

Summary of policy recommendations to reduce the risk of West Nile Virus Introduction in to Galapagos

(From the Proceedings of the Galapagos West Nile Virus Workshop, Galapagos National Park Headquarters, Puerto Ayora, 29th April 2004)

WNV is projected to reach Ecuador by 2008. When WNV reaches Ecuador there is a high probability of its introduction in to Galapagos unless rigorous control measures are implemented prior to the arrival of the disease. If WNV is introduced in to Galapagos it is likely to cause catastrophic mortality of endemic birds, reptiles and mammals, leading to irreparable ecological and economic damage to the archipelago. WNV can cause disease and death in humans, thus further threatening livelihoods on Galapagos. Disease introduction is most likely to occur through the human transport of infectious mosquitoes, particularly via inadvertent transport in airplanes. Transport of mosquitoes by boat or of infected vertebrate hosts are also significant risks for WNV introduction. To minimise these risks, the following control measures are required.

1. Aircraft pose the highest risk, so the most critical control measure is to enforce the insecticide treatment of transport to Galapagos to prevent the incidental transport of mosquitoes. Provision in law already exists for this.
 - 'residual disinsection' (a residual insecticide applied to the interior surface of aircraft) methods must be employed, as traditional fog fumigation has been shown to be ineffective in aircraft cabins.
 - No aircraft should be allowed to fly to Galapagos unless they have an up to date residual disinsection treatment, including private and military aircraft.
 - There should be no direct flights other than from mainland Ecuador, as direct flights from areas that are already affected by WNV (e.g. the continental USA) greatly increase the risk of WNV introduction.
2. All sea transport, including cargo ships and private boats should be quarantined until inspected and fumigated at a central port.
 - Cargo, such as tyres and machinery, must be stored and packed to minimise the collection of rainwater that acts as breeding sites for mosquitoes or otherwise enhances mosquito survival.
3. Transport of vertebrate WNV hosts to Galapagos must be conducted under the strictest quarantine conditions.
 - Before WNV reaches Ecuador, the current transport of chicks to Galapagos should be reevaluated as this poses another significant introduction risk. At the least, chicks on the mainland must be hatched and reared in mosquito free conditions and kept in mosquito proof containers during transport to the islands.
4. Early surveillance for WNV should be initiated at major ports of travel to and from Galapagos (e.g Baltra, Guayaquil).

While some financial commitment is required to implement these policy measures, these costs are insignificant compared to the ecological and economic losses that would be experienced should WNV become established in Galapagos.

Proceedings of the Galapagos West Nile Virus Workshop, Galapagos National Park Headquarters, Puerto Ayora, 29th April 2004

This workshop was convened as part of the “Building capacity and determining disease threats to endemic Galapagos fauna” project; a partnership between the Institute of Zoology (Zoological Society of London), the University of Guayaquil and the Galapagos National Park Service (GNPS), funded by the UK Government’s Darwin Initiative programme.

The purpose of the workshop was to gather together experts in West Nile virus (WNV) biology and epidemiology, experts in Galapagos fauna and potential WNV vectors in Galapagos, and experts in disease threats to wildlife conservation to examine the likely threat of WNV to Galapagos fauna and to examine possible preventative and responsive measures to this threat.

The workshop was opened by Edwin Nuala, Director, GNPS who welcomed all the participants from Ecuador and from overseas. He noted the timeliness of the workshop and the importance of addressing disease threats to Galapagos fauna. The support of the GNPS was promised to assist with the areas of work raised as important in the workshop output.

Overview of West Nile Virus (WNV) and its Spread Across the Americas (led by Laura Kramer)

- WNV is a positive sense RNA virus: i.e. the viral RNA is infectious
- WNV is part of the Japanese Encephalitis serogroup of flaviviruses; other members of this subgroup include: Saint Louise Encephalitis (SLE) virus, Japanese Encephalitis (JE) virus, MVE and Kunjin (KUN) virus. KUN virus is a sub-type of WNV.
- Plaque reduction neutralization tests are required for the confirmation of the presence of antibodies specific to WNV because of the high degree of cross-reactivity among flaviviruses..
- The USA strain of WNV can kill some types of bird (e.g. corvids, gulls, house finches, and many more), horses, and selected other animals (e.g., farmed alligators; squirrels) , but a vaccine currently is available for equines. Humans are generally at low risk of severe disease unless very young or old or immunocompromised.
- As the virus has spread across the USA (1999-2004), nucleotide sequence analysis of virus isolated from crows (without cell culture passage) indicates that there is evidence of minimal, but directed, genetic change in the envelope region of the viral genome, , although it is still highly conserved.

