

Short Communications

Targeted surveillance for Usutu virus in British birds (2005–2011)

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The geographical range of many arboviruses is expanding, with viruses of the genus *Flavivirus* comprising some of the most widely reported examples. Most concerning is the ability of these viruses to evolve rapidly to exploit new ecological niches, leading to large-scale disease outbreaks (Vazquez and others 2011).

Usutu virus (USUV) is a mosquito-borne flavivirus that illustrates this changing threat. When USUV was first isolated from mosquitoes (*Culex univittatus*) in South Africa in 1959 it was not associated with disease in animals or humans (Vazquez and others 2011). However, when USUV emerged in Austria in 2001 it caused seasonal epidemic mortality of blackbirds (*Turdus merula*) that continued into the summers of 2002 and 2003 (Chvala and others 2007). Subsequent spread of USUV associated with wild and captive bird mortality, has occurred in continental Europe, with a northward-range expansion to south-west Germany in 2011 (reviewed by Becker and others 2012). USUV has also been associated with human disease, confirming its zoonotic potential (reviewed by Vazquez and others 2011). This rapid spread, combined with the public and animal health implications, highlights the need for vigilance for this virus.

Mosquito species involved in USUV outbreaks in Europe are present in the UK (Medlock and others 2005) but minimal surveillance for USUV has been undertaken. Serological evidence for exposure to USUV in British birds was reported in 2001–2002 (Buckley and others 2003), but no virus was isolated and no associated bird mortality was reported. In a random sample of 160 bird brains collected in 2001–2009, and tested using a pan-flavivirus RT-PCR assay, no USUV was detected (Johnson and others 2010). Although UK government-funded surveillance schemes for avian influenza and West Nile virus are designed to detect and investigate wild bird mortality (Brugman and others 2012), they do not currently include surveillance for USUV. Here, we have undertaken retrospective targeted surveillance for USUV in England and Wales.

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A total of 1623 birds were examined postmortem between 2005 and 2011, submitted from a total of 956 sites with a widespread distribution across England and Wales. A subset of birds was selected for USUV testing, using the following risk criteria from USUV epizootics in continental Europe. Birds that died between 1st March and 30th November 2005–2011 (to include the period of peak vector abundance) and were (1) species known to be susceptible to USUV infection, including residents and summer migrants; (2) birds of other species submitted with either splenomegaly or hepatomegaly or (3) birds of other species with traumatic injuries (due to the possibility of neurological disease precipitating trauma). A further random sample of birds (n=51) of a variety of species was selected, particularly from southeast England and East Anglia due to their geographical proximity to continental Europe.

Tissue samples of one or more of brain, spleen, liver and kidney (3–5 mg) were pooled for each bird and homogenised. RNA was extracted from clarified lysates either manually (RNeasy Protect Mini procedure, Qiagen) or automatically (RNeasy Mini QIAcube kit, Qiagen), following the manufacturer's protocols. Extracted RNA was quantified and stored at –70°C. All samples were tested in duplicate for the presence of USUV RNA using a Sybr-based pan-flavivirus PCR assay, as previously described (Johnson and others 2010).

A total of 201 cases were selected for USUV testing (Table 1). Hepatomegaly and/or splenomegaly were present in 12 per cent (24/201) of cases, and trauma was considered to be the cause of death in

TABLE 1: Total number of dead birds from England and Wales, tested for Usutu virus 2005–2011 (by species)

