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Humoral and cellular response to BoHV-1 in buffalo and cattle treated with an inactivated marker vaccine

S. Cavirani[1], F. Ghidini[1], S. Taddei[1], C.S. Cabassi[1], G. Donofrio[1], C. Piancastelli[1], M. Bonfanti[2], A. Marocchi[3]

[1] Dipartimento di Salute Animale, Università di Parma, Italy

[2] Merial Italia

[3] DVM, Practitioner

Corresponding author: S. Cavirani, Dipartimento di Salute Animale – Università di Parma, Via del Taglio, 8 - 43100 Parma (Italy) - Tel. 0039521902667 - Fax: 0039521902672 - Email: sandro.cavirani@unipr.it

ABSTRACT: The study is aimed at assessing and comparing the immune response to BoHV-1 elicited by an inactivated marker vaccine in buffaloes and cattle. Vaccination did not produced any local or general reactions in buffaloes. Seroneutralizing antibodies and cellular response by IFN-γ- test have been detected in buffaloes and cattle after a prime/booster vaccination strategy. Humoral and cellular responses were significantly higher in cattle than in buffaloes. Data pointed out the possibility to use the marker vaccine in buffaloes. However, further studies must be planned to assess the immune pressure of marker vaccines in terms of IBR eradicative attitude in infected buffalo herds.

Key words: Buffalo, IBR, Marker vaccine.

INTRODUCTION - Buffaloes (Bubalus bubalis) are susceptible to various viral and bacterial pathogens causing infectiuos disease in cattle. In particular, among the bovine herpesviruses, buffaloes are susceptible to BoHV-1 (Rao *et al*, 1991; Reda *et al*, 1985; St.George *et al*, 1972), BoHV-2 (Schiemann *et al*, 1971), and BoHV-4 (Rossiter *et al*, 1989). In addition the existance of a species-specific herpesvirus, bubaline herpesvirus type 1 (BuHV-1) has been demonstrated (4, 12). Regarding BoHV-1, serological investigations have demonstrated that the infection is widespread in buffaloes reared in different areas of Italy (Cavirani *et al*, 1995; Cavirani *et al*, 1997). However the presence of this virus has not be related to clinical signs that are usually observed in infectious bovine rhinotracheitis (IBR) outbreaks occurring in cattle.

By 1995 the European Commission approved IBR eradication plan. Following the european trend, also Italy started moving the first steps towards the IBR eradication presenting compulsory programs in the provinces of Bolzano, Trento and Belluno and voluntary programs in some Regions, namely Lombardia, Triveneto, Piemonte and Lazio. In some cases the eradication program involves both dairy cattle and buffalo herds. IBR eradication is of concern mainly for the herds rearing dairy cattle and buffaloes together. The availability of marker (gE-negative) vaccines, that allow the vaccinated animals to be distinguished from infected ones, has promoted the adoption of eradication program at herd level indipendently from the presence or not of official eradication plans. Regarding the efficacy of marker vaccines to control IBR infection in dairy cattle there are a lot of informations. Conversely there are no

evidences concerning safety and efficacy on marker vaccine treatments in buffaloes.
This study is aimed at proving data on safety and immunogenicity (humoral and cellular response) of an inactivated marker vaccine applied in buffaloes, producing a comparison with the immune reaction elicited by the same vaccine in dairy cattle.