Phylogenetic tree analysis based on genome sequence data of WNV shows that there are two distinct lineages (Lanciotti et al. 2002):

- Lineage 1, causes disease in humans and is spreading across Europe and the Americas.
- Lineage 2, found only in sub-Saharan Africa, where it does not cause disease in humans.
- Until recently, most research on WNV had been done on the Egyptian Strain (e.g. 101), but, although this virus is in lineage 1, it is quite different to the strain introduced to the Americas.

Spread of WNV through the Americas (from CDC documentation of human and avian infections)

- First reported in New York in August 1999.

- Subsequent years report focus in the SE states in addition to a continued focus in NE states, (although a lack of monitoring in intervening coastal states may account for lack of cases reported there)
- 2002 saw an explosion in reported human cases of WNV disease (from approx. 29 human cases in 2001 to approx. 4,156 in 2002, and approx. 14,000 horses died of WNV in 2002) as the virus spread from eastern foci through to most of Eastern and Central USA (a lot of human cases were in the Chicago area).
- 2002 was the first year a human case was reported on the West coast (in California) – the person, who worked for FedEx, had not travelled and no infected mosquitoes or birds were found in the area – this person was possibly infected by a single infected mosquito which had been shipped with a FedEx parcel?
- 2003 reported human cases of virus have shifted Westward to new locations (note that human cases follow bird cases reported in previous year), incl. southern California
- Horse cases were 14,000 in 2002; 4,000 in 2003 (probably fewer in 2003 since many were vaccinated by then)
- WNV arrived in Mexico in 2002 and subsequently has spread throughout the Mexican states
- Also reported in birds on the island of Jamaica (where it appears to have become established in resident birds); recently also spread to El Salvador and Guadeloupe. Infections (Ab + ve) now have been reported in Puerto Rico in resident and migratory bird species. There is also an unconfirmed report of a seropositive flamingo in Chile.

Wildlife Infections of WNV - Birds

- Birds show high morbidity, mortality, with viral shedding from cloacal and oral cavities (can have high concentrations of virus shed from the oral cavity).
- Clinical signs of infected birds include weakness, recumbency and ataxia.
- WNV causes meningoencephalitis and necrotizing myocarditis.
- 15,000 dead birds were surveyed 2000-03 in US. 12,000 were passeriformes, and 30% were WNV +ve; of these 45% were corvids
- Of top 10 WNV positive bird species in US, 5 were corvid species
- A total of 225 species of birds have now been documented as being WNV positive in the USA.
- Migratory birds as well as residents are infected in the USA
- Crows infected with 10pfu all die; have not defined an LD₅₀ (ie are very, very sensitive). Note that infected crows display titres of up to 10¹⁴ pfu/ml in their blood (so are highly infectious)
- It has been shown that crows can start to die as early as day 4 post-infection, but more usually by day 6 or day 7. All infected crows are dead by day 10.
- Crow to crow transmission has been observed – possibly an important route of transmission for gregarious species and for birds on the nest
- Infection can also occur via eating infected carrion (demonstrated by feeding infected mice to crows).
- Morbidity and mortality experimentally determined for 3 species:
 - Doves: 18% morbidity, 0% mortality
 - Sparrows: 19% morbidity, 19% mortality
 - Crows: 100% morbidity, 100% mortality
- There is a high variance in the WNV titres reached in the blood of infected sparrows. The mean titer is approx. 10⁸ pfu, sufficiently high to be infectious to mosquitoes.

- Viral RNA can persist in random tissues (e.g. spleen, heart, spinal cord) of infected birds (eg. sparrows and pigeons) for at least up to 27 weeks, but no infectious virus has been recovered from these RNA-positive tissues.
- Sparrows and pigeons can maintain protective Ab titres for a long time (for at least 28 weeks post-infection).

