Epidemiol. Infect. (2013), **141**, 1134–1142. © Cambridge University Press 2012
doi:10.1017/S095026881200177X

Epidemiological perspectives on West Nile virus surveillance in wild birds in Great Britain

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Received 7 March 2012; Final revision 20 July 2012; Accepted 24 July 2012; first published online 5 September 2012

SUMMARY

West Nile virus (WNV) is a zoonotic arthropod-borne pathogen with continued geographical expansion in Europe. We present and evaluate data on the temporal, spatial and bird species focus of the WNV surveillance programme in dead wild birds in Great Britain (2002–2009). During this period all bird samples tested negative for WNV. Eighty-two per cent of the 2072 submissions occurred during the peak period of vector activity with 53% tested during April–July before human and equine infection would be expected. Samples were received from every county, but there was significant geographical clustering (nearest neighbour index = 0·23, $P < 0{\cdot}001$). Over 240 species were represented, with surveillance more likely to detect WNV in resident bird species (92% of submissions) than migrants (8%). Evidence indicates that widespread avian mortality is not generally a reported feature of WNV in Europe and hence additional activities other than dead bird surveillance may maximize the ability to detect WNV circulation before the onset of human and equine infections.

Key words: Epidemiology, surveillance, veterinary epidemiology, West Nile virus, zoonoses.

INTRODUCTION

West Nile virus (WNV) is a globally important arthropod-borne flavivirus of the Japanese encephalitis antigenic complex which is maintained in enzootic transmission cycles between birds and mosquitoes principally of the genus *Culex* [1]. Public and animal health concerns lie with the incidental infection of humans and horses that may lead, in a small proportion of cases, to neuroinvasive disease [2].

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Following an apparent expansion in range across the Americas and Europe that began in the 1990s, WNV is now the world's most widely distributed arbovirus (arthropod-borne virus) [3]. The number of human and equine WNV outbreaks reported in Europe has increased over the past decade [4], and phylogenetic studies on WNV strains isolated from regions of Greece, Italy, Spain, Portugal, France and Romania indicate that the virus has become established in Europe [4–6].

Planning for WNV surveillance and response to outbreaks is complicated by a relatively poor knowledge of the relationship between virus, multiple vectors, hosts, and the environment [3]. Apparent

differences in WNV disease ecology between North America and Europe, which may be due to differences in historical exposure of wild bird populations to WNV, also may confound the interpretation of previous studies [3]. Birds are the primary reservoir hosts for WNV but vary in their susceptibility to infection [7]; infection in European birds has not usually been associated with the substantial detectable mortality that characterizes outbreaks in the USA; however, die-offs of wild birds have been observed [8]. Evidence indicates the incursion of WNV into Europe is most probably occurring through the migratory movements of birds (reviewed in [4]); however, molecular genetic studies carried out on a US WNV vector, *Culex tarsalis*, suggest mosquito movements could act as a means of local virus dispersal in the USA [9]. Moreover, other routes of possible introduction of infected mosquitoes to Europe such as translocations of vectors in aircraft have been highlighted as an additional risk to Great Britain (GB) [10].

Global climate change leading to warmer and wetter conditions in parts of Europe may facilitate the establishment of WNV in new areas through the range expansion and seasonal abundance of vector species, and by directly increasing competence for transmission [11]. Alternatively, it could improve virus survival in overwintering mosquitoes [12]. Warmer and wetter conditions have been implicated in facilitating recent WNV outbreaks in both Greece [13], and Romania [14].

Surveillance for human cases of WNV in GB has taken place every year between 1 June and 31 October since 2002 [15]. While WNV infection has not been reported from indigenous cases of humans or horses in GB, some evidence of exposure to WNV, or closely related viruses, has been reported in British migratory and resident birds [16, 17]. Conditions in GB are such that they have the potential to support the introduction and subsequent establishment of WNV transmission and possibly that of other flaviviruses. Thirty-four species of mosquito have been reported in GB [18], several of which have been identified as potential vectors of WNV according to their host preference and known vector status in Europe and elsewhere [19, 20]. Each year birds travel on migration between GB and areas of Africa where WNV is likely to be endemic, and therefore may import the virus when they return. Since 2001, in light of the ongoing threat posed by WNV in Europe, WNV surveillance has been undertaken in GB, using an approach primarily based on WNV dead bird (passive) surveillance [21].

