APPENDIX D – HAWAIIAN MONK SEAL VACCINATION RESEARCH AND RESPONSE PLAN

Vaccination – Objectives and Justification

Current information suggests infectious disease is not limiting recovery of the Hawaiian monk seal. However, the species is rare, has very low genetic diversity and may have been buffered from exposure to many mammalian diseases due to its isolation in the Hawaiian Archipelago for millions of years. Together, these factors raise great concern that outbreaks of diseases to which monk seals have not been previously exposed could have devastating impacts.

Proactive efforts to mitigate the potential or eventual negative effects of infectious disease on monk seals include vaccination studies to determine the safety and efficacy of vaccines against specific pathogens considered most likely to spread to monk seals (e.g., morbillivirus and West Nile Virus). Captive studies would include both monk seals and surrogate species, and potentially free-ranging Hawaiian monk seals. If such research indicates that the vaccines are safe and effective, they may be administered preventatively or in response to an outbreak, to wild or rehabilitating seals.

Epidemic diseases (referred to as epizootics when occurring in animals rather than humans) are diseases that occur at a time or place that they do not usually occur, or with a greater frequency than expected in a certain period. Severe epidemics may reduce host population density to such an extent that stochastic events or previously unimportant ecological factors may further reduce the host population size (Harwood and Hall 1990). For example, canine distemper dramatically reduced black-footed ferret (*Mustela nigripes*) populations in Wyoming, bringing them to extinction in the wild (Thorne and Williams 1988); and, avian malaria reduced native Hawaiian honeycreeper (*Hemignathus parvus*) populations to such small numbers that many were finally eliminated by predation or habitat loss (Warner 1968).

Infectious diseases, especially those that are newly introduced to naïve populations of animals, can cause mass illness and mortality. The best means of preventing the spread of infectious disease among animals are vaccinations. Vaccines are available for two viruses that have been identified as high risks to Hawaiian monk seals: morbillivirus and West Nile virus. Background surveys conducted on Hawaiian monk seals support that they remain naïve to both viruses. These two viruses are the current focus of vaccination research and response planning for Hawaiian monk seals.

Morbilliviruses—These viruses, specifically phocine distemper virus (PDV) and canine distemper virus (CDV), have caused mass die offs of phocids. During 1988, approximately 18,000 (70% of the population) harbor seals (*Phoca vitulina*) in Europe died from PDV infection (Heide-Jørgensen *et al*. 1992). A second outbreak of PDV occurred in the North Sea in 2002, which killed over 20,000 harbor seals (Jensen *et al*. 2002). Outbreaks of canine distemper (CDV) killed 5-10,000 Baikal seals (*Pusa sibirica*) in 1987-1988 (Grachev *et al*. 1989), 10,000 Caspian seals

(P. caspica) in 2000 (Kennedy *et al*. 2000) and may have been responsible for the deaths of 2,500 crabeater seals (*Lobodon carcinophagus*) in the Antarctic in 1955 (Laws and Taylor 1957). While a morbillivirus was isolated from Mediterranean monk seals (*Monachus monachus*) that died during an epidemic, its importance relative to biotoxins in causing mortality remains controversial (Hernandez *et al.* 1998). While the susceptibility of Hawaiian monk seals to morbilliviruses is unknown, due to the devastating effects these viruses can have on phocids, there is a need to better understand and prepare for such an event in Hawaii.

West Nile Virus—This virus caused the death of a captive monk seal at SeaWorld San Antonio, Texas, and has caused mortality in captive harbor seals in the mainland U.S. To date this virus has not been identified in wild marine mammals, although it is present along the eastern seaboard and southern California. This mosquito-borne virus is currently not present within Hawaii, and the State has rigorous surveillance and response plans for this virus due to its public health importance. Although neither single cases of disease nor epidemics of West Nile Virus have been reported in wild marine mammals to date, the death of a monk seal in Texas from this infection indicates monk seals are susceptible. Thus, the possibility of extensive mortality in monk seals exists if the virus were to be introduced to Hawaii, warranting a response plan to such a scenario.

