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# 2016 Report on Hawaiian Monk Seal Vaccination Program[1]

Report period: January 1, 2016 – December 31, 2016

## I. BACKGROUND

Morbilliviruses have caused mass die offs of wild phocid populations in some parts of the world, namely the Atlantic Ocean (Europe and N. America), North Sea, Caspian Sea and Lake Baikal (Grachev et al. 1989, Harwood and Hall 1990, Heide-Jorgensen et al. 1992, Jensen et al. 2002, Earle et al. 2011). Although this pathogen has not been associated with phocid morbidity or mortality in the North Pacific to date, it has been detected in marine mammals in this region (Duignan et al. 2014, Fujii et al. 2006, Goldstein et al. 2009, Ohashi et al. 2000, Van Bressem et al. 2014, West et al. 2013).

Health surveillance of wild Hawaiian monk seals (HMS) indicates that there is no pre-existing immunity to morbilliviruses in this endangered species (Kaufman 2016). Hence, introduction of morbillivirus could rapidly lead to an outbreak with devastating impacts, a realization that led research teams to consider protection through vaccination.

Several tools, including a contact network analysis and epidemiological model, have been recently developed to evaluate the effectiveness of vaccinating wild HMS in response to an outbreak (Baker et al. in review, Baker et al. 2016). These analyses indicate that vaccination of seals in response to an outbreak would be ineffective in preventing spread. This is largely the result of the time lag between initial vaccination and presumed immunity (Quinley et al. 2013). Hence, prophylactic vaccination of monk seals appears to be a necessary action to protect the species.

The Merial monovalent *Purevax*® canarypox-vectored recombinant distemper vaccine, labeled for ferrets, was previously tested for safety and seroconversion in captive harbor seals (Quinley et al. 2013) and Hawaiian monk seals (Yochem and Gulland, pers. comm.) before going on indefinite backorder in 2013. In late 2015, Merial made a limited quantity of Purevax available again. Given the 2015 window of availability and results of the epidemiological model, it was decided that use on wild HMS was appropriate in order to begin implementing a prophylactic vaccination strategy to protect this species from a devastating morbillivirus outbreak.

Thus, the 2016 vaccination program was designed to: (a) re-confirm safety in HMS in controlled settings (i.e., rehabilitation) using the currently available Purevax product; (b) establish capacity to further evaluate long-term antibody titer levels in

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a subset of seals; and (c) implement prophylactic vaccination of free-ranging HMS, with an initial focus on achieving herd immunity on Oahu. This document summarizes 2016 Hawaiian monk seal vaccination efforts (Johanos, 2016a) and serum antibody findings (Kaufman, 2016). Monk seal demographic data (Johanos, 2016 b-e) were utilized during study design and implementation, and provided context for results. All activities were conducted under NMFS Permits 16632 and 16632-01.

## II. KE KAI OLA SEALS

In January 2016, 7 HMS nearing completion of rehabilitation were the first vaccination candidates. These seals were in rehabilitation from Sept. 2015-Apr. 2016 and treated for malnutrition at The Marine Mammal Center's Ke Kai Ola (KKO) monk seal rehabilitation facility. Patients were rescued from the main (n=1) and Northwestern Hawaiian Islands (n=6). Six of the female seals were prematurely weaned pups and one (Y70F) was a malnourished yearling at the time of admission. All patients were vaccinated in January 2016 after recovering from malnutrition and awaiting release to the wild. These individuals presented an opportunity for vaccination and monitoring with direct, daily visual monitoring by trained professionals in a controlled setting as well as follow-up physical examinations and bloodwork.

### *KKO Seals: Methods*

All seals were determined to be healthy by a veterinarian prior to vaccination. Beginning in January 2016, KKO seals were vaccinated in two groups. Each seal was given two 1 mL doses of the Merial Purevax canarypox vectored recombinant distemper vaccine several weeks apart. The injection was given intramuscularly in the gluteal muscle as done in previous studies (Quinley et al. 2013). Injections were given by hand and, whenever possible, concurrent with routine veterinary examinations.

The interval between initial vaccine and booster was 22 days for Group 1 (n=4 seals) and 54 days for Group 2 (n=3 seals). The booster window in Group 2 was intentionally lengthened in order to permit an opportunity to examine antibody titers with a longer window between sequential vaccines.

