUCTION

Bovine viral diarrhoea virus is a positive single-stranded RNA virus of 12.3 kb encoding a single polyprotein. It is of the *Pestivirus* genus in the Flaviviridae family. The genus also includes classical swine fever virus (CSFV) and border disease virus (BDV) of sheep with similar genome organisation and replication strategy to each other but different transmission mode. Previous studies on BVDV RNA sequence suggested the existence of 2 viral genotypes, BVDV type 1 (BVDV-1) and BVDV type 2 (BVDV-2) (Ridpath, 2003). Phylogenetic studies to differentiate and classify BVDV isolates have made use of multiple regions of the virus genomic DNA such as the Npro, E2 and NS3 and 5′UTR which is more widely utilised due to its sequence conservation (Vilcek *et al*., 1994; Harasawa, 1996; Becher *et al*., 1999; Sakoda *et al.,* 1999; Neill *et al*., 2019;). Both genotypes are able to cause acute and persistent infection but BVDV-2 infection can trigger more severe symptoms as compared to type-1 (Houe, 2003). The genotypic difference of these viruses also contributed to their antigenic differences, consequently affecting accurate diagnostics of BVDV genotypes which is crucial for infection control via vaccination. Diagnostic tests and vaccines targeting BVDV-1 strains have been observed to fail in detecting and controlling BVDV-2 (Ridpath *et al*., 2010). BVDV-1 and

BVDV-2 can be found globally, although their prevalence varies even in different areas in the same country. In Australia, type 1 is the only genotype found, while in India, type-1 is more predominant, while type-2 occurrence is sporadical (Mishra *et al.*, 2004; Ridpath *et al.*, 2010).

Classically, BVDV was thought to mainly infect cattle. However, it is currently known to also infect ruminants such as goats, sheep, deer, pig and also wild animals (Ridpath, 2010). Varying disease severity have been observed from subclinical to acute. BVDV can cause mucosal disease, acute infections of respiratory system, gastrointestinal tracts infection and reproductive disorders such as congenital malformations and abortion, consequently being regarded as an economically important disease in livestock (Lanyon *et al.*, 2014). Due to this, BVDV infection in cattle is listed as a notifiable disease by the World Organisation for Animal Health or Office International des Epizooitic (OIE) (Sayers *et al.*, 2015). In pigs however, the course of infection is based on the strain virulence and the infected pig immune response. BVDV in pigs sometimes presented clinical signs closely similar to CSFV and also generate cross reactivity in serological tests that diagnosis becomes a challenge, thus interrupting CSFV surveillance programmes (Terpstra & Wensvoort 1988; de Oliviera *et al.*, 2020). On the other hand, the presence of anti-BVDV antibodies were observed to give pigs protection against CSFV infection due to cross-reactions, consequently preventing CSFV outbreaks in herds with high prevalence anti-BVDV antibodies (de Oliviera *et al.*, 2020).

In 1973, the first case of BVDV isolation from naturally infected swine was reported in Canada (Fernelius *et al.*, 1973). In England, an outbreak of BVDV in pigs had been reported by Paton *et al.* (1992) while the presence of BVDV in pigs had also been recorded in the Netherlands (Loeffen *et al.*, 2009), China (Deng *et al.*, 2012), Poland (Lipowski, 2014) and Brazil (Gatto, 2015 and Almeida, 2015; Almeida *et al.*, 2017). Here, we report the first detection of BVDV in swine in Malaysia.

MATERIALS AND METHOD

Sample processing

A pooled of swine tonsil (VRI 7505) from a pig abattoir in Sarikei, Sarawak was received by Veterinary Research Institute (VRI) in June 2017 for CSFV surveillance in 2017. The samples were first ground using sterile mortar and pestle with a small amount of sand. The grounded samples were then centrifuged at 3000 rpm for 10 minutes. The supernatant was collected for RNA extraction using QIAamp Cador Pathogen Kit (QIAGEN) as per manufacturer's instruction.

