Predicting Pathogen Introduction: West Nile Virus Spread to Galápagos

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Abstract: *Emerging infectious diseases are a key threat to conservation and public health, yet predicting and preventing their emergence is notoriously difficult. We devised a predictive model for the introduction of a zoonotic vector-borne pathogen by considering each of the pathways by which it may be introduced to a new area and comparing the relative risk of each pathway. This framework is an adaptation of pest introduction models and estimates the number of infectious individuals arriving in a location and the duration of their infectivity. We used it to determine the most likely route for the introduction of West Nile virus to Galápagos and measures that can be taken to reduce the risk of introduction. The introduction of this highly pathogenic virus to this unique World Heritage Site could have devastating consequences, similar to those seen following introductions of pathogens into other endemic island faunas. Our model identified the transport of mosquitoes on airplanes as the highest risk for West Nile virus introduction. Pathogen dissemination through avian migration and the transportation of day-old chickens appeared to be less important pathways. Infected humans and mosquitoes transported in sea containers, in tires, or by wind all represented much lower risk. Our risk-assessment framework has broad applicability to other pathogens and other regions and depends only on the availability of data on the transport of goods and animals and the epidemiology of the pathogen.*

Keywords: disease, invasive species, model, mosquito, risk assessment

Predicción de la Introducción de Patógenos: Dispersión del Virus del Nilo a las Galápagos

Resumen: *Las enfermedades infecciosas emergentes son una amenaza crucial para la conservación y la salud pública, sin embargo la predicción y prevención de su emergencia son notoriamente difíciles. Diseñamos un modelo predictivo de la introducción de un patógeno zoonótico transmitido por vectores en el que consideramos todas las vías por las que pudiera ser introducido a una nueva área y comparamos el riesgo relativo de cada vía. Este marco es una adaptación de modelos de introducción de plagas y estima el número de individuos infecciosos que llegan a una localidad y la duración de su infectividad. Utilizamos el modelos para determinar la ruta más probable para la introducción del virus del Nilo a las Galápagos y las medidas que se pueden tomar para reducir el riesgo de introducción. La introducción de este virus altamente patógeno a este Sitio de Patrimonio Mundial único podría tener consecuencias devastadoras, similares a las vistas después de la introducción de patógenos en otras faunas insulares endémicas. Nuestro modelo identificó el transporte de mosquitos en aviones como el mayor riesgo de introducción del virus del Nilo. La diseminación del patógeno*

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por medio de la migración aviar y la transportación de pollos de un día de edad parecieron ser las vías menos importantes. Humanos infectados y mosquitos transportados en contenedores marinos, en neumáticos o por el viento representaron un riesgo mucho menor. Nuestro marco de evaluación de riesgo es aplicable con otros patógenos y en otras regiones y solo depende de la disponibilidad de datos sobre el transporte de mercancía y animales y de la epidemiología del patógeno.

Palabras Clave: enfermedad, especies invasoras, modelo, mosquito, evaluación de riesgo

Introduction

Emerging infectious diseases are a key threat to conservation as well as public health (Meffe 1999; Daszak et al. 2000). The majority of programs that deal with these threats involve surveillance, outbreak control, and vaccine and drug development, and are by nature reactive, occurring after the introduction of diseases (Smolinski et al. 2003). A growing number of researchers have proposed new approaches to combating emerging diseases, however, based on forecasting outbreaks (Linthicum et al. 1999; Davis et al. 2004), predicting pathogen dynamics once an outbreak has occurred (Keeling et al. 2001), or predicting broad patterns in pathogen evolution or the underlying causes of emergence (Burke 1998; Taylor et al. 2001; Moya et al. 2004).

We developed a predictive model for the introduction of a zoonotic vector-borne pathogen by considering each of the pathways by which it may be introduced to a new area and comparing the relative risk of each pathway. Our model is an adaptation of an established approach for assessing the risk of introducing pest insects through the transport of commodities (Yamamura & Katsumata 1999; Stanaway et al. 2001; Hennessey 2004; Work et al. 2005). Pest risk assessments generally consider the volume of a commodity transported, the infestation rate of the pest, the efficacy of inspection, and the probability of establishment (Wearing et al. 2001). We applied this approach in a conservation context to assessing the risk of introduction of an emerging pathogen, West Nile virus (WNV), which is lethal to a wide range of species, into an important World Heritage Site, the Galápagos Islands. Our model can be easily applied to other pathogens and locations.