### *KKO Seals: Results*

The paramount objective of vaccinating seals at KKO was to confirm safety with the currently available vaccine prior to vaccinating wild seals. **No adverse effects were detected, either at the sites of injections or systemically, in any of the individuals vaccinated at KKO**. Physical examinations and hematological analyses were conducted on each individual at KKO during the vaccination period. Hematological analyses and physical exam findings were within acceptable limits for each individual throughout the remainder of their care. All 7 seals were released into the wild in the NWHI in spring 2016 and visually monitored by field staff for

3-4 months; all 7 individuals were alive at the end of the field season in August 2016.

At admission to rehabilitation in September 2015, all 7 patients were screened by serology and nasal swab PCR for exposure to or shedding of morbillivirus and all were negative. Serum antibody titers were evaluated in KKO seals after vaccination to enable rough comparisons to previous studies on captive phocids using this vaccine (Quinley et al. 2013). Serum samples were collected at day 0, on the day of booster administration (22 or 53 days post-initial), and at least one later time point (75-102 days post-initial), as described in Tables 1 and 2. The complete time series of samples was available for 6 seals. Later time point samples from the 7$^{th}$ seal were misplaced but have been relocated and will be analyzed in 2017.

Titers to canine and phocine distemper (CDV and PDV, respectively) were measured by serum neutralization at the University of Georgia, Athens Veterinary Diagnostic Laboratory. Titers of 16 or higher indicate the presence of antibody to morbillivirus in the serum sample tested (Tables 1 and 2). For the 6 seals with a complete time series of samples, 6/6 (100%) mounted CDV antibody titers ≥16 after vaccination; all but one required booster administration before mounting a titer at or above 16. Titers subsequently declined at later time points in 3 of these seals (50%).

Heterologous CDV antibody responses against PDV were also evaluated: 3/6 seals (50%) with a complete time series of samples had antibody responses that cross-neturalized PDV; all 3 required booster administration before showing cross-neutralization (≥16). These values are expected to be lower than those for CDV and this is consistent with the Quinley et al. 2013 study on harbor seals in captivity; however the PDV antibody titers in the KKO seals were higher than the PDV antibody titers measured in the harbor seals. This could reflect a more robust response or could be attributed to the different sampling interval used at KKO. Serum antibody titers were measured as coarse indicators of vaccination success. They provide a metric that the vaccine was delivered effectively. However, caution must be used in their interpretation in light of what these antibody titers would mean in the face of actual pathogen exposure. Efficacy will not be measured in this study given that it would require exposing vaccinated seals to the virus. Thus, we cannot conclude from these data that seals are definitively protected nor that a decline in titers at later time points indicates a lack of protection.

Nasal swabs were evaluated (PCR) and determined to be negative for shedding of canarypox virus or morbillivirus (pan-morbillivirus, CDV and PDV), consistent with the controlled vaccine trials conducted in monk seals and harbor seals.

Table 1. Canine distemper serum antibody titers from 7 KKO seals vaccinated in rehabilitation. Titers of 16 or higher indicate the presence of antibody to morbillivirus in the serum sample tested.

| Seal ID | Booster window (#days) | CDV serum antibody titer | | | | |
|---|---|---|---|---|---|---|
| | | Day 0 | Day 22 | Day 53 | Day 75-80 | Day 102 |
| **Group 1** | | | | | | |
| LG06 | 22 | Neg | Neg | | 128 | |
| NG99 | 22 | Neg | Neg | | 128 | 64 |
| Y70F | 22 | Neg | Neg | | 64 | 32 |
| AG18 | 22 | Neg | 256 | | Neg | 128 |
| **Group 2** | | | | | | |
| KG56 | 53 | Neg | | 4 | *Pending* | *Pending* |
| LG36 | 53 | Neg | | 8 | 256 | |
| AG34 | 53 | Neg | | 128 | >512 | |

Table 2. Phocine distemper serum antibody titers from 7 KKO seals vaccinated in rehabilitation. Titers of 16 or higher indicate the presence of antibody to morbillivirus in the serum sample tested.

| Seal ID | Booster window (#days) | PDV serum antibody titer | | | | |
|---|---|---|---|---|---|---|
| | | Day 0 | Day 22 | Day 53 | Day 75-80 | Day 102 |
| **Group 1** | | | | | | |
| LG06 | 22 | Neg | Neg | | 4 | |
| NG99 | 22 | Neg | Neg | | Neg | Neg |
| Y70F | 22 | Neg | | | 4 | 32 |
| AG18 | 22 | Neg | Neg | | 16 | Neg |
| **Group 2** | | | | | | |
| KG56 | 53 | Neg | | 4 | *Pending* | *Pending* |
| LG36 | 53 | Neg | | Neg | Neg | |
| AG34 | 53 | Neg | | Neg | 32 | |

## III. WILD, FREE-RANGING SEALS

After safety was ensured through vaccination of the 7 seals at KKO, efforts expanded to vaccinate free-ranging HMS in the main Hawaiian Islands. This phase of the program began in February 2016.