Available vaccines—Vaccines currently used for prevention of viral diseases in domestic animals can be divided into three types:

- Vaccines based on a dead inactivated virus;
- Vaccines using live attenuated viruses; and
- Vaccines consisting of recombinant viruses.

Vaccines using a dead virus are considered the safest because the virus cannot replicate in the host or cause disease; however, this lack of replication often means that the immune response generated following vaccination is short-lived and may not be protective. Live vaccines typically generate the most effective immune response. When used in species other than the one for which the vaccine was developed, live vaccines present the risk of the virus replicating in the host and either causing disease in the vaccinated animal, or being shed in secretions and becoming infective to contact animals. One vaccine proposed for use under this permit is an inactivated West Nile virus vaccine (Innovator, Fort Dodge) that has been used regularly to date on Hawaiian monk seals in captivity in San Antonio, Texas, with no adverse reactions observed (Workshop to Evaluate the Potential for Use of Morbillivirus Vaccination in Hawaiian Monk Seals, Final Report 2005).

Recombinant virus vaccines use a vector virus that does not typically infect the target host but expresses antigens from the pathogen of interest to stimulate an immune response against it. A recombinant vaccine to CDV (monovalent recombinant canary pox vector expressing canine distemper virus antigens, Purevax, Merial) licensed for use in ferrets in the U.S., is now used extensively in zoological collections (Bronson et al. 2007) and is proposed for use in research and enhancement activities under this permit. It is the only distemper vaccine recommended by the American Association of Zoological Veterinarians for use in non-domestic carnivores

including mustelids (http://www.aazv.org). It is approved generically for animal use in the State of Hawaii. Safety and efficacy trials with this CDV vaccine have been conducted on four captive harbor seals and on one captive Hawaiian monk seal. These preliminary studies demonstrated that the vaccine is safe, and antibodies to canary pox were detected after a second (booster) dose. This vaccine has also proven to be a safe and effective prophylactic treatment for captive southern sea otters (*Enhydra lutra nereis*) (Jessup et al. 2009).

Research and Enhancement—Vaccination

Vaccination Methods: Up to 1,100 monk seals (essentially the entire species) could be vaccinated if the need were to arise and safe, effective vaccines were available to meet that need. The following describes the proposed approach to vaccine studies and vaccination.

Vaccine research

To prepare for and respond to an epidemic caused by morbilliviruses or West Nile virus, the following research is proposed.

Surveillance for morbillivirus and West Nile infections—To enable detection of novel viral infections in the Hawaiian monk seal population, there is a need to routinely and actively monitor for infections. Monitoring wild monk seals for these viruses may include tests for antibodies against the virus in blood (e.g., enzyme linked immunosorbent assays), tests for actual virus in blood, feces, or nasal swabs (e.g., polymerase catalyzed reaction assays), and syndrome-based surveillance. Sample and data collection for these tests would be covered by health assessment studies described in the Final PEIS.

Assess the safety and efficacy of the recombinant CDV vaccine—Currently, one captive Hawaiian monk seal has been vaccinated against morbillivirus. Vaccination of additional Hawaiian monk seals would better elucidate their ability to mount a proper immune response, the number of vaccines (including boosters) needed to generate this response, and the duration of immunity against morbilliviruses. Vaccination of additional captive Hawaiian monk seals will be pursued with partners under separate permits, including the Waikiki Aquarium and Sea World San Antonio, which have both applied to conduct this research under their own permits. Authorization to conduct vaccine research on monk seals in other facilities that do not have permits to conduct the research are being sought by NMFS.

Post-Vaccination Antibody Response (PVAR) Methods for Permanently Captive Monk Seals

Captive seals can serve as a model to establish vaccine antibody response for Canine distemper virus (CDV) and West Nile virus (WNV). For CDV, the use of Purevax (Meriel) would be used (a monovalent recombinant canary pox). Recombinant vaccines pose less risk than use of a live virus. The WNV vaccine is a product made by Fort Dodge of inactivated WNV. As an inactivated virus, it cannot be shed and therefore does not require a closed system. In addition, the recombinant canary pox has been tested in harbor seals at Sea World (by Pam Yochem) and no virus shedding was detected (Dr. Frances Gulland, personal communication).