Here we present and evaluate data on WNV surveillance in wild birds in GB between 2002 and 2009 and identify potential improvements. We consider the bird species sampled and the spatio-temporal pattern of sampling, with comparison to available data on candidate mosquito vectors for WNV in GB. These data are discussed in view of accumulating knowledge of the changing epidemiology of WNV in Europe, and reported surveillance activities.

MATERIALS AND METHODS

Surveillance for WNV in wild birds in GB has been undertaken since 2001, through the AHVLA Diseases of Wildlife Scheme and additionally through the GB Wildlife Disease Partnership. Carcasses of dead wild birds have been submitted to AHVLA regional Laboratories in England and Wales, Scottish Agricultural College (SAC) laboratories and other non-governmental partner organizations (details available on the GB Wildlife Surveillance Partnership website [22]). Sources include wildlife hospitals, conservation organizations, gamekeepers, farmers, private veterinary practices, zoos, and the general public. Surveillance was targeted at species associated with WNV mortality, including corvids, sparrows and other small passerine species, raptors and water birds and has included samples from mass mortalities of wild birds and those showing neurological signs. Reverse transcription–polymerase chain reaction (RT-PCR) and virus isolation (VI) testing on brain and kidney samples taken at post mortem were undertaken, as previously reported by Phipps *et al.* [21]. Briefly, organ homogenates were prepared in tissue culture medium and used to inoculate Vero C1008 cells (ATCC). Two passages were undertaken and virus was detected on development of cytopathic effect. Total RNA was extracted from tissue using the RNeasy column method (Qiagen, UK). Nested RT–PCR was performed as described previously [23] with positive samples being visualized by agarose gel electrophoresis.

Information on the species of bird, sampling dates and locations was collated from sample submission documents and diagnostic reports for this analysis. Birds were classified into species groups and their migratory status determined. Results for the Carrion Crow (*Corvus corone*), and Hooded Crow (*Corvus cornix*), were combined for the purposes of the

