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Article *in* Journal of the American Veterinary Medical Association · July 2008

DOI: 10.2460/javma.233.2.299 · Source: PubMed

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Evaluation and comparison of the health status of Atlantic bottlenose dolphins from the Indian River Lagoon, Florida, and Charleston, South Carolina

John S. Reif, DVM, MSc; Patricia A. Fair, PhD; Jeffrey Adams, MS; Brian Joseph, DVM; David S. Kilpatrick, DVM, DABVP; Roberto Sanchez, DVM; Juli D. Goldstein, DVM; Forrest I. Townsend Jr, DVM, DABVP; Stephen D. McCulloch; Marilyn Mazzoil; Eric S. Zolman, MS; Larry J. Hansen, MS; Gregory D. Bossart, VMD, PhD

Objective—To conduct health assessments and compare outcomes in 2 populations of Atlantic bottlenose dolphins.

Design—Repeated cross-sectional study.

Animals—171 Atlantic bottlenose dolphins.

Procedures—During June and August of 2003 through 2005, 89 dolphins from the Indian River Lagoon (IRL), Florida, and 82 dolphins from estuarine waters near Charleston, SC, were evaluated. A panel of 5 marine mammal veterinarians classified dolphins as clinically normal, possibly diseased, or definitely diseased on the basis of results of physical and ultrasonographic examinations, hematologic and serum biochemical analyses, and cytologic and microbiologic evaluations of gastric contents and swab specimens.

Results—Prevalence of dolphins classified as definitely diseased did not differ significantly between the IRL (32%) and Charleston (20%) sites. Proportions of dolphins classified as possibly diseased also did not differ. Lobomycosis was diagnosed in 9 dolphins from the IRL but in none of the dolphins from Charleston. Proportions of dolphins with orogenital papillomas did not differ significantly between the IRL (12%) and Charleston (7%) sites. From 2003 through 2005, the proportion classified as definitely diseased tripled among dolphins from the Charleston site but did not increase significantly among dolphins from the IRL. Dolphins from the Charleston site were more likely to have leukocytosis, lymphocytosis, and low serum concentrations of total protein and total γ-globulins than were dolphins from the IRL.

Conclusions and Clinical Relevance—High prevalences of diseased dolphins were identified at both sites; however, the host or environmental factors that contributed to the various abnormalities detected are unknown. (*J Am Vet Med Assoc* 2008;233:299–307)

Bottlenose dolphins (*Tursiops truncatus*) inhabit estuarine, coastal, and offshore waters along the mid Atlantic coast of the United States.[1] As apex predators, bottlenose dolphins can serve as a sentinel species for monitoring environmental conditions.[2–4] Clinicopathologic data are used routinely by marine mammal veterinarians in clinical practice to perform health assessments of bottlenose dolphins, and data obtained from free-ranging marine mammals such as bottlenose dolphins can be used to evaluate relationships between exposure to biological and chemical agents and adverse health effects.[5,6]

Abbreviations

CI	Confidence interval
DDE	Dichlorodiphenyldichloroethylene
HERA	Health and Risk Assessment
IRL	Indian River Lagoon
OR	Odds ratio
PBDE	Polybrominated diphenyl ether
PCB	Polychlorinated biphenyl

The bottlenose dolphin HERA project was designed as a multidisciplinary, integrated, collaborative effort to

From the Department of Environmental and Radiological Health Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523 (Reif); the Division of Marine Mammal Research and Conservation, Harbor Branch Oceanographic Institute at Florida Atlantic University, 5600 US 1 North, Fort Pierce, FL 34946 (Reif, Kilpatrick, Goldstein, McCulloch, Mazzoil, Bossart); the National Oceanic and Atmospheric Administration, National Ocean Service, Center for Coastal Environmental Health and Biomolecular Research, 219 Ft Johnson Rd, Charleston, SC 29142 (Fair, Adams, Zolman); Living Exhibits Inc, 2339 Royal Crest Dr, Escondido, CA 92025 (Joseph); Dolphin Discovery, Blvd Kukulkkan, Plaza Quetzal Local 11, Quintana Roo, Cancun, Mexico (Sanchez); Bayside Hospital for Animals, 251 Racetrack Rd NE, Fort Walton Beach, FL 32547 (Townsend); and the National Marine Fisheries Service, 101 Pivers Island Rd, Beaufort, NC 28526 (Hansen).

Conducted under National Marine Fisheries Service Permit No. 998-1678-01, issued to Dr. Gregory Bossart.

Supported by the Florida Protect Wild Dolphins Program and the Fisheries Marine Mammal Health and Stranding Response Program of the National Oceanic and Atmospheric Administration.

The authors thank Wayne McFee for analyses of dental enamel to estimate age and Larry Fulford for dolphin captures.

Address correspondence to Dr. Reif.

assess the health of dolphin populations within 2 estuarine regions in the southeastern United States: the IRL in Florida and Charleston in South Carolina.[7] Although a complex stock structure (including a single stock with a large home range, multiple year-round resident stocks with small home ranges, multiple seasonally resident stocks with large home ranges, and multiple migratory groups with long-range movements[8]) has been proposed for bottlenose dolphins along the southeast coast of the United States, dolphins at the Charleston and IRL sites appear to be predominantly year-round residents.[9,10] Because of their limited home range, these populations of dolphins are suitable for assessing risks attributable to environmental factors. Differences in patterns of land use, degree of industrialization, and concentrations of anthropogenic contaminants between sites provide additional rationale for selection of the IRL and Charleston populations of dolphins for health assessment.

Long-term goals of the HERA project are to develop methods for assessing health of and risks to dolphins and to explore associations between health status and environmental stressors. The first phase of the HERA project included conducting physical examinations of dolphins and measuring several clinicopathologic variables to determine the health status of dolphins in the Charleston and IRL regions from 2003 through 2005. The objectives of the study reported here were to conduct health assessments and compare outcomes in 2 populations of Atlantic bottlenose dolphins. Specifically, we sought to use the data collected in the first phase of the HERA project to compare proportions of dolphins classified as definitely and possibly diseased at the IRL and Charleston sites, assess the prevalence of specific disorders in the 2 populations, and determine the major clinicopathologic abnormalities that contributed to dolphins being classified as diseased.

Materials and Methods

Animals—Bottlenose dolphins were captured in the IRL in June of 2003 (year 1), 2004 (year 2), and 2005 (year 3) and in coastal locations near Charleston in August of 2003 through 2005. The number captured in each area at each site was in approximate proportion to the total number of dolphins in the area, as estimated on the basis of photo-identification surveys. Target sample size was 25 dolphins/y for each site, which was chosen on the basis of available resources. These sample sizes were reduced in years 2 and 3 after targets for year 1 were exceeded at each location. Sampling success was dependent upon permit restrictions, safety considerations (lightning and high wind velocity), local environmental conditions (water depth and tidal flow), and age and group size of targeted dolphins as well as the presence of previously captured dolphins.

Ecosystems—The IRL is a 250-km, shallow-water ecosystem that constitutes 40% of the waters along the east coast of Florida.[11] The lagoon is an aggregate of 3 estuarine water bodies: the Indian and Banana Rivers and the Mosquito Lagoon. Because the lagoon is shallow and rates of tidal exchange between the lagoon and the Atlantic Ocean are low, the IRL tends to harbor pollutants for long periods. Extensive residential development along the eastern coast of Florida and intense agricultural activity have resulted in incursion of freshwater into the IRL and altered quality of water (eg, chemical contamination, high nutrient input, decreased salinity, decreased sea grass habitat, and eutrophication), which is exacerbated by low rates of tidal exchange.[12,13] All of Florida's sugarcane, approximately 38% of citrus fruits, and 42% of vegetable crops are grown in an area that drains into the IRL.[14]

The Charleston site is an estuarine ecosystem in the central region of the coastal zone of South Carolina that includes the Charleston Harbor as well as portions of the Ashley, Cooper, and Wando Rivers and the Stono River estuary. The Charleston ecosystem has more effective tidal exchange than does the IRL, but it is surrounded by rapidly expanding urban development and a growing human population, serves as an active seaport, and contains a former US naval base.[15] Analysis of data from long-term monitoring of water quality suggests that non–point-source runoffs, contaminated sediments, and pollutant concentrations are increasing.[16] High concentrations of heavy metals, PCBs, and pesticides have been detected in sediments of the Charleston Harbor and the Ashley and Cooper Rivers.[16] Two hazardous waste sites included on the National Priority (Superfund) List by the US Environmental Protection Agency, which are contaminated with polycyclic aromatic hydrocarbons, lead, chromium, copper arsenic, zinc, and dioxin, are located on the estuary.[17,18]

Dolphin capture—Standard operating protocols and techniques used for capture, sample collection, and release of dolphins are described in detail elsewhere.[19] Methods used for capture and tissue collection were approved under a National Marine Fisheries Service Scientific Research Permit as part of the bottlenose dolphin HERA project and by the Harbor Branch Oceanographic Institutional Animal Care and Use Committee.

In the IRL, dolphins were captured in the Mosquito Lagoon; Banana River; and north, central, and southern portions of the IRL. At the Charleston site, dolphins were captured in the Cooper, Stono, and Wando Rivers as well as in Charleston harbor. At both locations, dolphins were captured when they were sighted in small groups. The number of dolphins captured during a single set of the net was usually limited to ≤ 3 dolphins. Dolphins with calves < 2 years old were not captured. Dolphins identified as eligible for inclusion were encircled with a net and restrained by teams of 5 handlers.[19]

Collection of tissue specimens—Adult female dolphins received an ultrasonographic examination while in the water to determine pregnancy status. Females identified as being in the last 4 months of pregnancy were released immediately without processing and were not included in the study. Blood samples were obtained from all other dolphins (including females judged to be in the first 8 months of pregnancy) while they were in the water and, generally, within 15 minutes after capture. A 19-gauge, 1.9-cm butterfly catheter[a] was inserted into periarterial venous rete in the flukes, and approximately 250 mL of blood was collected into evacuated tubes containing lithium heparin, EDTA, or serum separator gel.[b] Dolphins were then moved to a

boat and positioned on a stretcher for the remainder of the sample collection process. Each dolphin received a complete physical examination and an ultrasonographic examination by use of a hand held ultrasonographic device[c] to evaluate organ health; blubber depth; and, for males, testicular size. Typically, 2 or 3 veterinarians performed procedures on each dolphin simultaneously to minimize time the dolphin spent out of the water. After physical examination, other specimens were collected in approximately the following sequence: swab samples were obtained from the blowhole for microbiologic and cytologic analysis; a urine sample was obtained via sterile placement of a urinary catheter (8-F catheter for males; 10-F self-catheter for females); gastric fluid for cytologic and microbiologic evaluations was obtained by suction via a flexible, lubricated foal orogastric tube; and rectal swabs were collected for microbiologic and cytologic evaluations. The tissue and root structures of the left 15th mandibular tooth were infiltrated with 3% mepivicaine hydrochloride solution[d] prior to removal with a dental elevator and an extractor. Age was estimated by counting postnatal dentine layers.[20] Prior to obtaining biopsy specimens of any cutaneous, genital, and oral lesions, the subcutaneous area surrounding and beneath the lesion was infiltrated with approximately 2 to 3 mL of 2% lidocaine hydrochloride with epinephrine.[e] Biopsy specimens were obtained by use of a sterile No. 10 scalpel blade[f] and forceps. After removal, the tissue was placed in jars containing neutral-buffered 10% formalin or in vials for flash freezing in liquid nitrogen. All dolphins were freeze branded and tagged for future identification and to prevent recapture during the collection process.[19]

Health assessment—Dolphin health assessments were a collaborative effort between the Harbor Branch Oceanographic Institution and the National Ocean Service, Center for Coastal Environmental and Biomolecular Research of the National Oceanic and Atmospheric Administration. Methods for hematologic, serum biochemical, and cytologic analyses have been described elsewhere.[21–23] Analyses were performed at a single laboratory or by a single investigator to ensure consistency and standardization of analytic techniques.

A panel of 5 marine mammal veterinarians was convened to evaluate health information for each dolphin. Clinicopathologic data from each dolphin were compiled into a series of case reports, each of which contained the results of physical examination, ultrasonographic examination, and analyses of collected specimens. Expert judgment was applied to classify each dolphin as clinically normal, possibly diseased, or definitely diseased. After discussing each case, the panel worked to attain a final classification of disease status via consensus. Dolphins classified as definitely diseased were those that had evidence of a condition that would require the dolphins to receive medical treatment in a captive setting. Those judged to require additional diagnostic evaluation were classified as possibly diseased. Panel members did not classify each dolphin independently; a weighted scoring system was not used.

Dolphins in which lobomycosis (a chronic fungal disease of the skin and epidermis of dolphins and humans caused by *Lacazia loboi*[24]) or orogenital papillomatosis (an emerging disease associated with a gamma herpesvirus[25,26] and a novel papillomavirus[26]) was detected during physical examination were considered to be definitely diseased. The clinical and pathologic features of 9 dolphins with histologically confirmed lobomycosis were described in another report.[24] Six of the dolphins in which orogenital papillomas were identified during the health assessment reported here were also described in another report.[25] These diagnoses were confirmed by use of results of histologic evaluation of tissue biopsy specimens. Cytologic evidence of severe neutrophilic gastric inflammation was considered diagnostic of definite disease. Dolphins with moderate-acute, subacute, or chronic-active gastric or blowhole inflammation were classified as definitely or possibly diseased on the basis of other results.

Statistical analysis—Statistical analyses were conducted by use of computer software.[g] Only the first examination of each dolphin was included in the statistical analyses. Period prevalence data were calculated for disease status (clinically normal, possibly diseased, or definitely diseased) stratified by site (IRL or Charleston), age group (3 to 6 years; > 6 to 10 years; > 10 years), and sex (male or female). Calculations of ORs with 95% CIs and χ^2 tests were used to compare proportions of possibly and definitely diseased dolphins between the Charleston and IRL sites. The Mantel-Haenszel procedure was used to calculate age-adjusted and sex-adjusted ORs for each site. The χ^2 test for linear trend was used to evaluate the significance of changes in proportions of dolphins classified as definitely diseased and possibly diseased for each year, from 2003 through 2005.

Cutoff points for hematologic and serum biochemical values were used to detect differences in distributions of dolphins classified as having abnormal values between sites. Cutoff points chosen for the study reported here were based on reported values for clinically normal captive dolphins,[27] reported values for clinically normal dolphins from the IRL[21] and Charleston[22] sites, and values used in an algorithm-based scoring system for dolphin health.[3] The χ^2 test was used to compare proportions of dolphins with hematologic and serum biochemical values higher or lower than specified cutoff points. Values of $P < 0.05$ were considered significant for all analyses.

Results

One hundred seventy-eight dolphins were captured and assigned an identification number (84 at the Charleston site and 94 at the IRL site) during the 3-year study period. Three dolphins were excluded because hematologic data were not available. Ultrasonographic examination of the 13 pregnant females evaluated (5 from Charleston and 8 from IRL) revealed that 4 were in the final 4 months of pregnancy; those 4 were released without additional evaluation. Fifteen dolphins were recaptured; 13 were evaluated during each of 2 years and 2 were evaluated during each of the 3 years of the study. The final sample for analysis of health status contained 171 individual dolphins evaluated on first capture and 13 dolphins with 1 or 2 additional captures.

During the 3 years of the study, 80 of the 171 (46.8%) dolphins were classified by the panel of marine mammal veterinarians as clinically normal, 47 (27.5%) were classified as possibly diseased, and 44 (25.7%) were classified as definitely diseased (**Table 1**). The proportion of dolphins from the IRL site classified as definitely diseased (31.5%) was not significantly (OR, 1.84; 95% CI, 0.81 to 4.20; $P = 0.11$) different from the proportion of definitely diseased dolphins from the estuarine waters around the Charleston site (19.5%).

Mean ± SD ages for 73 dolphins from the IRL site (11.5 ± 5.6 years) and 70 dolphins from the Charleston site (13.8 ± 8.1 years) were not significantly ($P = 0.05$) different. Age estimates were unavailable for 28 of 171 (16.4%) dolphins. Distribution of sex was not significantly ($P = 0.58$) different between dolphins from the IRL and Charleston sites. Adjusting for age, dolphins from the IRL site were 1.82 times as likely to be classified as definitely diseased, compared with dolphins from the Charleston site, although that difference was not significant (95% CI, 0.73 to 4.64; $P = 0.24$). The odds of being classified as possibly diseased were also not significantly (OR, 0.93; 95% CI, 0.42 to 2.03; $P = 0.83$) different between dolphins from the IRL (24.7%) and Charleston (30.5%) sites; statistical adjustment for age had no effect on this association (OR, 1.02; 95% CI, 0.43 to 2.38; $P = 0.88$). When data from the possibly diseased and definitely diseased dolphins were combined, the total proportion of dolphins classified as diseased was not significantly (OR, 1.28; 95% CI, 0.67 to 2.45; $P = 0.42$) different between the IRL (56.2%) and Charleston (50.0%) sites.

Differences between proportions of dolphins classified as possibly diseased and definitely diseased were evaluated further by stratifying the data by age and sex (**Table 2**). Dolphins > 6 to 10 years old were 4.2 times as likely to be classified as definitely diseased as dolphins ≤ 6 years old. High proportions of dolphins > 6 to 10 years old were classified as definitely diseased at both the Charleston (63.6%) and IRL (40.0%) sites. The overall odds of being classified as diseased among dolphins that were > 10 years old were not higher than those among dolphins ≤ 6 years old at either site. Proportions of dolphins classified as possibly diseased were similar for all age groups. Associations between sex and disease classification status were not significant.