West Nile virus represents the most imminent threat to Galápagos fauna. In only 6 years it has spread west across North America and south into the Caribbean and Central and South America (Marra et al. 2004; Mattar et al. 2005). In the New World WNV has shown low host specificity and high virulence in a wide range of vertebrate species (Marra et al. 2004). The susceptibility of endemic Galápagos avifauna is unknown but Galápagos does not have any endemic corvids, the avian order most susceptible to West Nile disease in the United States (Komar et al. 2003*a*). Their small populations and evolution in the absence of WNV and other blood-borne pathogens suggest, however, that they would be highly susceptible (Wikelski et al. 2004), as Hawaii's avifauna was to avian malaria (Van Riper et al. 1986). Galápagos' unique reptile fauna, including land iguanas (*Conolophus subcristatus* and *C. pallidus*), marine iguanas (*Amblyrhynchus cristatus*), lava lizards (seven species of *Tropidurus*), and giant tortoises (*Geochelone elephantopus*), may also be under threat because WNV has caused significant mortality in some, but not all, reptiles (Klenk & Komar 2003; Miller et al. 2003; Steinman et al. 2003).

Galápagos has three mosquito vectors capable of transmitting WNV (*Culex quinquefasciatus, Aedes aegypti,* and *Ochleratatus taeniorhynchus*; Peck et al. 1998; Komar 2003) and many bird species that are closely related to known competent avian hosts (Komar et al. 2003*a*) and abundant throughout the areas where mosquitoes are present (e.g., Puerto Ayora, Santa Cruz Island). Thus the establishment of WNV in the Galápagos would be highly likely if the virus reached the islands. To reduce the probability of WNV introduction and its likely disastrous consequences, we performed a quantitative risk assessment of the pathways by which WNV could reach Galápagos.

Methods

We considered the risk of the introduction of a pathogen by six pathways: mosquitoes by (1) airplane, (2) wind, and (3) boat; (4) humans; (5) human-transported birds or other vertebrates; and (6) migratory birds. Pathways 1–4 are modes of introduction for many vector-borne pathogens with vertebrate hosts and pathway 6 is applicable to pathogens that have migratory birds as the primary hosts (e.g., flaviviruses such as St. Louis encephalitis virus).

For each pathway, we estimated the number of individuals arriving each year and the fraction likely to be infectious for the pathogen. We multiplied this by the duration of infectiousness to determine the number of infectious bird- or mosquito-days, I_d, for each pathway. An infectious bird-day and an infectious mosquito-day are not necessarily equivalent. The number of blood meals a mosquito will take in its lifetime depends on the length of the gonotrophic cycle (feeding through egg laying)

and biting rate. The maximum number of feedings by a mosquito is likely to be 1 every 3 to 4 days (Spielman & D'Antonio 2001), unless feeding is interrupted (Mitchell et al. 1979). In contrast, many mosquitoes can feed on an infectious bird in a single day; the realized number depends on mosquito densities, mosquito-to-bird ratios, and the defensive behavior of birds. The probability of pathogen introduction by an infected vector depends on that vector finding a susceptible host in an environment in which the reproductive ratio of the pathogen (R_0) is >1. Similarly, an infectious vertebrate host must be bitten by a competent vector in an environment in which $R_0 > 1$.

