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MORBILLIVIRUS INFECTION IN STRANDED COMMON DOLPHINS FROM THE PACIFIC OCEAN

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ABSTRACT: From August 1995 to August 1997, six of 18 common dolphins (*Delphinus delphis*) that stranded along beaches of southern California (USA) tested antibody positive for dolphin morbillivirus (DMV). Titers ascertained by virus neutralization ranged from 1:50 to 1:910 while those determined by ELISA ranged from 1:80 to 1:195. The first individual to strand survived and was released back into the Pacific Ocean 14 mo later. Histopathologic examination of tissues from the other five dolphins did not reveal lesions characteristic of morbilliviral disease; however, morbilliviral RNA was detected in three of the five by reverse transcriptase-polymerase chain reaction testing. This is the first report of morbilliviral infection in any marine mammal species in the northern hemisphere of the Pacific Ocean. These data indicate that DMV, or a closely related morbillivirus, is present in the Pacific Ocean and infection of common dolphins may not be associated with morbillivirus disease.

Key words: *Delphinus delphis*, enzyme linked immunosorbent assay, morbillivirus, polymerase chain reaction, rehabilitation, virus neutralization.

INTRODUCTION

The first established marine mammal morbilliviral epizootic began in June 1987 and involved bottlenose dolphins (*Tursiops truncatus*) along the Atlantic coast of the United States (Lipscomb et al., 1994; Schulman et al., 1997). During 1988, morbillivirus infections occurred epizootically in harbor seals (*Phoca vitulina*) and gray seals (*Halichoerus grypus*) in northwestern Europe and continued in Atlantic bottlenose dolphins (Kennedy et al., 1989; de Swart et al., 1995) Since that time, epizootics have occurred in striped dolphins (*Stenella coeruleoalba*) along the western Mediterranean Sea (Duignan et al., 1992; Domingo et al., 1995), and Atlantic bottlenose dolphins in the Gulf of Mexico (Lipscomb et al., 1996; Taubenberger et al., 1996).

Most marine mammals with morbilliviral disease wash ashore dead or strand in a moribund state and die shortly thereafter. Many present with overwhelming secondary bacterial and fungal infections due to the immune suppressive nature of the virus (Lipscomb et al., 1994; Schulman et al., 1997). Prominent lesions are interstitial pneumonia, nonsuppurative meningoencephalitis and lymphoid depletion: eosinophilic intranuclear and intracytoplasmic inclusion bodies and syncytial cells may be found in affected tissues (Kennedy et al., 1989; Domingo et al., 1995; Duignan et al., 1992; Schulman et al., 1997).

To date, there is only one report of morbilliviral infection in six dusky dolphins (*Lagenorhynchus obscurus*) one common dolphin (*Delphinus capensis*) and three bottlenose dolphins in the Pacific Ocean, off the coast of Peru (Van Bressem et al., 1998). In the present study, from August 1995 to August 1997, eighteen common dolphins (*Delphinus delphis*) stranded along beaches of southern California (USA); (33°25′N, 117°35′W to 34°15′N, 119°15′W) and six tested positive for exposure to dolphin morbillivirus (DMV) by virus neutralization (VN) and a newly developed enzyme linked immunosorbent assay test. Morbilliviral RNA was detected in

tissues of three of five individuals by reverse transcription-polymerase chain reaction (RT-PCR). One individual survived, developed immunity to the morbilliviral infection, and was rehabilitated and then reintroduced back into the Pacific Ocean.

MATERIALS AND METHODS

Serum samples were tested for the presence of antibodies to morbilliviruses by two methods, virus neutralization (VN) and enzyme linked immunosorbant assay (ELISA). The VN tests were conducted as previously described (Duignan et al., 1994) using dolphin morbillivirus (provided by A. D. M. E. Osterhaus, Erasmus University, Rotterdam, The Netherlands), porpoise morbillivirus (PMV, provided by C. Lyons, The Queen's University, Belfast, Northern Ireland, UK), phocine distemper virus (PDV, provided by A. D. M. E. Osterhaus), and Onderstepoort strain of canine distemper virus (CDV) (provided by M. Appel, Cornell University, Ithaca, New York, USA).

