

October 25, 2011

Memorandum for the Record

From: Nicole Adimey, USFWS, Manatee Rescue, Rehabilitation and Rescue Program Coordinator

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 enclosure 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.

Nicole M. Adimey

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Attachment 1

Expert Panel: Manatee Papilloma Virus Conference Call Minutes

## Manatee Papilloma Conference Call Notes April 14, 2004

Participants: Stephanie Wong, Dave Jessup, Thierry Work, James Casey, Francis Gulland, Jonna Mazet, and Terry Spraker.

### **General Comments:**

- It is acceptable to put manatees that are exposed to manatee PV back in areas where manatee PV is located/documented.
- Some PV have geographic restrictions (e.g., turtles in Hawaii)
- If we can show that lesions resolve themselves in the wild then it would be ok to release animals in that area where PV has been observed.
- Captivity is stressful, make sure facilities are not serving as hot spots for PV
- PV may not have the same type of “latency” as other viruses such as herpes
- Caution that ELISA may show positive for many animals; could have many “subtypes” of PV; there are downfalls with ELISA.
- If short term/long term effect is not lethal than this (PV) may not be worth the hype, however, it may just be the tip of the iceberg to what is happening in the environment.
- Could release exposed animals in other areas where they are not from (i.e., location where they were not rescued); let genetics mix as the population has already gone through a “bottleneck”; this should be done over keeping an animal in captivity.
- Should be screening continually for other diseases.
- Believe vertical transmission is unlikely, as compared to land animals.
- PV should not kill animals as long as other environmental stressors do not increase.
- PV has been in land animals for hundreds of years (e.g., cattle, coyotes, etc.) and has never been the cause of death.
- At this time literature and information reveal no concern over serious negative effects at a population level.
- Little evidence to show that PV is an emerging pathogen; it appears that it is not new or of diverse origin.
- Papillomatosis maybe an indicator or marker that a more serious health problem may be developing.
- If papillomatosis is revealed to be widespread, does not cause serious health problems by itself, or does not presage mortality due to some other cause, then concerns over releasing infected animals would not be warranted.
- Caution should be taken when developing vaccines with animals that have immunologic problems or testing the vaccine on healthy animals that will be applied to animals with other health problems.

### **Unanimous/Frequent Comments:**

- Move animals out of the spring run at HSSP.
- Develop an ELISA to test for virus, antibodies, immunities; use pair titers for individuals.
- Must know/document epidemiology of the PV in Florida manatees; standardize surveillance.
- Can release “exposed” animals to areas where PV s known to exist.
- Would not release exposed animals to other areas outside of CR/HSSP until ELISA or additional information became available.
- Management plans should be based on some type of “exposure test”.

### **Recommendations:**

- Monitor animals in the wild with PV (10-20) from various age classes, break down into Kaplan-Meier curves
- Compare manatee PV with the PV found in other cetaceans
- Exposed animals/animals with lesions should be moved to another facility to see if move/new environment will stress animal/reduce stress and change appearance of lesion or viral load; identify a quarantine period and add additional stress to see if lesions occur.
- Keep captive quarantine and keep “clean” facilities as well.
- Do a two-pronged approach for testing PV (tissue and ELISA)
- The progression and regression of lesions in infected animals should be documented and understood before changes in management decisions.
- Maybe screen for retrovirus (as it could be the underlying cause as to my papillomatosis is clinically expressed).
- Develop a test to assess exposure to PV
- Look at disease induced by PV to determine if it manifests itself differently in wild vs. captive manatee; determine if disease manifests itself differently in captives once they are released
- Determine if disease affects survivorship, and if so, what co-factors may influence survival
- Determine if PV measurable affects recruitment by age-prevalence data, disease manifestation in various age groups, recruitment to dams infected with PV or manifesting the disease.

**Questions:**

- 1) What is the difference between manatee PV and killer whale PV? Does manatee PV jump to other marine mammals?
- 2) What is the epidemiology of PV in Florida manatees? What happens to manatees with PV as they get older?
- 3) Lesions may be indicating changes in the environment and showing overall changes in the animal's environment/population. Is there some environmental stressor that is surfacing which is causing immune suppression?
- 4) Can a skin test for PV be done post-mortem using PCR be done in the path lab?
- 5) Do PV lesions regress partially or completely as most papillomas do?