### *Wild seals: Methods*

Oahu was chosen as the initial site for free-ranging seal vaccinations for several reasons. First, disease outbreak modeling indicates that the spatial structure of the MHI monk seal population makes herd immunity achievable with a relatively low number of vaccinations because of the small number of seals using specific islands or island clusters. Immunizing 17-26 seals in a total population of 43 seals on Oahu was estimated to provide herd immunity in 90-100% of simulated scenarios. Second, GPS-tagged seals moving between Kauai/Ni'ihau and Maui Nui typically landed on Oahu as an intermediate step. Thus, Oahu is likely a central node in the movement of seals within the MHI so that achieving herd immunity on Oahu yields the best chance of containing an outbreak that may occur anywhere in the MHI. Finally, it was logistically most feasible to achieve follow-up observations and conduct sampling of a subset of seals on Oahu because this is the island on which most staff are based. Select individuals may be sampled approximately 1 year from vaccination to re-examine serum titers and again every 1-2 years.

Efforts were expanded to Kauai and to a limited extent, Molokai, in June 2016. Vaccinations on Molokai were limited to weaned pups due to the ease of vaccination of this age class opportunistically, during tagging and the high likelihood of re-locating those seals in the same area a month later for booster administration.

Protocols are not specifically included in this document but are available online and can be provided upon request. In brief, routes of vaccine administration included hand injection and pole syringe injection, consistent with the draft Vaccination Implementation Strategy disseminated by webinar in winter 2014 and a Vaccination Drill conducted in summer 2015 on Oahu. To date, most seals vaccinated using the pole syringe have shown minimal responses (i.e., vocalize/shift body position but do not flush).

### *Wild seals: vaccination candidate selection*

Seals identified by social network analysis as having a high connectivity with other seals (in other words, potential disease spreaders) were given high priority for vaccination. Among these, seals that are often sighted on Oahu and can be handled and sampled at multiple time points were prioritized for sampling, particularly in the early stages of the effort. Any seal with a known health concern was avoided.

We did not anticipate any difference in response between males and females, but males and immature females were preferentially captured for sampling to avoid risk of capture stress for potentially breeding females. To be highly conservative at this

stage of the effort, females that could be pregnant based on reproductive history or age (> 4 years old) were avoided, with the exception of adult females during the 1-2 month period immediately after weaning a pup.

*Wild seals: number of seals vaccinated*

Table 3. Seals in this table received a booster within 8 weeks of the initial vaccine administration. Seals vaccinated in rehabilitation are not included.

| # Seals fully vaccinated: | Male | Female | Adult | Juvenile, suabdult | Weaned Pups | Total |
|---|---|---|---|---|---|---|
| Oahu | 11 | 10 | 12 | 5 | 4 | 21* |
| Kauai | 14 | 4 | 4 | 12 | 2 | 18 |
| Molokai | 1 | 2 | 0 | 0 | 3 | 3 |
| Total | 26 | 16 | 16 | 17 | 9 | 42 |

* Two female seals, 1 adult and one juvenile, died from unrelated causes following vaccination.

Table 4. Seals in this table were either not boostered or were given a booster >8 weeks after the initial vaccine was administered.

| # Seals partially vaccinated: | Male | Female | Adult | Juvenile, suabdult | Weaned Pups | Total |
|---|---|---|---|---|---|---|
| Oahu | 2 | 1 | 2 | 1 | 0 | 3* |
| Kauai | 1 | 1 | 1 | 0 | 1 | 2 |
| Molokai | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 3 | 2 | 3 | 1 | 1 | 5 |

*One adult male died prior to booster administration.

*Wild seals: Serum antibody titers*

Baseline serum samples were collected and archived from wild, free-ranging seals prior to vaccination (n=5) and follow-up samples have been collected from one individual. These samples are routinely collected as part of the HMSRP's epidemiology sampling protocol, which was followed as opportunities allowed and resulted in the collection of a full suite of samples for baseline population health. While we acknowledge that serum antibody titers are not a direct reflection of immunity in the face of natural exposure, this research will add to our growing body of knowledge about changes in serum titers over time and inform future booster vaccination protocols.

