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Angiomatosis, A Newly Recognized Disease in Atlantic Bottlenose Dolphins (*Tursiops truncatus*) from the Gulf of Mexico

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Abstract. A new disease, angiomatosis, was recognized in 25 of 54 (46.3%) Atlantic bottlenose dolphins (*Tursiops truncatus*) necropsied after being stranded along the Texas Gulf coast during 1991–1996. Angiomatosis was first recognized by the authors in 1992 and has increased in incidence and severity, affecting 100% of juveniles and adults. This disease is characterized by proliferation of small, thick-walled blood vessels diffusely throughout the lungs, without inflammation, exudation, or alveolar hemorrhage. The vascular proliferation also occurs in lung-associated and other visceral lymph nodes. Hemangiomas frequently occur in affected lymph nodes and occasionally in the lungs. The vascular proliferation reduces airspace and may occlude small airways. Angiomatosis appears to be a broad-field defect of vascular endothelium. Although this process appears to be an increasingly important factor in the morbidity of *T. truncatus*, its etiology has not been determined.

Key words: Angiomatosis; Atlantic bottlenose dolphin; hemangioma; lungs; lymph nodes; pathology; *Tursiops truncatus*; vascular proliferation.

Benign, diffuse proliferation of blood vessels in the lung is rare in any species. In our experience, chronic lung diseases of any sort, apart from various degrees of local inflammation associated with lung worms and bacterial, fungal, or viral pneumonitis, are also rare in free-ranging dolphins.[3,4] During the course of necropsy of cetaceans stranded along the Texas Gulf Coast from April 1991 through September 1996, we observed a high incidence of a peculiar vascular proliferation in the lungs and associated lymph nodes of Atlantic bottlenose dolphins, *Tursiops truncatus.* This process was characterized by diffuse micronodular proliferation of blood vessels originating from preexisting small veins. Marked thickening and vascularization of the pleura was usually accompanied by hypervascularity. A more diffuse vascular proliferation was typical in lymph nodes of animals with lung involvement, and hemangiomas frequently developed in involved lymph nodes. Our purpose here is to report and illustrate this process, apparently unique to the dolphin, that we call ''angiomatosis.''

Materials and Methods

Animals

The dolphins in this study were stranded or net-captured animals collected by the Texas Marine Mammal Stranding Network under the auspices of the National Marine Fisheries Service. The collection area ranges from Cameron County, Texas, at the Mexican border to Calcasieu Parish, Louisiana (i.e., the entire Texas Gulf coast and part of western Louisiana).

From April 1991 through September 1996, the total number of *T. truncatus* strandings, including live, recently dead, and decomposed animals, was 1,042. We performed postmortem examinations on 54 (34 female, 20 male) of these animals that were fresh (without bloating or decay). Four were examined in 1991 (9 months), 16 in 1992, 10 in 1993, 11 in 1994, 6 in 1995, and 9 in 1996 (9 months).

Selection and sampling

Well-preserved animals were taken to a central laboratory at Texas A&M University at Galveston for necropsy, including gross examination and systematic histologic sampling of all organs and tissues. All organs were removed and weighed on a Sartorius model 4800P electronic platform scale before sampling. For histologic analysis by light microscopy, tissues were fixed in 10% neutral buffered formalin, dehydrated through a series of alcohol solutions, embedded in paraffin, sectioned at 5 μm, and stained with 1) hematoxylin and eosin; 2) hematoxylin, phloxine, and saffron (HPS), a trichrome stain used mainly to discriminate muscle from collagen[9]; 3) Masson's trichrome; and 4) Movat's pentachrome. A labeled antibody against smooth muscle actin (clone A4, Dako Corporation, Carpinteria, CA) was also used on selected tissues. Standard immunohistochemical procedures were used.[13,15]

Sexual maturity

Sexual maturity of the animals in this study was determined by histologic examination of the testes or ovaries. Males with active spermatogenic cells and spermatozoa in

Table 1. Distribution of angiomatosis in *T. truncatus.*

	Total No. of *T. truncatus*	No. of *T. truncatus* with Angiomatosis	Percentage of *T. truncatus* with Angiomatosis	No. of Sexually Mature *T. truncatus* with Angiomatosis
Female	34	14	41	10*
Male	20	11	55	8†
Total	54	25	46	18

* One female was pregnant, and two were lactating.
† All mature males had large testes with active spermatogenesis.

the seminiferous tubules were considered sexually mature. Females with developing ovarian follicles, corpus luteum, or corpus albicans were considered mature. Body size of the animals generally related to maturity status. In this population of *T. truncatus,* animals with body lengths less than 225 cm were sexually immature, and animals with body lengths greater than 225 cm were sexually mature. In males, testicular weights also correlated with sexual maturity. A range of 9 to 29 g was typical of immature animals, whereas weights of 187–1,092 g was typical of mature animals.