Enzyme linked immunosorbent assays tests were carried out using a specific indirect antibody method, in which 96-well immunoassay plates (Probind, Falcon, BD, Oxnard, California, USA) were coated overnight at 1 μg/ml in phosphate buffered saline (PBS: 145 mM NaCl, 7.5 mM Na_2HPO_4, 2.5 mM NaH_2PO_4, pH 7.2) with rinderpest virus recombinant N-protein (RPV-N expressed in baculovirus, kindly donated by S. Ahmad and T. Yilma, Univeristy of California, Davis, California, USA; Ismail et al., 1994). After washing the wells with PBS-0.1% tween, serum samples were added and incubated for 1 hr at room temperature. After further washing, bound serum immunoglobulin was visualized by incubations with 100 μl HRP-conjugated protein A (Bio-Rad, Hercules, California, USA) for 1 hr followed by 100 μl of substrate buffer (0.1 M citric acid-phosphate buffer, pH 5 containing 0.67 μg/ml O-phenylenediamine dihydrochloride and 0.44 μl/ml of 30% hydrogen peroxide) for 20 min. Enzyme reaction was stopped by addition of 150 μl 1M H_2SO_4, after which optical density (490 nm) of wells was read (UV-max, Molecular Devices, Menlo Park, California, USA). Titer values were calculated by interpolation from serum dilution curves using OD value three times background signal.

Whole blood was examined for the presence of morbilliviruses by polymerase chain reaction (PCR) using methods described by Barrett et al. (1993). Briefly, peripheral blood mononuclear cells were isolated from whole blood by discontinuous density centrifugation (Isolymph, Gallard-Schlesinger, Carle Place, New York, USA). Cells were washed in DMEM media (GIBCO-BRL, Grant Island, New York, USA) and pelleted. Total RNA was isolated from cells by RNAzol method (TM Cinna Scientific, Friendswood, Texas, USA) according to manufacturer's instructions. cDNA was synthesized using annealed random hexamers, 4 μg of total RNA and M-MLV reverse transcriptase (Promega, Madison, Wisconsin, USA). Conserved primers (5′ ATGTTTATGATCACAGCGGT-3′) and (5′ ATTGGGTTGCACCACTTGTC-3′) used for polymerase chain reaction (PCR) were derived from a lineup of morbillivirus P genes. These primers amplify a fragment of 429 base pairs. Two PCR reactions with conditions as follows were performed: 94 C for 90 sec, annealing at 50 or 55 C for 120 sec and 72 C chain elongation for 180 sec. In experiments, a positive canine distemper virus cDNA and negative (no cDNA) controls were used.

Polymerase chain reaction testing of formalin fixed, paraffin-embedded tissue was performed on tissues from five stranded common dolphins, described by Krafft et al. (1995). Four to eight 6-μm sections were deparaffinized, extraction buffer (20 mM Tris-HCL, pH 7.6, 20 mM ethylene-diamine-tetraacetic acid, 1% sodium dodecyl sulfate [SDS], and 0.5 mg/ml proteinase K) was added (600 μl pellet), and the sample was incubated in a 55 C water bath for 4 hr, followed by two phenol/chloroform extractions. The aqueous phase from the final extraction was placed in a fresh tube and nucleic acids were precipitated. Reverse transcription was performed with a specific primer (primer 1) from a conserved region of the morbillivirus phosphorprotein (P) gene as previously described (Krafft et al., 1995). PCR was performed on the first strand cDNA. A PCR reaction mixture containing 14 pmol primer 2 (5′-ATTAAAAAGGG(G/C)ACAGGAGAGAGATCAGCC-3′), 1.5 U Taq polymerase, and 3 μl 10-fold concentrated PCR buffer was added to each tube. Amplification was carried out in a thermal cycler (Perkin-Elmer, Norwalk, Connecticut, USA) using the following conditions: 94 C for 300 sec; 40 cycles of 94 C/60 sec, 72 C/60 sec; followed by a final 420 sec 72 C extension. The products were analyzed by gel electrophoresis. Southern blots (Krafft et al., 1995) were performed using a dolphin morbillivirus specific oligonucleotide probe (5′-CGGAGACCGAGTCTTCATT-3′), which hybridized to a sequence contained within the amplified 78-base pair product. As a control for amplifiable RNA, RT-PCR for beta actin was performed in each case as previously described (Krafft et al., 1995).

