Bluetongue virus serotype 3 in Western Sicily, November 2017

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Summary
Bluetongue virus serotype 3 has been detected in a sheep in the Western part of the island of Sicily (Italy). This area is 150 km away from the peninsula of Cap Bon (Tunisia), where recent activities included in the Bluetongue National Surveillance plan demonstrated the widespread circulation of the same BTV strain.

Sierotipo 3 del virus della Bluetongue identificato in Sicilia occidentale, novembre 2017

Parole chiave
Bluetongue,
Sierotipo 3 del virus della Bluetongue,
Italia,
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Riassunto
Il sierotipo 3 del virus della Bluetongue è stato identificato in una pecora in provincia di Trapani (Sicilia occidentale, Italia). Le coste siciliane della provincia trapanese distano 150 km dalla penisola di Capo Bon in Tunisia, dove la presenza dello stesso virus è stata dimostrata grazie a recenti attività previste nei piani nazionali di sorveglianza disegnati per monitorare la circolazione del virus della Bluetongue.

Alert: Bluetongue virus serotype 3 in Sicily

In December 2016, the General Direction of the Veterinary Services of the Tunisian Ministry of Agriculture notified a bluetongue [BT, a vector-borne notifiable disease included in the World Organization for Animal Health (OIE) list] outbreak[1] to the OIE. The outbreak occurred in Cap Bon, a peninsula in far Northeastern Tunisia. The *Institut de la Recherche Vétérinaire de Tunisie* (IRVT), the BT Tunisian National Reference Laboratory, in collaboration with the OIE Reference Laboratory for BT of the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM) of Teramo, Italy, characterized the etiological agent as a novel Western bluetongue virus serotype 3 (BTV-3) strain. It was the first time that a BTV-3 strain was reported in Tunisia. Through next generation sequencing (NGS, Marcacci *et al.* 2016) the whole genome sequence of the new strain (BTV-3 TUN2016) was obtained directly using RNA purified from a blood sample of a symptomatic

[1] http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=22088.

sheep (Sghaier *et al.* 2017). Sequences were immediately released on a public database (NCBI, KY432369-KY432378). Further surveillance activities conducted in collaboration between the Tunisian and Italian authorities, revealed a widespread presence of BTV-3 RNA and antibodies in sheep of the entire Tunisian territory. Moreover, an additional Western BTV-3 strain, named BTV-3 TUN2016/Zarzis (NCBI, MF124292-MF124301), was identified in the Southeastern regions of Tunisia, nearby the border with Libya. The 2 viruses can be easily differentiated as the Seg-2 sequences show 92% of nucleotide identity. Seg-10 sequences of both strains are remarkably different since they show only 80% of nucleotide identity (Lorusso *et al.* 2018). In November 2017, a 3-year old female crossbred sheep belonging to a flock of nearly 400 animals located in the surroundings of Trapani (Western part of the island of Sicily, facing the peninsula of Cap Bon) showed clinical signs consistent with BT infection. Symptoms included fever, oedema of the head, nasal discharge, and depression. The veterinary services of local health unit (ASL) were immediately alerted. They visited the entire flock and collected EDTA-blood and serum samples from the symptomatic sheep. Samples were sent to the Istituto Zooprofilattico Sperimentale of Sicily for BT diagnosis. The RNA was purified from the whole blood sample and a real time reverse transcriptase-polymerase chain reaction (RT-PCR) molecular assay detecting Seg-10 of BTV genome [Hofmann *et al.* 2008 (RT-qPCR$_{NS3}$)] was performed. As for the serum sample, c-Elisa for the detection of BTV specific antibodies was also conducted (Lelli *et al.* 2003). Whole blood and serum samples resulted positive for BTV RNA and antibodies, respectively. As required by the Italian legislation, BTV positive samples were sent to the BT National Reference Laboratory (NRL) at the National Reference Centre for Foreign Diseases of Animals (CESME) of the IZSAM in order to confirm the BTV infection and characterize, by serological and molecular assays, the strain. The BT NRL confirmed the results obtained in Sicily. Genotyping by using the VetMAX European BTV Typing kit for detection of BTV 1-2-4-6-8-9-16 serotypes (BTV$_{European\ typing}$, LSI, Thermo Fisher Scientific, Waltham MA-USA) and serotyping by serum-neutralization assay using the same reference BTV serotypes (SN, Savini *et al.* 2004) were therefore conducted. Both tests were negative.

Considering the epidemiological scenario of BTV-3 in Tunisia (Lorusso *et al.* 2018), the origin of the previous BT incursions in Southern Europe (Calistri *et al.* 2004), and the proximity (nearly 150 km) of Cap Bon to Sicily, RNA purified from whole blood was also tested for the presence of BTV-3 RNA by a specific real time RT-PCR (RT-qPCR$_{BTV-3}$, Lorusso *et al.* 2018) while the serum sample was tested by SN assay using all BTV reference serotypes. Blood and serum samples resulted positive for BTV-3 RNA (Threshold cycle, C_T 20) and BTV-3 neutralizing antibodies (titre 1:180), respectively. Additional molecular and serological typing assays for the remaining BTV serotypes were negative. A portion of Seg-2 of BTV-3 genome was also amplified by RT-PCR (Lorusso *et al.* 2018) to identify of which BTV-3 variant the Italian strain belongs. The Seg-2 of BTV-3 identified in Sicily was identical to that of BTV-3 TUN2016 first detected in Cap Bon in November 2016. On December the 4th, the Italian Ministry of Health notified the outbreak to the OIE[2]. In order to identify the genome constellation, isolation attempts and whole genome analysis of the Italian BTV-3 strain are currently in progress.

These events further emphasize the importance for European countries in general, and for Italy in particular due to its geographical location, of having in place robust collaborations with Northern African authorities on public and animal health. The prompt detection of BTV-3 in Sicily is just an example of the benefits that could derive from such relationships. Thanks to the Italian and Tunisian Government initiatives and through the OIE laboratory twinning programmes, it was indeed possible to build and strengthen a fruitful and long-lasting relationship between the IRVT and IZSAM. The possibility of sharing scientific expertise and diagnostic issues between the 2 institutions led to organizing common surveillance programs and monitor the BTV situation in Tunisia. This was crucial, as it facilitated the development of a specific and accurate RT-qPCR for the detection of BTV-3, which was used both to detect BTV-3 circulation in Tunisia and to detect promptly this serotype in Sicily. It is then critical that European and Northern African authorities collaborate in organizing common surveillance programs to detect early novel strains or emerging serotypes and to set up proper preventive measures, including the development of specific vaccines and coordinated vaccination campaigns. In this regard, however, as inactivated vaccines against this serotype are not currently available, BTV-3 in Sicily is certainly a great concern for both Italian and European animal health.

[2] http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review/viewsummary?fupser=&dothis=&reportid=25341.

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