Serum samples from all sampled seals have been archived and await measurement of antibody titers once samples from all desired time points have been collected. All time points must be analyzed in the same assay at the same time to minimize the effect of inter-assay variability on results interpretation. Hence, no preliminary data are available on serum antibody titers for these seals at this time. Ultimately, serum samples will be analyzed by serum neutralization for antibodies to CDV and PDV (University of Georgia and duplicates sent to Cornell University for blind comparisons between laboratories). Remaining serum will be archived.

Five Hawaiian monk seals in permanent captivity at the Minnesota Zoo were previously vaccinated and boostered while housed at Sea World San Antonio (Yochem and Gulland, pers. comm.) in 2013. Serum was opportunistically collected from these 5 seals in 2016 during routine annual examinations and was archived for future analysis. These samples will add to our data set to determine long-term immune response to vaccination.

*Wild seals: Mortalities*

While some vaccinated seals died in the weeks/months after vaccination, based on post-mortem analyses and population-level statistics, there is no evidence to suggest that the mortalities were vaccine related (Barbieri, 2016, Kashinsky, 2016). A total of 5 non-pup monk seals were found dead in the MHI in 2016 (n=3 juveniles, n=2 adults; 3 pup deaths not included in analysis). Three of the observed deaths occurred in vaccinated seals (3 of 47), while the other two deaths occurred in the non-vaccinated population (2 of 98). A Fisher's exact test indicates no significant difference in odds of death within the vaccinated vs non-vaccinated groups (odd ratio confidence range from 0.34-38.2, p value 0.33). It is also important to bear in mind that not all mortalities are observed, and since only routinely-seen and accessible seals could be vaccinated, these seals may be more likely to be observed upon death, so that the number of mortalities is more likely to be underestimated in the non-vaccinated group.

RT12

RT12 was seen swimming, interacting with another seal, and hauled out in his usual area the day before he was found dead. The seal was vaccinated 20 days prior to mortality and was seen regularly post-vaccination without any sign of illness. The seal was in robust nutritional condition.

Radiographs were taken to look for foreign bodies but we were limited because of bloating of the carcass; no fractures were identified. The ventral neck tissues and back of the skull were grossly hemorrhagic; this was confirmed by histopathology, indicating that trauma was likely sustained pre-mortem. Edema was noted in the lungs, which is a non-specific finding. The throat, esophagus and stomach were full of partially digested fish and hard parts, indicative of recent foraging. The area where the vaccine was given was thoroughly examined at necropsy and there was

no evidence of local infection or inflammation around the injection site. Routine histopathology did not identify any underlying disease in this seal. Overall, this seal was in good health and all findings indicate that this was an acute mortality.

Blood tests were negative for *Toxoplasma gondii* as well as other protozoa. Nasal swabs were negative for shedding of phocine distemper virus.

Therefore, the key findings we have to help us in understanding the mortality in RT12 were the evidence of trauma, large volume of stomach contents, pulmonary edema, and the absence of signs of ill health. Together, these findings suggest that this seal may have died from being trapped underwater, and possibly entrapped in a net while foraging. One of the fish that was identifiable from the stomach was a bonefish (species targeted in recreational gill net fishing). These cases are very difficult to definitively classify given that lesions are not exclusive to underwater entrapment.

R912

R912 was vaccinated on August 18 and boostered on September 8. She was observed behaving normally and hauled out in a typical location on September 13, and reported as molting. The carcass was found on September 23 in a state of advanced decomposition (early code 4). No definitive cause of death was evident on necropsy, but nutritional condition was robust and there was evidence of recent feeding. No fractures were observed but a large opening in the skin/blubber and muscle was present along the right chest and abdomen. It is uncertain whether this occurred ante- or post-mortem. Cranial radiographs did not reveal any metallic foreign bodies. Tissues were collected for histopathology to look for underlying disease but most samples were too decomposed for suitable evaluation. The main finding was a mild to moderate aspiration pneumonia. The material that was aspirated into the lungs was skeletal muscle and other necrotic (dead) cellular material. The pneumonia did not have any characteristics of a bacterial or viral infection. This seal had been previously vaccinated, but nothing suggests an adverse reaction related to the vaccine. Together, the necropsy and histopathology findings suggest that she may have sustained trauma, which led to difficulty breathing, regurgitating/aspirating, and developing acute aspiration pneumonia just prior to death. R912 was last seen alive on Sept. 13 (and an unconfirmed sighting report on Sept. 20) - no abnormal behavior or trauma was reported, which further supports a diagnosis of acute trauma.