To assess the effectiveness of the vaccines, serum antibody samples must be taken throughout the year. It is proposed to collect serum on days 0, 28, 42 and 365 to monitor antibody formation. Day 0 serum collection will occur prior to vaccination to provide baseline values for each animal. Vaccination for both CDV and WNV will occur after the serum is collected. Along with serum samples, duplicate nasal swabs will be obtained. A follow up vaccine will be given on day 14, but no blood sample will be taken at this time. Each vaccine is given subcutaneously in a 1 ml dose, administered twice, fourteen days apart. To minimize restraint and handling time of the seals, the serum collections on days 0 and 365 may also serve as annual blood sampling for the seals regular health monitoring. Additional handling and sedation will occur on days 28 and 42 post-vaccination to obtain the serum and nasal swabs only.

For both routine health monitoring and the PVAR study, blood samples will be obtained through the use of chemical sedation if deemed necessary by the attending veterinarian and light physical restraint. Sedation would be achieved with either diazepam (0.2 mg/kg IV) or midazolam (0.2 mg/kg IM) and blood collected from the extradural sinus or interdigital webbing vein. Flumazenil will be kept on hand for emergency use to reverse diazepam or midazolam sedation if necessary. However, it will not be used routinely as the half-life is less than that of the sedative drugs. Blood samples and nasal swabs will be obtained. At some facilities, seals may be trained for voluntary blood sampling. In addition, vaccination of future monk seals brought into temporary captive care (under the MMHSRP permit) may be conducted during the research phase.

Outbreak response for seals in the wild

Vaccination of monk seals may occur either in response to an outbreak or prophylactically in the absence of disease in Hawaii. Once a minimum of five captive seals has been vaccinated with no adverse effects identified, a prophylactic vaccine trial should be developed in the MHI. However, until this trial has been performed, a response plan is needed in case of disease events that could significantly increase the risk of morbillivirus disease in monk seals, due to their critically endangered status. A series of different disease parameters in Hawaiian monk seals, other marine mammals and domestic animals have been identified that could trigger a vaccination response in Hawaiian monk seals.

HMSRP proposes to vaccinate in response to disease outbreaks as diagnosed by a series of triggers described below. If the risk of morbillivirus or West Nile virus epidemics to monk seals changes from the current situation, this approach may be modified.