Reverse transcriptase polymerase chain reaction (RT-PCR)

RT-PCR was carried out using Superscript™ III One-step RT-PCR System with Platinum™ Taq DNA Polymerase (Invitrogen, Thermo Scientific) following manufacturer's instruction with primers 324 (5'ATGCCCTTAGTAGGACTAGCA-3') and 326 (5'-TCAACTCCATGTGCCATGTAC-3') from Vilcek *et al.* (1994). Thermocycling conditions

were as follows: Reverse transcription at 55 °C for 30 minutes, 94 °C for 2 minutes, followed by 40 cycles of denaturation at 94 °C for 15 sec, primer annealing at 55 °C for 30 sec and primer extension at 68 °C for 1 min. This is followed by an extension step at 68 °C for 5 min. The amplicon was consequently sent for sequencing and results were compared with sequences in the GenBank® database using Basic Local Alignment Search Tool (BLAST) algorithm.

Phylogenetic analysis

Sixteen sequences of BVDV-1, 29 of BVDV-2 and 7 of CSFV which contained a complete sequence of 5'UTR were obtained from the GenBank database, representing isolates from different regions of the world. Using MEGA 7 (Kumar, Stecher and Tamura, 2016), these sequences and the sequence from VRI 7505 were subjected to phylogenetic analysis and phylogenetic tree was constructed using Neighbor-Joining method with the Kimura-2 -parameter distance model. 1,000 bootstrap replications were used to estimate the probabilities of internal branches and evaluate the robustness of nodes. CSFV sequences were included as an outgroup in this analysis.

RESULTS AND DISCUSSION

The RT-PCR was carried out successfully using a Pestivirus universal primer, amplifying 288bp amplicons of the 5' untranslated region (5'UTR) of the viral genome (Figure 1). However, RT-PCR using a CSFV specific primer results in no amplification. Using BLAST, sequencing results of these amplicons were found to have at most 98% similarities with many isolates of BVDV-1 and BVDV-2. A further analysis was subsequently performed on a 261 bp partial sequence of the 5'UTR to generate a phylogenetic tree and grouped our samples in the BVDV-2 cluster (Figure 2). The sequence of VRI 7505 was deposited in the GenBank with accession number MH814636.

This is the first BVDV-2 case recorded in pig in Malaysia. As the sample was obtained from an abattoir in a pool of tonsils mixed from different pigs, it is not possible to trace the origin of the sample and investigate the likely source of the virus. However, a plausible cause may include direct contact with cattle infected by BVDV, as some local farmers, especially in small or backyard farms, usually kept a mix of livestock in the same premises. According to de Oliviera *et al.* (2020), direct contact between cattle and pigs is the main source of BVDV transmissions to positive pig herds. An earlier study by Terpstra and Wensvoort (1991) found that in farms with cattle, the percentage of pigs with BVDV seropositive results was significantly higher. This was also observed by Fernelius *et al.* (1973) as the first BVDV isolate in Canada was found in a farm where swine were kept in close proximity to cattle while isolates from BVDV outbreak in England was also found in cattle reared on the same farm (Paton *et al.* 1992). As such, farmers are encouraged to continue developing specialised animal farms and segregate different animal species as a strategy to halt further possible interspecies transmission of BVDV and also other viruses.

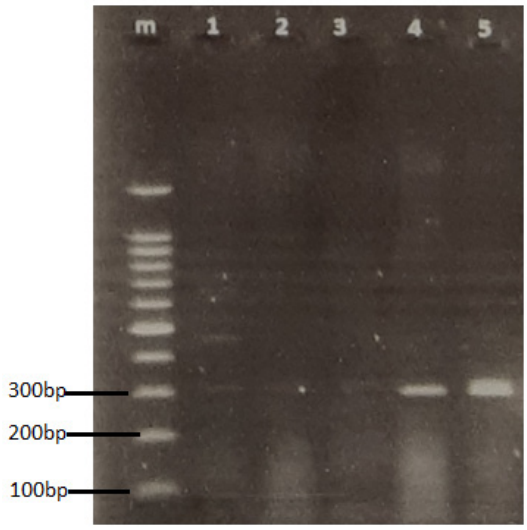

Figure 1. Agarose gel electrophoresis of BVDV-2 amplified with Pestivirus universal primers by RT-PCR. Lane M: 100bp DNA ladder (Promega, USA); Lane 1, 2 & 3 are samples with negative results (Negative control); Lane 4: VRI 7505; Lane 5: Positive control CSFV.