TABLE 1. Signalment, stranding date, stranding location, and titers to dolphin morbillivirus (DMV) and rinderpest virus (RPV-N) on six stranded common dolphins (*Delphinus delphis*) from the coast of California (USA).

Animal	Age	Gender	Stranding date	Location	DMV VN titer[a]	RPV-N titer[a]
95-59	adult	female	8-21-95	33°56′N; 118°28′W	1:50	1:80
95-132	subadult	male	12-7-95	33°35′N; 118°28′W	1:100	ND[b]
96-6	subadult	female	2-26-96	34°15′N; 119°15′W	1:110	ND
96-9	adult	male	3-16-96	33°56′N; 118°28′W	1:190	ND
96-61	subadult	female	9-24-96	34°20′N; 118°40′W	1:130	1:135
97-35	subadult	male	6-13-97	33°25′N; 117°35′W	1:910	1:95

[a] See text for explanation.
[b] ND-not determined.

RESULTS

Three of the six common dolphins presented with neurologic signs characterized by seizures or uncontrolled trembling (96-9, 96-61, and 97-35), two of the six presented with dyspnea (95-59 and 96-6), and the other was normal (95-132) (Table 1). Each of the dolphins that presented with clinical abnormalities, except 95-59, were euthanized within 24 to 48 hr due to worsening clinical signs. One of the two normal individuals (95-132) developed seizures within 48 hr and was euthanized while the other (95-59) survived and was later released.

Antibody VN titers for the six common dolphins ranged from 1:50 to 1:910 (Table 1). Comparable changes also were recorded with rinderpest N-protein virus ELISA titers using sera samples collected from this animal (Fig. 1). Each individual had negative virus neutralization titers to canine and phocine distemper virus, and slightly positive to porpoise morbillivirus and highly positive to dolphin morbillivirus.

One of the five necropsied dolphins had mild lymphocytic meningoencephalitis (96-9), but neither inclusion bodies nor synctia were found. A single protozoan tissue cyst was present in gray matter of the brain stem. No inflammation was directly associated with the intact cyst. It was 160 μm in diameter, had an amorphous cyst wall that was less than 1 μm, and was filled with closely packed bradyzoites that were 1 × 2 to 3 μm. Additional histologic sections cut from the same tissue block did not contain protozoa, so additional techniques could not be used to more completely characterize the organism. The other four necropsied dolphins did not have lesions that suggested viral infection. Morbilliviral RNA was detected in brain tissue from the individual with meningoencephalitis (96-9) and in normal spleen and heart (95-132 and 96-6). By Southern blots, a dolphin morbillivirus-specific oligonucleotide probe hybridized to the PCR products.

Dolphin 95-59 stranded on 21 August 1995 in Marina del Rey, (California 33°56′N; 118°28′W), and was taken to Sea World of California's (San Diego, California, USA) cetacean quarantine pool where she was treated for pneumonia with trimethomprimsulfadiazine at 15 mg/kg SID for 1 mo and then enrofloxacin at 5 mg/kg PO BID for 2 mo. At presentation, the dolphin's VN titer was 1:50 which rose to 1:140 by day nine and then stabilized at 1:720 by 30 October 1995, 70 days after presentation (Fig. 1). Similarly, the presenting ELISA titer was 1:25 which stabilized at 1:100 (Fig. 1).

