

EVALUATION OF REPLICATION KINETICS AND CYTOKINE RELEASE OF DIFFERENT STRAINS OF BLUETONGUE VIRUS SEROTYPE 1 ON SHEEP MACROPHAGES



Elisabetta Coradduzza¹, Rosario Scivoli¹, Angela M. Rocchigiani¹, Davide Pintus¹, Ciriaco Ligios¹, Roberto Bechere¹, Annalisa Oggiano¹, Giulia Franzoni¹, Anna P. Murtino¹, Giovanni M. Pala¹, Lorena Mura¹, Alessandra Leoni², Liana Teodori², Giovanni Savini², Giontonella Puggioni¹.

¹ Istituto Zooprofilattico della Sardegna, Sassari, Italy.
² Istituto Zooprofilattico dell'Abruzzo e del Molise, Teramo, Italy.

Introduction

Bluetongue (BT) is an infectious haemorrhagic disease caused by the bluetongue virus (BTV), genus Orbivirus, family Sedereoviridae. Mainly transmitted by some species of Culicoides, BTV infects domestic and wild ruminants. In Sardinia, BT has been reported since 2000 and currently, it is endemic causing significant losses in the livestock industry, especially in the ovine production systems. The severity of infection depends on various factors, such as species, breed, age, immune status of animals, and environmental stresses, as well as the virulence of the BTV strain involved. In a previous study, we found different clinical outcomes in rams experimentally infected with 2 different strain of BTV-1 (BTV-1 IT₂₀₀₆ and BTV-1 IT₂₀₁₃). In this work, we set up an in vitro study based on infection of ovine monocyte-derived macrophages (MDMs) with BTV-1 IT₂₀₀₆ and BTV-1 IT₂₀₁₃ to evaluate viral load and cytokine release, to better investigate the pathogenicity of both BTV-1 strains.

Methods

MDMs cultures from 3 BTV-free ovine were obtained. On day 5, they were infected with 1 moi of either BTV-1 IT₂₀₀₆ or BTV-1 IT₂₀₁₃. At different time-points (0, 6, 12, 24, 48, and 72 hours post infection [p.i.]) cells and supernatants were tested for virus load by using qRT-PCR targeting the segment 10 of the virus. Interleukin 1 β (IL-1 β), Interleukin 6 (IL-6), Interferon α (INF- α), Interferon β (INF- β), Tumor necrosis factor α (TNF- α), antiviral protein release were also assessed by commercial ELISA kits.

To detect BTV in the cells, an immunofluorescence assay targeting BTV-NS2 protein was performed. Furthermore, we monitored viral expression in macrophages by flow cytometry using BTV-NS2 antibody; VERO-infected and Mock-infected cells were used as positive and negative control, respectively.

Results

Immunofluorescence detected BTV-NS2 signal in macrophages (fig. 1A). qRT-PCR showed that viral RNA concentration was significantly higher in cells infected with BTV-1 IT₂₀₁₃ than in those with BTV-1 IT₂₀₀₆ in both, supernatants, at 6-12-24-48 h p.i. and in cells at 6-24 h p.i. (fig. 1B, D). Inherent to the quantification of cytokines (TNF- α , INF- α , INF- β , IL-1 β and IL-6) and antiviral proteins (MXI), the levels of TNF- α , INF- α , IL-1 β , IL-6 and MXI showed no statistically significant changes between infected cells and negative controls at all testing times. Instead, significantly higher INF- β values was shown at 48 h p.i. in cells infected with BTV1 IT₂₀₀₆, compared with those infected with BTV-I IT₂₀₁₃ and negative controls (Fig 1C). Using flow cytometry, no NS2 protein expression in macrophages was detected at 24, 48 and 72h p.i.