BVDV cases in pigs have also been associated with vaccination against CSFV. Fetal bovine serum used in the production of CSFV life vaccine can be contaminated by BVDV if sourced from infected bovine herds (de Oliviera *et al.* 2020). In contrast to the generally low prevalence of BVDV in pig herds in the previous years, high prevalence of BVDV in pigs (23.1% to 33.6%) were recorded in China from 2007 to 2010 (Deng *et al.* , 2012). These were later related to the widespread use of live vaccines against CSFV which were confirmed to be contaminated with BVDV (Deng *et al.* , 2012). In Malaysia, CSFV is managed through regular vaccination using live attenuated vaccines from Chinese GPE strain, Lapinized Chinese strain Thiveral strain and LPC-PRK strain (DVS: List of Approved Veterinary Vaccines, 2018). Vaccines of both strains are currently not subjected to any compulsory screening test to certify the absence of other non-seed virus thus poses risks of contamination.

In addition, pig-to-pig transmission has also been recorded (Wieringa-Jelsma et.al., 2006). Terpstra & Wenssvoort (1997) have detected BVDV from oropharyngeal fluid, urine, and semen of persistently infected boar while intermittent pattern of nasal shedding was observed on challenged piglets 5-24 days post-inoculations with BVDV (Santos *et al.* 2017). The presence of BVDV in these bodily fluids gives indication that infected pigs too can be a source of infection to other animals and create its own loop of infection if occurs on a mixed farms of susceptible animal species.

Another possibility for virus transmission is through the use of milk or its derivatives from infected cattle to feed pigs and fomites (Carbrey et al., 1976; Terpstra and Wensvoort, 1988). Cattle infected with