Wildlife Infections of WNV - Other vertebrate orders

- Mammals can be infected by WNV, the most commonly affected being horses, squirrels, dogs, cats, and a range of other species, including bats and marine mammals (e.g. seals). Zoo surveys have shown that a large range of species can be infected and seroconvert, although the range of species that show evidence of disease or mortality is not known.
- Reptiles have also been shown to be Ab positive and are symptomatic (alligator, 1 turtle and 1 crocodile monitor with symptoms)
- In 2003, huge death rates in farmed alligators in FL observed: 10-50% mortality

Risk Analysis of WNV Reaching Galapagos (led by Marm Kilpatrick)

Both known vectors and known vertebrate hosts of WNV are present on the Galapagos Islands, therefore only the virus is required for it to threaten the Galapagos fauna. If it reaches the archipelago, there is plenty of opportunity for it to become established and for it to be spread widely throughout the islands. Therefore, an analysis of the likelihood of the virus reaching the islands is required in order to estimate the degree of threat posed by WNV. In order to conduct such an analysis, and with a lack of certain data on WNV ecology, several important assumptions were made, based on work conducted on WNV in the USA and elsewhere.

Important assumptions for risk analysis:

- WNV is established at sources for potential introduction (mainland Ecuador for human transport, sites from which birds migrate)
- Mosquitoes will be the primary vectors of WNV in the Galapagos.
- A minimum viraemic titre of 10^4 pfu is required for infection to be passed on to a biting mosquito (although it is unknown if this is the case for mosquitoes on Galapagos).
- Only 20% of infected mosquitoes will successfully transmit WNV (also unknown if this is the case for mosquitoes on Galapagos).

Five potential routes of introduction have been identified:

- 1) Introduction by infected human
- 2) Wind blown mosquitoes
- 3) Mosquitoes transported by humans (sea or air)
- 4) Human transported animals
- 5) Infected migratory birds

1. Infected humans

- Viraemic titres in humans probably only reach $\sim 2.1 \log_{10}$, therefore infected humans would not be as source of virus transmission if bitten by mosquitoes. (Titres may be higher in immunocompromised humans, however, and a small risk may be posed by such people.)

2. Wind blown mosquitoes

- Based on other studies, such as the introduction of Japanese Encephalitis to Papua New Guinea presumably via wind-borne mosquitoes, the maximum range is about 155km. Thus wind blown mosquitoes are unlikely to arrive on Galapagos from mainland. This is also borne out by the presence of only one endemic species of mosquito on Galapagos (*Ochleratatus taeniorhynchus*), suggesting that colonisation from the mainland is a rare event. Wind dispersal of *Oc. taeniorhynchus* (including *Oc. taeniorhynchus* eggs) from the mainland might pose a very minor risk, but the rate of WNV infection via vertical transmission is low.

3. (i) Mosquitoes transported by humans – boats

- Highest risk for mosquito introduction is via tyres or machinery (including cars), where water collects and in which larvae can survive. For adults, closed spaces (e.g. containers, cabins, possibly cars) are most important for their incidental transportation via shipping.
- A study in New Zealand showed that 7 of 10,000 containers had mosquitoes
- Given 10,000 tons of goods shipped to Galapagos pa; a 0.2% infection rate in progeny and 0.22 transmission rate - predict 0.002 to 0.21 infected adults imported by boat each year.

3. (ii) Mosquitoes transported by humans – aeroplanes

- Large studies have been carried out of mosquitoes arriving in planes to Japan and Australia (note, that many planes were also spray fumigated). They showed an average of 1-2.2 mosquitoes arriving per plane
- Most mosquitoes were transported in the cargo hold and not in the passenger cabin.
- There are 1,910 flights to Galapagos p.a. and assuming a similar infection rate as above this leads to an estimated 1.3-13 live, infectious mosquitoes introduced by aeroplane every year (note, this translates to 26-260 “infectious mosquito days”, assuming an adult mosquito lives for 30 days and becomes infectious on day 10 as an adult).
- Spray fumigation did not have a significant impact on the numbers of mosquitoes successfully transported by aeroplanes.