Morbillivirus

Triggers

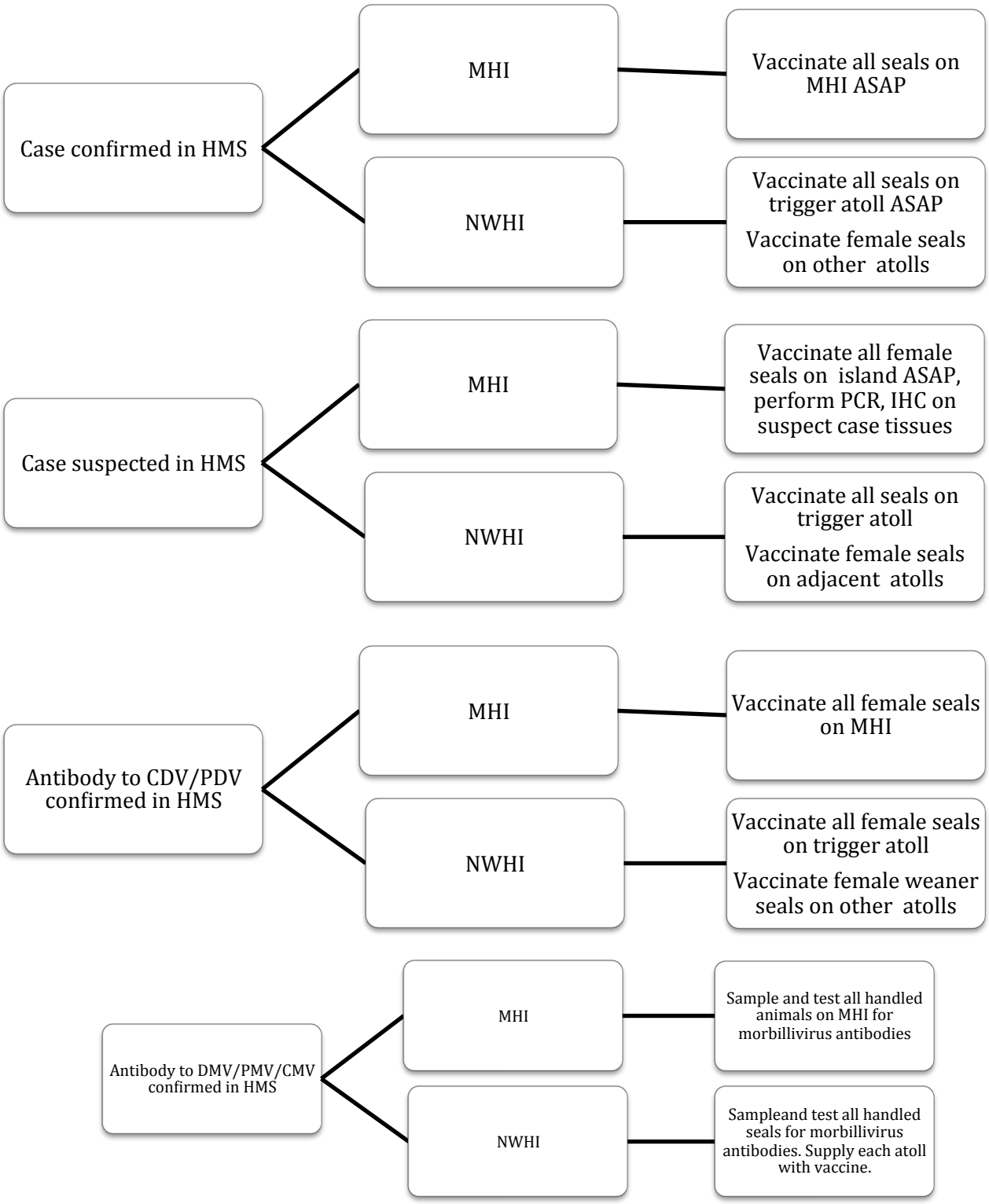
A *confirmed* case is an animal with pneumonia, or encephalitis, or lymphadenitis, or dermatitis, with morbillivirus detected in tissues by PCR or immunohistochemistry, and its identity confirmed by nucleic acid sequencing.

A *suspect* case is an animal with severe pneumonia or encephalitis associated with syncitial cells and inclusion bodies detected on histology, with either a positive PCR or immunohistochemistry result. Detection of antibody occurs when serum neutralization test results are greater than 1:16.

Responses to each disease parameter are summarized in the decision tree below. Each response is made by weighing the advantages and disadvantages, and recognizing that a second trigger occurring during a response may increase the level of response. Detection of antibody implies that exposure is occurring, but lack of disease would imply seals have developed resistance to the exposure. Thus vaccination response would be at a lower level than that to a detected case.