Common name	Latin name	Number of birds (sites)
Blackbird*	<i>Turdus merula</i>	41 (33)
House sparrow*	<i>Passer domesticus</i>	15 (12)
Robin*	<i>Erithacus rubecula</i>	15 (11)
Greenfinch*	<i>Carduelis chloris</i>	14 (14)
Song thrush*	<i>Turdus philomelos</i>	10 (7)
Barn swallow*	<i>Hirundo rustica</i>	8 (4)
Dunnock	<i>Prunella modularis</i>	7 (5)
Sparrowhawk	<i>Accipiter nisus</i>	7 (7)
Tawny owl	<i>Strix aluco</i>	6 (6)
Yellowhammer	<i>Emberiza citrinella</i>	6 (5)
Blackcap	<i>Sylvia atricapilla</i>	5 (5)
Goldfinch	<i>Carduelis carduelis</i>	5 (5)
Chaffinch	<i>Fringilla coelebs</i>	4 (4)
Common buzzard	<i>Buteo buteo</i>	4 (4)
Feral pigeon	<i>Columba livia</i>	4 (3)
Goldcrest	<i>Regulus regulus</i>	4 (4)
Starling*	<i>Sturnus vulgaris</i>	4 (4)
Blue tit*	<i>Parus caeruleus</i>	3 (3)
Carion crow	<i>Corvus corone</i>	3 (2)
Collared dove	<i>Streptopelia decaocto</i>	3 (3)
Great spotted woodpecker	<i>Dendrocopos major</i>	3 (2)
Long tailed tit	<i>Aegithalos caudatus</i>	3 (3)
Ring necked parakeet	<i>Psittacula krameri</i>	3 (3)
Rook	<i>Corvus frugilegus</i>	3 (2)
Great tit*	<i>Parus major</i>	2 (2)
Mistle thrush	<i>Turdus viscivorus</i>	2 (2)
Nuthatch*	<i>Sitta europea</i>	2 (2)
Redwing	<i>Turdus iliacus</i>	2 (2)
Siskin	<i>Carduelis spinus</i>	2 (2)
Spotted flycatcher	<i>Muscicapa striata</i>	2 (1)
Wren	<i>Troglodytes troglodytes</i>	2 (2)
Barn owl	<i>Tyto alba</i>	1
Coal tit	<i>Periparus ater</i>	1
Green woodpecker	<i>Picus viridis</i>	1
Jackdaw	<i>Corvus monedula</i>	1
Lesser redpoll	<i>Carduelis flammea</i>	1
Marsh tit	<i>Parus palustris</i>	1
Redstart	<i>Phoenicurus phoenicurus</i>	1

*Species reported susceptible to USUV in Europe (Chvala and others 2007, Vazquez and others 2011, Becker and others 2012)

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38 per cent (76/201) of cases. Pooled tissue samples from all 201 birds tested were negative for USUV using RT-PCR (Johnson and others 2010). All positive controls showed unambiguous amplification plots and dissociation curves.

No evidence of USUV infection was detected in the 201 wild birds tested in this study. Traditional sample-size-based approaches to proving absence of infection are often not applicable to wildlife diseases due to the difficulty in satisfying inherent assumptions regarding homogeneity of populations, random sampling and consistent disease prevalence. The non-random approach used here, where surveillance is targeted at disease detection, has been effectively used for other wildlife diseases (Walsh and others 2010). A feature of the majority of USUV outbreaks in Europe has been mortality in wild birds, with certain species and lesions over-represented. Therefore, we focused on susceptible species and birds with suspect pathological features. Although cost-effective, this approach is dependent on the reporting of wild bird mortality which is, in turn, dependent on numerous factors that cannot easily be quantified, including bird and human population densities and public vigilance. The Garden Bird Health initiative, however, has successfully identified outbreaks of other emerging infectious diseases of garden birds (eg, Robinson and others 2010).

An additional limitation to the approach used here is the assumption that USUV will cause detectable disease. Since its emergence in Austria in 2001, bird mortality due to USUV has apparently declined while seroprevalence has increased, suggesting continued circulation and the development of herd immunity (Chvala and others 2007). Establishing whether this zoonotic virus could already be circulating in the UK without detectable bird mortality has immediate implications for human blood donor schemes and would help guide public health policy. Detecting an infection circulating in wildlife without clinical effect, however, can be challenging. Serological assays and age-related seroprevalences can be used (eg, Buckley and others 2003) but interpretation is limited by cross-neutralisation between related viruses (Mansfield and others 2011), and a lack of agreement on cut-offs for positive results. Hence, our results are not a priori a contradiction of previous evidence for USUV antibodies in birds in the UK (Buckley and others 2003), but further work is required to confirm the presence of this virus in the UK.

Assuming that Great Britain is USUV-free, and that incursion would cause bird mortality as it has in Europe, prospective surveillance for the detection of USUV should focus on blackbirds, a highly visible and common garden visitor, which has experienced the greatest mortality in USUV outbreaks in continental Europe (Chvala and others 2007, Vazquez and others 2011, Becker and others 2012). Furthermore, a weighted surveillance approach could be developed, following the model adopted for chronic wasting disease detection in the USA to maximise cost-effectiveness (Walsh and others 2010). The potential importance of captive avian collections, particularly raptors, for surveillance should also be emphasised since captive birds have featured in the majority of European outbreaks, for example, (Becker and others 2012).

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Competing interests None.

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