

Attachment 2
Letters of Recommendation



College of Veterinary Medicine PO Box 100126
Department of Small Animal Clinical Sciences Gainesville, FL 32610-0126
352-392-2226 ext 5700
352-392-6125 Fax
www.vetmed.ufl.edu/sacs

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,

A handwritten signature in blue ink, appearing to read 'H. Nollens', with a long horizontal flourish extending to the right.

Hendrik Nollens, DVM, PhD

College of Veterinary Medicine PO Box 100126
Department of Small Animal Clinical Sciences Gainesville, FL 32610-0126
352-392-2235
352-392-6125 Fax
www.vetmed.ufl.edu/sacs

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,



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

References:

1. Antonsson, A., McMillan, N.A.J., 2006. Papilloma virus in healthy skin of Australian mammals. *J. Gen. Virol.* 87, 3195-3200.
2. Michael KM, Waterboer T, Sehr P, Rother A, Reidel U, Boeing H, Bravo IG, Schlehofer J, Gärtner BC, Pawlita M. 2008. Seroprevalence of 34 human papilloma virus types in the German general population. *Plos Pathog.* 20;4(6):e1000091.

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:

- Bossart, G., Ewing, R., Lowe, M., Sweat, M., Decker, S., Walsh, C. Ghim, S., Jenson, B. (2002). Viral papillomatosis in Florida manatees (*Trichechus manatus latirostris*). *Experimental and Molecular Pathology* 72, 37-48.
- Marine Mammal Pathobiology Lab. "Manatee Mortality Statistics". 1974 - 2008. Fish & Wildlife Research Institute. 26 November 2008
<http://research.myfwc.com/features/category_sub.asp?id=2241>.
- Rector, A., Bossart, G., Ghim, S., Sundberg, J., Jenson, B., Van Ranst, M. (2004). Characterization of a novel close-to-root papillomavirus from a Florida manatee by using multiply primed rolling-circle amplification: *Trichechus manatus latirostris* papillomavirus type 1. *J. Virol.*78; 12698-12702.
- Sundberg, J., Van Ranst, M., Burk, R., Jenson, B. (1997). The nonhuman papillomaviruses: Host range, epitope conservation, and molecular diversity. *In* "Human papillomavirus Infections in Dermato-venereology" (G. Gross and G. Von Krogt, Eds.), pp 47-68. CRC Press, Boca Raton, Fl.
- Sundberg, J., Van Ranst, M., Montali, R., Homer, B., Miller, W., Rowland, P., Scott, D., England, J., Dustan, R., Mikaelian, I., Jenson, B. (2000). Feline papillomas and papillomaviruses. *Vet. Pathol.* 37; 1-10.
- Woodruff, R., Bonde, R., Bonilla, J., Romero, C. (2005). Molecular identification of a papilloma virus from cutaneous lesions of captive and free-ranging Florida manatees. *J. Wildlife Dis.* 41(2) 437-441.



United States Department of the Interior

U.S. GEOLOGICAL SURVEY

Florida Integrated Science Center

Sirenia Project

2201 N.W. 40th Terrace
Gainesville, Florida 32605-3574
e-mail: Robert_Bonde@usgs.gov

Phone: (352) 264-3555

Fax: (352) 374-8080

8 September 2008

Nicole Adimey
U.S. Fish and Wildlife Service
7915 Baymeadows Way, Suite 200
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,

A handwritten signature in black ink, appearing to read "Bob", written in a cursive style.

Robert K. Bonde
Biologist

HARBOR BRANCH
OCEANOGRAPHIC INSTITUTION, INC.
Marine Mammal Research and Conservation Program

5600 U.S. 1 NORTH
FORT PIERCE, FL 34946

(772) 465-2400, Ext. 556
Fax (772) 466-4853
gbossart@hboi.edu
<http://www.hboi.edu>

September 19, 2008

Nicole Adimey
U.S. Fish and Wildlife Service
Fish & Wildlife Biologist
6620 Southpoint Drive, South #310
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 passed 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

Gregory D. Bossart, V.M.D., Ph.D.
Research Professor
Chief Marine Mammal Veterinarian and Head of Pathology
Marine Mammal Research and Conservation Program
Harbor Branch Oceanographic Institute at Florida Atlantic University
5600 US 1 North
Ft. Pierce, Florida 34946
and
Adjunct Professor
Department of Pathology
Miller School of Medicine
University of Miami
Miami, Florida

772.465.2400 (Ext 556)
772.466.4853 (Fax)
gbossart@hboi.fau.edu

References:

Rector A, Bossart GD, Ghim SJ, Sundberg JP, Jenson AB, and Van Ranst M.
Characterization of a novel close-to-root papillomavirus from a Florida manatee by using multiply primed rolling-circle amplification: *Trichechus manatus latirostris* papillomavirus type 1. *J. Virol.* 78: 12698-12702, 2004.

Bossart GD, Ewing R, Lowe M, Sweat M, Decker S, Walsh C, Ghim S and Jenson AB.
Viral papillomatosis in Florida manatees (*Trichechus manatus latirostris*). *Experimental and Molecular Pathology* 72:37-48, 2002.

Bossart GD, Ghim S, Rehtanz M, Goldstein J, Varela R, Ewing R, Fair P, Lenzi R, Joseph B, Hicks C, Schneider L, McKinnie CJ, Reif JS, Sanchez R, Lopez A, Novoa S, Bernal J, Goretti M, Rodriguez M, Defran RH, and Jenson A. B. Orogenital neoplasia in Atlantic bottlenose dolphins (*Tursiops truncatus*). *Aquatic Mammals* 31(4): 473-480, 2005.

Rehtanz M, Ghim S-J, Rector A., Van Ranst M, Fair P, Bossart GD and Jenson A.B. Isolation and characterization of the first American bottlenose dolphin papillomavirus: *Tursiops truncatus* papillomavirus type 2. *Journal of General Virology* 87: 3559-3565, 2006.

Rehtanz M, Bossart GD, Doescher B, Rector A, Van Ranst M, Fair P, Jenson AB, Ghim S. Bottlenose dolphin (*Tursiops truncatus*) papillomaviruses: Vaccine antigen candidates and screening test development. *Veterinary Microbiology* (2008), doi: 10.1016/j.vetmic.2008.06.017.

Rector A, Stevens H, Lacave G, Lemey P, Mostmans S, Salbany A, Vos M, Van Doorslaer K, Ghim S, Rehtanz M, Bossart G. D. Jenson A. B, Van Ranst M. Genomic characterization of novel dolphin papillomaviruses provides indications for recombination within the Papillomaviridae. *Virology* 378: 151–161, 2008.

JAMES GRAHAM BROWN CANCER CENTER
Vaccinology Laboratory

University of Louisville
529 South Jackson Street
Louisville, Kentucky 40202

October 16, 2008

Nicole Adimey
U.S. Fish and Wildlife Service
7915 Baymeadows Way, Suite 200
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.

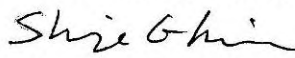
Four years ago we isolated TmPV-1 from the captive manatees at HSWSP and published our findings in *J Virol.* 12698-702, 2004. A phylogenetic tree was established for TmPV-1 which revealed TmPV-1 to be a novel close-to-root papillomavirus that has been passed from generation to generation of manatees 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,


Alfred Bennett Jenson, MD


Shin-je Ghim, PhD