

Flaviviruses in Game Birds, Southern Spain, 2011–2012

To the Editor: Certain arthropod-borne epornitic flaviviruses, namely, West Nile virus (WNV) and Usutu virus (USUV), have spread recently in parts of Europe (1,2). In southern Spain, the emergence of a third virus of this type, known as Bagaza virus (BAGV), is of concern (3). Because of the outbreaks in 2010

in Cádiz (southern Spain) of WNV infection, which affected birds, horses, and humans, and of BAGV infection, which affected game birds (partridges and pheasants), and the reported presence of USUV in mosquitoes in this area (4), a surveillance program was implemented in partridges and pheasants during the next hunting season (October 2011–February 2012) to assess the possible circulation of these 3 flaviviruses in the area.

Serum samples and brain tissue from 159 hunted-harvested wild red-legged partridges (*Alectoris rufa*) and

13 common pheasants (*Phasianus colchicus*) were collected on 12 hunting properties from Cádiz (online Technical Appendix Figure, wwwnc.cdc.gov/EID/article/19/6/13-0122-Techapp1.pdf). All sampled birds were reared and shot in the wild. The age of the partridges was determined according to plumage characteristics.

Presence of antibodies against WNV was tested with a commercial epitope-blocking ELISA (Ingezym West Nile Compac, INGENASA, Madrid, Spain) (5). Virus-neutralization titers against WNV (strain

Table. Results of serologic studies in red-legged partridges and common pheasants, southern Spain, 2011–2012*

Species, no.	Age	ELISA, WNV	VNT titers†			Interpretation‡
			WNV	BAGV	USUV	
Partridges (<i>Alectoris rufa</i>), n = 159						
6	Juvenile	+	10–20	≤5	≤5	WNV
7	Adult	+	10–20	≤5	≤5	WNV
1	Adult	+	160	20	20	WNV
4	Not determined	+	10–80	≤5	≤5	WNV
1	Not determined	+	80	≤5	10	WNV
2	Juvenile	+	10	40	≤5	BAGV
1	Juvenile	+	20	80	10	BAGV
1	Juvenile	–	≤5	160	10	BAGV
2	Adult	+	20, 40	80, 320	10	BAGV
1	Adult	+	≤5	20	≤5	BAGV
1	Adult	+	≤10‡	40	≤10‡	BAGV
1	Adult	–	≤5	40	≤5	BAGV
1	Adult	+	≤5	≤5	40	USUV
2	Juvenile	+	10, 20	20, 40	≤5	Flavivirus
3	Adult	+	≤5	≤5	≤5	Flavivirus
2	Adult	+	20, 80	80, 160	40	Flavivirus
1	Adult	+	20	≤5	10	Flavivirus
1	Adult	+	160	≤20‡	80	Flavivirus
1	Adult	+	40	40	≤10‡	Flavivirus
1	Adult	–	≤10‡	20	≤10‡	Flavivirus
3	Not determined	+	≤5	≤5	≤5	Flavivirus
1	Not determined	+	≤5	10	≤5	Flavivirus
Pheasants (<i>Phasianus colchicus</i>), n = 13						
1	Adult	+	80	20	10	WNV
2	Not determined	+	10, 20	≤5	≤5	WNV
2	Adult	+	10, 80	80, 640	20	BAGV
1	Adult	–	≤5	20	≤5	BAGV
3	Adult	+	20–40	20–160	40–160	Flavivirus
1	Adult	+	10	≤5	≤5	Flavivirus
1	Adult	–	≤5	10	≤5	Flavivirus
1	Adult	Not determined	≤20‡	≤20‡	40	Flavivirus
Positive, no. (%)						
Partridges			40 (25)	31 (19)	17 (11)	11 (7)
Pheasants			9 (69)	9 (69)	8 (62)	7 (54)
Total			49 (29)	40 (23)	25 (15)	18 (10)

*WNV, West Nile virus; VNT, virus neutralization test; BAGV, Bagaza virus; USUV, Usutu virus; +, positive; –, negative. **Boldface** indicates VNT-positive serum.

†Serum samples were titrated from 5 to 1,280 dilutions and neutralization titers of ≥10 were considered positive.

‡Differentiation was based on comparison of VNT titers obtained in parallel against the 3 flaviviruses: the neutralizing immune response observed was considered specific when VNT titer for a given virus was ≥4-fold higher than titers obtained for the other viruses; samples showing VNT titer differences <4-fold between the viruses examined were considered positive for flavivirus but not conclusive for any specific virus.

‡VNT at the indicated (or lower) dilution(s) could not be determined because of cytotoxic effect caused by the sample.