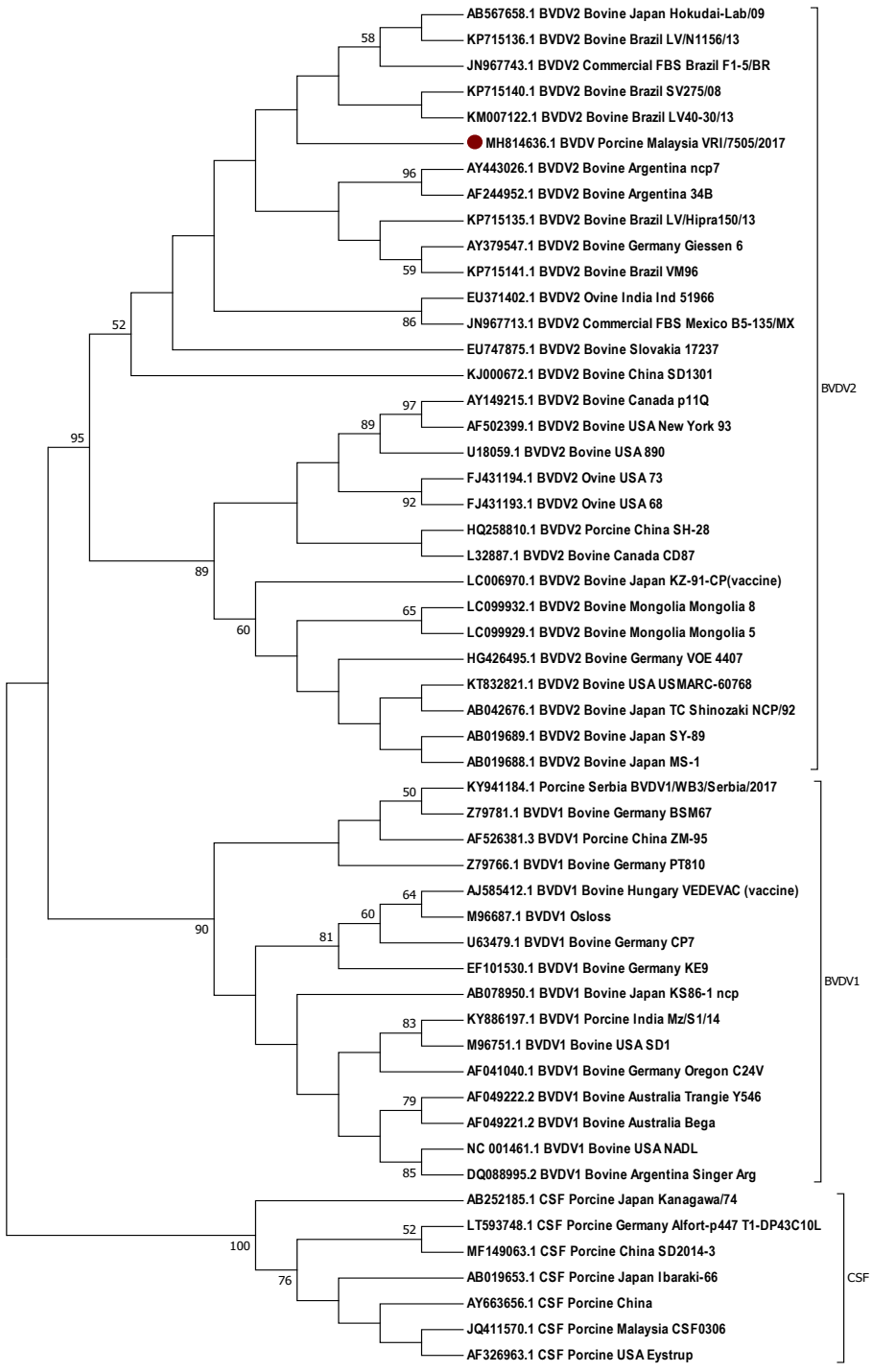

Figure 2. Phylogenetic tree constructed based on 261 bp 5'UTR of Malaysia BVDV isolate and other BVDV isolates of several countries.

the virus are able to shed BVDV at least intermittently at low titer through bodily fluids secretion, sufficient to contaminate feed, milk and fomites and result in vertical transmission to other animals (Lanyon *et al.*, 2014).

As limited study has been carried out on BVDV, not much is known of the epidemiology, occurrence and prevalence of this virus infection in cattle and pigs in Malaysia. The only report available on BVDV status was by Daves *et al.* (2016) on 5 cattle farms in Selangor involving 407 animals, revealing 33.2% seroprevalence of BVDV antibodies. The authors reported that the seroprevalence was contributed mostly from imported dairy breed from endemic Thailand and Australia while farms with mainly local Kedah-Kelantan breed showed only 3% prevalence, suggesting the local breed could have been free of BVDV, if not for the lack of BVDV importation restriction of cattle into the country. Cattles are the main infection source of BVDV in pigs and the prevalence rate of BVDV in cattle, such as reported in Daves *et al.* (2016) are sometimes used to infer the presence and prevalence of the disease in pigs in the same area (de Oliviera *et al.*, 2020). Similar inference should also be applied for the findings of this study, whereby the detection of this virus in swine might be indicative of the presence of BVDV in cattle of the same area. Currently, the National Surveillance Programme is conducted only involving dairy cattle. However, it is suggested that beef cattle and swine are also included in the programme for a more thorough disease monitoring.

CONCLUSION

This paper reports the chance encounter with BVDV as we tested swine samples for CSFV. Although the presence of BVDV itself might not cause a significant economic impact to pig farming in general, it can still interfere with CSFV diagnosis and control in swine herds, hence its epizootiological importance.

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