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Bovine herpesvirus type 1 marker vaccine induces cross-protection against bubaline herpesvirus type 1 in water buffalo

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ABSTRACT

Water buffalo (*Bubalus bubalis*) are susceptible to bovine herpesvirus type 1 (BoHV-1) and a species-specific herpesvirus, bubaline herpesvirus type 1 (BuHV-1). In this study, an attenuated marker BoHV-1 based vaccine against BuHV-1 challenge was evaluated to determine whether it induces protection from viral replication. One group of water buffalo calves was immunized with an attenuated BoHV-1 marker vaccine. A second group was not vaccinated and used as the control. During the post-vaccination period, we monitored the humoral immune response. The efficacy of the vaccine was tested after intranasal challenge of the calves with a BuHV-1 strain. The experiment showed that after vaccination, BuHV-1 replication was significantly reduced by approximately three titer points compared to the controls. The control animals showed high levels of viral shedding and mild signs associated with BuHV-1 infection. Therefore, our study provides evidence for the existence of cross-protection between BoHV-1 and BuHV-1 in buffalo calves.

1. Introduction

In Italy, the water buffalo (*Bubalus bubalis*) has become an important zootechnical and economical animal, were buffalo herds are an important economic resource for production of the milk-derived PDO (protected designation origin) product, the mozzarella cheese. This species is closely related to bovines (*Bos taurus*), is primarily reared in the central and southern part of Italy with a population of approximately 350,000 animals (National Livestock Database - B.D.N., 2010). In these areas, buffaloes and bovines are occasionally reared together on the same farm. Although these two species belong to the *Bovidae* family they are not classified in the same genus, yet conclusions from cattle research have been extrapolated to water buffalo without appropriate verification. For example, Fosgate et al. (2003) demonstrated that the *B. abortus* strain RB51 (SRB51) commercial live vaccine, administered at the recommended calfhood dose, failed to protect water buffalo from infection following natural exposure to *B. abortus biovar 1*.

Bovine herpesvirus type 1 (BoHV-1) and bubaline herpesvirus type 1 (BuHV-1) are two closely related herpesviruses with a recognized narrow host range, rapid growth in cell culture, ability to lyse infected cells and establishment of latency. BoHV-1 is a member of the genus Varicellovirus of the subfamily Alphaherpesvirinae and is antigenically and genetically related to other ruminant alpha-herpesviruses (Thiry et al., 2006a; Muylkens et al., 2007). Herpesviruses are primarily associated with a

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single host species but BoHV-1 and related ruminant alphaherpesvirus have been reported to adapt to other species (Thiry et al., 2006a). However, BoHV-1 is distributed worldwide and it is the causative agent of infectious bovine rhinotracheitis (IBR) in cattle. In cattle, it is responsible for considerable economic losses due to decreased milk production, weight loss and abortion. The wide spectrum of clinical symptoms includes rhinotracheitis, infectious pustular vulvovaginitis, balanopostitis, conjunctivitis, abortion, enteritis and encephalitis (Muylkens et al., 2007). The susceptibility of water buffalo to BoHV-1 infection has been demonstrated both serologically and virologically (Scicluna et al., 2010). However the presence of this virus in water buffalo is not associated with the severe clinical signs that are usually observed during BoHV-1 infection in cattle (Scicluna et al., 2010).

The prevention and control of BoHV-1 infections are based on thorough farm management practices, including hygienic measures, vaccination schedules, BoHV-1 seronegative status for new animals, and removal of infected animals from the herd. Vaccines usually prevent the development of clinical signs and markedly reduce the shedding of virus after infection, thereby leading to a reduction in economic losses; however, vaccines do not completely prevent infection. Several eradication campaigns have been previously performed or are currently being performed in different countries including test-and-removal programs and/or vaccination campaigns (OIE Manual, 2010).