Attachment 2  
Letters of Recommendation



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13 October 2008

Dear Fish and Wildlife Officer,

The results of the serosurvey study conducted by Bonde *et a* suggest that the manatee papilloma virus (TmPV-1) is endemic in the Florida manatee population. The seroprevalence and degree of reactivity in affected and clinically unaffected animals is very similar to what has been observed with papilloma viruses that are endemic in other species. Further, whole genome sequencing indicates that TmPV-1 is a virus that has co-evolved with its manatee host.

Based on these findings I see no reason to restrict movement of manatees that have been in direct or indirect contact with TmPV-1 and that are otherwise clinically healthy.

Kind regards,

/s/

Hendrik Nollens, DVM, PhD



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9 Oct 08

Dear Nicole,

I would caution against clinical over interpretation of the presence of manatee papilloma virus 1 (TmPV1). In mammals, papilloma viruses are widely found in skin of normal animals (Antonsson and McMillan, 2006). In humans, the best investigated host species, there are well over 80 papilloma virus species, and a relatively small proportion of these have any clinical significance. It is highly probable that a large number of additional papilloma viruses remain to be discovered in manatees. These viruses appear to have frequently coevolved with their hosts, and it is probable that TmPV1 has been widely dispersed in manatee populations for centuries. Serological data shows that many human papilloma viruses are widely dispersed (Michael et al, 2008), and weak humoral immune responses to papilloma viruses suggest that these studies underestimate virus prevalence. The serological data that was shown to me by Bob Bonde indicates that TmPV1 is dispersed in wild manatee populations. Given this, I see no reason to restrict manatee movement based on the presence of virus or serological data.

While TmPV1 may have the potential to become more significant in immunosuppressed animals, and the presence of papilloma viral lesions may be an indication for clinical assessment of immune function, this should not be mistaken for a primary papilloma viral disease.

Sincerely,

/s/

James F.X. Wellehan Jr., DVM, MS, DACZM, DACVM

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26 November, 2008

Dave Hankla  
United States Fish and Wildlife Service  
6620 Southpoint Drive, South #310  
Jacksonville, Florida 32216-0958

RE: Manatee Papilloma virus Quarantine

Dear Mr. Hankla,

The Captive Manatee Veterinary Group would like to thank the United States Fish and Wildlife Service (USFWS) and the State of Florida for the current efforts to address the manatee papilloma issue. The Manatee Rescue, Rehabilitation, and Release Program is tasked by the Florida Manatee Recovery Plan to maintain a rescue network with rehabilitation centers and to develop a better understanding of manatee health factors.

Six of eleven participating rescue and rehabilitation institutions are currently hampered by a 10 year USFWS imposed facility quarantine for those animals positively diagnosed with, as well as those with known exposure to manatee papillomavirus (TmPV). During this time a great deal has been learned about the pathological behavior of TmPV in manatees. Papilloma viruses are ancient viruses that have coevolved and cospeciated with their host species; in fact, animals that are closely related to each other have PVs that are also closely related (Rector et al. 2004). They are transmissible and can induce benign tumors (warts) in a wide range of mammalian and bird species (Sundberg et al. 1997, Sundberg et al. 2000). Papilloma viruses are generally species-specific, with interspecies transmission being very rare.

The PV linked to manatees has been characterized as a close-to-root PV (meaning it coevolved with manatees for millions of years) and appears to be typical of its family. This evidence that this species of PV has coevolved with manatees over time provides support that it is unlikely to have been transmitted to manatees from other animals in recent history (Bossart et al. 2002; Rector et al. 2004). The majority of cutaneous PVs, including the one found in manatees, appear to inhabit healthy skin as a part of their normal flora and only become clinically apparent when the individual becomes immuno compromised (Bossart et al. 2002; Rector et al. 2004). In a few captive individuals infection has produced cutaneous benign tumors (warts) but these benign tumors have never been positively identified in free-ranging animals despite an intensive effort by the state of Florida to necropsy every carcass (Marine Mammal Pathobiology Lab, 2008).