Sensitivity Analysis

As in other prospective analyses, many of the parameter estimates in our model are approximate or derived from work in other locations. We addressed the uncertainty inherent in our analyses in three ways. First, we used a range of values for parameter estimates for which we were unable to obtain local data. Second, we incorporated error in the parameter estimates into a confidence interval for the estimate of I_d. We calculated the upper and lower bounds of the confidence interval of I_d by selecting the 2,500th and 97,500th value from 100,000 sorted estimates of I_d (corresponding to the upper and lower 2.5% of the estimates). For each estimate we drew parameter values from a uniform distribution for parameters that we assumed had a range and from a normal distribution for parameters for which we were able to obtain estimates of the standard error. Although this does not strictly produce a 95% confidence interval, it does represent the variability inherent in the parameter estimates. Third, we performed a sensitivity analysis on all parameters in the model by considering the change in the estimated risk (in infectious days) from a 25% change in each parameter estimate. Although the choice of 25% was arbitrary, it illustrates the sensitivity of the risk calculations to variation in each parameter estimate. Because of the simplicity of the risk equations for most pathways (they are simple products; see Mosquitoes section), any percent increase in a parameter in the equation would be matched by the same percent increase in the estimated risk I_d.

WNV Risk Assessment

In our analysis we made two key assumptions: (1) WNV would eventually reach Ecuador and become established in local mosquito populations and (2) WNV-infectious birds would continue to migrate and would survive the overseas trip to Galápagos. Evidence of WNV transmission has recently been documented in horses in Colombia (Mattar et al. 2005), and WNV is likely to be present in Ecuador very soon if it is not there already. Recent work has also shown that WNV infection does not inhibit migratory movements in three species of birds—Gray Catbirds (*Dumetella carolinensis*), Wood Thrushes (*Hylocichla mustelina*), and Swainson's Thrushes (*Catharus ustulatus*) (N. Komar, personal communication)—although it is still unknown whether migrating birds could survive the overseas migration to Galápagos. We used these assumptions and a modeling framework to consider the risk of WNV introduction into the Galápagos as follows.

Infected Humans

Human WNV infections in immunocompetent individuals show peak viremias $< 10^3$ plaque forming units (PFU)/mL (Biggerstaff & Petersen 2002), which are insufficient to infect mosquitoes (Sardelis et al. 2001). This may not, however, be the case for other zoonotic pathogens.

Mosquitoes

We conservatively estimated the rate of mosquitoes reaching Galápagos by wind as <1 per 1000 years. Only one species of mosquito, *Oc. taeniorhynchus*, appears to have colonized Galápagos unaided (Hardy 1960) in the 4 million years that suitable habitat has been available. If our assumed rate is correct, approximately 4000 mosquitoes reached Galápagos by wind in the last 4 million years and resulted in a single species establishment. Increasing the colonization rate by five orders of magnitude (10^5) would not have affected our conclusion that this pathway represents a relatively low risk for WNV introduction compared with mosquitoes on airplanes (Table 1). Although long-distance, wind-aided flights have been documented for Culicoides (biting midges) and Simuliids (blackflies), fewer have been documented for mosquitoes (Lounibos 2002). Once WNV-infected mosquitoes are established in the archipelago, however, wind transport may be an important pathway for transporting them between islands.

Results of two large-scale studies of inadvertent mosquito transport on commercial airplanes landing in Australia (307 planes; Russell et al. 1984) and Japan (928 planes; Takahashi 1984 showed that on average 0.9 and 2.2 live mosquitoes were transported on each flight, respectively, and that 95% of the mosquitoes were *Culex pipiens* or *Cx. quinquefasciatus* (the latter was introduced to Galápagos during the 20th century; Peck et al. 1998). We used a range of 0.84 to 2.45 mosquitoes per airplane, which represent the lower and upper bounds of a 95% confidence interval from the two studies, respectively. In another large-scale study, searches of 11,265 shipping containers on boats arriving in New Zealand found 4 live *Culex* mosquitoes, or 0.00036 live *Culex*/container (New Zealand Ministry of Agriculture and Forestry 2003). We used this estimate to calculate the risk from mosquitoes traveling to Galápagos in cargo by sea.

Table 1. Estimated risk of West Nile virus introduction to Galápagos.