In countries with a high prevalence of field-virus infections, BoHV-1 control is achieved by vaccination of cattle with glycoprotein E (gE)-negative marker vaccines (Strube et al., 1996; de Wit et al., 1998) analogous to the previously successful pseudorabies DIVA (differentiating infected from vaccinated animals) vaccination campaign. Different vaccination strategies have been evaluated in the past, and it was shown that the application of modified live vaccines (MLVs) could reduce the excretion of challenge virus more efficiently than that of inactivated vaccines (Bosch et al., 1996). Therefore, in the framework of a control or eradication program, it appears adequate when the herd is infected to a high degree, to vaccinate cattle on a regular basis during a certain period with DIVA-vaccines and to monitor the number of infected cattle. For this purpose, conventional vaccines are not adequate, because the antibody response they induce cannot be differentiated from that of the infection (EFSA, 2005).

A field study in The Netherlands concerning the efficacy of MLVs revealed that both the incidence and transmission of BoHV-1 infections in marker-vaccinated herds were markedly reduced; however additional measures such as housing purchased cattle in quarantine are necessary to reduce field-virus introduction into the herd (Bosch et al., 1998; Mars et al., 2001).

BuHV-1 is a recently characterized species-specific herpesvirus of water buffalo. Virological evidence of the susceptibility of buffaloes to the species-specific BuHV-1 has been described in an experimental pharmacological reactivation study in naturally infected buffaloes (De Carlo et al., 2004) and evidence of BuHV-1 infection in the buffalo population has been demonstrated serologically (Scicluna et al., 2007).

BuHV1 has been mainly associated with subclinical disease in water buffalo (St George and Philpott, 1972; Thiry et al., 2007a; Scicluna et al., 2010) but recent reports show that BuHV-1 infection is associated with abortion and severe respiratory distress (Petrini et al., 2012; Amoroso et al., 2013).

Phylogenetic analysis has repeatedly classified BoHV-1 and BuHV-1 as distinct viruses but with a high homology (De Carlo et al., 2004; Ros and Belák, 2002; Thiry et al., 2007b) with a sequence nucleotide identity of approximately 96.6% for glycoprotein B (gB) and 86.5% for glycoprotein E (gE).

This close genetic and antigenic relationship between BoHV-1 and BuHV-1 presents implications for diagnosis and eradication programs, because routine serologic tests do not discriminate between antibodies against BoHV-1 or BuHV-1. Therefore, because there is no commercially available vaccine against BuHV-1, it is possible to take advantage of a immunization against BoHV-1, which may potentially protect ruminants from becoming infected by their own herpesviruses. It has been demonstrated that vaccination with BoHV-1 induces protection against virus shedding and neurological signs associated with BHV-5 infetion (Del Médico Zajac et al., 2006). Partial cross-protection also occurred in goats that were vaccinated intranasally with a live attenuated gE-negative BoHV-1 and subsequently showed a reduced viral titer after CpHV-1 challenge (Thiry et al., 2006b).

The objective of this study was to quantify the level of cross-protection induced by an attenuated gE-negative BoHV-1 vaccine against a BuHV-1 challenge..

2. Materials and methods

2.1. Virus and cells

BoHV-1 Cooper strain and BuHV-1 b6 strain (St George and Philpott, 1972) were kindly provided by Dr. Scicluna M.T., Experimental Zooprophilactic Institute of Lazio and Toscana, and were propagated in Madin Darby bovine kidney (MDBK) cells grown in Dulbecco Minimal Essential Medium (DMEM), supplemented with 5% fetal bovine serum (FBS). This cell line was maintained free of both mycoplasma and of bovine viral diarrhea virus (BVDV).

2.2. Experimental design

Twenty 4–6 month-old buffalo calves (10 male and 10 female) serologically negative for BoHV-1 and BuHV-1, as determined by virus neutralization and gB and gE ELISA, were used in the experiment. The animals were virologically negative for BVDV when their leucocytes were tested in a commercial BVD antigen ELISA (Idexx, France). The calves came from a commercial breeding farm without history of BoHV-1 related problems and were maintained on antibiotic-free commercial feed, supplemented with hay and water ad libitum.

The animals were randomly divided in two groups. The first group ($n = 10$, 5 male and 5 female) consisted of buffalo