Infection of the Florida manatee with PV was first identified in captive rehabilitated individuals but since has been observed in a number of wild manatees. Woodruff *et al.* (2005) found molecular evidence of manatee papilloma virus in both captive and free-ranging populations of manatees inhabiting Florida waters. More recently, Dr. Alfred Bennett Jensen and colleagues from James Brown Cancer Center in Louisville, Kentucky collaborated with United States Geological Survey's Sirenia Project and the Manatee Veterinary Group to complete a seroprevalence study for TmPV. That study demonstrated positive titers in captive as well as free-ranging manatees indicating that TmPV exposure is evident in manatees in all four designated manatee management units. Clearly, TmPV not only exists in individuals living in rehabilitation facilities but indeed in free-ranging animals. These recent findings suggest that a new protocol is needed to address manatee papilloma virus in captive animals. Based on these new data, the Captive Manatee Veterinary Group recommends lifting the current quarantine on

rehabilitation facilities and instead substituting a revised protocol for those animals with clinically apparent papillomas or those manatees that have been in direct contact with active papilloma virus:

1. Case definition- if a lesion is suspected to be a papilloma virus infection it should be considered histological consistent with TmPV **and either**
  - a. Positive for TmPV on Polymerase Chain Reaction testing
  - b. Positive for TmPV on Immuno histo chemistry staining
  
2. Before considering a manatee for release back into the wild the following criteria must be met:
  - a. Meet current release criteria for ages and release classifications
  - b. Have no active TmPV lesions for 6 months
  - c. Have no direct contact with manatees with active TmPV lesions for 6 months prior to release
  
3. Continued TmPV disease surveillance within manatee populations will include:
  - a. Antemortem rehabilitation surveillance for TmPV
  - b. Postmortem carcass salvage surveillance for TmPV

Sincerely,

The Captive Manatee Veterinary Group: Drs. Michael Barrie, Mark Campbell, Lara Croft, Martine DeWit, Chris Dold, Scott Gearhart, Mark Lowe, Charlie Manire, Maya Menchaca, Dave Murphy, Gwen Myers, Elizabeth Nolan, Tom Reidarson, Michael Renner, Todd Schmitt, Judy St. Leger, Andrew Stamper

CC: Ken Haddad, Nicole Adimey, Colleen Castille

## References:

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# United States Department of the Interior

U.S. GEOLOGICAL SURVEY

## Florida Integrated Science Center

### Sirenia Project

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8 September 2008

Nicole Adimey  
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Jacksonville, Florida 32256-7517

Dear Nicole,

Within the last 15 years a novel, close-to-root papilloma virus (TmPV-1; Bossart et al. 2002) has been identified in a few captive manatees. Under current considerations all manatees known to have contact with manatees that have been identified with the virus are placed under quarantine conditions. This viral infection has persisted for a number of years predominantly in a small group of manatees housed at the Homosassa Springs Wildlife State Park (HSWSP). Evidence suggests that this exposure, resulting in active cutaneous viral shedding lesions, has been the result of lower immunological compromise and suboptimal captive environmental conditions. This form of stress to these captive manatees with active viral lesions has, through horizontal direct contact, allowed for this virus to self perpetuate within the confines of the facility. Recently, three manatees removed from this facility were able to resolve the cutaneous papillomatous lesions when relocated to facilities with much better environmental conditions (increased water temperature, exposure to salinity, removal of contact with other manatees with lesion, etc.). It is therefore recommended that all the manatees currently housed in the HSWSP be relocated to facilities with more adequate holding conditions for manatees.

Studies of the papillomatosis has identified a virus (TmPV-1) that has been successfully circulating in the wild population under latent conditions for possibly thousands of years (Rector et al. 2004). Given this condition, several manatees in the wild population were examined and monitored by field researchers. Lesions very similar to those observed in captive manatees known to have been caused by TmPV-1 were observed in the free-ranging manatee population in and around Crystal River, Florida. Some of these lesions were biopsied from individuals that resulted in positive verification of live TmPV using PCR amplification technology of the L1 capsid protein gene (Woodruff et al. 2005). This information was scrutinized and did not convince the program officials in the captive manatee community. Therefore, additional studies were instigated using seroprevalence titer detection.

