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**Evaluation of replication kinetics and cytokine release of different strains of Bluetongue virus serotype 1 on sheep macrophages**

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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 (1-2). In a previous study, we found different clinical outcomes in rams experimentally infected with 2 different strain of BTV-1 (BTV-1IT2006 and BTV-1IT2013) (3). In this work, we set up an in vitro study based on infection of ovine monocyte-derived macrophages (MDMs) with BTV-1IT2006 and BTV-1IT2013 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 BTVIT2013. 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? (IL-1?), Interleukin 6 (IL6), Interferon ? (INF-?), Interferon ? (INF-?), Tumor necrosis factor ? (TNF-?), antiviral protein MX1 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-1IT2013 than in those with BTV-1IT2006 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-?, INF-?, INF-?, IL-1? and IL-6) and antiviral proteins (MX1), the levels of TNF-?, INF-?, IL-1?, IL-6 and MX1 showed no statistically significant changes between infected cells and negative controls at all testing times. Instead, significantly higher INF-? values was shown at 48 h p.i. in cells infected with BTV-1IT2006, compared with those infected with BTV-1IT2013 and negative controls (Fig 1C). Using flow cytometry, no NS2 protein expression in macrophages was detected at 24, 48 and 72h p.i.
Conclusions
This study demonstrated that sheep macrophages were susceptible and permissive to BTV infection. The replicative efficiency of BTV-1IT2013 was significantly higher than that of the BTV-1IT2006 strain. However, in cells infected with the BTV-1IT2006, more INF-? was produced at 48 ore p.i. As already observed in in vivo studies, this finding confirmed the higher degree of virulence of BTV-1IT2006 compared with BTV-1IT2013.