Pathway	*Number arriving in Galápagos/year*[a]	*Infectiousness × duration*[a]	*Infectious host or mosquito days/year*[b]
Mosquito by			
wind	$<10^{-3}$	(0.0098[c])(0.22[d])(15[e])	$<1 \times 10^{-6}$
air	(1910[f])(1.65[g])	(0.0098[c])(0.22[d])(15[e])	101.7 (8.3–272.9)
sea (adults)	(9750[f])(0.00036[b])	(0.0098[c])(0.22[d])(15[e])	0.11 (0.00–0.35)
sea (larvae in tires)	see text	see text	see text
with day-old chickens	see text	see text	see text
Human	90,533[i]	0	0
Human-transported vertebrates (day-old chickens)	see text	see text	see text
Migratory birds (shorebirds)	12,500[j]	(0.00125[k]/5)(1.75[l])	5.5 (2.2–11.8)

[a] *Parameter estimates are mean values or the middle of the range used.*
[b] *Mean estimated risk* I_d *and confidence interval based on the ranges and error estimates.*
[c] *SE = 3.0.*
[d] *SE = 0.098.*
[e] *Range: 10–20.*
[f] *Galápagos National Park Service 2004.*
[g] *Range: 0.84–2.45.*
[b] *Binomially distributed with* n = *11,265.*
[i] *Galápagos Department of Transportation and Commerce, personal communication.*
[j] *Wiedenfield 2004.*
[k] *Binomially distributed with* n = *12,000.*
[l] *SD of integral estimates = 0.55 (see Methods).*

The shipment of tires also presents a risk for the introduction of mosquitoes in the larval stage (Lounibos 2002). For larvae in tires to present a risk for the introduction of WNV, the maternal parent must have been infected with WNV and must pass WNV through vertical transmission to her offspring. Vertical transmission of WNV has been documented in *Cx. quinquefasciatus* with a minimum filial infection rate of 3.0/1000 (Goddard et al. 2003). No published data exist, however, on the number of mosquitoes transported per tire or per ship. Consequently we determined the number of larvae per tire such that the risk from this pathway was equivalent to that for airplane-transported adult mosquitoes. We used the following expression to calculate the risk of WNV introduction by mosquito larvae in tires:

Risk (infectious mosquito-days) = 1250 tires/year × no. mosquito larvae/tire × 0.0098 (mosquito WNV prevalence) × 0.22 (infectiousness of infected mosquito) × 15 days (lifespan of infectious mosquito) × 0.003 (probability of vertical transmission).

We estimated the fraction of mosquitoes that would be WNV infectious, once WNV reaches Ecuador, as the product of the fraction of infected *Cx. quinquefasciatus* mosquitoes that are able to transmit the virus with a bite (0.22; 95% CI: 0.064–0.48; Sardelis et al. 2001), and the WNV minimum infection rate (MIR = 1000 × no. WNV positive pools/no. individuals tested). We used an estimate for the MIR of mosquitoes based on data from 2232 pools of *Cx. quinquefasciatus* trapped in California between July and September 2004 (MIR = 9.8 ± SE 0.7 or 0.0098 of mosquitoes tested; Kramer 2005). We increased the SE for our confidence interval to 3.0 to reflect the variation in MIRs observed in other areas such as New York (*Cx. pipiens*, MIR = 3.5; Bernard et al. 2001) and Colorado (*Cx. tarsalis* MIR = 50; Pape 2004). We used data for *Cx. quinquefasciatus* because it is present in Galápagos and Guayaquil, Ecuador, where all flights to Galápagos currently originate or pass through before landing in Galápagos. Finally, we conservatively estimated that mosquitoes would be WNV infectious for approximately 10–20 days, based on an average lifespan of 30–60 days for *Cx. quinquefasciatus* in the laboratory (Oda et al. 2002) and 7–14 days needed for viral development within the mosquito (Dohm et al. 2002).