Data were analyzed by year of dolphin capture to evaluate temporal trends in proportions of dolphins classified as definitely diseased (**Table 3**). Prevalence of dolphins classified as definitely diseased (excluding

Table 1—Prevalence of bottlenose dolphins (*Tursiops truncatus*) classified as clinically normal, possibly diseased, and definitely diseased as determined via initial health evaluations at 2 sites (Charleston, SC, and IRL, Fla) from 2003 through 2005 (n = 171).

Health classification	No. (%) of Charleston dolphins	No. (%) of IRL dolphins	Total No. (%) of dolphins	OR	95% CI	*P* value*
Clinically normal	41 (50.0)	39 (43.8)	80 (46.8)	1.0	Referent	—
Possibly diseased	25 (30.5)	22 (24.7)	47 (27.5)	0.93	0.42–2.03	0.83
Definitely diseased	16 (19.5)	28 (31.5)	44 (25.7)	1.84	0.81–4.20	0.11

*Values of $P < 0.05$ were considered significant.
— = Not applicable.

Table 2—Prevalence of dolphins classified as possibly diseased and definitely diseased as determined via initial health evaluations, stratified by age and sex, from 2 sites (Charleston, SC [n = 82], and IRL, Fla [89]) from 2003 through 2005.

Category	Prevalence for Charleston dolphins (%)	Prevalence for IRL dolphins (%)	Prevalence for all dolphins (%)	OR	95% CI	*P* value*
Possible disease						
Age						
3 to 6 y†	33.3	40.0	36.4	1.0	Referent	—
> 6 to 10 y	27.3	20.0	22.6	0.97	0.23–4.03	0.96
> 10 y	34.2	29.0	31.6	0.82	0.30–2.26	0.67
Sex						
Female	26.5	18.2	22.3	1.0	Referent	—
Male	33.3	28.6	30.8	1.50	0.66–3.43	0.29
Definite disease						
Age						
3 to 6 y†	11.1	26.7	18.2	1.0	Referent	—
> 6 to 10 y	63.6	40.0	48.4	4.17	1.01–18.06	0.02
> 10 y	12.2	29.0	20.3	1.05	0.31–3.93‡	0.93
Sex						
Female	17.7	39.4	28.4	1.0	Referent	—
Male	20.8	26.8	24.0	0.92	0.41–2.08	0.83

†Data on age were unavailable for 28 of 171 dolphins (12 from the Charleston site and 16 from the IRL site).
‡Confidence interval estimated by use of an exact test.
See Table 1 for remainder of key.

Table 3—Prevalence of dolphins classified as definitely diseased among first captures from 2 sites (Charleston, SC, and IRL, Fla) from 2003 through 2005, by capture year and site.

	Charleston*			IRL†		
Year	No. of diseased dolphins	No. of dolphins evaluated	Prevalence (%)	No. of diseased dolphins	No. of dolphins evaluated	Prevalence (%)
2003	4	47	8.5	12	42	28.6
2004	6	17	35.3	9	33	27.3
2005	6	18	33.3	7	14	50.0

*Significant ($P < 0.01$) temporal trend detected by use of χ^2 test for trend. †Temporal trend was not significant ($P = 0.23$).

data from recaptures) increased at both sites between 2003 and 2005. At the Charleston site, a significant ($P < 0.01$) 3-fold increase was detected in proportions of definitely diseased dolphins. In the IRL, a 0.7-fold increase was detected, although that increase was not significant ($P = 0.23$).

Lobomycosis and orogenital papillomatosis accounted for 25 of the 44 (56.8%) diagnoses of definite disease. Nine of 89 (10.1%) dolphins captured in the IRL had lobomycosis; the disease was not identified among dolphins at the Charleston site. Orogenital papillomatosis was diagnosed in 10 of 104 (9.6%) male and 6 of 67 (9.0%) female dolphins from both capture sites. The prevalence of orogenital neoplasia did not differ significantly (OR, 1.60; exact 95% CI, 0.50 to 5.63; $P = 0.38$) between dolphins at the IRL site (10/89 [11.2%] affected) and dolphins from the Charleston site (6/82 [7.3%] affected). Histologic examination of biopsy specimens revealed that all orogenital neoplasms were sessile papillomas. The lesions were located primarily on genital mucosae of male and female dolphins (n = 14) as well as on oral mucosae and tongues (2).

Because diagnoses of lobomycosis and orogenital papillomatosis constituted a substantial proportion of all diagnoses of definite disease, analyses were repeated to exclude data from dolphins with lobomycosis or orogenital papillomatosis. The remaining 19 of 44 (43.2%) dolphins with definite disease were classified on the basis of findings from hematologic, serum biochemical, and cytologic analyses. In this subset of 19 dolphins, the prevalence of definite disease was also not significantly (OR, 0.76; 95% CI, 0.26 to 2.22; $P = 0.38$) different between 8 of 70 (11.4%) dolphins from the IRL site and 11 of 76 (14.5%) dolphins from the Charleston site. No diseases or organ abnormalities were detected via ultrasonographic examination. Results of urinalysis contributed to disease classification in only 2 dolphins. Organisms recovered from bacteriologic cultures of blowhole, rectal swabs, and gastric contents were considered normal flora; no primary pathogens were isolated.

Repeated captures of 15 dolphins (13 dolphins were captured twice and 2 dolphins were captured 3 times, for a total of 17 recaptures) during the 3-year study period provided an opportunity to evaluate the stability of disease classification over time. Six dolphins were classified identically when recaptured 1 year after initial evaluation. Six other dolphins had developed an oral or genital papilloma when examined 1 or 2 years later. Four of the new orogenital papillomas were detected in dolphins from the IRL site, and 2 were detected in dolphins from the Charleston site. Two dolphins were reclassified from clinically normal to possibly diseased; 3 were reclassified from possibly diseased to clinically normal.

