Research in Veterinary Science 94 (2013) 813–816

Contents lists available at SciVerse ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Bubaline herpesvirus 1 associated with abortion in a Mediterranean water buffalo

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ARTICLE INFO

Article history:
Received 12 April 2012
Accepted 23 December 2012

Keywords:
Foetus
Bubaline herpesvirus 1
Glycoprotein E
Sequencing

ABSTRACT

During routine analysis of water buffalo foetuses, one sample was positive for herpesvirus and negative to all the other abortive agents investigated. Sequencing of the herpesvirus glycoprotein E gene identified the virus as bubaline herpesvirus 1, showing few differences with the published sequences. This represents the first finding of bubaline herpesvirus in a water buffalo foetus associated with abortion.

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 responsible for a wide range of clinical syndromes in cattle, such as rhinotracheitis, abortion, pustular vulvovaginitis, encephalitis (Nandi et al., 2009). BuHV1, however, has only been associated with subclinical disease in water buffalo (St George and Philpott, 1972; Thiry et al., 2007; Scicluna et al., 2010). Seroprevalence in water buffalo to both BuHV-1 and BoHV-1 is widely documented (Peshev and Christova, 2000; De Carlo et al., 2004; Scicluna et al., 2007, 2010), however, virus 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).

The current study describes the first detection of a field strain of BuHV-1 in a water buffalo foetus in Europe. This study was carried out by analysing 22 samples of aborted buffalo foetus collected from four water buffalo farms in southern Italy during a 1 year period of routine state surveillance. Samples were investigated for the presence of pathogens typically involved in abortion. Analyses were carried out on the following organs: liver, brain, fourth stomach, kidney, lung, and placenta. Bacterial abortive agents were isolated by microbiological methods (Quinn et al., 2011). Isolated strains where further typed biochemically using a VITEK 2 instrument (Biomerieux Italia s.p.a.). The samples were tested for the presence of *Chlamydophila* spp., *Coxiella burnetii*, *Leptospira* spp., Bovine viral diarrhoea virus (BVDV), *Neospora caninum*, *Toxoplasma gondii* 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).

The 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 h 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 the foetuses recovered from this herd in the last 5 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 (7 years before the episode of abortion here described), against infectious bovine rhinotracheitis (IBR) and with a BoHV-1 gE deleted vaccine.

After the survey of herpesvirus in the foetus, the herd was serologically tested for anti-BoHV-1 antibodies in order to estimate viral circulation. A competitive ELISA was used to determine seropositivity to both gB and gE (IBR gE Ab Test and IBR gB Ab Test, respectively; IDEXX) in 36 animals. The number of samples tested was determined considering a prevalence of 10% and a confidence

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http://dx.doi.org/10.1016/j.rvsc.2012.12.009

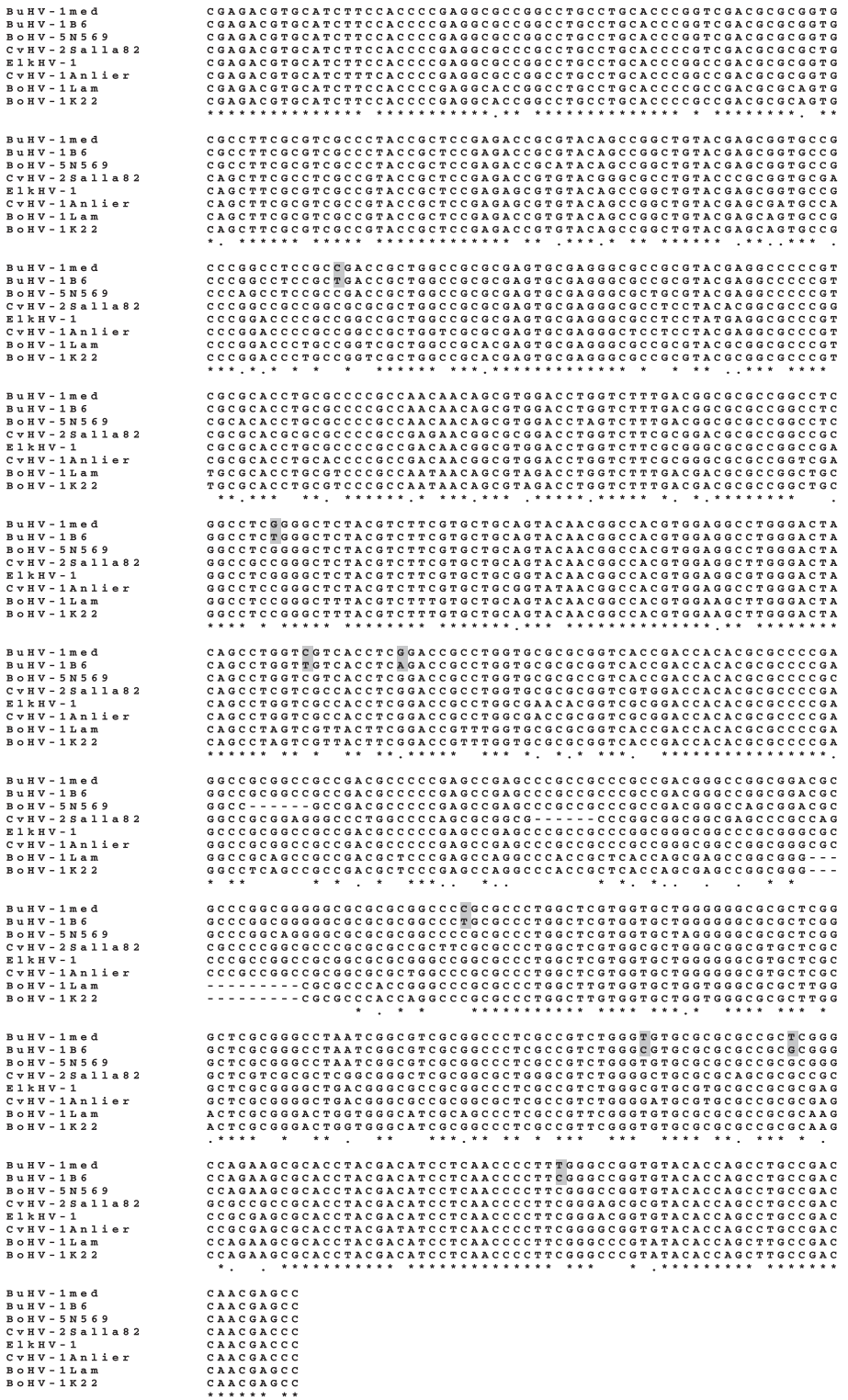

Fig. 1. Nucleotide alignment of BuHV US8 sequence with the Mediterranean isolate and ruminant Alphaherpesviruses. Differences between BuHV-1med and BuHV-1B6 are marked in grey.

interval >95% (Pfeiffer, 2010). Of the samples analysed, 86% were positive to gB and 25% to gE. In particular, the buffalo that had the abortion possessed both gB and gE antibodies. The high percentage of seropositivity to gE demonstrated the wide dissemination of herpesvirus in the farm under study. Moreover, when physically examined, none of the animals of the herd showed any clinical signs attributable to herpesvirus infection.

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: