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Memorandum for the Record

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Subject: U.S. Fish and Wildlife Service position regarding captive manatees and papilloma virus

Introduction
The purpose of this memo is to present the history of papilloma virus (PV) within the captive and wild manatee population, present results of research conducted to better understand PV, outline recommendations made by an expert disease panel, and document the actions and decisions made by the Service regarding PV and captive manatees.

History
The North Florida Ecological Services Office in Jacksonville, Florida has recovery lead and administers the daily management of the Florida manatee. One recovery activity for this species is the implementation of the Manatee Rescue, Rehabilitation and Release Program (Rehab Program). This program began in the early 1970s with the goal of assisting injured and distress manatees in the continental United States and the Caribbean. To date the Rehab Program has successfully rescued, medically treated, and released hundreds of injured and distressed manatees.

In 1996 a small 4mm raised lesion was removed from the facial disc of a manatee in rehabilitation at Sea World Orlando. Pathology interpretation indicated a possible papilloma lesion; it was assumed that this finding was a normal expected issue in the species, so no action was taken. The animals with the lesion had been in captivity for many years and came in contact with a number of sick and injured animals during rehabilitation. A number of manatees taken to Homosassa Springs Wildlife State Park (HSWSP) for rehabilitation originated from the SeaWorld Orlando facility and had either been exposed to this animal or to other animals that had come into contact with the first biopsied manatee.

On July 23, 1997 researchers announced the discovery of a papilloma virus (PV) in Florida manatees undergoing rehabilitation at the HSWSP. PVs are transmissible viruses that are generally species-specific and form usually benign tumors in numerous mammalian and avian species (Sundberg et al. 1995; Sundberg et al. 2000). After the discovery of this virus a quarantine was implemented in 1998 at the facility prohibiting any direct contact between captive manatees housed in the facility and wild manatees just outside the exclosure fence.
HSWSP, in collaboration with Dr. Greg Bossart and colleagues, began to monitor the infected manatees noting lesion numbers, size, location and overall health. In 1999 several biopsies were taken from wild captive manatees and tested for PV. These samples did not indicate the presence of PV, however, these data were based on bovine PV screens, which at the time, was the most advanced technology in use to accurately detect exposure to the virus.

In 2003, a papilloma virus was identified using a screening assay adapted for use specifically in manatees. The researchers successfully sequenced the entire manatee papilloma virus genome, and designated this virus as a novel species TmPV-1 (Rector et al. 2004) and the first virus recognized in any members of the Order Sirenia. This research suggested that TmPV-1 was not transmitted from other hosts to manatees, but rather co-evolved with the species and was present in a latent form in manatee skin tissue. In 2004, the production of virus-like particles was also completed allowing for the development of a sensitive and specific ELISA sera test (these virus-like particles were also potential vaccine antigen candidates). The ELISA test was designed as a tool to determine exposure to TmPV-1 in manatees; a seroprevalence study was then initiated by Dr. Greg Bossart and colleagues from the James Graham Brown Cancer Center in Louisville, Kentucky. Subsequently, in 2005 archived samples were tested for serum sensitivity to antibody titers for TmPV-1 using the ELISA. Both wild and captive manatees (n = 21 and 20 respectively) from Florida were screened, as well as 23 samples from manatees collected in Mexico. Results demonstrated positive titers for four captive manatees known to have had TmPV-1; however, free-ranging manatees had low levels of sera sensitivity detection for the TmPV-1 antibody.

In parallel, in the winter of 1998, biologists from the US Geological Survey Sirenia Project (USGS) began collecting manatee skin samples from Homosassa and Crystal Rivers as part of a preliminary study to investigate for the presence of PV in wild manatees. Samples were opportunistically collected through 2003, then subject to DNA extraction and analyzed using polymerase chain reaction (PCR) amplification. Results from this study (Woodruff et al. 2003; Woodruff et al. 2005) supported the presence of PV in six wild manatee samples collected in the winter of 2003. Subsequently, concerns were raised regarding the integrity of the laboratory and possible contamination of the samples collected. An internal review determined the research and its findings were valid, however, concerns and doubts were still expressed within the manatee research and management community, prohibiting the necessary confidence to support a change in management practices or lifting the existing quarantine.

In the fall of 2003 the Service discovered manatees in captivity at Sea World San Diego possessed lesions associated with PV. Following this discovery, the Service was also informed about a previously PV infected manatee at Sea World Orlando (SWF). This animal had been housed with many other captive
manatees that subsequently were released or transferred to other holding facilities. This new information resulted in an immediate quarantine for both Sea World facilities.

A series of meetings were organized in the spring of 2004 with manatee veterinarians, biologists, caregivers and managers to address the issue of PV in the captive manatee population. An outside panel of disease experts was also convened (Attachment #1) to advise the Service on the best approach for research and management actions to address manatee PV. Unanimous recommendations included: (1) the development of an ELISA screening technique for both the captive and wild manatee populations; (2) the initiation of an epidemiology study to determine prevalence of PV in the wild; (3) the standardization and continued surveillance for PV in the wild population; (4) the creation of a management plan based on scientific findings of the ELISA test; and (5) the prohibition on releasing infected manatees with “active” lesions caused by PV. The Service was also advised to limit the release of “exposed” manatees to only the Homosassa River area; however, the Service took a conservative approach and decided not to release “exposed” manatees until it was scientifically proven that PV did exist in free-ranging manatees.

