

Bagaza Virus in Wild Birds, Portugal, 2021

Appendix

Appendix Table. Bagaza virus–positive specimens analyzed in a study of wild birds in Portugal, 2021*

Code	Species	Status	Collection date	Sex	Age class	Real time RT-PCR (RT-qPCR)								Pan-Flavivirus conventional RT-PCR	
						Duplex RT-qPCR ¹ (3'NTR, 172 bp amplicon)				Uniplex RT-qPCR ² (NS5, 61pb amplicon)				nested RT-PCR ³ (NS5, 143 bp amplicon)	RT-PCR ⁴ (NS2b, 209 bp amplicon)
						Feather	Brain	Kidney	Spleen	Heart	Kidney	Brain	Intestine	Feather/spleen	Heart
Partridge 1	<i>A. rufa</i>	Found dead	01/09/21	Male	Adult	+	–	–	–	nt	nt	nt	nt	+(#)	nt
Partridge 2	<i>A. rufa</i>	Found dead	01/09/21	Male	Adult	–	–	–	+	nt	nt	nt	nt	+(#)	nt
Partridge 3	<i>A. rufa</i>	Found dead	28/09/21	Female	Juvenile	+	+	–	nt	nt	nt	nt	nt	–	nt
Partridge 4	<i>A. rufa</i>	Found dead	29/09/21	Female	Juvenile	+	+	–	nt	nt	nt	nt	nt	+(#)	nt
Corn Bunting	<i>E. calandra</i>	Found dead	09/10/21	Unknown	Unknown	–	–	–	+(#)	nt	nt	nt	nt	–	nt
Partridge 7	<i>A. rufa</i>	Found dead	13/10/21	Male	Adult	nt	nt	nt	nt	+	+	+	+	nt	+(#)
Partridge 8	<i>A. rufa</i>	Found dead	13/10/21	Male	Adult	nt	nt	nt	nt	+	+	+	+	nt	+(#)
Partridge 9	<i>A. rufa</i>	Found dead	13/10/21	Female	Juvenile	+(#)	+(#)	+	nt	nt	nt	nt	nt	+(#)	nt
Partridge 11	<i>A. rufa</i>	Found dead	14/10/21	Female	Juvenile	+	+	+	nt	nt	nt	nt	nt	+(#)	nt
Partridge 14	<i>A. rufa</i>	Live-trapped	03/10/21	Male	Adult	+	na	na	na	na	na	na	na	+(#)	na
Partridge 16	<i>A. rufa</i>	Live-trapped	03/10/21	Male	Adult	+	na	na	na	na	na	na	na	–	na
Partridge 33	<i>A. rufa</i>	Live-trapped	03/10/21	Male	Adult	+	na	na	na	na	na	na	na	–	na
Partridge 37	<i>A. rufa</i>	Live-trapped	03/10/21	Male	Juvenile	+	na	na	na	na	na	na	na	–	na

*+, positive; -, negative; nt, not tested; na, not applicable; # - samples for which successful sequences were obtained. 1- dRT-qPCR developed for the detection of Japanese Encephalitis virus and virus from the Ntaya serocomplex (108 nt-long sequences obtained from Partridge 9 and corn bunting showed 100% similarity with BAGV sequence HQ644143, position 10,510 to 10,680); 2- RT-qPCR developed for the specific detection of BAGV (not sequenced), 3- nested RT-PCR developed by Sánchez-Seco et al. (2005) for the detection of *Flavivirus* (110 nt-long sequences obtained from Partridge 1, 2, 4, 11 and 14 showed 99.1% similarity with BAGV sequence HQ644143, position 8,996 to 9,139); 4- RT-PCR, developed by the INIAV team (unpublished, 171 nt-long sequences obtained from Partridge 7 and 8 showed 98.8% similarity with BAGV sequence HQ644143). Sizes of the sequences do not include the primers' annealing sequences. HQ644143 is the reference BAGV genome strain from the 2010 outbreak in Spain.