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Short communication

Reactive oxygen metabolites in alpha-herpesvirus-seropositive Mediterranean buffaloes (*Bubalus bubalis*): a preliminary study

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Abstract

In the present study on *Bubalus bubalis* of the Campania Region (Italy) the serum levels of derivatives of reactive oxygen metabolites (d-ROMs), anti-ROM and oxidative stress index (Osi) were evaluated. These data were then related to the seropositive status of the animals against alpha-herpesviruses, precisely Bubaline herpesvirus 1 (BuHV-1) and Bovine herpesvirus 1 (BoHV-1). Clinically healthy Mediterranean buffaloes were selected for this study. The serum samples of these animals were taken, and d-ROMs, anti-ROM and Osi were measured using commercially available tests. The preliminary data demonstrated that animals seropositive to both BuHV-1 and BoHV-1 present more oxidative stress than seronegative animals, as revealed by a significant increase in d-ROMs. Our results provide, for the first time, insight into the reactive oxygen species (ROS) modulation induced by the herpesvirus in *Bubalus bubalis*.

Key words: Bubalus bubalis, Bubaline herpesvirus 1 (BuHV-1), Bovine herpesvirus 1 (BoHV-1), reactive oxygen metabolites (ROM), oxidative stress index (Osi).

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Introduction

In recent years, oxidative stress has been postulated as an important factor in the pathogenesis and development of diseases. Reactive oxygen species (ROS), which are defined as oxidized molecules containing oxygen, such as O_2^-, hydrogen peroxide (H_2O_2) and HO^- (El-Benna et al. 2009), are well known for being both advantageous and injurious, and have long been known to be a component of the killing response of immune cells to microbial attack. Viruses can alter oxidative balance by either increasing the formation of free radicals or inhibiting the synthesis of enzymes involved in oxidative defence within host cells (Lang et al. 2013, Williams et al. 2014). Herpesvirus infections are frequently associated with the generation of oxidative stress in infected cells. Herpes simplex virus type 1 (HSV-1) has been reported to induce the depletion of glutathione, the main endogenous antioxidant defence, and to increase ROS levels and lipid peroxidation (Kavouras et al. 2007). Recently, it has been demonstrated that cows with natural bovine herpesvirus 1 (BoHV-1) infection seemed to have more oxidative stress and low antioxidant defence (Durgut et al. 2013).

Bubalus bubalis, a species closely related to bovines, is mainly infected by bubaline herpesvirus 1 (BuHV-1), which has been isolated in Italy after pharmacological reactivation (De Carlo et al. 2004); however, it has been demonstrated experimentally the susceptibility of buffaloes even to the BoHV-1 (Scicluna et al. 2010), showing their possible role as host/reservoirs of BoHV-1. In any case, both BuHV-1 and BoHV-1 generally induce only subclinical disease in Bubalus bubalis (Thiry et al. 2006).

Active oxygen and free radicals play an important role in host defences. Many viruses are evolved to manipulate the delicate pro- and antioxidant balance, and it has been documented that these disorders can not only cause cellular damage but also stimulate the processes to reduce the treatment of damage.

The aim of this study was to determine the association between the status of seropositivity to BuHV-1 and BoHV-1 and oxidative stress in Mediterranean buffaloes (*Bubalus bubalis*).

Materials and Methods

For this study we chose, in the Campania Region (Italy), clinically healthy Mediterranean buffaloes (*Bubalus bubalis*) which, since birth, had shown absence of bacterial/viral infections, infestations or injuries, as reported by the owner. The commercial breeding farm's owner reported also no history of BoHV-1 related problems. Moreover, these animals were not vaccinated for herpesvirus infection, and they were also virologically negative for BVDV as tested by a commercial BVD antigen ELISA (Idexx, France). For sampling, only buffaloes up to 5 years of age were selected since they are considered to be at higher risk of infection. Blood samples collected from the tail veins, were centrifuged (750 g for 5 min) and the serum was divided into aliquots and stored at -20°C until use.

The presence of antibodies against BuHV-1 or BoHV-1 was investigated in serum samples using an ELISA serological kit.

To assess serum total oxidant and antioxidant levels, commercially available d-ROMs and anti-ROM tests were utilized. These tests were performed according to the manufacturers' instructions and by using Free Carpe Diem, a dedicated spectrophotometer (Diacron International, Grosseto, Italy).

As indicator of the degree of oxidative stress, the oxidative stress index (Osi) levels were calculated using the following formula: Osi = d-ROM/anti-ROM x 100 (Abuelo et al. 2013).