On 9 October 1995, whole blood was collected from 95-59 and tested for the presence of morbillivirus by PCR in two separate laboratories. Both tests were negative, so believing that the common dolphin was aviremic and was unable to shed virus to other individuals, we decided to

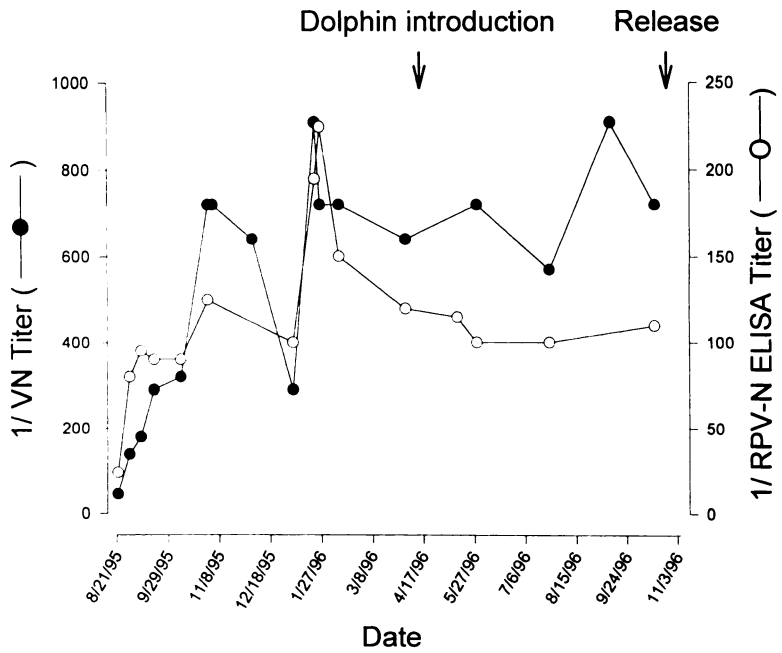

FIGURE 1. Rising and stabilizing virus neutralization and ELISA titers to dolphin morbillivirus and rinderpest virus, respectively, in a stranded common dolphin (*Delphinus delphis*; 95-59) from the coast of California (USA). An Atlantic bottlenose dolphin (*Tursiops truncatus*) and a Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) were introduced to the common dolphin on 12 April 1996. During the next 6 mo neither seroconverted and the common dolphin was released on 16 October 1996.

introduce a 15-yr-old Atlantic bottlenose dolphin (*Tursiops truncatus*) and a 28-yr-old female Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) to this individual on 4 May 1996. The three lived together in a 760,000 L pool for >6 mo while each was monitored by CBC/chemistries and VN for morbillivirus infection. Both of the introduced dolphins remained healthy and neither seroconverted during this period, while the common dolphin's VN and ELISA titer rose to 1:910 and 1:225, respectively, and then stabilized over the next 5 mo (Fig. 1). On 16 October 1996, 14 mo after stranding, the common dolphin was released back into the Pacific Ocean.

DISCUSSION

These findings are the first demonstration of morbillivirus infection in common dolphins from the north Pacific ocean. To date, the only other reported morbilliviral infections in common dolphins occurred in 11 from the Western Atlantic (Duignan et al., 1996) and one from the coast of Peru (Van Bressem et al., 1998). Using differential virus neutralization tests, it appears that all were infected by a morbillivirus similar to PMV and DMV, which is consistent with reports that these isolates are probably strains of the same virus (Taubenberger et al., 1996, Barrett et al., 1993, Visser et al., 1993a, b, Bolt et al., 1994).

The detection by RT-PCR of morbilliviral RNA in tissues of three dolphins is consistent with infection at the time of death. In two of these dolphins, no lesions that suggested infectious disease, including that caused by morbillivirus, were found. The mild lymphocytic meningoencephalitis in one dolphin may have been caused by morbillivirus, but characteristic lesions such as syncytia and inclusion bodies were not present. The morphologic features of the protozoan cyst found in the brain are consistent with those of *Toxoplasma gondii* (Dubey et al., 1988). The

protozoan infection might have been the cause of the inflammation. Toxoplasmosis has been found in morbillivirus-infected striped dolphins and has been considered a result of immunosuppression caused by the virus (Domingo et al., 1992); however, toxoplasmosis also has been found in dolphins that did not have evidence of morbillivirus infection (Migaki et al., 1990; Inskeep et al., 1990). Morbilliviral lesions were not present in other tissues that are characteristically affected such as lung, spleen, and lymph node. Thus, the relationships among the morbillivirus infection, the protozoan infection and the encephalitis are uncertain.