Migratory Birds

Because of uncertainties about the ability of WNV-infected birds to migrate successfully we made assumptions to maximize the risk from this pathway. We estimated the fraction of migrating birds that would be viremic (have WNV in their blood) from a 3-year study of migrating birds in the eastern United States that found 15 of 12,000 birds infected with WNV (R. McLean, personal communication). We assumed it required only a single day for migration to Galápagos from the area where the migrating bird became infected with WNV and that 100% of viremic birds would survive the migration. We calculated the infectiousness of a bird to a mosquito with the viremia-infectiousness relationship for *Cx. quinquefasciatus* (see below), which is thought to have a more restricted range in Galápagos than the less competent vector *Oc. taeniorhynchus* (Turell et al. 2001). Finally, we assumed that all migrants came from areas where WNV

was fully established, despite the fact that it has yet to become established in parts of northwestern North America (CDC 2004) where many Galápagos migrants come from or pass through after breeding.

We estimated the number of days that each migratory bird landing in Galápagos would be infectious as:

$$\frac{\varphi}{n}\sum_{i=1}^{n}\sum_{j=i}^{n}\left(\int_{5}^{15.3} I_m(v)N(v_i,\sigma)dv+\int_{15.3}^{\infty} N(v_i,\sigma)dv\right), \quad (1)$$

where φ is the fraction of birds that are viremic with WNV, and the summation is over the viremic period n (in days) for that species (Komar et al. 2003*a*). The terms in parentheses represent the integral of the probability distribution of an animal's viremia on day i, assuming a normal distribution, $N(v_i,\sigma)$ (see below for parameter estimates), after log transformation with mean $\log_{10}$(viremia), v_i, and variance, σ^2, multiplied by the probability of a bite leading to a virus-transmitting mosquito, I_m, given the host's viremia, v. The first summation and $1/n$ terms account for the possibility that infectious migratory birds may arrive in Galápagos on any of the n days they are viremic. The second summation calculates the number of infectious days for the remaining j to n days in the viremic period.

More than 95% of the birds that migrate to Galápagos are shorebirds in the family Charadriidae (Castro & Phillips 1997). Thus we used data from experimental infection of Killdeers (*Charadrius vociferous;* in the order Charadriiformes and family Charadriidae) to estimate mean WNV viremia (v_i = 6.2, 7.5, 8.1, 4.9, 2.6, respectively, on days 1–5 postinfection; Komar et al. 2003*a*) and data from Charadriiform birds to estimate $\sigma^2 = 1.90$ (based on $n = 4$ individuals over 4 or 5 days postinfection or 18 bird days; N. Komar, unpublished data). For the confidence interval we drew values for the mean viremia from a normal distribution with the means stated above for each day of the 5-day viremic period and variance $\sigma^2 = 1.90$.

The probability, I_m, was based on a vector competence study of *Cx. quinquefasciatus* (Sardelis et al. 2001):

$$I_m = 0 \text{ for } \log_{10}(v) < 5.0,$$
$$I_m = 0.097^{*}\log_{10}(v) - 0.48 \text{ for}$$
$$5.0 < \log_{10}(v) < 15.3, \text{ and}$$
$$I_m = 1 \text{ for } \log_{10}(v) > 15.3.$$

This viremia-infectivity relationship was based on viremias ranging from 10^5 to 10^7 PFU/mL, which are slightly lower than the range of mean viremias that we used in the calculation, $10^{4.9}$–$10^{8.1}$, and necessitated an extrapolation of the fitted line.

Human-Transported Host Vertebrates

Current regulations ban the import of live animals into Galápagos except for day-old chickens, which are shipped in mosquito-proof containers. Some illegal transport of domestic animals occurs, however, and it is possible that the containment for the day-old chicks could be breached. If so, mosquitoes could bite and infect the chicks or hide in the box. We could not accurately estimate the risk from the transport of these animals because there are no data on the frequency of these events. We assessed the risk from infected day-old chickens, however, by setting the risk of this pathway equal to that of migratory birds and calculating the frequency of chick infection necessary for the pathways to have equal risk. We estimated the mean and variance for the WNV viremia of day-old chickens based on data from experimental infections (Turell et al. 2001). We calculated the risk from day-old chicks as follows:

risk (infectious bird-days)
= 114,243 chicks/year
× probability of chicks becoming infected with WNV
× 0.36 days (infectious days/infected chick based on Eq. 1).

We estimated the risk from mosquitoes hiding in the boxes of day-old chicks by calculating the number of mosquitoes that would need to be transported with chickens to equal the number of mosquitoes transported by airplanes.