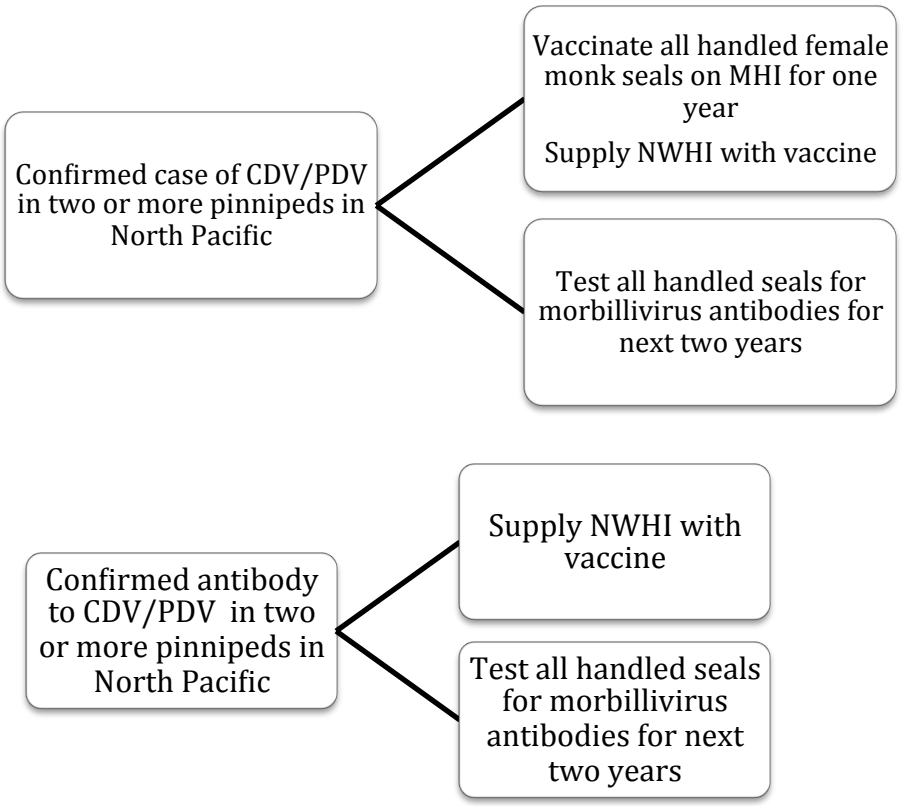
All vaccination responses would be maintained for one year. During response, surveillance for morbillivirus infection through necropsy of dead animals and serology of handled live animals will be prioritized by NMFS. Following vaccination, all vaccinated animals would be blood sampled and tested for morbillivirus antibodies within one year of vaccination unless pregnant.