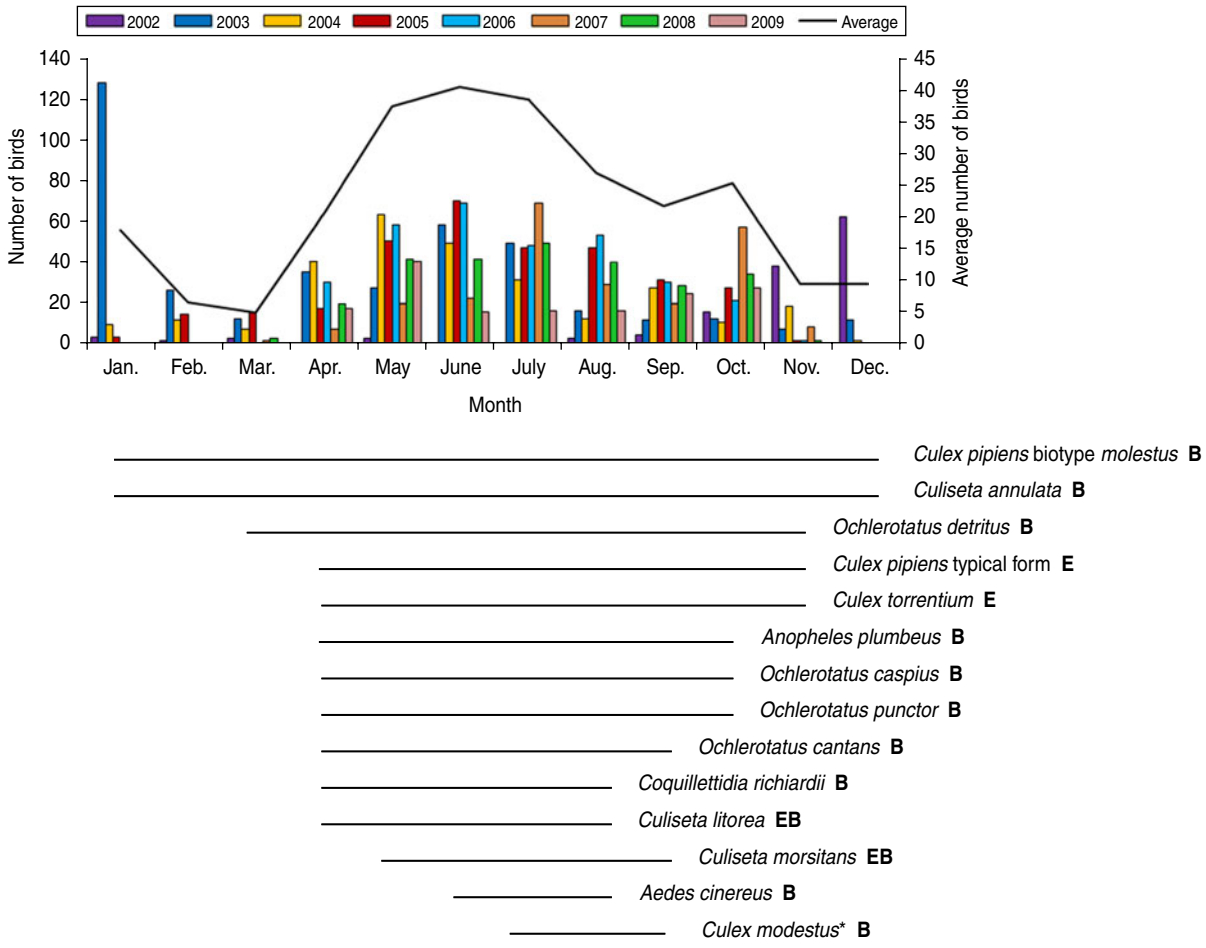

Fig. 1 [*colour online*]. The number of birds received for West Nile virus (WNV) testing per month, in each year from 2002 to 2009 (bars), and the monthly average across the years (line), above the reported seasonal adult activity for 14 candidate WNV vectors and their potential role as enzootic vectors (E), bridge vectors (B), or both (EB). * Information inferred from European studies [25], due to insufficient GB data.

analyses due to lack of sufficient distinguishing information in submission reports. A total of 14 candidate vectors for WNV were identified that have sufficient populations in GB and a role in WNV transmission elsewhere. These were taken from detailed assessments of candidate WNV vectors in GB that were conducted previously [19, 20], with the addition of *Culex modestus*, which was previously considered too rare in GB to be involved in WNV transmission until the recent discovery of a population in the North Kent marshes [24]. Information was obtained on the vectors' adult seasonal activity and role as enzootic vectors (bird–bird), bridge vectors (bird–human/horse), or both (summarized in Fig. 1). As little published information on the ecology of *Cx. modestus* in GB exists, its adult seasonal activity was inferred from European studies [25]. The geographical distribution of samples was assessed using the ArcGIS Desktop 9.2 platform (ESRI ArcGIS, UK) and distribution maps were produced using this program by linking samples to the county in which they were taken, and by kernel density analysis to show relative sampling intensity across GB. Sample location information to at least town level was provided for 77% of submissions ($n=1595$), with the remaining 23% ($n=477$) identified only to the level of county or containing no location information at all. In these cases, the coordinates of the nearest town, or if not available, of the submitting laboratory, were used for the purposes of the analysis. The analysis was also undertaken with the omission of submissions identifiable only to the location of the submitting laboratory. Nearest-neighbourhood analysis was performed in ArcGIS to statistically test the spatial distribution of sample locations collated from all years, for all samples and omitting samples identified only to county/laboratory

https://doi.org/10.1017/S095026881200177X Published online by Cambridge University Press

Table 1. *The number* (*percentage*) *of birds in the most frequently sampled avian families in WNV surveillance 2002–200*