Infection also was inferred by the presence of neutralizing antibodies in serum, with sequential samples during the initial phase of infection showing rising titers. Until recently the only accepted method for detection of antibodies was virus neutralization. In this paper, we have demonstrated that ELISA also may be a useful tool, allowing the screening of large numbers of wild free-ranging dolphins for exposure to this morbillivirus. Virus neutralization titers which are ≥1:45 are considered suspicious and titers >1:80 are considered positive. Alternatively, it appears that ELISA titers which are ≥1:25 are positive (D. P. King, unpubl. data).

In this study, six of 18 (33%) common dolphins had evidence of morbilliviral infection. Antibody titers were present in all six, and morbilliviral RNA was detected by RT-PCR in three of six individuals. One recovered and the other five did not have postmortem lesions characteristic of morbilliviral disease such as syncytia and inclusion bodies although one had mild meningoencephalitis and cerebral toxoplasmosis. These findings indicate that morbillivirus infection occurs in common dolphins of the Pacific Ocean, that infected common dolphins may not have characteristic pathologic features of morbilliviral disease and that recovery is possible. Although the number of dolphins in this study is relatively small, evidence of infection in 33% suggests that morbilliviral infection may be frequent in common dolphins. Additional studies are needed to determine the significance of common dolphins in the epizootiology of marine mammal morbilliviruses.

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LITERATURE CITED

BARRETT, T., I. K. G. VISSER, L. MAMEY, L. GOATLEY, M. F. VAN BRESSEM, AND A. D. M. E. OSTERHAUS. 1993. Dolphin and porpoise morbilliviruses are genetically distinct from phocine distemper virus. Virology 193: 1010–1012.

BOLT, G., M. BLIXENKRONE-MOLLER, E. GOESCHLALACK, R. A. WISHAUPT, J. J. WELSH, J. A. P. EARLE, AND B. RIMA. 1994. Nucleotide and deduced amino acid sequences of the matrix (M) and fusion (F) protein genes of cetacean morbilliviruses isolated from a porpoise and a dolphin. Virus Research 34: 291–304.

DE SWART, R. L., T. C. HARDER, P. S. ROSS, H. W. VOS, AND A. D. M. E. OSTERHAUS. 1995. Morbilliviruses and morbillivirus diseases of marine mammals. Infectious Agents and Disease 4: 125–130.

DOMINGO, M., M. VILAFRANCA, J. VISA, N. PRATS, A. L. TRUDGETT, AND I. VISSER. 1995. Evidence of chronic morbillivirus infection in the Mediterranean striped dolphin (*Stenella coeruleoalba*). Veterinary Microbiology 44: 229–239.

———, J. VISA, M. PUMAROLA, A. J. MARCO, L. FERRER, R. RABANAL, AND S. KENNEDY. 1992. Pathologic and Immunocytochemical studies of morbillivirus infection in striped dolphins (*Stenella coeruleoalba*). Veterinary Pathology 29: 1–10.

DUBEY, J. P., J. L. CARPENTER, C. A. SPEER, M. J. TOPPER, AND A. UGGLA. 1988. Newly recognized protozoan disease of dogs. Journal of the American Veterinary Medical Association 192: 1269–1285.

DUIGNAN, P. J., J. R. GERACI, J. A. RAGA, AND N. CALZADA. 1992. Pathology of Morbillivirus infection in striped dolphins (*Stenella coeruleoalba*) from Valencia and Murcia, Spain. Canadian Journal of Veterinary Research 56: 242–248.

———, P. J., J. T. SALIKI, D. J. ST. AUBIN, J. A. HOUSE, AND J. R. GERACI. 1994. Neutralizing antibodies to phocine distemper virus in Atlantic Walruses (*Odobenus rosmarus rosmarus*) from Arctic Canada. Journal of Wildlife Diseases 30: 90–94.

———, P. J., C. HOUSE, J. R. GERACI, N. DUFFY, B. K. RIMA, M. T. WALSH, G. EARLY, D. J. ST. AUBIN, S. SADOVE, H. KOOPMAN, AND H. RHINHART. 1996. Morbillivirus infection in cetaceans of the western Atlantic. Veterinary Microbiology 44: 241–249.