RG06

This yearling female seal died from a mesenteric volvulus after surgical intervention for an intussusception, or invagination of one segment of the intestine into another. Intussusceptions are rarely reported in pinnipeds. Risk factors for intussusceptions

in domestic species include young age, high parasite burdens and intestinal hypermotility. Endoscopy did not reveal a high parasite burden (subsequently, this was supported by gross necropsy findigns). In surgery, approximately 100 cm of intestine was resected and the serosal surfaces of the anastomosed loops of intestine were pexied together to reduce the chance of a repeat occurrence. Post-operative complications are common following any disturbance of the GI tract, including surgical intervention, and unfortunately the volvulus (twisting of the intestines around the mesenteric root) occurred approximately 2 days into post-operative recovery and the patient died a few hours later.

The underlying cause of the intussusception in this case is not definitively known and was not identified by histopathology. Intussusceptions are thought to develop secondary to altered intestinal motility; in the case of RG06, intestinal motility could have been impacted by a fish hook that was found in her tongue, leading to impaired feeding for 7 days or more. She was first reported with a suspect hook on 9/30/16 and was dehooked on 10/6/16 (total duration of hooking unknown). A prophylactic dose of antibiotics was administered at that time, along with a booster vaccine, and she was immediately released. Nutritional condition was thin/average, and typical for a yearling seal. On Oct. 15, RG06 was reported with weight loss and suspect lethargic behavior; she was monitored the following day and captured and brought into rehabilitation on Oct. 20, at which time the intussusception was diagnosed.

A standard suite of samples was collected for histopathology and examined. No evidence of infection or concurrent disease was identified.

## CONCLUSIONS

In 2016, 49 wild Hawaiian monk seals were fully vaccinated (2 vaccines, ≤ 8 weeks apart) with no adverse effects. Of these, 7 were released in the NWHI and 40 are alive and contributing to herd immunity in the MHI. The 3 vaccinated free-ranging Hawaiian monk seals that died during 2016 (2 fully vaccinated, one partially vaccinated) were closely examined; there is no evidence from post-vaccination sighting histories or from post-mortem examinations that these mortalities were linked to vaccination. The number of mortalities in 2016 is not significantly different between vaccinated and unvaccinated seals. However, these individuals no longer contribute to herd immunity and are not considered in the total vaccinated pool.

Thus, 40 individual seals in the MHI presently contribute to herd immunity in this subpopulation. On Oahu and Kauai, where most seals were vaccinated, we estimate that our efforts conferred sufficient herd immunity to prevent an outbreak of morbillivirus in > 90% of possible scenarios at these 2 sites.

The serum antibody titers measured in the seals in rehabilitation at KKO provide evidence that vaccinations were administered effectively; titers were consistent with those measured in captive seals (Quinley et al., 2013, Yochem and Gulland,

pers. comm.). Post-vaccination physical examinations, CBC and chemistry panels for these seals were within normal limits, and all were subsequently released in the NWHI.

Overall, this effort greatly expanded the number of phocids vaccinated with the Merial Purevax recombinant CDV vaccine, which was previously limited to safety trials on seals in permanent captivity (5 harbor seals and 7 Hawaiian monk seals) (Quinley et al., 2013, Yochem and Gulland, pers. comm.). Activities to-date have therefore demonstrated continued safety and that methods of vaccine delivery are adequate.

## NEXT STEPS

The vaccination program in 2016 established a high likelihood of herd immunity on Oahu and Kauai, however we recognize that it will be affected by births and mortalities in the population over time. Vaccination efforts on these 2 islands in the future will primarily target recently weaned pups at the time of flipper tagging. Incompletely or unvaccinated juveniles, subadults and adults will be vaccinated opportunistically. In addition, efforts in the MHI will expand to all age classes of seals on Molokai, as opportunities permit.

While the vaccine was unavailable between October and November 2016, a large supply of product is now available with a shelf life through late July 2017. Hence, plans and protocols for vaccinating seals in the remote NWHI during the summer 2017 field season as well as a continuation of vaccination efforts in the MHI are in preparation.

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