Early seroprevalence studies by Bossart and colleagues at the University of Louisville in 2005 utilized 64 individuals and documented through ELISA the serum sensitivity to antibody titers for TmPV-1. That study only used samples from 21 wild Florida manatees and did not identify any positives in the wild population. Subsequently, another more encompassing project utilizing 176 individuals from throughout their range did identify positive titers in the wild population under even more stringent conditions than those employed by the Bossart team. This new information has convincingly documented that there is very strong titer evidence to suggest that TmPV-1 is indeed in the wild, which also supports the proposed condition of inheritable latency throughout the population.

One should realize that there is also benefit in employing vaccine trial studies on manatees that have come into contact with TmPV-1 and those that have not in both captives and free-ranging animals. Shedding light on this aspect of latency/exposure and the etiology of the significance of papillomatosis in manatees would be helpful. Use of the vaccine in its present form should not be administered to wild manatees in an effort to provide prophylactic protection against the antigen.

By all indications, TmPV-1 papillomatosis in manatees does not present a known risk to overall manatee health and appears to readily resolve under non-stressful conditions. Care should continue to ensure that manatees with active TmPV-1 lesions remain in quarantine until the lesions completely heal. TmPV-1 is suspected to be benign in nature and poses no serious complications as have been observed in other, rare forms of papilloma viruses in other species. This wart-like disease, as with most other forms of the papilloma virus, is host specific and generally latent in historic and present populations of manatees.

Determining with both PCR and seroprevalence data that the virus is in the wild, and has been for thousands of years, it is recommended that any captive manatees free of active lesions and currently housed under quarantine conditions be allowed to move out of quarantine contact. These animals will then be considered eligible for release candidacy if they remain free of lesions for at least 6 months. Additionally, these manatees must meet general release guidelines as maintained by the U.S. Fish and Wildlife Service, as well as satisfy research criteria for advancing our understanding of post-release success. Prior to release, concurrent approval from the Clinical Manatee Veterinarian Working Group should be obtained for each animal under consideration for release and appropriate release plans be implemented.

Field researchers at the USGS Sirenia Project will continue wild manatee surveillance studies in the NW region of Florida with respect to identification of individuals with suspicious lesions. Additionally, wild manatee health and risk assessments to obtain base-line biological data will be conducted periodically throughout Florida under various types of habitat regimes. It is imperative that clinicians and handlers at captive manatee facilities remain on alert for individuals with unusual lesions. Presently, laboratory facilities with forensic capabilities for TmPV-1 detection (histology, immunohistochemistry, molecular analysis, etc.) such as the University of Florida,

College of Veterinary Medicine should be identified and sample submission protocol standardized. Finally, it is recommended that the FWRI Pathobiology Lab remain vigilant to unusual or suspicious cutaneous lesions on manatee carcasses collected through their necropsy program and continue to submit samples for examination.

Please feel free to contact me should you choose to discuss these points further. Look forward to working with you on the facilitation of this project.

Sincerely,

— /s/ —

Robert K. Bonde  
Biologist

**HARBOR BRANCH  
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September 19, 2008

Nicole Adimey  
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Fish & Wildlife Biologist  
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Jacksonville, Florida 32216-0958

Dear Nicole:

Per your request, this letter describes my review and suggestions for the Florida manatee quarantine proposal previously provided.

First, I would like to briefly summarize the research conducted by our laboratory. Non-federal funding sources have allowed us to conduct research on manatee cutaneous viral papillomatosis since 1997. Our research of this first described viral disease in the endangered manatee resulted in numerous scientific publications and the collaboration of Drs. Bennett Jenson and Shin-je Ghim (internationally renowned papillomavirus scientists from the Brown Cancer Center, University of Louisville) and Dr. John Reif (Chief of Epidemiology at Colorado State University who has extensive experience with infectious diseases in marine mammals and other species). Laboratories at the University of Miami School of Medicine and Harbor Branch Oceanographic Institute at Florida Atlantic University provided the necessary infrastructure for the published research (Bossart et al., 2002; Rector et al. 2004).