Family	GB species (common names)	Number (%)
Corvidae	Crows, ravens, magpies	473 (23)
Anatidae	Ducks, geese, swans	397 (19)
Fringillidae	Finches	284 (14)
Columbidae	Pigeons, doves	197 (10)
Accipitridae	Hawks, eagles, kites	112 (5)
Laridae	Gulls	102 (5)
Sturnidae	Starlings	68 (3)
Turdidae	Blackbirds, thrushes	65 (3)
Alcidae	Puffins	54 (3)
Passeridae	House sparrows	50 (2)
Phasianidae	Pheasants, partridges	48 (2)
Falconidae	Falcons, kestrels	34 (2)
Hirundinidae	Swallows, house martins	27 (1)
Tytonidae	Barn owls	16 (1)
Strigidae	Little owls	12 (1)

level. This analysis produced a nearest neighbour index (NNI), a ratio of the observed distance between neighbouring points (sampling locations) divided by the expected distance (from a hypothetical random distribution) between neighbours. A NNI of <1 indicated a clustered pattern of points and a value >1 indicated that the points were dispersed. A Z score (0·01 significance) was then calculated to assess the significance of these data.

RESULTS

In the period between January 2002 and December 2009, a total of 2072 birds were tested as part of WNV surveillance in GB, with a mean annual total of 259 birds. All samples tested were negative for WNV using both testing methods; results from 2002 to 2006 previously reported in Phipps *et al.* [21]. There was some variation in the numbers of birds tested per year: 2002 (129), 2003 (392), 2004 (278), 2005 (322), 2006 (310), 2007 (231), 2008 (255), 2009 (155). Birds tested represent 19 orders, 43 families and at least 244 species. Table 1 provides details of the most frequently sampled Families. The most sampled orders were the Passeriformes (49% of submissions), Anseriformes (19%) and Columbiformes (10%). The most sampled families were the Corvidae (23%), Anatidae (19%) and Fringillidae (14%). The most sampled individual species over the 8-year period were British residents: the Mute Swan (*Cygnus olor*, 9%, $n=167$), Magpie (*Pica pica*, 8%, $n=149$), Crow (*Corvus corone*/*C. cornix*, 8%, $n=146$), Greenfinch, *Carduelis chloris* (7%, $n=142$) and Rock Pigeon (*Columba livia*, 6%, $n=114$). Overall, British resident birds represented 92% ($n=1911$) of submissions, while migratory species accounted for only 8% ($n=161$) of submissions. Twenty-three species with reported international migratory behaviour were sampled (Supplementary Table S1), ten of which are classed as 'summer visitors' arriving in GB from Africa to breed in late spring/early summer.

The distribution of submissions received each month between 2002 and 2009 was observed to be strongly associated with the reported seasonal activity of 14 candidate mosquito vectors for WNV in GB (Fig. 1). The majority of submissions tested (82% overall) fall within the assumed period of peak vector activity (April–October) as targeted [21]. The submissions during this period increased annually, with 100% of samples falling within this period in 2009 (Supplementary Table S2).

Submissions were received from every GB county between 2002 and 2009 but the number of submissions from each varied considerably (Fig. 2*a*). Mapping samples according to the density of submissions (Fig. 2*b*) indicated significant clustering of samples to certain locations across the 8-year period, and this was confirmed by nearest neighbour analysis (NNI $=0{\cdot}23$; Z score $=-67{\cdot}48$, $P<0{\cdot}001$). The omission of submissions identifiable only to the location of the submitting laboratory resulted in significant clustering (NNI $=0{\cdot}25$; Z score $=-58{\cdot}31$, $P<0{\cdot}001$) indicating that these samples alone were not responsible for skewing the distribution of submission data.

DISCUSSION

While some evidence of exposure to WNV, or closely related viruses, has been reported in British migratory and resident birds [16, 17], the surveillance data reported in this study did not find any evidence of WNV infection in wild British birds. Furthermore, panflavivirus screening of 160 bird and 1000 mosquito samples collected in GB was negative for WNV [26]. Additionally, no horses have been reported with WNV in GB and no GB-acquired human infections have been reported [15, 21]. Although each year many thousands of birds return from their wintering sites in Africa to breed in GB, with the risk of importing a WNV infection that could be transmitted by