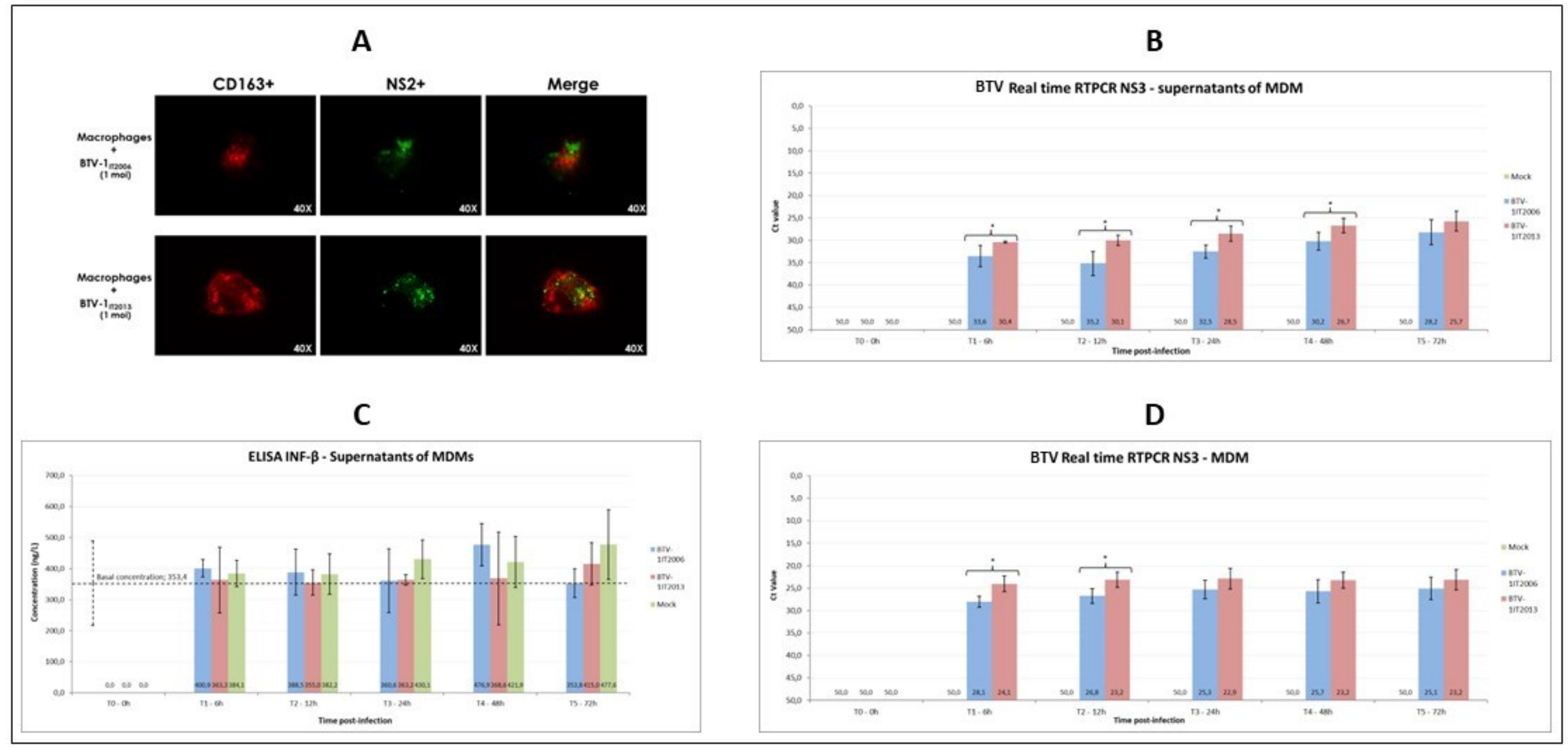


Fig 1: 1A; Immunofluorescence. 1B; BTV NS3 Real Time RTPCR -supernatants of MDM. 1C; ELISA INF- β -Supernatants of MDMs. 1D; BTV NS3 Real Time RTPCR -MDM.



Conclusions

This study demonstrated that sheep macrophages were susceptible and permissive to BTV infection. The replicative efficiency of BTV-1 IT₂₀₁₃ was significantly higher than that of the BTV-1 IT₂₀₀₆ strain. However, in cells infected with the BTV-1 IT₂₀₀₆, more INF- β was produced at 48 ore p.i. As already observed in in vivo studies, this finding confirmed the higher degree of virulence of BTV-1 IT₂₀₀₆ compared with BTV-1 IT₂₀₁₃.