Biological Conservation 165 (2013) 79–85

Contents lists available at SciVerse ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon

Predicted and observed mortality from vector-borne disease in wildlife: West Nile virus and small songbirds

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ARTICLE INFO

Article history:
Received 29 April 2013
Accepted 20 May 2013

Keywords:
Disease
Population
Birds
Epidemiology
West Nile virus
Conservation

ABSTRACT

Numerous diseases of wildlife have recently emerged due to trade and travel. However, the impact of disease on wild animal populations has been notoriously difficult to detect and demonstrate, due to problems of attribution and the rapid disappearance of bodies after death. Determining the magnitude of avian mortality from West Nile virus (WNV) is emblematic of these challenges. Although correlational analyses have shown population declines coincident with the arrival of the virus, strong inference of WNV as a cause of mortality or a population decline requires additional evidence. We show how integrating field data on mosquito feeding patterns, avian abundance, and seroprevalence can be used to predict relative mortality from vector-borne pathogens. We illustrate the method with a case study on WNV in three species of small songbirds, tufted titmouse (*Baeolophus bicolor*), Carolina wrens (*Thryothorus ludovicianus*), and northern cardinals (*Cardinalis cardinalis*). We then determined mortality, infectiousness, and behavioral response of wrens and titmouse following infection with WNV in laboratory experiments and compared them to a previous study on WNV mortality in cardinals. In agreement with predictions, we found titmouse had the highest mortality from WNV infection, with 100% of 11 birds perishing within 7 days after infection. Mortality in wrens was significantly lower at 27% (3/11), but still substantial. Viremia profiles indicated that both species were highly infectious for WNV and could play roles in WNV amplification. These findings suggest that WNV may be killing many small-bodied birds, despite the absence of large numbers of dead birds being observed and testing positive for WNV. More broadly, they illustrate the utility of a framework for predicting relative mortality in hosts from vector-borne disease.

1. Introduction

The impact of disease on wild animal populations has been notoriously difficult to detect and demonstrate, due to problems of attribution and the rapid disappearance of bodies after death (McCallum, 2005; McCallum and Dobson, 1995). The clearest examples of disease-caused impacts on wildlife populations come from epidemics in large abundant animals such as anthrax and Rinderpest in African mammals (Holdo et al., 2009), experimental or purposeful viral introductions such as myxomatosis and Australian rabbits (Ratcliffe et al., 1952), and experimental studies that remove pathogens from hosts through treatment (Hudson et al., 1998). For many other diseases and populations, impacts are inferred from long term monitoring and observations of sudden declines, and in rare cases scientists have been able to observe a wave of mortality as a pathogen arrives (Hochachka and Dhondt, 2000; Kilpatrick et al., 2010; Langwig et al., 2012; Lips et al., 2006; Vredenburg et al., 2010). However, in many cases mortality due to disease is difficult to detect and even striking patterns, such as distributional limits coincident with disease boundaries, required experimental infection studies to confirm impacts of disease (e.g., avian malaria and Hawaiian birds; (Van Riper et al., 1986; Warner, 1968)).

A recent introduction of a pathogen to North America, *West Nile virus* (WNV; *Flaviviridae*; *Flavivirus*) in 1999, was also accompanied by waves of mortality in wild birds, with large numbers of dead American crows and Blue jays testing positive for WNV in the northeast USA (Bernard et al., 2001; Nemeth et al., 2007). A decade later, transmission still occurs annually in many bird communities throughout North and South America (Kilpatrick, 2011). Several retrospective analyses have shown population declines in birds coincident with the arrival of WNV as it spread south and west

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http://dx.doi.org/10.1016/j.biocon.2013.05.015

from New York, with impacts being largest on corvids (Hochachka et al., 2004; LaDeau et al., 2007; Wheeler et al., 2009). Evidence of WNV-caused mortality in corvids was also provided by experimental infection in laboratory studies (Komar et al., 2003; Reisen et al., 2005). However, evidence of WNV mortality in smaller passerines has been far sparser, with relatively few WNV-infected dead birds collected. The extent to which this is due to poor detectability (Ward et al., 2006) or lack of mortality is not clear.

Two families of small passerines that past studies have suggested may suffer population level impacts from WNV are Paridae (chickadees and titmouse) and Troglodytidae (wrens). Multiple studies have observed declines in one or more species in the family Paridae and Troglodytidae coincident with the arrival of WNV (Bonter and Hochachka, 2003; LaDeau et al., 2007), and several other studies have demonstrated feeding on parids and wrens by WNV mosquito vectors (Hamer et al., 2009; Hassan et al., 2003; Kilpatrick et al., 2006a). However, these data are only suggestive and supportive evidence in the form of WNV-infected dead chickadees, titmouse or wrens is mostly lacking.

The gold standard to determine whether a species suffers mortality from a pathogen, part of Koch's postulates (Koch, 1893), is through experimental infection. There are far too many species of birds in North America to do this for all taxa, and these studies cannot determine whether in fact birds are exposed to a pathogen in nature. For effective conservation planning, there is clearly a need for a framework to determine whether WNV and other vector-borne diseases cause mortality in small avian hosts, and other small wildlife species that are difficult to detect.