4. Animals transported by humans

- Assume that current quarantine laws are continued and are 100% effective (i.e. there are no illegal or incidental introductions of animals).
- 10-15,000 day old chicks are transported to Galapagos annually. Adult chickens are not a threat as they have a very low viraemia and are not killed by WNV. Vertical transmission in hens is highly unlikely (as infected embryos die). Young chickens (up to about 1 week old), however, are highly susceptible to infection and produce a very high viraemia.

5. Migratory Birds

- Assuming a worst case scenario of 60,000 migrants per year- (probably an overestimate – see David Wiedenfeld’s presentation below in which it is estimated that only 15,000 migrants come near-shore or on-shore).
- Assuming migration from a WNV infected area, a day for migration (and all migrants survive), the fraction of viraemic birds is 0.00046. Translates to 1.25 infectious bird days per year.

CONCLUSION: plane flights pose high risk, with secondary risks through cargo arriving by ship and bird migration.

WNV Threats to Galapagos Fauna
(led by David Wiedenfeld)

Should WNV reach the Galapagos, the species potentially at risk can be classified into five groups:

1. Species present in low numbers and with restricted distributions, e.g. Galapagos hawk, flightless cormorant, lava gull
2. Non-endemic species which are known to be susceptible to WNV infection in other countries, e.g. pelican, heron, flamingo, yellow warbler, short-eared owl, plus chickens, dogs, horses
3. Migratory species in which WNV has been detected elsewhere, e.g. grebe, turnstone, purple martin, swallow
4. Endemic species closely related to animals known to be susceptible to WNV infection elsewhere, e.g. penguins, cormorants, hawks, mockingbirds, finches. (It is highly likely that these species will also be susceptible to infection.)
5. Other members of orders known to be susceptible to WNV elsewhere (i.e. reptiles) and which may also be susceptible to infection, e.g. tortoises, iguanas, lava lizards.

Survey of migrant numbers

- 25,000 regular migrants come to Galapagos, but 15,000 are phalaropes and only come within 250m of shore (so probably don't get bitten because mosquitoes don't fly over water much and flight over the ocean would be desiccating. Also, it is doubtful that they would need to fly offshore to feed as hosts on land are unlikely to be limiting.)
- A few migrants occur in low numbers and there are also a few species that exchange with the continent
- Overall estimate is that 12,500 migratory birds will be near mosquitoes.

Mosquitoes on the Galapagos
(led by Helmuth Rogg)

Three species of mosquito are known to occur on the Galapagos Islands:

- *Oc. taeniorhynchus*, which is endemic and is widely distributed throughout the archipelago.
- *Culex quinquefasciatus*, which is introduced and which has only been reported from Puerto Ayora, Santa Cruz and San Cristobal, where it is much wetter and where there is an airport.
- *Aedes aegypti*, which is introduced and which is limited to the inhabited areas of Santa Cruz. (Note – this species of mosquito is specific to humans and doesn't bite other animals, therefore it is of low risk for WNV transmission.)

Other mosquito species that are at risk of introduction to the Galapagos are:

- *Aedes albopictus*, the introduction of which could be particularly problematical as it feeds on both humans and other animals, is a known WNV transmitter and is extant in Central and South America.
- *Anopheles*, which is unlikely to establish in Galapagos as it needs swamps.

Note there is no correlation between *Aedes aegypti* numbers and rainfall, but there is a positive correlation with temperature.

- Permanent Dengue control program here in Puerto Ayora via the use of “Abate” (an organochlorine pesticide) in water.

Note: By law, aeroplanes and boats arriving in Galapagos must be sprayed with insecticide, BUT this law is not being enforced

Non-mosquito vectors of WNV may also be present on Galapagos, such as the blackfly (which is present in high numbers on San Cristobal. The competence of these other potential vectors is unknown.

Pathology and Training for WNV on Galapagos (led by Nicole Gottdenker)

- WNV causes lesions and neurological signs, but other diseases can cause similar lesions; furthermore some other viruses (e.g. Eastern Equine Encephalitis, Western Equine Encephalitis, Venezuelan Equine Encephalitis) can also cross-react serologically with WNV.
- Specific diagnostics for WNV include immunohistochemistry (IHC), *in situ* hybridization, virus isolation (the gold standard), RT-PCR, RT and sequencing, Taqman, EM, ELISA and serology
- Several of these techniques e.g. PCR, IHC will be utilised at the new Galapagos Epidemiology and Pathology Laboratory
- Proper equipment for the handling of potentially infected animals, tissues and carcasses (e.g. Microbiological Safety Cabinet) will also be in place at the new laboratory.
- Proper education and training for personnel who may come into contact with WNV-infected animals will need to be implemented.