Typically, abnormalities in > 1 clinicopathologic variable were detected in the 91 dolphins that were classified as diseased (definitely diseased or possibly diseased). The most common abnormalities detected were in hematologic values (data not reported). Leukocytosis with high absolute numbers of lymphocytes, neutrophils, or eosinophils was the most consistent finding. Other hematologic abnormalities included mild regenerative anemia characterized by low values of erythrocyte and hemoglobin concentrations, Hct, and mean corpuscular volume as well as polychromasia and reticulocytosis. Cytologic evidence of severe neutrophilic gastric inflammation was detected in samples of gastric fluids obtained from 3 dolphins from each site; these 6 dolphins were classified as definitely diseased. Evidence of moderate neutrophilic gastric inflammation was detected in samples obtained from 11 dolphins; 4 of these dolphins were classified as definitely diseased on the basis of other findings, and 7 were classified as possibly diseased. Cytologic findings in swabs obtained from the blowholes of 2 dolphins at the Charleston site were indicative of mild to moderate neutrophilic inflammation; these dolphins were classified as possibly diseased. The remaining 49 dolphins that were classified as diseased had low serum alkaline phosphatase activities; low serum iron concentrations; abnormal serum immunoglobulin concentrations; or abnormalities in values of other serum analytes, particularly elevations in activities of hepatic transaminases (alanine aminotransferase, aspartate aminotransferase, or γ-glutamyltransferase).

The proportion of dolphins with abnormal hematologic and serum biochemical values as determined by preestablished cutoff points was compared between sites (**Table 4**). Leukocytosis (defined as WBC count > 12.3×10^3 WBCs/μL) was detected more frequently ($P = 0.03$) in dolphins from the Charleston site, compared with that in dolphins from the IRL site. Dolphins from the Charleston site were significantly ($P < 0.001$) more likely than those from the IRL site to have lymphocytosis (defined as lymphocyte count > 3,000 cells/μL) and low serum concentrations of total protein (defined as a value < 7.0 g/dL). A higher proportion of dolphins from the Charleston site also had low serum concentrations of total globulin (defined as a value < 2.5 g/dL);

Table 4—Proportions of dolphins with specific hematologic and serum biochemical abnormalities among first captures from 2 sites (Charleston, SC, and IRL, Fla) from 2003 through 2005.

	Charleston		IRL		
Analyte	No. of dolphins evaluated	No. of dolphins affected (%)	No. of dolphins evaluated	No. of dolphins affected (%)	*P* value*
WBCs > 12.3 × 10^3/μL	77	25 (32.5)	85	15 (17.6)	0.03
Neutrophils > 6.0 × 10^3/μL	77	10 (13.0)	85	15 (17.6)	0.42
Lymphocytes > 3.0 × 10^3/μL	77	23 (29.9)	85	6 (7.1)	< 0.001
Hct < 38%	77	8 (10.4)	85	14 (16.5)	0.26
Hemoglobin < 13 g/dL	77	8 (10.4)	85	6 (7.1)	0.45
SUN > 70 mg/dL	80	10 (12.5)	89	25 (28.1)	0.13
Creatinine > 2.0 mg/dL	80	1 (1.3)	89	0 (0.0)	NC
Iron < 80 μg/dL	80	12 (15.0)	89	35 (39.3)	< 0.001
Alkaline phosphatase < 100 U/L†	40	10 (25.0)	43	6 (14.0)	0.20
Alanine aminotransferase > 60 U/L	36	4 (11.1)	47	6 (12.8)	0.82
Aspartate aminotransferase > 300 U/L	80	9 (11.3)	89	17 (19.1)	0.16
γ-Glutamyltransferase ≥ 40 U/L	80	0 (0.0)	89	0 (0.0)	NC
Total protein < 7.0 g/dL	62	22 (35.5)	75	9 (12.0)	< 0.001
Albumin < 3.5 g/dL	80	21 (26.3)	89	23 (25.8)	0.95
Total globulins < 2.5 g/dL	80	10 (12.5)	89	0 (0.0)	NC
Total γ-globulin < 1.0 g/dL	80	14 (17.5)	89	1 (1.1)	< 0.001
Fibrinogen ≥ 300 mg/dL	77	6 (7.8)	85	11 (12.9)	0.29

†Alkaline phosphatase calculated for dolphins ≥ 10 years of age.
NC = Not calculated.
See Table 1 for remainder of key.

however, a value of *P* was not calculated because none of the dolphins from the IRL site had low values of that variable. Furthermore, low serum concentrations of total γ-globulin (defined as a value < 1.0 g/dL) were more common ($P < 0.001$) in dolphins from the Charleston site, compared with values in dolphins from the IRL site. In contrast, dolphins from the IRL site were more likely ($P < 0.001$) than those from the Charleston site to have low serum concentrations of iron (defined as a value < 80 μg/dL), which is an important marker for disease in dolphins.

Discussion

The IRL and Charleston sites represent unique ecosystems that are affected to differing extents by anthropogenic pollutants and other environmental hazards. Although neither site is pristine, differences in the prevalence of various diseases among dolphins may be useful in associating the patterns of disease at each site with local environmental conditions and specific exposures. The finding that less than half (47%) of the dolphins captured at both sites were classified as clinically normal in our study raises concerns about the overall health of these populations of dolphins and about the environmental conditions that may have contributed to poor health status. As an initial step in that risk assessment, health assessments of a large sample of bottlenose dolphins were undertaken in the present study.

Historical patterns of morbidity and death among dolphins from the IRL and Charleston sites are disparate, but comparison between sites is hindered because of uncertainty regarding the size of populations at each site. Data concerning stranded dolphins in the IRL have been collected since the 1970s. For that population, higher numbers of seasonal strandings have been reported since 1996, with peak numbers of strandings reported in 2000 and 2001.[28] In 2001, an unusual fatality event of unknown etiology took place in which at least 35 dolphins died during a 2-month period in the northern portion of the IRL.[29] Along the coastal region of South Carolina, the mean number of bottlenose dolphin strandings was 43/y, with a range of 28 to 68/y between 1997 and 2003.[30] Of the 302 stranded dolphins in South Carolina during this interval, 25% appeared to have had an adverse interaction with humans, such as trauma from a boat strike, but causes of most strandings are unknown.

