Bubaline Herpesvirus 1 Associated with Abortion in a Mediterranean Water Buffalo

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ABSTRACT

The current study describes the first detection of a field strain of BuHV-1 in a water buffalo foetus in Europe. The study was carried out by analysing samples of aborted buffalo foetus collected from water buffalo farms in southern Italy during a one year period of routine state surveillance. Foetus tissues were investigated for the presence of pathogens typically involved in abortion. In all samples analysed, the presence of a single pathogen was observed with no cases of co-infection. One foetus, positive for herpesvirus, was further investigated to typify the virus identified. In particular, we characterised the herpesvirus by sequencing the gE gene (US8) and we carried out phylogenetic analysis to assess the relationship between our isolate and other ruminant Alphaherpesviruses. The herpesvirus discovered in this research, that we called BuHV-1 Mediterranean isolate, showed the highest degree of homology with BuHV-1 strain b6. To our knowledge, the current study represents the first survey of BuHV-1 in buffalo abortive tissues suggesting its involvement in an abortion episode. Genetic analysis demonstrated that the virus isolated is closely related to, but genetically distinct from the BuHV-1 b6. These data suggest the presence of a BuHV-1 subtype typically found in Mediterranean water buffalo and/or the existence of a more virulent strain, possibly associated with abortion.

Keywords: Foetus, Bubaline herpesvirus 1, Glycoprotein E, Phylogenetic analysis.

INTRODUCTION

Bubaline herpesvirus 1 (BuHV-1) is a virus antigenically and genetically related to bovine herpesvirus 1 (BoHV-1) (Thiry et al., 2007). BoHV-1 is considered responsible of a wide range of clinical syndromes in cattle (rhinotracheitis, pustular vulvovaginitis, encephalitis and abortion) (Nandi et al., 2009) and BuHV1 has been related only to subclinical disease in water buffalo (St. George and Philpott, 1972; Thiry et al., 2007; Scicluna et al., 2010). Water buffalo serum positive to herpesvirus is widely documented by serological surveys (Peshev and Christova, 2000, De Carlo et al., 2004; Scicluna et al., 2007; Scicluna et al., 2010), however, viral isolation has only been described in Australia (St. George and Philpott, 1972) from the prepuce of buffalo, and many years later from water buffalo in southern Italy after pharmacological reactivation (De Carlo et al., 2004).

MATERIALS AND METHODS

The study was carried out by analysing 22 samples of buffalo foetus collected in four water buffalo farms in southern Italy during one year routine analysis. Samples were investigated for the presence of pathogens usually involved in abortion. Analysis were carried out on liver, brain, fourth stomach, kidney, lung and placenta. Bacterial abortive agents were investigated by microbiological methods (Quinn et al., 2011). Chlamydophila spp., Coxiella burnetii, Leptospira spp., bovine viral diarrhoea virus (BVDV), Neospora caninum, Toxoplasma gondii was investigated by polymerase chain reaction (PCR) using previously described protocols (Ossewaarde and Meijer, 1999, Perugini et al., 2009, Marianelli et al., 2007, Martucciello et al., 2009, Magnino et al., 2000).

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Occurrence of herpesvirus was investigated by a PCR protocol able to detect a broad range of herpesvirus species (van Devanter et al., 1996). In all samples analysed, the presence of a single pathogen was observed with no cases of co-infection. In particular, six foetuses were positive for C. burnetii, six for N. caninum, five for Chlamydophila spp., one for BVDV, one for B. subtilis, one for Salmonella spp., one for Proteus spp., and one for herpesvirus. Herpesvirus DNA was recovered from the placenta and lung from the foetus, while other tissues were negative.

The mother of the foetus carrying BuHV-1 was a pluriparous, lactating and in the fourth month of pregnancy. As indicated by the attending veterinarian, the animal was clinically normal before the abortion with only a slightly elevated body temperature for 48 hours after the abortion. During abortion there were no evident signs of gross pathological lesions, only exudate in the pelvic cavity. The animal belonged to a farm of 460 animals (350 adults) with a yearly percentage of abortion of around 2%. All foetuses recovered from this herd in the last five years had been analysed (for all the possible causes of abortion) by our laboratories and this case was the first case of herpesvirus identification. The animals on the farm had been vaccinated only once (seven years before the episode of abortion here described), against infectious bovine rhinotracheitis (IBR) and with a BoHV-1 gE deleted vaccine.

