

## Identification of co-infections of Bluetongue virus serotypes 3, 4, and 8 through animal and entomological surveillance in Sicily, Italy

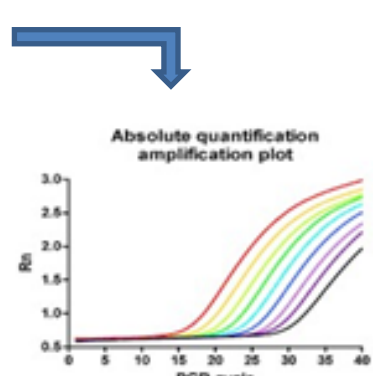
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### INTRODUCTION & AIM

Bluetongue virus (BTV), an *Orbivirus* from the *Reoviridae* family, is characterized by a segmented double-stranded RNA responsible for high genetic plasticity. A pathogen mainly transmitted by biting midges belonging to the *Culicoides* genus, it causes viral disease in ruminants with economic and health impacts on livestock and wild animals (1). There are more than 30 BTV serotypes that differ in antigenic and pathogenicity characteristics and can also undergo reassortment phenomena, producing new variants (2). The first outbreak of BTV infection in Sicily was reported in 2000 (3). In this study, we analyzed cases of co-infections in animals and insects by BTV 4-8, BTV 3-8, which occurred in Sicily (Italy) from January 2024 to May 2025, highlighting the risk of genetic reassortment between strains with different pathogenic and diffusion characteristics.



BTV-8 and BTV-4 Outbreaks 2024-2025



BTV-8 and BTV-3 Outbreaks 2024-2025

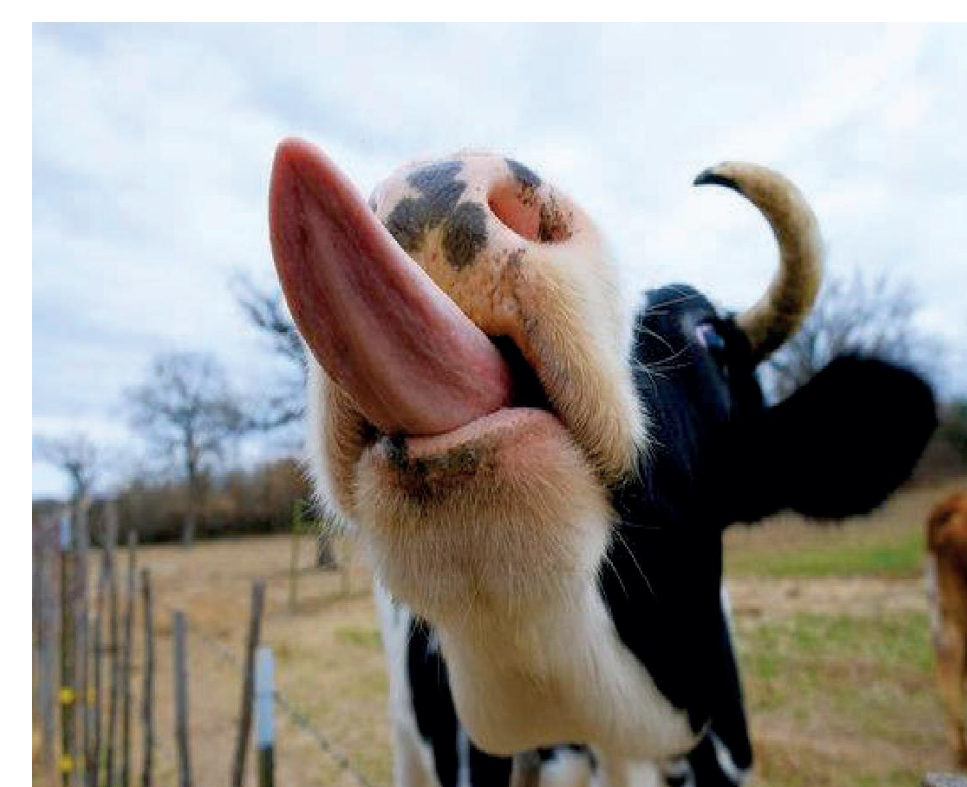


### METHOD

Blood, tissue samples and insect traps were collected. Insect vectors were sampled and morphologically identified at the species level and grouped into pools. All samples were then subjected to RNA extraction and amplification by real-time RT-PCR for the BTV NS3 gene. Specific real-time RT-PCR for serotypes 1-3-4-8 was carried out on positive samples by the WOA Reference Laboratory for Bluetongue, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM).



Culicoides Traps



### RESULTS & DISCUSSION

Co-infection with BTV 4-8 was detected in 4/27 (14.81%) positive insect pools of the species *Culicoides imicola*, *Culicoides newsteady* and *Culicoides* spp. Co-infection with BTV 4-8 was also found in a total of 21/77 (27.27%) positive blood samples from cattle; initially, only 2 blood samples were genotyped and subsequently a further 19 positives were confirmed by IZSAM. Co-infection with BTV 3-8 was identified in an additional 8 blood samples, following confirmation by WOA Reference Laboratory, and in 4/32 (12.5%) sheep spleens.

### CONCLUSION

The work carried out allowed us to the identification of BTV serotypes circulating and also documented cases of co-infections in animals and insects during the 2024-2025 transmission seasons in southern Italy. Some serotypes, such as BTV-8, have a greater impact on health and economy, so expanding epidemiological knowledge is essential to strengthen surveillance systems and methods for the early detection of new or more virulent strains.

### REFERENCES

- 1) Erram, D., McGregor, B., Acevedo, C. et al. Epizootic hemorrhagic disease virus oral infection affects midge reproduction and is vertically transmitted to offspring in *Culicoides sonorensis*. *Sci Rep* 15,16078 (2025). <https://doi.org/10.1038/s41598-025-00849-y>
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- 3) Caracappa S et al. Epidemiological surveillance of bluetongue in Sicily. *Vet Ital* 2004 Jul-Sep;40(3):124-9. PMID: 20419648.