Our research has revealed a complex pathogenesis for manatee cutaneous viral papillomatosis, which is still largely undefined, resulting in difficulty with respect to resource management decisions. In other species, papillomaviruses (PVs) cause warty growths that spontaneously regress, disseminate or undergo malignant transformation,

depending on the biology of the virus and associated cofactors. PVs are highly species-specific, small double stranded DNA of 7 to 8 kilobase pairs in size. Many vertebrates and nonvertebrate species are susceptible to PV infections. Interestingly, PVs have the same genetic organization, are highly species-specific, yet evolve from the phylogenetic scale with the natural host.

Transmission of PVs occurs from one generation of the natural host to another by viral shedding from productive infections associated with clinical PV lesions or subclinical infections. This is important from the standpoint of infection control and potential transmission. PVs that primarily cause subclinical infections frequently co-exist with the host in a latent state over extended periods of time, activated by different stressors to produce lesions. Subclinical infections appear to become more widely disseminated as intermittent latent/subclinical infection over time, most likely by self-inoculation. In other species, a latent infection that is activated then becomes capable of horizontal transmission, even to an animal that already has latent infection. The latent infection is held in check by other mechanisms intrinsic to innate immunity and the capability of individual cells to continue suppressing activation of a latent infection. The latent phenotype is maintained by the immune response of the host, appearing to wax (become active) and wane (revert to a latent state) in response to either host or environmental stressors. Some PVs cause active clinical infections that are maintained (without latency) as warts or papillomas. These infections are most often focal, rarely latent, and spread by reinoculation, sometimes along lines of trauma (pseudokobner effect). Most PVs need cofactors to help spread the virus infection by self-inoculation or by transmission to susceptible hosts. These co-factors usually induce clinically active lesions or prepare the susceptible host for infection.

PVs can induce severe disease including neoplasia in many terrestrial mammals. PVs cause cancer deaths in snow leopards, where both cutaneous and mucosal cancers develop from PV-induced papillomas. Horses are euthanized on daily basis as a result of BPV-1 and -2 induced cutaneous sarcoid. Shope PV kills rabbits by causing cutaneous cancers. Cows die of BPV-4 induced mucosal cancers usually associated with bracken fern ingestion. Although cows are not killed by BPV-1 and -2 induced fibropapillomas, there is interstate restriction on shipping beef from infected animals. COPV causes cancer deaths in 5% of dogs. HPV causes cervical cancer, the 2nd leading cause of death from cancer in women worldwide. We suspect that oral papillomas of Atlantic bottlenose dolphins are caused by a PV (Bossart et al, 2005; Rehtanz et al. 2006; Rehtanz et al., 2008; Rector et al., 2008). These benign oral tumors undergo malignant transformation to squamous cell carcinoma, which can be fatal.

The manatee PV (TmPV-1) has likely been transmitted as a subclinical infection among manatees for millions of years. Our phylogenetic analysis indicated that TmPV-1 is a novel close-to-root papillomavirus that is only distantly related to other papillomavirus sequences and is probably an ancient virus (i.e., it is unlikely that the virus has been transmitted to manatees in recent history). As in the case of many animal species,

manatees are probably latently infected with TmPV-1 through their skin. Latent infections are typically contained by the immune system (hence the absence of productive viral lesions in most free-ranging manatees) and tend to become clinically apparent upon acquired, genetic or iatrogenic deficiencies in cell-mediated immunity (see references below). Papillomas caused by TmPV-1 have only been visible since 1997, presumably because of a strong cofactor or stressor in the environment that is responsible for activating virus replication. Immune suppression appears to be a cofactor in the Homosassa Springs manatees (Bossart et al., 2002). The recent results from our seroepidemiologic study of TmPV-1 based on ELISA support the speculation that TmPV-1 infection is present in free-ranging manatees although at a lower prevalence than anticipated. The co-factors involved that are apparently necessary for the development of clinical disease in manatees need to be clearly defined if we are to understand the pathogenesis of this infection. I am particularly interested in the immunologic suppression co-factor in the pathogenesis of this disease. In other species with PV infection, the frequently observed regression of tumors is a direct result of an intact, functional and appropriate immunologic response.