Here we describe how one can use field data on the transmission ecology of a vector-borne disease – specifically the feeding patterns of WNV mosquito vectors, avian abundance, and the WNV antibody prevalence of wild-caught birds – to generate hypotheses about differences in mortality from WNV infection between hosts. We illustrate this method with a study on three species of small songbirds, tufted titmouse (*Baeolophus bicolor*), Carolina wrens (*Thryothorus ludovicianus*), and northern cardinals (*Cardinalis cardinalis*). We generated and tested hypotheses about the relative mortality of three species and measured morbidity and mortality following experimental infection with WNV. Our experimental infection studies also provide data on infectiousness for WNV that can be integrated with the aforementioned data on mosquito preferences to determine the role of species in WNV transmission (Hamer et al., 2009; Kilpatrick, 2011; Kilpatrick et al., 2006a).

2. Methods

2.1. Framework for predicting relative host mortality from a vector-borne pathogen

This framework generates a prediction about the relative mortality from infection with a vector-borne pathogen between two or more species and can be applied to any vector-borne pathogen and host taxa with the data described.

The seroprevalence, S, or fraction of a population with antibodies against a pathogen at a point in time is equal to the fraction of the population exposed, e, multiplied by the probability of survival ($1 - m$, where m is the probability of mortality given infection), divided by the fraction of the original population size after exposure, ($e(1 - m) + 1 - e$):

$$S = e(1 - m)/(1 - em) \quad (1)$$

The fraction of the population exposed, e, will increase asymptotically with the average number of infective bites, I, each host receives (Smith et al., 2005):

$$e = 1 - (1 + I/k)^{-k} \quad (2)$$

where k is parameter describing the degree to which mosquitoes feed more on some individuals of a species than others (Dye and Hasibeder, 1986). Previous work suggests that in some populations k is approximately 0.25 (Smith et al., 2005). Simulations suggest that using $k = 0.25$ produces qualitatively correct predictions about which species suffers higher mortality as long as k is not too small (i.e. <0.1, as long as bites are not extremely concentrated on just a few individuals).

The number of bites that a subset of the population is exposed to will increase with the host utilization index (sometimes termed mosquito preference, forage ratio, or host selection index) of vectors, U, on that subpopulation, where the utilization index is the fraction of bloodmeals, b, from that subpopulation divided by the relative abundance of that subpopulation, a (e.g. the fraction of all hosts made up by a species):

$$U = b/a \quad (3)$$

Thus, if data on host utilization, U, and seroprevalence, S, is available for two or more species at the same site(s), they can be used to predict which species has a higher mortality probability, m, given infection. First, it is necessary to invert Eq. (2) and derive an approximate value of infective bites, I, using the measured seroprevalence, S:

$$I \cong k[(1/(1 - S))^{1/k} - 1] \quad (4)$$

where a value of $k = 0.25$ is often valid. We then computed the ratio(s) and confidence bounds of predicted mortality for each of the two or more species ($i = 1, 2, \ldots$):

$$m_1/m_2 \propto (U_1/U_2)/(I_1/I_2) \quad (5)$$

A ratio greater than one indicates that species 1 suffers higher mortality once infected than species 2. It is worth noting that the ratio derived cannot be used to estimate the exact mortality in a species due to the approximations made in Eq. (4), but it does indicate the relative difference in mortality (i.e., a larger ratio indicates a larger difference in mortality, all else being equal).

2.2. Sites

We determined mosquito feeding patterns, avian abundance, and WNV antibody prevalence in ~1 km diameter areas at three urban sites (Foggy Bottom, DC, Baltimore, MD, and the National Mall, DC), two residential sites (Takoma Park, MD and Bethesda, MD) and two park sites surrounded by residential development (Rock Creek Park Meadowside Nature Center in Rockville, MD, and Fort Dupont Park in southeast DC) (Kilpatrick et al., 2006a,b) from 2004 to 2008. Evidence of WNV transmission (infected mosquitoes or antibody-positive resident (non-migratory) hatch-year birds) was present at all sites except Rock Creek Park in 2005 (Kilpatrick et al., unpub. data).

2.3. Mosquito feeding patterns

We trapped mosquitoes at each site with at least eight CDC light traps, four CDC gravid traps and by aspirating the surfaces of vegetation with a large backpack mounted aspirator. Sites were trapped for two nights approximately every 2–3 weeks between May and mid-October each year. Mosquitoes were identified to species and all partially or fully engorged mosquitoes were stored in a freezer at −80 °C for subsequent host identification. We used PCR to molecularly identify engorged *Culex* mosquitoes to distinguish between *Cx. pipiens*, *Cx. restuans*, *and Cx. salinarius* (Crabtree et al., 1995). We only used data from *Cx. pipiens* or *Cx. restuans* to estimate feeding utilizations, because these two species have similar feeding patterns, whereas *Cx. salinarius* feeds on a very