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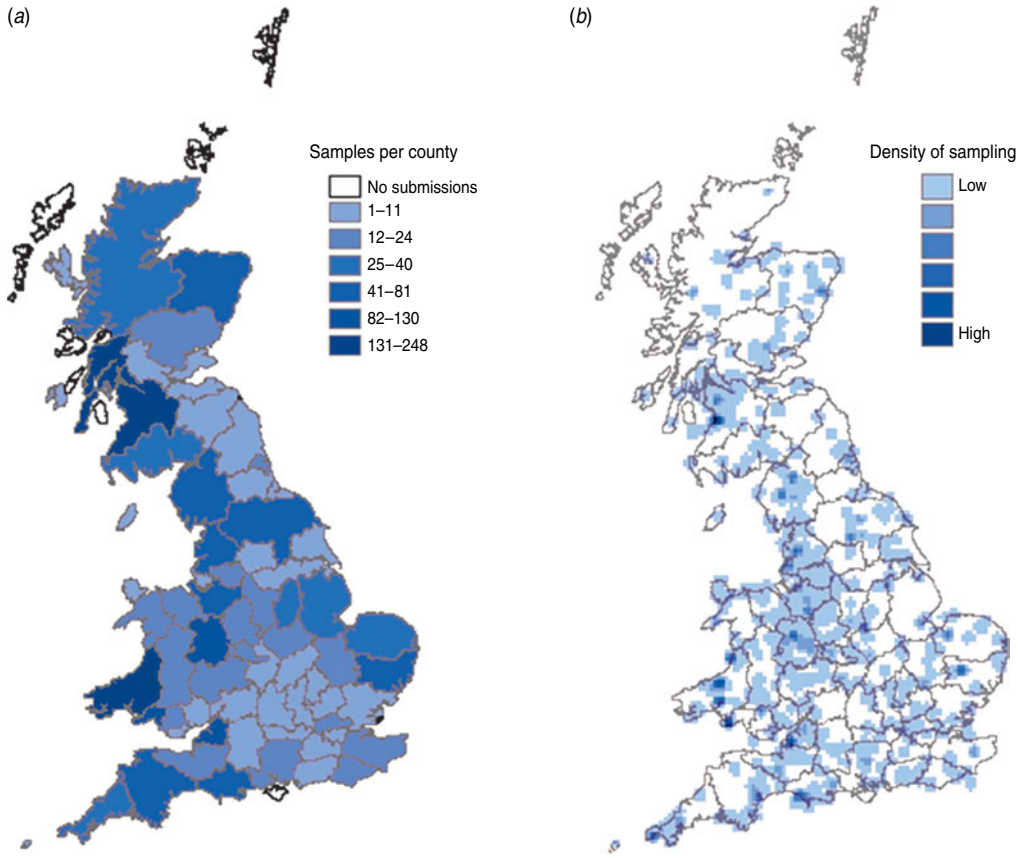

Fig. 2 [*colour online*]. (*a*) Number of samples received per county in GB, 2002–2009. (*b*) Kernel density analysis indicating the relative intensity of dead bird submissions tested for WNV 2002–2009 across GB.

mosquitoes in GB, a 2009 risk assessment suggested that the likelihood of this occurring was very low [27]. However, recent evidence from Italy and Greece suggests that outbreaks of WNV were being observed with increasing frequency in Southern Europe and that WNV may have become endemic in this area [4–6]. In addition lineage 2 strains, previously not associated with neuroinvasive disease, have been identified as the cause of fatal infection in humans [28] and horses [29], and viral adaptation for increased pathogenicity and virulence may be occurring [13]. The evolving epidemiological picture of WNV in Europe indicates that the risk of WNV incursion to GB remains real and may increase in the future, and suggests that it is prudent to regularly review surveillance activities to ensure their continued efficacy.

During the years evaluated there was considerable variation in the numbers of birds sampled from different orders. That the Passeriformes were the most sampled was perhaps unsurprising as birds of this order represent approximately half of extant bird diversity worldwide, including in GB [30]. Despite this, the Mute Swan (*Cygnus olor*, order Anseriformes), was the most sampled species. This is likely to be a result of convenience testing of samples submitted under the GB Avian Influenza Wild Bird Surveillance Project [31] and the large size and high visibility of swan carcasses. Corvidae such as the magpie (*Pica pica*), have remained the focus of bird surveillance in Italy (number tested 607, positive 4·4%), but species in other orders sampled less frequently, such as the Charadriiformes (gulls, waders, auks), had a higher prevalence of infection (number tested 8, positive 38%) albeit with wider confidence intervals due to smaller sample sizes [32]. Calistri *et al*. [33] reported high prevalence of WNV by RT–PCR in magpies, carrion crows and rock pigeons, which are among the most sampled species in GB surveillance.