Discussion Session/ Summary West Nile Virus Workshop

Timescale of Introduction

It was agreed that the most important factor threatening introduction would be the arrival of WNV in mainland Ecuador. Although the establishment of WNV in western North America could be an important stage before introduction to Ecuador is likely, there was agreement that this stage would not be a necessity: Ecuadorian migrants also come from the eastern USA and Central America), so WNV could arrive in Ecuador via this route. An unconfirmed report of WNV in Chilean flamingo raised the possibility that WNV may already be in mainland South America.

Extrapolating from the spread of WNV through North America and the Caribbean, WNV will almost certainly be established in Ecuador within 5 years (possibly arriving in 2 or sooner), after which its arrival in Galapagos will be inevitable unless preventative measures are taken.

Reducing the risk of WNV Introduction

The main risk is from infected mosquitoes arriving in aeroplanes. This threat will be high once WNV become established in Ecuador as (almost) all air travel to the Galapagos comes from mainland Ecuador. The transport of infected mosquitoes in cargo shipped to the Galapagos is a second significant risk and infected migratory birds pose a third significant risk. Risks posed by imported domestic animals (especially day-old chicks) were also recognized.

Currently, all aircraft arriving in Galapagos should be fumigated with insecticide, but this is not being carried out. Elsewhere, fumigation has been shown to be pretty ineffective in preventing the import of live mosquitoes, whereas coating the inside of aircraft with an insecticidal residue has been shown to be highly effective. Efforts should, therefore, be made to bring about enforcement of the “disinsection” law, but with the use of an effective means of killing on-board mosquitoes. This will have additional benefits of reducing the risks of importing other (alien) insects to the islands.

It was suggested that the addition of a small \$ charge to the cost of (tourist) ticket would be enough to cover the costs of aircraft disinsection. It was recognised, however, that this additional charge could be politically difficult.

Fortunately, both the Ecuadorian aviation authority and the Port Authority in Guayaquil are in favour of enforcement of the disinsection law, so we are hopeful that we may be pushing against an open door. It was recommended that SICGAL and SESA should be approached to take this plan forward.

One area of particular concern that was raised was the proposed changes to the regulation of air travel to the Galapagos and to the numbers of tourists visiting the islands. Currently, there are political demands on the table requesting a de-regulation of flights to the islands, with an increase in numbers of flights from the mainland and the introduction of flights directly to the Galapagos from other countries (e.g. USA, Panama). The latter would greatly increase the risk of WNV introduction to the islands whether or not the virus had reached mainland Ecuador as infected mosquitoes could be transported directly from countries where the virus is already endemic. Strict enforcement of the ban on introduced animals and of the most effective methods of disinsection would be required to minimize this risk.

An increase in the number and size of tourist boats is also being demanded, which would also increase the numbers of flights to the islands (as all tourists arrive by plane and then transfer to boats in the Galapagos). In order to accommodate the projected increase in air travel, some people are calling for the development of an airport on Santa Cruz (close to Puerto Ayora) to replace the airport on Baltra. The siting of Baltra for the main airport has turned out to be propitious as far as WNV introduction goes because (i) it is arid, thus the population of mosquitoes there is not high, and (ii) it is leeward to Santa Cruz, thus prevailing winds will tend to blow vectors away from Santa Cruz and out to sea. Should it be introduced, the chances of WNV (or any other introduced arthropod-borne disease) becoming established would be much greater if the airport was on Santa Cruz rather than on Baltra (especially if it was near Puerto Ayora). It is likely that only luck has prevented matters on San Cristobal (where the airport is close to town) from being much worse than they have been so far. It was pointed out, however, that the authorities should not be complacent about this and that even Baltra could provide highly suitable mosquito habitat during El Nino years.