While we now have seroepidemiologic evidence of latent TmPV-1 infection in free-ranging manatees, the presence of **productive** tumors (i.e., skin tumors actively producing TmPV-1 virus) appears to be limited to the Florida manatees that originated from Homosassa Springs or those that had exposure to those manatees. This is important to remember since a latent infection that is activated can then lead to horizontal transmission, even to an animal that already has latent infection. The latent infection is not kept at bay by the immune response but by other mechanisms intrinsic to innate immunity and capability of individual cells to continue suppressing activation of a latent infection. In other words, although the PV in Florida manatees has most likely been vertically passaged through the centuries as an activated latent infection, an active **productive** infection can be horizontally transmitted with equal ease as demonstrated at Homosassa Springs. Thus a manatee with cutaneous tumors producing virus could spread the virus to other manatees (even those manatees latently infected) inducing tumor formation in those individuals. Unsolved questions include the identification of co-factors necessary for this to happen and the eventual course of the tumors (i.e., do the tumors regress, persist or undergo malignant transformation to cancer). Based on this knowledge, item number 1 in your email needs clarification. While it is true that serologic evidence of viral infection is present at a low prevalence in free-ranging manatees (latent infection) the presence of productive infection (cutaneous tumors with virus) is rare. Because of this fact and our incomplete understanding of the pathogenesis of this infection (other likely co-factors), a quarantine period of 6 months with no known exposure to manatees with viral productive cutaneous tumors and negative Tm-PV-1 serum ELISA results might represent a conservative requirement for removing the quarantine. Based on our recent studies in other marine mammal species and studies in terrestrial mammalian species as summarized above, it would be against my better judgment at this time to say flatly that the TmPV-1 virus does not present any appreciable risk to the free-ranging Florida manatee population.

I hope this information I helpful. If you have any questions please do not hesitate to contact me.

Sincerely,

Greg

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October 16, 2008

Nicole Adimey  
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Jacksonville, Florida 32256-7517

Dear Nicole,

The papillomavirus research group at the James Graham Cancer Center, University of Louisville, for the last 6 years has been studying the role of TmPV-1 infection in manatees at Homosassa Springs Wildlife State Park (HSWSP) with Greg Bossart from Harbor Branch Oceanographic Institute (HBOI). During the last year we have been closely collaborating with Robert Bonde, U.S. Geological Survey, Department of the Interior, on seroepidemiological studies of free-ranging and captive manatees.

Four years ago we isolated TmPV-1 from the captive manatees at HSWSP and published our findings in *J Virol.* 12698-702, 2004. A phylogenetic tree was established for TmPV-1 which revealed TmPV-1 to be a novel close-to-root papillomavirus that has been passed from generation to generation of manatees for millions of years. This by definition means that TmPV is present in the free-ranging population of manatees, most likely as a latent infection. Moreover, Woodruff et al. (2005) reported that some active PV lesions had been observed in free-ranging manatees in and around Crystal River, Florida. These lesions were positive for TmPV-1, the only PV ever detected in manatees. The development of the phylogenetic tree, the report by Woodruff et al., and our recent serological study of captive and free-ranging manatees, with Robert Bonde that showed positive antibody titers for TmPV-1 in both wild and captive manatees, is sufficient evidence that free-ranging manatees carry TmPV-1.

Captive manatees that are positive for TmPV-1 lesions have been shown to resolve the lesions when removed from Homosassa Springs and relocated to zoos and aquaria where environmental conditions are not as harsh. Natural conditions found in waters inhabited by wild manatees do not appear to be as harsh either. Therefore, we support the decision to open up Homosassa Springs and other estuaries for general release of manatees that have been free of lesions for at least 6 months.

Since we have the laboratory facilities and the capacity to make large quantities of serological substrate and vaccine antigen we request to be the co-recipients of sample submission protocols that are standardized for the continued monitoring and surveillance of papillomavirus in manatees.