Evidence in support for the role of migratory birds in the translocation of WNV into and around Europe is compelling (reviewed in [4]). Birds that return to GB to breed in spring/summer ('summer visitors') from international wintering grounds may be the most likely to successfully introduce a WNV transmission cycle, as adults of the majority of candidate vector species present are active at this time (Fig. 1). Ten

https://doi.org/10.1017/S095026881200177X Published online by Cambridge University Press

summer visitor species with an overwintering ground in Africa were tested under WNV surveillance (Supplementary Table S1). However this comprised a total of 44 birds sampled and therefore there is insufficient data to draw conclusions on the risk of WNV incursion via bird migration. If resident (i.e. non-migratory) populations of bird species in GB [e.g. Eurasian Sparrowhawk (*Accipiter nisus*), Carrion Crow, Magpie, Mute Swan] are naive to WNV infection they may be more likely to experience increased mortality than migratory birds should a WNV incursion occur. Hence species such as these may be the most appropriate to target in surveillance of birds found dead, particularly if the surveillance programme targets clusters of mortality (i.e. die offs).

The focusing of surveillance effort between April and October is appropriate as it coincides with the presence of the adults of 14 candidate vectors in GB. These include two species considered to be major European vectors of WNV: *Cx. pipiens* typical form and *Cx. modestus*, implicated in enzootic and bridge transmission, respectively [25, 34]. Overall, 53% of samples were tested between April and July (Supplementary Table S2), with a general trend of the highest number of samples submitted during May, June and July (Fig. 1). This is before most human or equine cases would be expected to occur based on evidence from European outbreaks [4], and surveillance of dead birds during this period may provide 'early warning' of such spillover infection.

Dead bird submissions were received from every GB county over the 8 years of surveillance analysed, showing broad geographical coverage (Fig. 2*a*). However, there was significant clustering of samples to certain locations across GB (NNI = 0·23, $P < 0{\cdot}001$). This is primarily due to multiple submissions being received from organizations such as wildlife rehabilitation centres rather than due to any deliberate geographical targeting of surveillance. Outbreaks of WNV in Europe most frequently occur in close proximity to wetland or marshy areas supporting large populations of competent mosquito vectors (primarily *Culex* spp.), large densities of wild birds, and serving as breeding or aggregation sites for migratory species [4]. Accordingly, analogous wetland areas in GB may be potential foci of WNV introduction and subsequent spread.

Despite good evidence that dead bird mortality, particularly of corvids, provides an effective early warning system for the onset of spillover WNV activity in the USA [35], the use of dead bird surveillance in Europe has been suggested to be less valuable, on the basis that noticeable bird mortality has not been a notable reported feature of most outbreaks of WNV in Europe. Detection of an increase in mortality of dead birds was also not observed in association with a recent WNV outbreak in Australia [36]. However, certain European bird species have been shown susceptible to experimental WNV infection [37], bird mortality has been seen in WNV outbreaks in Europe [8] and may be increasing [4], and the susceptibility of birds in different regions of Europe is unknown. Furthermore, the detection of mortality in wild populations depends upon numerous factors including species and environmental variables. For example, a simulated mortality event using 1-day-old chicks in grazed grassland habitat showed systematic searching grossly underestimated the total mortality, indicating that some mortality events of small bird species may not be detected by surveillance activities [38].

While evidence exists that increased mortality of wild birds may occur in European WNV outbreaks [4], testing dead birds for WNV remains a pivotal component of an early warning system for WNV disease in horses and humans. Dead bird surveillance has the added advantage that it can be logistically and financially synergistic with surveillance programmes for other diseases, and sampling on a convenience basis by co-sampling with other surveillance programmes, for example for avian influenza [39] or the Garden Bird Health Initiative, could offer added value given limited resources.