In the winter of 2005-06 USGS biologists, in coordination with the Service, initiated an epidemiology study in the Crystal River/Homosassa Springs region to determine the prevalence of PV in the wild manatee population and investigate the health of individuals with suspicious lesions through the change of seasons. To date, the study has been conducted for three field seasons with a total of 37 manatees (24-males, 13-females) captured and examined. Although skin lesions have occasionally been observed, no papillomavirus infections have been confirmed using any of the validated, gold-standard tests (i.e., immunohistochemistry, PCR, and general histology).

Since the discovery of PV, Dr. Bossart, Dr. Jensen and colleagues continued to investigate TmPV-1. A cyclical nature of the lesions on the captive manatee population was eventually observed (similar to that observed by field biologists from suspect lesions on wild manatees), suggesting the probability of immune compromise and activation of a latent virus infection, as seen in other species infected with similar PVs (Jensen 2008 pers. comm.). This collaboration continued with vaccine trials in 2006 on horses to ascertain the efficacy of virus-like particles as a vaccine antigen. Results from the trials indicated a positive immune response and no adverse reactions. Due to permitting and financial constraints, this collaborative work that was intended to test the vaccine antigen and adjuvants in a vaccine format and validate the ELISA on captive manatees known to possess TmPV-1 was not completed. Later discussions with clinical veterinarians, researchers and managers concluded a limited applicability of the vaccine, as it was not feasible both financially and logistically to consider vaccinating large numbers of wild manatees.
In the spring of 2008, a recommendation was made to the Service to use existing ELISA methodology to determine the existence of PV in free-ranging manatees. This recommendation resulted in a joint effort among partners within the Rehab Program and, the James Graham Brown Cancer Center. Fresh samples were collected from all captive manatees (held in captivity for > 1 month). Additionally, archived samples were obtained from wild manatees in all four designated management units in Florida (i.e., St. Johns River area, Atlantic coast, Northwest region and Southwest region) and from wild manatees in Belize. Drs. Jensen, Ghim, Dona and colleagues used the same ELISA that was previously conducted on captive manatees. Results from the study at the most conservative detection levels revealed that many free-ranging manatees had been exposed to TmPV-1 (Dona et al. 2011). Additionally, this work supported the established theories that PV co-evolved with the species and that manatees can host latent PV infections which, under certain conditions where a co-factor is present, can become active and eventually result in non-lethal lesion expression.

The research from Dona and colleagues resulted in letters to the Service supporting the release of previously infected or “exposed” manatees, pending that certain conditions are to be met; these letters are presented in Attachment 2 of this memo.

On September 1, 2011 the Manatee Veterinary Group recommended changes to the Service’s current position regarding manatee PV. The recommended changes reflected information obtained from additional studies (Dona et al. 2011) that demonstrated TmPV1 antibodies are prevalent in wild manatees throughout Florida, as well as an updated opinion from Dr. James Wellehan with recommended changes to the current PV position. These recommendations are presented in Attachment 3 of this memo.

Service Position

Given the scientific evidence presented and recommendations from the expert panel, the Service now supports the release of captive manatees that are free of PV lesions, and have been free from lesions or any contact with animals possessing lesions for at least three months.

Our position is based on the following factors: (1) the species-specific nature of PVs which eliminates concerns that PV could infect other species in the wild; (2) research by Drs. Jensen and Bossart which supports the theory that TmPV-1 is a subclinical infection that manatees have possessed for millions of years and has co-evolved with species; (3) the recent findings by Dona et al. that prove manatees in the wild are exposed to TmPV-1; (4) no manatee has been rescued with a known active PV lesion (n > 900, USFWS unpublished data); (5) the benign nature of many PV lesions, supported by the fact that no manatee has been known to die as a result of PV (April 1974 through 23 September 2011: 8100 total manatee carcasses reported and examined from Florida, and an
additional 269 of which were unrecovered); and (6) professional opinions from the Manatee Veterinary Group and other field experts.

To date there is no evidence that manatee papillomaviruses have had an impact on the health of the population however, the Service will continue to support research to enhance our understanding of this virus. It is believed that wild, healthy manatees have the ability to resolve PV on their own. Monitoring of both the captive and wild populations will continue to ensure that any changes within the wild manatee population are promptly addressed and evaluated.

The following approach had been recommended by the Manatee Veterinary Group and is supported by the Service:

1. If a lesion is suspected to be a papillomavirus infection, the animal should be considered positive for TmPV if the lesion:
   a. is histologically consistent with TmPV and
   b. is positive for TmPV on a Polymerase Chain Reaction test.
2. Before considering a manatee for release back into the wild the following criteria must be met:
   a. must meet current release criteria for age and release classifications,
   b. have had no active TmPV lesions for at least 3 months, and
   c. have no direct contact with manatees with active TmPV lesions for at least 3 months prior to release.
3. Continued TmPV disease surveillance within captive and wild manatee populations will include:
   a. antemortem surveillance for TmPV lesions and
   b. postmortem carcass salvage surveillance for TmPV lesions.

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Relevant References


*manatus latirostris*. Veterinary Immunology and Immunopathology 103:247-256.