Pathologic findings in stranded dolphins from the IRL suggest that a substantial portion of deaths among the stranded dolphins is attributable to infectious diseases and that dysfunction of the immune system may contribute to the pathogenesis of disease.[31] Various skin lesions have been diagnosed in IRL dolphins, including proliferative ulcerative dermatitis caused by ciliated protozoans and fungi, dolphin pox dermatopathy, and vesicular dermatopathy of unknown etiology.[31] Photo-identification surveys confirm the high prevalence of skin disease and suspected lobomycosis among dolphins in the IRL, particularly among those that inhabit the southern zone of the lagoon.[h] That zone is characterized by higher water temperatures and freshwater incursion with lower salinity, compared with conditions that are typical of the northern zones of the IRL. The requirements for maintenance and growth of *L loboi* in the marine environment have not been established because the organism has not been isolated in vitro. Human cases of infection with *L loboi* are most common in people with occupational exposure to water, soil, and vegetation in rural areas of Latin America.[32] Local trauma (eg, lacerations or abrasions) appears to create a portal of entry for the organism in dolphins and humans.[24,32]

In the study reported here, orogenital papillomas were diagnosed in 16 dolphins from the IRL and Charleston sites at initial evaluation. These papillomas have been detected in dolphins from both sites.[25] A novel papillomavirus (designated TtPv2) has been isolated from a genital lesion of a Charleston dolphin and characterized.[26] Such lesions may also contain a genital papilloma-associated gamma herpesvirus or no evidence of virus expression with either agent.[25,26] To the authors' knowledge, the specific role of these papillomaviruses in oncogenesis and tumor progression remains to be defined. In our study, 6 new cases of orogenital papillomas were identified among 15 dolphins reexamined in the 2 years following the initial evaluation, which represents an incidence of 40% during a period of 1 to 2 years in a small sample of dolphins. This finding suggests that the infectious agent or agents responsible for the orogenital papillomas were spreading rapidly or that an unknown environmental factor may have contributed to the pathogenesis of these papillomas. The prevalence of dolphins with orogenital papillomas increased substantially from 2003 to 2005. Dolphins from the IRL were unaffected in 2003; however, in 2004 and 2005, the prevalence of affected dolphins was 10% and 42%, respectively.

Leukocytosis and lymphocytosis, as well as low serum concentrations of total protein, total globulin, and γ-globulins, were more common in dolphins from the Charleston site, compared with respective values in dolphins from the IRL. The cause of these differences is not known; however, substantial evidence exists to indicate that dolphins from the Charleston site are exposed to higher concentrations of contaminants than are dolphins from the IRL. For example, concentrations of organochlorine contaminants (eg, PCBs and DDE) in blubber samples obtained by remote biopsy are higher in dolphins from the Charleston site, compared with those in dolphins from the IRL site.[33] Previous findings of the Bottlenose Dolphin HERA Project indicate that concentrations of PCBs in dolphins from the Charleston site in 2003 and 2004 were approximately twice as high (mean concentration of total PCBs, 223 ng/g of wet wt) as those obtained from the IRL (mean total PCBs, 122 ng/g of wet wt).[34] Exposures to PCBs (and DDE) are associated with suppressed immune responses in dolphins,[35] and increased blubber concentrations of PCBs have been associated with risk of death from infectious disease in harbor porpoises.[6] Thus, exposure to higher concentrations of PCBs at the Charleston site versus the IRL site could be responsible for the differences in total and γ-globulin concentrations in the dolphins of this report. New chemicals of concern in the marine environment, such as perfluorinated chemicals and PBDEs, bioaccumulate in dolphins.[36,37] In laboratory animals, perfluoroalkyl compounds affect prenatal and postnatal development and the neuroendocrine system.[38] To the authors' knowledge, the toxic effects of these compounds in marine mammals have not been established. Results of other research[36] indicate that dolphins from the Charleston site have the highest concentrations of perfluoroalkyl compounds in blood, compared with concentrations determined in other surveys of dolphin populations. Polybrominated diphenyl ethers, which are used as flame retardants, are widely distributed in the marine environment and bioaccumulate in fish and marine mammals.[37] In experimental exposure studies,[39] PBDEs induced various pathologic conditions, including cancer; disorders of the reproductive, endocrine, and central nervous systems; and developmental abnormalities. The concentrations of PBDEs in blubber from dolphins from the Charleston site are some of the highest measured in marine mammals to date (approx 5 times as high as concentrations in dolphins from the IRL site).[37] The potential effect of exposure to environmental contaminants on hematologic and serum biochemical values as well as on immune function is under investigation.

Despite the aforementioned differences in the concentrations of anthropogenic chemicals in the environment and in dolphin tissues, we did not find significant differences in the proportions of definitely diseased or possibly diseased dolphins from the Charleston and IRL sites in the study reported here. Exclusion of cases of lobomycosis and orogenital neoplasia, which have specific microbiologic causes, had no significant effect on the results. There are several explanations for the lack of an apparent effect of site. First, the data were subject to survivor bias, by which the sickest and most debilitated members of a cohort may die selectively. The finding that the highest risk of disease was in dolphins aged > 6 to 10 years, rather than in those aged > 10 years, provided support for this hypothesis. Second, the results reported here represent only the first phase of risk assessment, which used standard methods of physical examination and clinicopathologic testing. The screening tools used in the present study may not have been adequately sensitive to detect subtle differences in health status. Third, a high proportion (26%) of dolphins evaluated was classified as definitely diseased. This high prevalence may indicate that environmental stressors, albeit of differing origins, are affecting dolphins at both sites. For example, results of other studies[40,41] indicated that concentrations of mercury are higher in skin and blood specimens obtained from dolphins from the IRL site, compared with those in dolphins from the Charleston site. The restriction of all cases of lobomycosis to dolphins at the IRL site may indicate an environmental exposure that is unique to that habitat. Similarly, the temporal trend of increasing proportions of dolphins classified as definitely diseased at Charleston may be indicative of deteriorating environmental quality for that site. No known environmental insults, such as oil spills or discharges or other infectious disease epizootics, that may explain the increases in proportions of dolphins classified as diseased took place immediately prior to or during the study period.