INSKEEP, W., C. H. GARDINER, R. K. HARRIS, J. P. DUBEY, AND R. T. GOLDSTON. 1990. Toxoplasmosis in Atlantic bottlenose dolphins (*Tursiops truncatus*). Journal of Wildlife Diseases 26: 377–382.

ISMAIL, T., S. AHMAD, M. D'SOUZA-AULT, M. BASSIRI, J. SALIKI, C. MEBUS, AND T. YILMA. 1994. Cloning and expression of the nucleocapsid gene of virulent kabele O strain of rinderpest virus in baculovirus: use in differential diagnosis between vaccinated and infected animals. Virology 198: 138–147.

KENNEDY, S., J. A. SMYTH, P. F. CUSH, P. DUIGNAN, M. PLATTEN, S. J. MCCULLOUGH, AND G. M. ALLAN. 1989. Histopathologic and immunocytochemical studies of distemper in seals. Veterinary Pathology 26: 97–103.

KRAFFT, A., J. H. LICHY, T. P. LIPSCOMB, B. A. KLAUNBERG, S. KENNEDY, AND J. K. TAUBENBERGER. 1995. Postmortem diagnosis of morbillivirus infection in bottlenose dolphins (*Tursiops truncatus*) in the Atlantic and Gulf of Mexico epizootics by polymerase chain reaction-based assay. Journal of Wildlife Diseases 31: 410–415.

LIPSCOMB, T. P., S. KENNEDY, D. MOFFETT, A. KRAFFT, B. A. KLAUNBERG, J. H. LICHY, G. T. REGAN, G. A. J. WORTHY, AND J. K. TAUBENBERGER. 1996. Morbilliviral epizootic in Atlantic bottlenose dolphins of the Gulf of Mexico. Journal of Veterinary Diagnostic Investigation 8: 283–290.

———, T. P., F. Y. SCHULMAN, D. MOFFETT, AND S. KENNEDY. 1994. Morbilliviral disease in Atlantic bottlenose dolphin (*Tursiops truncatus*) from the 1987-1988 epizootic. Journal of Wildlife Diseases 30: 567–571.

MIGAKI, G., T. R. SAWA, AND J. P. DUBEY. 1990. Fatal disseminated toxoplasmosis in a spinner dolphin (*Stenella longirostris*). Veterinary Pathology 27: 403–404.

SCHULMAN, F. Y., T. P. LIPSCOMB, D. MOFFETT, A. E. KRAFFT, J. H. LICHY, M. M. TSAI, J. K. TAUBENBERGER, AND S. KENNEDY. 1997. Histologic, immunohistochemical, and polymerase chain reaction studies of bottlenose dolphins from the 1987–1988 United States Atlantic Coast epizootic. Veterinary Pathology 34: 288–295.

TAUBENBERGER, J. K., M. TSAI, A. E. KRAFFT, J. H. LICHY, A. H. REID, R. Y. SCHULMAN, AND T. P. LIPSCOMB. 1996. Two morbilliviruses implicated in bottlenose dolphin epizootics. Emerging Infectious Diseases 2: 213–216.

VAN BRESSEM, M. F., K. VAN WAEREBEEK, M. FLEMING, AND T. BARRETT. 1998. Serological evidence of morbillivirus infection in small cetaceans from the Southeast Pacific. Veterinary Microbiology 59: 89–98.

VISSER, I. K. G., M. VAN BRESEEM, R. L. DE SWART, M. W. G. VAN DE BILDT, H. W. VOS, R. W. J. VAN DER HEIJDEN, J. T. SALIKI, C. ORVELL, P. KITCHING, T. KUIKEN, T. BARRETT, AND A. D. M. E. OSTERHAUS. 1993a. Characterization of morbilliviruses isolated from dolphins and porpoises in Europe. Journal of General Virology 74: 631–641.

———, ———, T. BARRETT, AND A. D. M. E. OSTERHAUS. 1993b. Morbillivirus infections in aquatic mammals. Veterinary Research 24: 169–178.

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