Sincerely,

/s/

Alfred Bennett Jenson, MD

/s/

Shin-je Ghim, PhD

Attachment 3  
Updated Recommendations

01 September 2011  
Dave Hankla  
United States Fish and Wildlife Service  
7915 Baymeadows Way, Suite 200  
Jacksonville, Florida 32256-7517

Subject: Manatee Papillomavirus Quarantine

Dear Mr. Hankla,

The Captive Manatee Veterinary Group would like to propose a revision to the manatee papillomavirus (TmPV) protocol. This letter follows up on our letter to the United States Fish and Wildlife Service dated 26 November 2008, in which TmPV history and literature were discussed. Further studies (Dona et al., 2011) demonstrated that TmPV1 antibodies are prevalent in wild manatees throughout Florida. There is no evidence that manatee papillomaviruses have had an impact on the health of the population. Moreover, lifting the quarantine of entire facilities and the ability to move animals between facilities has contributed to a more efficient use of available critical care space.

The following information and recommendations were provided in an email by Dr. James Wellehan with the University of Florida's College of Veterinary Medicine on 5 April 2011.

*"There are at least 125 known human papillomavirus types, and only a small handful of them cause significant clinical disease. It took very little investigation of bottlenose dolphins for us to find over 25 types. It is probable that dozens of manatee papillomaviruses remain to be described. Papillomaviruses are commonly found in skin of normal animals. They are generally widely dispersed in their endemic hosts.*

*My opinion is that I see no reason to restrict manatee movement based on the presence of TmPV1 or TmPV2. The behavior of papillomaviruses is generally benign and I am aware of no papillomaviruses that have had significant impacts on wild populations of any species. If this were an RNA virus which would be expected to behave more explosively, such as a paramyxovirus, rhabdovirus, or picornavirus, I would advise greater caution."*

*My recommendations for an actionable TmPV case definition include fulfillment of all of the following three criteria:*

- 1.) A lesion that is considered clinically significant by a veterinarian.*
- 2.) Histology consistent with a papillomavirus lesion.*
- 3.) Sequence of papillomavirus DNA from the lesion.*

*The first criterion is important. If the associated lesions are not clinically significant, there's really no point holding things up. If there are squamous cell carcinomas popping up, that's a different story. The second criterion is a key part of the current criteria and should stay. The third criterion is important, since there are likely diverse papillomaviruses present in the species, and only a small subset are likely*

*to be clinically relevant. Immunohistochemistry is likely to cross react with different, as of yet unknown related papillomaviruses, with differing clinical relevance. If clinically significant lesions are seen, sequence-based identification of viruses is critical for understanding.”*

Based on the aforementioned information, the Captive Manatee Veterinary Group recommends reducing the quarantine period to three months instead of six months and a revised protocol for the testing of suspected papilloma lesions:

1. Case definition- if a lesion is suspected to be a papillomavirus infection, it should be considered positive for TmPV if:
  - a. Histological consistent with TmPV **and**
  - b. Positive for TmPV on Polymerase Chain Reaction testing
2. Before considering a manatee for release back into the wild the following criteria must be met:
  - a. Meet current release criteria for ages and release classifications
  - b. Have no active TmPV lesions for 3 months
  - c. Have no direct contact with manatees with active TmPV lesions for 3 months prior to release.
3. Continued TmPV disease surveillance within manatee populations will include:
  - a. Antemortem rehabilitation surveillance for TmPV
  - b. Postmortem carcass salvage surveillance for TmPV

Sincerely,

The Captive Manatee Veterinary Group: Drs. Ray Ball, Christopher Bonar, Mark Campbell, LaraCroft, Luis Figueroa-Oliver, Deirdre Fontenot, Scott Gearhart, Gwen Myers, Natalie Mylniczenko, David Murphy, Donald Neiffer, Elizabeth Nolan, Maya Rodriguez, Andrew Stamper, Scott Terrell, Michael Walsh, Martine de Wit  
cc: Nicole Adimey, Drs. James Wellehan, Gregory Bossart, Bennett Jenson

Reference:

Dona, M.G., Rehtanz, M., Adimey, N.M., Bossart, G.D., Jenson, A.B., Bonde, R.K., Shin-je G. 2011. Seroepidemiology of TmPV1 infection in captive and wild Florida manatees (*Trichechus manatus latirostris*). J. Wildlife Dis. 47(3): 673-684.