The apparent absence of WNV from GB [15, 21] would appear to suggest that surveillance could provide an early warning system for increased threat of human or equine disease. This could facilitate implementation of government WNV contingency plans to minimize the impact of an incursion. Evidence from recent European outbreaks suggests that a surveillance system that includes avian, equine and vector surveillance can detect WNV circulation up to 3 weeks before the onset of human cases [32], and therefore may be more sensitive than dead-bird scanning surveillance alone.

Serological sampling of birds is labour and resource intensive, but may be more sensitive than dead bird testing given the apparent low mortality in European birds and could also provide valuable data on the ecology of vector-borne diseases in birds. Results from serological testing could help focus dead bird surveillance geographically and temporally [40]. However there is potential complication of

cross-reactivity with co-circulating antigenically related viruses which could lead to difficulty in accurate diagnosis of virus-specific response. For these reasons serological sampling of birds has not been routinely undertaken in GB. Interpreting previous serological evidence [16, 17] suggesting a low level of enzootic WNV circulation in England is complicated by a lack of consensus on the level of neutralization required for a positive test and the absence of virus detection. If WNV is, or has been circulating in GB, and resident birds may have a degree of acquired immunity, this would have direct impact and implications for control strategies. The application of serological surveillance of wild birds in GB could therefore be a sensitive method to identify exposure to WNV in resident birds following incursion and local circulation, and contribute valuable information for the targeting of future surveillance activities by identification of migratory species that have been exposed to WNV elsewhere.

The GB equine population is widely distributed, closely observed and accessible [41]. Passive surveillance could be enhanced throughout the vector activity season by increased communication with first-line clinicians and testing of suspect cases. Active surveillance via seasonally focused serological testing of naive sentinel horses could also be implemented as performed in other European counties. Capture and testing of mosquitoes is time and resource intensive but has proved useful, particularly for public health risk management during outbreaks of WNV and other arboviruses that may pose a threat to GB [34, 42]. The value of capture and identification of mosquitoes is highlighted by the recent discovery in GB of populations of *Cx. modestus*, a major WNV vector in Europe [24], suggesting that changes in the mosquito fauna of GB may be occurring.

The epidemiological situation with respect to WNV has changed during the timespan of this surveillance programme in wild birds and hence regular evaluation of current and future activities is appropriate. At the commencement of the WNV surveillance plan in GB, there was a limited understanding of the ecology of WNV in Europe, with most data available from outbreak and experimental studies in the USA. There is a growing body of evidence from Europe that WNV has become established and is continuing to increase its distribution across Europe and Asia, and that the virus may be undergoing genetic changes resulting in increased virulence. Evidence shows that wild bird mortality is not a common feature of WNV outbreaks in Europe in contrast to observations in North America. A surveillance system that integrates current methods of dead bird surveillance with serological sampling of birds and horses, and includes vector surveillance, may provide both a more sensitive method for detecting a viral incursion and information that could inform policy and control strategies, not only for WNV but for other exotic mosquito-borne flaviviruses that could be introduced into Europe.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S095026881200177X.

ACKNOWLEDGEMENTS

The authors thank Fiona McCracken and Claire Jeffries (AHVLA) for technical assistance; David Rae, Adnan Younas and Peter Sewell at the AHVLA for their assistance with GIS analysis; Felicity D'Mello and Ruby Chang (Royal Veterinary College) for guidance and useful discussion; Kim Stevens (Royal Veterinary College) for providing the base map used in the GIS analysis; and Jolyon Medlock (HPA) for advice and granting access to information from the Mosquito Recording Scheme. Some of this work was completed in part fulfilment of the M.Sc. in Control of Infectious Diseases in Animals at the Royal Veterinary College.

D.L.H., L.P.P., N.J., A.R.F. and A.C.B. were funded by the UK Department for Environment, Food and Rural Affairs (Defra) Projects SV3040 and SE0530. The work published in this article has received partial funding from the European Commission Seventh Framework Programme under ANTIGONE with project number 278976.

DECLARATION OF INTEREST

None.

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