The techniques described in the present report may be applied to health assessments of other populations of bottlenose dolphins for which more limited data are available. The methods we used for classification of health status have several aspects in common with those used in algorithm-based, weighted-scoring systems.[3,i] Both systems use findings from physical examination and hematologic and serum biochemical analyses to assess health status. However, in our study, the use of expert judgment by a panel of marine mammal veterinar-

ians provided a more comprehensive assessment than other approaches that assign values to each variable for use in an algorithm. We incorporated age, physical condition, and the relationship between variables to assess health status for each dolphin. Furthermore, the use of ultrasonographic examination, urinalysis, and cytologic and microbiologic evaluations in the study reported here provided a more extensive clinical assessment of health, compared with data used in other studies.[3,i] The panel of marine mammal veterinarians used findings from cytologic examination of gastric contents to identify diseased dolphins because severe gastric inflammation has good predictive value as an indicator of disease.[23] Interpopulation comparisons of clinicopathologic data will require standardization of laboratories and analytic methods to ensure comparability.

a. Becton-Dickinson, Franklin Lakes, NJ.
b. Vaccutainer, Becton-Dickinson, Franklin Lakes, NJ.
c. SonoSite 180plus, Bothell, Wash.
d. 3% Carbocaine solution, Cooke-Waite, Rochester, NY.
e. Lidocaine HCl 2% and epinephrine 1:100,000, Cooke-Waite, Rochester, NY.
f. No. 10 scalpel blade, Becton-Dickinson, Franklin Lakes, NJ
g. EPI INFO, version 6.0, CDC, Atlanta, Ga.
h. Reif JS, Murdoch E, Mazzoil M, et al. Lobomycosis in bottlenose dolphins: spatial aggregation of an emerging infectious disease (abstr). *Epidemiology* 2006;17:S434.
i. Reif JS, Hansen L, Galloway S, et al. Chlorinated hydrocarbon contaminants and selected health parameters in bottlenose dolphins (abstr), in *Proceedings*. 11th Biennial Conf Biol Mar Mammals. *Soc Mar Mammal* 1995;95.