The results are expressed as mean ± standard deviation (SD). Levels of oxidative stress between the different groups were compared using Student t-tests, as the data were normally distributed. p less than 0.1 was considered to be statistically significant. All the above-mentioned analyses were performed using the SPSS II 11.0 for Windows software package (SPSS Japan Inc., Tokyo, Japan).

Results and Discussion

We collected sera from unvaccinated buffaloes that did not show clinical signs referable to an alpha-herpesvirus infection or to other infectious diseases. The screening of samples by anti-gB/gE blocking ELISA allowed us to select three groups of animals, as below: group 1, BuHV-1 seropositive animals (n = 5), group 2, BoHV-1 seropositive animals (n = 8) and group 3, seronegative animals (n = 6).

As shown in Fig. 1, the results indicated a significant increase of d-ROMs in seropositive BuHV-1 animals (group 1) compared with seropositive BoHV-1 (group 2) ($p<0.05$) and seronegative animals (group 3) ($p<0.01$). An increase, though not significant, was highlighted in seropositive BoHV-1 animals (group 2) compared with seronegative control animals (group 3). To assess the antioxidant capacity, the anti-ROM test was also performed on the sera of the same animal. We detected no significant difference when groups 1 and 2 values were compared with control group 3.

The oxidative stress index (Osi) was significantly

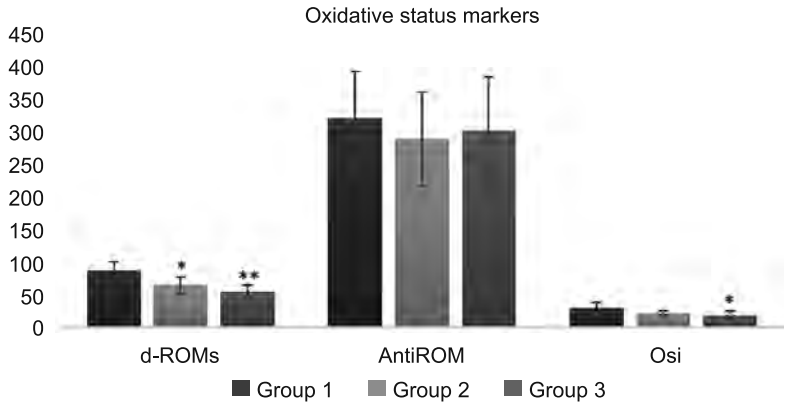

Fig. 1 – Mean values of oxidative status markers (d-ROMs expressed as Carr U = 0.08 mg hydrogen peroxide/dL; Anti-ROM expressed as μEq/L; Osi expressed as arbitrary unit calculated as d-ROM/anti-ROM × 100) of the studied animal groups: group 1 - BuHV-1 seropositive animals; group 2 - BoHV-1 seropositive animals; group 3 – seronegative animals.
* $p<0.05$ - group 1 versus group 2 (d-ROMs); group 1 versus group 3 (Osi); ** $p<0.01$ - group 1 versus group 3 (d-ROMs).

higher ($p<0.05$) in group 1 compared with control group 3, whereas no significant difference was detected between group 2 and control group 3 (Fig. 1).

Oxidative stress through virus infections can contribute to several aspects of viral disease pathogenesis including apoptosis, loss of immune function, viral replication, inflammatory response and loss of body weight. In fact, research has shown that many retroviruses, DNA viruses and RNA viruses can cause cell death by generating oxidative stress in infected cells (Reshi et al. 2014).

Previously, it has been demonstrated that bovine herpesvirus type 4 (BHV-4), belonging to the gamma-2-herpesviruses of the gamma-herpesvirinae subfamily, induces apoptosis *in vitro*, and in the early phases of apoptosis the infected cells showed an increase in the intracellular level of ROS. It has also been reported that oxidative stress is a crucial event in the sequence leading to apoptotic cell death; nevertheless, apoptosis is not required for the multiplication of BHV-4 (Pagnini et al. 2004). However, the interplay between oxidative stress and herpesviruses infection has not been extensively studied either in human or veterinary medicine. In particular, very little information exists about the pathological aspects of a biological relationship in *Bubalus bubali* infected by alpha--herpesviruses. Taken together, our results provide, for the first time, insight into the ROS modulation induced by the herpesvirus in buffalo. However, further studies are needed to elucidate the exact mechanisms of the oxidative stress in *Bubalus bubalis* in relation to the pathophysiology of both BuHV-1 and BoHV-1 infections.

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