It was noted that WNV is a zoonosis and has killed a large number of people (mainly elderly) in the USA. If WNV was to reach the Galapagos, associated increased morbidity and mortality would have a major impact on healthcare costs for the islanders. Also, should a tourist die from WNV contracted on the islands, the negative impact on the tourist industry is likely to be significant.

Large numbers of day-old (or two-to-three-day old) chicks are imported to the Galapagos for rearing as broilers (there being no commercial laying enterprise on the islands). This provoked much discussion and concern, as young chicks are susceptible to WNV, developing viraemia high enough for virus transmission via mosquitoes. It was thought that the import of large numbers of young chicks could pose a conduit for WNV introduction to Galapagos if/when the virus becomes established on mainland Ecuador. Suggestions to mitigate this included hatching and packing birds in mosquito-proof caging; banning the import of chicks if/when WNV reaches the mainland, and banning the import in favour of establishing egg production on the islands. (The latter might be favourably received by locals as it would provide employment and help the local economy, but the supply of fresh water may be a limiting factor.) It was suggested that a working group with SESA should be set up to look into this further, perhaps with a cost-benefit analysis being conducted on the local production of chicks.

Minimising the risk of WNV becoming established

It was recognized that, should WNV be introduced to the islands, the only effective way of preventing it from becoming established would be via a mosquito control programme. In addition to having a major mosquito control programme on stand-by to be implemented very rapidly after WNV introduction, the continued control of mosquito populations at the most likely portals of WNV entry should be conducted. Such portals of entry are the towns (where cargo is imported; where there is a high throughput of people and their belongings; and where the highest species complement and populations of mosquitoes are found), the airports and the cargo port on Baltra. Such control is important at all times from now on, but will be particularly important once WNV reaches mainland Ecuador and especially so during El Nino years.

Surveillance: Which species, who, when, where and how much will it cost?

The group identified surveillance for WNV as a high priority and there was much discussion as to how this could best be done. One way would be to regularly trap and test mosquitoes around the most likely entry points (see above), but the percentage of positive mosquitoes can be very small (and almost undetectable) while the disease is just getting established in a new location as the prevalence is low. (During the midst of an epidemic, however, the % of mosquitoes infected is usually 0.3-5%, which is easy to pick up when testing pools of 50 mosquitoes.) Therefore, testing of birds and possibly other vertebrates was considered to be important. Again, the sample size required for testing if the virus causes no mortality needs to be very high. Testing of birds and other wildlife was, therefore, considered important for detecting the extent of spread of WNV, but not a useful tool for early warning of WNV introduction. Testing should be done on purposefully-obtained samples and also on any samples obtained opportunistically from any species) from other studies. Such testing could be carried out in the new pathology laboratory using standard protocols, and this would also allow rapid feedback of the results.

The best way to do conduct surveillance for early warning of WNV introduction would be via the regular testing of sentinel species (and preferably the use of a sentinel species that is highly susceptible to WNV, in which the infection causes rapid onset mortality). Horses were

considered likely to be the best sentinel species. These could be bled regularly for WNV serology. Also, in the event of mortality, a local mosquito control programme could be initiated while awaiting necropsy results. Such a response plan would be an integrated part of the surveillance strategy as it would be crucial for control to be instigated as rapidly as possible after WNV introduction. It was suggested that sentinels could be held at the main ports on Baltra, Santa Cruz and San Cristobal, and possibly also at Guayaquil.

Funding Plans

- revisit Princeton proposal for baseline
- note possible tourism risk – selling points for CDC and human oriented company grants such as (OFF-makers J&J)
- various private US foundations
- companies (e.g. J&J), tourists and tour companies
- new proposal to Darwin Initiative
- GEF?
- SESA, SICGAL and GNPS, health and agriculture depts. should lead (but note they have no funds)
- estimated cost for entomological monitoring alone may be ~ \$50K (HR)

May be better to fundraise for endowment fund rather than grants (but potential for political fighting over who controls it?)

Other notes:

- 1) costs may be lowered by collaborations, free testing e.g. CDC, St. Luis, Cornell, NYDoH
- 2) Sell a well packaged plan: since risk is high, prevention and surveillance is a cheaper long term solution

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(names in italics – uncertain spelling)

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