References

1. Waring GT, Pace RM, Quintal JM, et al. *US Atlantic and Gulf of Mexico marine mammal stock assessments–2003*. NOAA Technical Memorandum NMFS-NE-182. Washington, DC: National Oceanic and Atmospheric Administration, 2004;250–260.
2. Reddy ML, Reif JS, Bachand A, et al. Opportunities for using navy marine mammals to explore associations between organochlorine contaminants and unfavorable effects on reproduction. *Sci Total Environ* 2001;274:171–182.
3. Wells RS, Rhinehardt HL, Hansen LJ, et al. Bottlenose dolphins as marine ecosystem sentinels: developing a health monitoring system. *EcoHealth* 2004;1:246–254.
4. Bossart GD. Marine mammals as sentinel species for oceans and human health. *Oceanography* 2006;19:44–47.
5. Schwacke LH, Voit EO, Hansen LJ, et al. Probabilistic risk assessment of reproductive effects of polychlorinated biphenyls on bottlenose dolphins (*Tursiops truncatus*) from the southeast United States coast. *Environ Toxicol Chem* 2002;21:2752–2764.
6. Hall AJ, Hugunin K, Deaville R, et al. The risk of infection from polychlorinated biphenyl exposure in the harbor porpoise (*Phocoena phocoena*): a case-control approach. *Environ Health Perspect* 2006;114:704–711.
7. Fair PA, Bossart GD. *Overview of the bottlenose dolphin health and risk assessment project. Synopsis of researcher meeting bottlenose dolphin health and risk assessment project*. NOAA Technical Memorandum NOS NCCOS 10. Washington, DC: National Oceanic and Atmospheric Administration, 2005;22–24.
8. Hohn AA. *Design for a multiple-method approach to determine stock structure of bottlenose dolphins in the mid-Atlantic*. NOAA Technical Memorandum NMFS-SEFSC-401. Washington, DC: National Oceanic and Atmospheric Administration, 1997;1–22
9. Mazzoil M, McCulloch SD, Defran RH. Observations on the site fidelity of bottlenose dolphins (*Tursiops truncatus*) in the Indian River Lagoon, Florida. *Fla Sci* 2005;68:217–227.
10. Zolman ES. Residence patterns of bottlenose dolphins (*Tursiops truncatus*) in the Stono River estuary, Charleston County, South Carolina, USA. *Mar Mamm Sci* 2002;18:879–892.
11. US Environmental Protection Agency, National Estuary Program, Indian River Lagoon. Available at: www.epa.gov/owow/estuaries/programs/irl.htm. Accessed May 24, 2007.
12. Scott GI, Fulton MH, Wirth EF, et al. Toxicological studies in tropical ecosystems: an ecotoxicological risk assessment of pesticide runoff in south Florida estuarine systems. *J Agric Food Chem* 2002;50:4400–4408.
13. Sigua GC, Steward JS, Tweedale WA. Water-quality monitoring and biological integrity assessment in the Indian River Lagoon, Fla: status, trends, and loadings (1988–1994). *Environ Manage* 2000;25:199–209.
14. Miles CJ, Pfeuffer RJ. Pesticides in canals of South Florida. *Arch Environ Contam Toxicol* 1997;32:337–345.
15. Tiner RW. *An inventory of South Carolina's coastal marshes. South Carolina Wildlife and Marine Resources Department*. Technical Report No. 23. Charleston, SC: South Carolina Wildlife and Marine Resource Department, 1977;1–33.
16. Long ER, Scott GI, Kucklick J, et al. *Magnitude and extent of sediment toxicity in selected estuaries of South Carolina and Georgia*. NOAA Technical Memorandum NOS ORCA 128. Washington, DC: National Oceanic and Atmospheric Administration, 1998;1–289.
17. US Environmental Protection Agency. National Priorities List site narrative for Koppers Co, Inc (Charleston Plant). Available at: www.epa.gov/superfund/sites/npl/nar1329.htm. Accessed May 24, 2007.
18. US Environmental Protection Agency. National Priorities List site narrative for Mcalloy Corporation. Available at: www.epa.gov/superfund/sites/npl/nar1577.htm. Accessed May 24, 2007.
19. Fair PA, Adams JD, Zolman E, et al. *Protocols for conducting dolphin capture-release health assessment studies*. NOAA Tech Memorandum NOS NCCOS 49. Washington, DC: National Oceanic and Atmospheric Administration 2006;1–83.
20. Hohn A, Scott M, Wells R, et al. Growth layers in teeth from free-ranging, known-age bottlenose dolphins. *Mar Mamm Sci* 1989;5:315–342.
21. Goldstein JD, Reese E, Reif JS, et al. Hematology, serum analyte and cytologic findings from Atlantic bottlenose dolphins (*Tursiops truncatus*) inhabiting the Indian River Lagoon, Florida. *J Wildl Dis* 2006;42:447–454.
22. Fair PA, Hulsey TC, Varela RA, et al. Hematology, serum chemistry and cytology findings from apparently healthy Atlantic bottlenose dolphins (*Tursiops* truncatus) inhabiting the estuarine waters of Charleston, South Carolina. *Aquat Mamm* 2006;32:182–195.
23. Varela RA, Schmidt K, Goldstein JD, et al. Evaluation of cetacean and sirenian cytologic samples. *Vet Clin North Am Exot Anim Pract* 2007;10:79–130.
24. Reif JS, Mazzoil M, McCulloch SD, et al. Lobomycosis in Atlantic bottlenose dolphins (*Tursiops truncatus*) from the Indian River Lagoon, Florida. *J Am Vet Med Assoc* 2006;228:104–108.
25. Bossart GD, Ghim S, Rehtanz M, et al. Orogenital neoplasia in Atlantic bottlenose dolphins (*Tursiops truncatus*). *Aquat Mamm* 2005;31:473–480.
26. Rehtanz M, Ghim JS, Rector A, et al. Isolation and characterization of the first American bottlenose dolphin papillomavirus: *Tursiops truncatus* papillomavirus type 2. *J Gen Virol* 2006;87:3559–3565.
27. Bossart GD, Reiderson TH, Dierauf LA, et al. Clinical pathology. In: Dierauf LA, Gulland FM, eds. *CRC handbook of marine mammal medicine*. 2nd ed. Boca Raton, Fla: CRC Press LLC, 2001;383–436.
28. Stolen MK, Durden WN, Odell DK. Historical synthesis of bottlenose dolphin (*Tursiops truncatus*) stranding data in the Indian River Lagoon system, Florida, from 1977–2005. *Fla Sci* 2007;70:45–54.
29. Marine Mammal Commission. *Annual report of the Marine Mammal Commission, calendar year 2002*. Bethesda, Md: Marine Mammal Commission, 2003;1–264.
30. McFee WS, Hopkins-Murphy S, Schwacke L. Trends in bottlenose dolphin (*Tursiops truncatus*) strandings in South Carolina, 1997–2003. Implications for the southern North Carolina and South Carolina management units. *J Cetacean Res Manag* 2006;8:195–201.
31. Bossart GD, Meisner R, Varela R, et al. Pathologic findings in stranded Atlantic bottlenose dolphins (*Tursiops truncatus*) from the Indian River Lagoon, Florida. *Fla Sci* 2003;6:226–238.
32. Rodriguez-Toro G. Lobomycosis. *Int J Dermatol* 1993;32:324–332.
33. Hansen LJ, Schwacke LH, Mitchum GB, et al. Geographic variation in polychlorinated biphenyl and organochlorine pesticide

concentrations in the blubber of bottlenose dolphins from the US Atlantic Coast. *Sci Total Environ* 2004;319:147–172.
34. Houde M, Pacepavicius G, Wells RS, et al. Polychlorinated biphenyls and hydroxylated polychlorinated biphenyls in plasma of bottlenose dolphins (*Tursiops trucatus*) from the Western Atlantic and the Gulf of Mexico. *Environ Sci Technol* 2006;40:5860–5866.
35. Lahvis GP, Wells RS, Kuehl DW, et al. Decreased lymphocyte responses in free-ranging bottlenose dolphins (*Tursiops truncatus*) are associated with increased concentrations of PCBs and DDT in peripheral blood. *Environ Health Perspect* 1995;103:67–72.
36. Houde M, Wells RS, Fair PA, et al. Polyfluoroalkyl compounds in free-ranging bottlenose dolphins (*Tursiops trucatus*) from the Gulf of Mexico and the Atlantic ocean. *Environ Sci Technol* 2005;39:6591–6598.
37. Fair PA, Mitchum G, Hulsey TC, et al. Polybrominated diphenyl ethers (PBDEs) in blubber of free-ranging bottlenose dolphins (*Tursiops truncatus*) from two southeast Atlantic estuarine areas. *Arch Environ Contam Toxicol* 2007;53:483–494.
38. Lau C, Anitole C, Hodes D, et al. Perfluoroalkyl acids: a review of monitoring and toxicological findings. *Toxicol Sci* 2007;99:366–394.
39. Birnbaum LS, Staskal DF. Brominated flame retardants: cause for concern? *Environ Health Perspect* 2004;112:9–17.
40. Stavros HC, Bossart GD, Hulsey TC, et al. Trace element concentrations in skin of free-ranging bottlenose dolphins (*Tursiops truncatus*) from the southeast Atlantic coast. *Sci Total Environ* 2007;388:300–315.
41. Stavros HC, Bossart GD, Hulsey TC, et al. Trace element concentrations in blood of free-ranging bottlenose dolphins (*Tursiops truncatus)*: influence of age, sex and location. *Marine Pollution Bulletin* 2007;56:371–379.