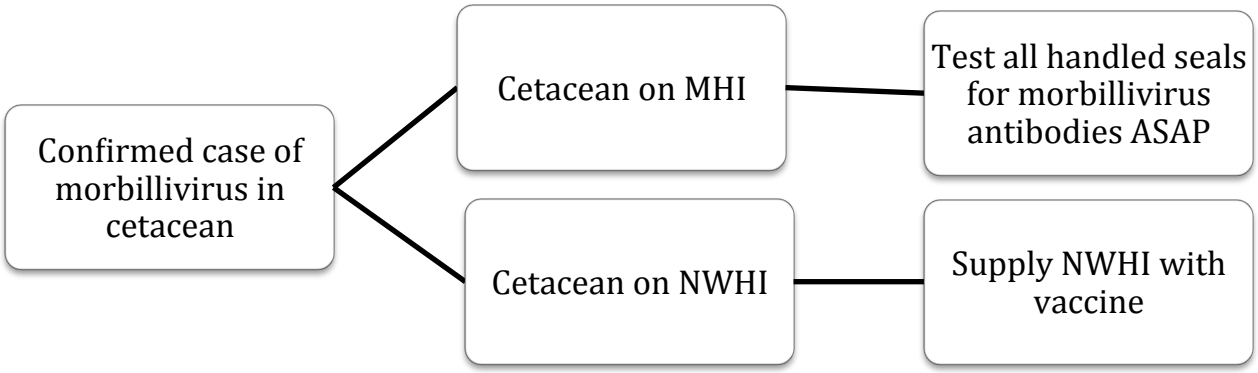
Triggers in Hawaiian Monk Seals

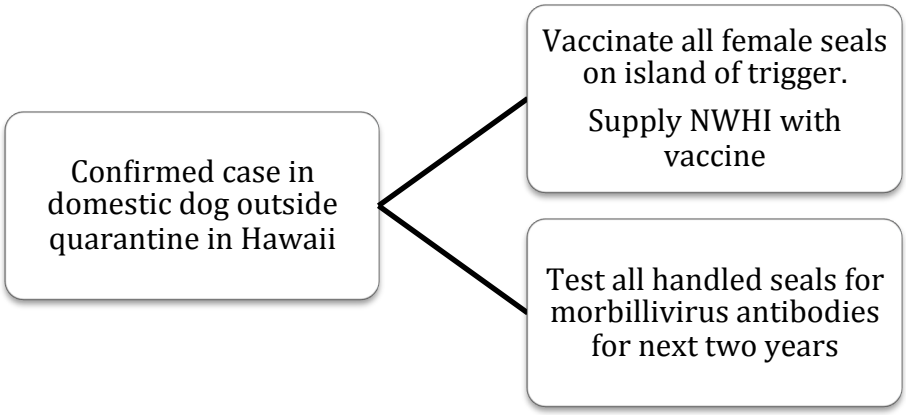


Triggers in Other Mammals

Morbillivirus associated disease in seals to date worldwide is believed to have resulted from transmission of virus from other seal species and domestic dogs (Grachev et al 1989, Jensen et al. 2002). Thus diseases in these species are considered risk factors for monk seals. Morbillivirus disease has not been reported to date in pinnipeds of the North Pacific, nor in mammals on the Hawaiian Islands, despite its prevalence in seals in Europe and the Atlantic (see above), and in domestic dogs in the continental United States. If morbillivirus disease was detected in pinnipeds in the North Pacific, the risk of Hawaiian monk seal exposure to morbillivirus infections would be heightened due to occasional movement of pinnipeds from other regions of the North Pacific to Hawaii. A small number of northern elephant seals have been documented in Hawaii and in 2012 a female northern fur seal landed on Oahu. Movement of other pinnipeds to Hawaii occurs unpredictably, and vaccination takes time to perform and achieve protective immunity. Thus, triggers that suggest pinniped morbillivirus disease could reach Hawaii at random times have been identified to trigger vaccination. Triggers that could occur in mammals other than pinnipeds have also been identified.







Results of the response to the first trigger event will be used to refine responses to subsequent trigger events. In particular, records will be taken on:

- Time between trigger and administration of first and second dose of vaccine;
- Number of seals vaccinated;
- Time required to vaccinate all or most animals on island;
- Age distribution of vaccinated animals; and
- Resightings of vaccinated animals
- Any indication of adverse reaction to vaccination.

West Nile Virus

The epidemiology of West Nile Virus differs significantly from that of morbilliviruses, as it is a vector borne zoonotic virus rather than a directly spread animal pathogen. This virus caused the death of a captive monk seal at SeaWorld San Antonio, Texas, and has caused mortality in captive harbor seals in the mainland U.S. To date this virus has not been identified in wild

marine mammals, although it is present along the eastern seaboard and southern California. As this mosquito-borne virus is currently not present within Hawaii, the State has rigorous surveillance and response plans for controlling this virus due to its public health importance. Although neither single cases of disease nor epidemics of West Nile Virus have been reported in wild marine mammals to date, the death of a monk seal in Texas from this infection indicates monk seals are susceptible. Thus, the possibility of extensive mortality in monk seals exists if the virus were to be introduced to Hawaii, warranting a response plan to such a scenario

Trigger

A case of West Nile virus in the Hawaiian Archipelago in humans or wildlife, with activation of the State emergency response for West Nile virus control could trigger implementation of West Nile virus vaccinations in wild Hawaiian monk seals.

Response

As vaccination of Hawaiian monk seals to WNV has occurred with proven safety for over 5 years in 8 captive monk seals in Texas, the risk of vaccination against WNV is minimal, apart from risks associated with approach and injection.

In response to a detected case of WNV in any species in Hawaii, all accessible seals on the MHI would be vaccinated with West Nile virus vaccine (Innovator, Fort Dodge), starting with the island on which the case was identified. Vaccine would be transported to each NWHI and used if the outbreak is not controlled in the MHI within 2 months.

Potential prophylactic vaccination

The best way to protect Hawaiian monk seals against these viral infections is to vaccinate prior to population-wide exposures. This is especially true if multiple doses of vaccines are required to gain immunity against infections, or if immunity responses take weeks to months to develop. Conversely, vaccines that mount short-term responses against infections or have higher risks of side effects may best be delivered only in the face of population-wide exposures. Based upon the information gained from research and any outbreak response, it will be determined whether prophylactic or solely response-driven vaccinations against morbillivirus and West nile virus are needed.

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