The herpesvirus identified in the foetus was further characterised by sequencing the gE gene (US8). This gene was chosen since its presence can differentiate wild-type herpesviruses from the vaccination strain, which lacks gE (Thiry et al., 2007). Viral DNA was first amplified as indicated by Ros and Belak (1999) with some modifications. In particular, DNA underwent two successive rounds of PCR with the following primers: buHV-gEF1: (5’-CGAGACGTGCATCTTCCACC-3’) and buHV-gER1: (5’-GGCTCGTTGGTCGGC-3’). The reaction mix consisted of: 100 ng DNA, 15 pmol each primer, 2 mM MgCl2, 10% DMSO, 0.2 mM each dNTP, 1 U Taq gold (Roche), 1× buffer (Roche). The thermal profile consisted of 95 °C for 10 min, 35 cycles of: 95 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min and a final elongation step of 72 °C for 7 min. PCR product (1 µl) was re-amplified using the protocol described above only varying annealing temperature (62 °C instead of 60 °C). Amplicons were purified using a Qiaquick purification kit (Qiagen) and bi-directionally sequenced using Big Dye Terminator cycle sequencing kit (ver. 3.1; Applied Biosystems) following the manufacturer’s instructions. After dye purification and formamide denaturation, sequenced samples were analysed by capillary electrophoresis (3130 Genetic Analyzer; Applied Biosystems). Sequences were analysed using the BioEdit software (BioEdit Sequence Alignment Editor version 7.0.5.2, Ibis Therapeutics; Carlsbad, California, USA) and the CLUSTALW alignment method. BuHV-1 and BoHV-1 reference strains were used as positive controls.

**RESULTS**

The multiple nucleotide sequence alignments against the US8 gE sequence, the herpesvirus identified in this study called BuHV-1 Mediterranean isolate (BuHV-1med, GenBank accession number KC202807), displayed 99% identity (601 out of 609 nucleotides) with the gE gene of the b6 strain of BuHV-1 (Thiry et al., 2007; GenBank accession number: EF624469.1). BuHV-1med also displayed 97% identity (592/609 identity and 6/609 gaps) with BoHV-5 gE (GenBank accession number: EF624468.1) and 87% identity (531/609 identity and 12/609 gaps) with BoHV-1 gE (GenBank accession number: EF624466.1). Amino-acid alignment of gE showed that BuHV-1med displayed two amino-acid substitutions compared with BuHV-1b6. One substitution was conservative to an amino-acid of the same charge (Ala→Val), the second substitution was non conservative from a non polar to a polar amino-acid (Ala→Ser). The gE sequence of controls matched with 100% identity to those deposited in GenBank. Phylogenetic analysis based on the US8 gE gene was carried out to assess the relationship between BuHV-1med and other ruminant Alphaherpesviruses and showed the highest degree of homology with BuHV-1 (Fig. 1).
DISCUSSIONS

To our knowledge, the current study is the first report of the first survey of BuHV-1 in buffalo abortive tissues suggesting its involvement in an abortion episode. This finding, corroborated by the absence of any other abortive pathogen in the sample, is in contrast with the existing literature that suggests bubaline herpesvirus only causes mild clinical respiratory symptoms (St. George and Philpott, 1972; Thiry et al., 2007; Scicluna et al., 2010). Genetic analysis demonstrated that the virus isolated is closely related to, but genetically distinct from the BuHV-1 b6 strain. Indeed, as shown in Fig. 1, the gE sequence of BuHV-1med differed from that other BuHV-1 isolates by eight nucleotides (Thiry et al., 2007). These data suggest that the presence of a BuHV-1 subtype typically found in Mediterranean water buffalo and/or the existence of a more virulent strain, may be associated with abortion. However, since no histopathologic investigation was performed on the foetal tissues, it is difficult to definitively link the presence of BuHV-1 to the cause of abortion and further studies, including molecular and histopathological analyses and virus isolation studies are needed to confirm this link.

REFERENCES


**Figure 1:** Phylogenetic tree (neighbor-joining method) based on the US8 gE gene, showing relationships between BuHVmed and other ruminant Alphaherpesviruses. The Suid herpesvirus type 1 (SuHV-1) Becker strain gE gene was used as an outgroup. Numbers at the nodes indicate the bootstrap confidence values obtained after 1000 replicates.