



**ID 2999.**

Detection of Usutu virus in Portugal through active surveillance for *Orthoflavivirus* in red-legged partridges

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**Tipo de comunicación/Type of communication:** Oral

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**Introducción y Objetivo/Background and objectives**

Usutu virus (*Orthoflavivirus usutuense*, USUV), a neurotropic arthropod-borne virus of the family Flaviviridae, is a zoonotic virus that was first isolated in Africa in 1959 and emerged in Europe in 1996. Since then, USUV has spread throughout the European continent and has been detected in several species of wild birds and mosquitoes, as well as in humans and other mammals. However, no cases of USUV have been detected in Portugal. Following an outbreak of Bagaza virus in southern Portugal in 2021, an active surveillance program for *Orthoflavivirus* in hunted red-legged partridges (*Alectoris rufa*) was implemented to identify the circulating viruses and characterize the strains.



### **Métodos/Methods**

We collected growing feather samples from 249 partridges hunted in the municipalities of Mértola and Serpa, during the hunting seasons 2021-2023. RNA was extracted and used to screen for orthoflaviviruses by RT-qPCR. Samples positive for USUV were subjected to whole genome sequencing and strain characterization. Phylogenetic trees were constructed using the obtained genomes and those available in the NCBI Virus online database.

### **Resultados/Results**

Two partridges tested positive for USUV, a juvenile female hunted in Serpa in November 2021 and an adult female hunted in Mértola in November 2023. Phylogenetic analyses of the complete and partial genomes assigned the USUV strains to the African 3 lineage, particularly the African 3.1 sub-lineage.

### **Conclusión y Relevancia/Conclusions and relevance**

Our study detected USUV for the first time in Portugal, confirming previous serological evidence of USUV circulation in the country. Given the ongoing circulation and the increasing risk of its spillover to other domestic and wild animals, and humans, additional efforts are needed to improve USUV surveillance in Portugal from a One Health perspective.