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Molecular characterization of Bagaza virus (BAGV) outbreaks in red-legged partridges from Spain (2010-2021)

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Introduction: Bagaza virus (BAGV), synonymous with Israel Turkey Meningoencephalomyelitis virus (ITMV) (1), is a flavivirus belonging to the Ntaya serocomplex. It is transmitted by mosquito bites and affects avian species. In Europe, it was detected for the first time in southern Spain in 2010, severely affecting game bird species (mainly red-legged partridges) in the province of Cádiz (2). BAGV reappeared in the same province after several years of epidemiological silence, causing important outbreaks in 2019 and 2021. BAGV had never been noticed in any other European country until 2021, when a primary outbreak occurred in Portugal in the same species. This study aims to characterize the Spanish BAGV strains and to elucidate if outbreaks have been due to a single or more virus introductions into the country.

Methods: Four recent Spanish BAGV isolates (2019-2021) were obtained from brain samples collected from red-legged partridges (*Alectoris rufa*) in Cádiz. After RNA extraction and confirmation by real time RT-PCR, samples were sequenced (by Sanger approach) and phylogenetic analyses were executed including all BAGV and ITMV complete genomes publicly available at GenBank. To allow the incorporation of additional African isolates to the study, further partial phylogenetic analyses targeting the NS5 coding region were carried out. Finally, a comparison of the viral polyproteins was also performed.

Results: The phylogenetic analyses showed that BAGV isolates from Spain (obtained in 2010, 2019 and 2021) belonged to two different clusters, here designated as BAGV cluster 1 (C1) and cluster 2 (C2). On the one hand, the 2021 isolate grouped to those that were firstly detected in Spain in 2010 (BAGV-C2). Additionally, the first BAGV isolate from Portugal also gathered this group. On the other hand, the Spanish isolates from 2019 belonged to BAGV-C1, closely related to isolates obtained in mosquitoes from Senegal for more than twenty years. The comparison of the viral polyprotein sequences supported and confirmed the phylogenetic results.

Conclusions: The phylogenetic analyses showed that at least two introductions of two clusters of BAGV have occurred in Spain. The first emergence occurred in 2010 due to BAGV-C2, which was closely related to the strains detected in Israel in the same period. A second introduction was reported in 2019, the isolates belonged to BAGV-C1 and it was the first introduction of this cluster in Europe. Finally, the Portuguese and Spanish isolates associated to the recent outbreaks occurred in 2021 are closely related and seem to be originated by a single strain. In summary, our results confirmed that BAGV is able to reach southern Europe from other regions occasionally. BAGV has only been detected sporadically during the past decade; however, a continuous circulation of the virus in these areas cannot be discarded.

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