Results

Infectious mosquitoes transported on airplanes carrying tourists represent the highest risk of WNV reaching Galápagos by a vector pathway (Table 1). Our assessment predicted that 6.8 (CI: 0.6–18) WNV-infectious mosquitoes will arrive in Galápagos each year after WNV is established in Ecuador, representing approximately 101.6 (CI: 8.3–272.9) infectious mosquito-days (Table 1). For larvae in tires to present an equal risk, an average of 838 (CI: 68–2250) larvae would need to be present in each tire. For mosquitoes transported with chickens to have a similar risk as airplanes, 3144 (1606–4682) mosquitoes would have to be transported with chickens, which is approximately 1 for every 24 chickens. Other mechanisms of WNV introduction by an infected vector pose a risk at least an order of magnitude lower than that due to airplane-transported mosquitoes (Table 1).

For hosts, we estimated that approximately 15.6 (CI: 7.9–25.2) viremic migratory birds will arrive in Galápagos each year, representing 5.5 (CI: 2.2–11.8) infectious bird-days (Table 1). The importation of day-old chicks would carry a similar risk of WNV introduction if approximately 1.3 (0.54–2.9) in 10,000 chicks were infected accidentally

with WNV en route to Galápagos. Finally, infected humans did not present a substantial risk for WNV introduction to Galápagos (Table 1).

Except for the infectious period of migratory birds, our sensitivity analysis revealed simple linear scaling: the risk from each pathway increased (or decreased) 25% for each 25% increase (or decrease) in each of the parameter estimates. This is due to the simplicity of the risk calculations, which are products of the parameter estimates for each component of the equations for these pathways. In contrast, the risk increased/decreased by the following amounts for a 25% increase/decrease in the other parameters for the migratory bird pathway: 81%/63% (mean host viremia); 2.4%/2.5% (variance in host viremia); 85%/69% (slope of mosquito infectivity-viremia relationship); 62%/46% (y-intercept of mosquito infectivity-viremia relationship). This analysis suggests that mean host viremia and mosquito infectivity-viremia relationships are key components in determining the risk from this pathway.

Discussion

We present a predictive model for the introduction of a vector-borne pathogen that also allows for assessment and mitigation of the risk of introduction. Our work builds on the risk assessment models for invasive species that have been used by many countries to reduce the introduction of pest species (Yamamura & Katsumata 1999; Simberloff 2005; Work et al. 2005). Our case study on WNV and Galápagos suggests that mosquito transport on airplanes represents a key pathway for introduction, with migratory birds presenting a lower but non-negligible risk. It was difficult to accurately compare the relative risk of host and vector pathways. Although the mean number of WNV-infectious days from mosquitoes on airplanes was estimated to be 17 times higher than those from migratory birds, an infected mosquito is unlikely to feed on more than one or two hosts every 3–7 days. In contrast, a bird could be bitten by several mosquitoes during each infectious day, although the precise number cannot be determined with currently available data. Nonetheless, we believe the risk from mosquitoes on airplanes is at least as high as, and most likely higher than, the risk from migratory birds, in part because we made assumptions in our analysis to maximize the risk from the migratory bird pathway (e.g., we assumed survival of viremic birds during migration would be 100%) and in part because the risk of WNV introduction by a mosquito on an airplane is likely to rise in the future (see below). Whether mosquitoes on airplanes will be the most important pathway for the introduction of WNV to other locations depends on the rate of movement of vectors, hosts, and the epidemiology of the pathogen between the two locations.

The framework outlined here can be used to determine the key pathways for pathogen introduction in other mainland-island systems and for the movement of pathogens between continents. Similarly, it can be adapted to model a broad range of pathogens such as avian influenza or airport malaria (Gratz et al. 2000; Karch et al. 2001) as long as data are available to estimate the flow of humans, transport, goods, and mobile animals and the epidemiology of the pathogen. The collection of local data to estimate epidemiological and transport parameters, including the number of mosquitoes on airplanes and the biting rate of mosquitoes on arriving migratory birds, is an important goal for future research. For Galápagos, this will allow for a refinement of our analysis, and for other pathogens and locations it will enable proactive action to prevent introduction.