MATERIAL AND METHODS - Animals and vaccination schedule. The survey involved forty mediterranean buffaloes and forty dairy heifer. All the animals, 10-12 months of age, belonged to IBR free herds. Twenty buffaloes and twenty dairy heifers were injected subcutaneously with an inactivated marker vaccine (IBRAXION – Merial) in accordance with a protocol of immunization including a priming and a booster treatment 21 days a part. Twenty buffaloes and twenty dairy heifers were kept unvaccinated as control for absence of viral circulation during the study. The occurrence of local and general reactions after each vaccination treatment on buffaloes were monitored. **Laboratory investigations.** A blood sample, with and without anticoagulant, was collected at priming (T0), at booster (T1) and 21 days after the booster (T2) from all the animals involved in the study. Humoral response to BoHV-1 was detected on serum samples by seroneutralization test (Cavirani *et al*, 1986). In addition, BoHV-1 gE antibodies were detected by an ELISA kit (Herd Anti-IBR gE, Idexx). Cellular response to BoHV-1 was detected on blood samples through the production of γ-Interferon (Bovine Gamma Interferon Test – Bovigam, CSL) after in vitro stimulation of the blood buffy-coat with an inactivated reference strain of BoHV-1 (BoHV-1 New York). Data regarding serumneutralizing antibody titres (SN) and Optical Density (OD) for γ-Interferon test detected in buffaloes and cattle at different times were submitted to statistical analysis by the Student t test.

RESULTS AND CONCLUSION: The treatment of buffaloes with the marker inactivated vaccine did not elicited any local or general reaction after a prime/boost vaccination regimen. All the animals (vaccinated and unvaccinated groups) did not showed BoHV-1 gE antibody response as demonstrated by ELISA tests carried out at T0, T1 and T2.
Data concerning the assessment panel measuring humoral and cellular response to BoHV-1 in vaccinated and unvaccinated animals (buffaloes and cattle) are indicated in Table 1.

Table 1. Humoral and cellular response to BoHV-1 (mean ± standard deviation) in buffaloes and cattle treated with an inactivated marker vaccine.

Sampling time	Seroneutralization (log2)		γ-Interferon (OD)	
	Buffaloes	Cattle	Buffaloes	Cattle
T0	0.00±0.00	0.00±0.00	0.07±0.05	0.08±0.04
T1	1.65±0.99	3.15±1.53	0.14±0.05	0.18±0.09
T2	3.70±0.86	5.05±1.36	0.58±0.28	0.99±0.31

The prime/boost vaccination regimen elicited seroneutralizing antibodies and cellular response (γ-Interferon production) either in buffaloes and cattle. Humoral and cellular response following vaccination were significantly higher in cattle than in buffaloes. In particular statistical analysis by Student t test showed :
at T1, seroneutralizing antibodies cattle vs buffaloes $p=0.0064$ (significant <0.01)

γ-Interferon cattle vs buffaloes p=0.0333 (significant p<0.05);
at T2, seroneutralizing antibodies cattle vs buffaloes p=0.0049 (significant p<0.01)
γ-Interferon cattle vs buffaloes p=0.0002 (significant p<0.01).

This evidence should be expected since BoHV-1 natural infection usually induces a higher immune response in cattle than in buffaloes as previously detected in IBR infected herds where the buffaloes and dairy cattle are reared together (Cavirani *et al*, 1986; Cavirani *et al*, 1997). The lack of clinical disease following BoHV-1 infection in buffaloes must be considered as an indirect demonstration that buffaloes is not the natural host for this virus. The isolation of BuHV-1 from buffaloes pointed out that there is a species-specific bovine herpesvirus (Thiry *et al*, 2002). The molecular characterization of BuHV-1 field isolates showed that the viral strains were more closely related to BoHV-5 than BoHV-1 (De Carlo *et al*, 2004).

Taken together our data pointed that an inactivated marker vaccine, originally devoted to control IBR infection in cattle, is safe and able to induce a specific immune response in buffaloes too. This evidence is a starting point to hypothesize an application of IBR eradication program in buffalo herds showing high BoHV-1 seroprevalence through the use of marker vaccines. We must highlight that the need of performing IBR control in buffaloes by using marker vaccine is of concern particularly in the areas where IBR eradication plans are in progress. At present it is difficult to foresee the possible interference of BuHV-1 in the context of a BoHV-1 eradication plan in buffaloes. Further studies must be planned to assess the efficacy of traditional and marker vaccines in terms of cross-protection to other bubaline herpesviruses, BuHV-1 in particular. In addition, the immune pressure (basic reproduction ratio, R_0) induced by marker vaccines in the context of an IBR eradication strategy in buffalo herds must be elucidated.

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