Modeling pathogen introductions as a predictive approach necessarily involves assumptions and analyses based on incomplete data. We outlined several of these assumptions in the Methods section. A final key assumption underlying our analysis is that WNV will invade and persist (i.e., R_0 will be > 1) if it is introduced to the Galápagos. To test this would require measuring the vector competence, feeding behavior, biting rate, and abundance; distribution of the vectors present; reservoir competence; antimosquito behavior; and distribution and abundance of the potential vertebrate hosts in Galápagos. We believe that uncertainties underlying our assumptions should not prevent analyses of the type we have performed here. In addition, our approach can be used to identify high-risk pathways that merit new research to refine risk estimates. The rate of spread of WNV, however, across North America and into the Caribbean (Dupuis et al. 2003) and Central America (Komar et al. 2003*b*) and South America (Mattar et al. 2005) suggests that its movement into Ecuador is likely to occur before sufficient data can be collected to address all uncertainties. As a result, we suggest that measures to reduce the risk of WNV introduction from the pathways we have identified should be implemented concurrent with research to refine risk assessments.

The most effective short-term action to reduce the risk of WNV introduction would be implementation of existing requirements that all airplanes landing in Galápagos be chemically treated to kill incoming insects. Previous research has shown that residual disinfection (using an insecticide coating on the interiors of planes) is much more effective than fog fumigants (Naumann & McLachlan 1999). In addition, because 82% of mosquitoes on airplanes were found in cargo holds (Takahashi 1984), the use of insecticides in only the cargo holds would have a substantial impact. Unfortunately, the risk of WNV introduction by mosquitoes on airplanes is likely to grow in the immediate future. The number of tourists visiting Galápagos increased steadily from 40,746 in 1991 to 90,533 in 2003, which represents a mean annual growth rate of 6.9% (Galapagos National Park Service 2004).

There is severe economic pressure within Ecuador to expand the number of tourists visiting Galápagos, and air travel to Galápagos is largely driven by ecotourism. In addition, plans for tourism expansion include the potential of allowing direct international flights to the Galápagos and developing an additional airport on Santa Cruz Island, where mosquito populations are larger (UCPPAPG 2001). Although larger planes could be used to increase the number of tourists without increasing the number of flights to Galápagos, current plans for expansion do not include this provision (UCPPAPG 2001) and allowing international flights to Galápagos would certainly add to the number of planes landing in Galápagos. Consequently, as ecotourism grows, the threat of WNV-infectious mosquitoes arriving on airplanes will increase and most likely make the risk from most other pathways negligible by comparison.

The two other pathways that appear to represent important risks are migratory birds and the transport of day-old chickens. The most effective strategy for reducing the risk from migratory birds would be to reduce the densities of mosquitoes (especially *Cx. quinquefasciatus*) near arrival areas in Galápagos through the elimination of larval habitat. This would decrease the probability that the pathogen would become established if an infectious bird reached Galápagos. In fact, reducing mosquito densities would reduce the risk of WNV invading and persisting on Galápagos should it reach there by any pathway. Reducing the risk of WNV introduction through day-old chicks could be accomplished by inspecting the boxes of chicks for mosquitoes before departure and after arrival, establishing a local supplier for chicks within Galápagos, and ensuring that the boxes the chicks are shipped in are robust to breakage or mosquito invasion.

Our results demonstrate that predictive approaches to disease emergence are possible and can be used to identify strategies to prevent, rather than react to, conservation or public health crises. In addition, as intervention strategies are implemented or new information becomes available our model framework allows for continuous reassessment. As a result it fits well with adaptive management strategies (Salafsky et al. 2002). Although none of the management actions we suggest will reduce the probability of pathogen introduction to zero, they will substantially reduce the risk and thereby increase the time available to develop and implement strategies to respond to an initial epidemic. We believe that taking a proactive approach to pathogen introduction may offer insight into how to stem the wave of emerging diseases linked to globalization of our planet.

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