Examination of tissues and lesions

Lungs were examined in situ and then removed by cutting through the bronchovascular pedicle. Each lung was sliced transversely at approximately 2-cm intervals, and the exposed surfaces were inspected for lesions. Bronchi were examined for evidence of lungworms. Four to six representative samples from each lung were collected for histologic examination. Additional samples were collected from identifiable lesions.

Dimensions of vascular clusters and the external diameter, lumen diameter, and wall thickness of individual vessels were measured in lungs with representative degrees of disease. The range of dimensions was determined using a calibrated eyepiece scale. Measured clusters were selected as representative within a section, and two maximal diameters were determined. Lesional vessels presenting as round profiles, suggesting true cross sections, were selected for measurement, whereas oblong or other nonround profiles were interpreted as tangential cuts of varying degree and thus not measured.

Results

Abnormal vascular proliferation (angiomatosis) was identified in 25 of 54 (46.3%) *T. truncatus* over the study period. Seven (28%) of the animals were sexually immature, and 18 (72%) were mature. All mature males had large testes with active spermatogenesis. Table 1 shows the distribution of disease by sex. The number of animals stranded with angiomatosis each season (January–March, April–June, July–September, and October–December) approximated the general pattern of total strandings along the Gulf coast, suggesting that there was no seasonal correlation with the recognition of angiomatosis. The incidence and severity of lung lesions by year is presented in Table 2. Angiomatosis was found in none of 4 animals examined in 1991, 6 of 16 (38%) in 1992, 4 of 10 (40%) in 1993, 3 of 11 (27%) in 1994, 6 of 6 (100%) in 1995, and 6 of 7 (86%) in 1996. One of the seven cases in 1996 without angiomatosis was a neonate. If this animal is excluded because of its age, then the 1996 incidence was 100%. That is, the incidence of the disease in juveniles and adults increased from 0 to 100% over the 6-year study period.

Table 2. Incidence and progression of severity of angiomatosis.

Year	No. of *T. truncatus* Necropsied	No. of Angiomatosis Cases				Percentage of Total *T. truncatus* with Angiomatosis
		Mild	Moderate	Severe	Total	
1991*	4	0	0	0	0	0
1992	16	3	2	1	6	38
1993	10	2	2	0	4	40
1994	11	0	0	3	3	27
1995	6	1	2	3	6	100
1996*	7	0	5	1	6	86

* 9 months.

In each of the 25 dolphins, the causes of stranding and death were highly variable and usually multifactorial, including trauma. One dolphin (No. 3) had a lesion consistent with segmental mediolytic arteritis,[14] a widespread degenerative change in small muscular arteries, in addition to angiomatosis. Another (No. 5) had amyloid deposition in the lungs as well as angiomatosis.[2] Several (Nos. 4, 5, 8, 9, 13, 17, and 21) had focal or extensive acute pneumonia independent of angiomatosis. Five (Nos. 8, 9, 14, 15, and 18) had unilateral fibrinous pleuritis (empyema). Fifteen dolphins (Nos. 1–7, 9, 10, 12, 13, 17, 21, 23, and 25) had demonstrable lungworms, and most of these had sclerotic nodules with fragments of identifiable lungworms. Dolphin Nos. 11, 14, 19, and 20 had very similar sclerotic nodules but no demonstrable lungworms in either the nodules or elsewhere. Only six dolphins (Nos. 8, 15, 16, 18, 22, and 24) had neither worms nor nodules.

Angiomatosis in the lungs

The normal gross *T. truncatus* lung was salmon pink, and the visceral pleura was thin and transparent. Lymph vessels coursing over the surface were easily recognized through the pleura. With angiomatosis, the earliest gross change was the appearance of a coarse, whitish reticulation of the entire pleural surface of both

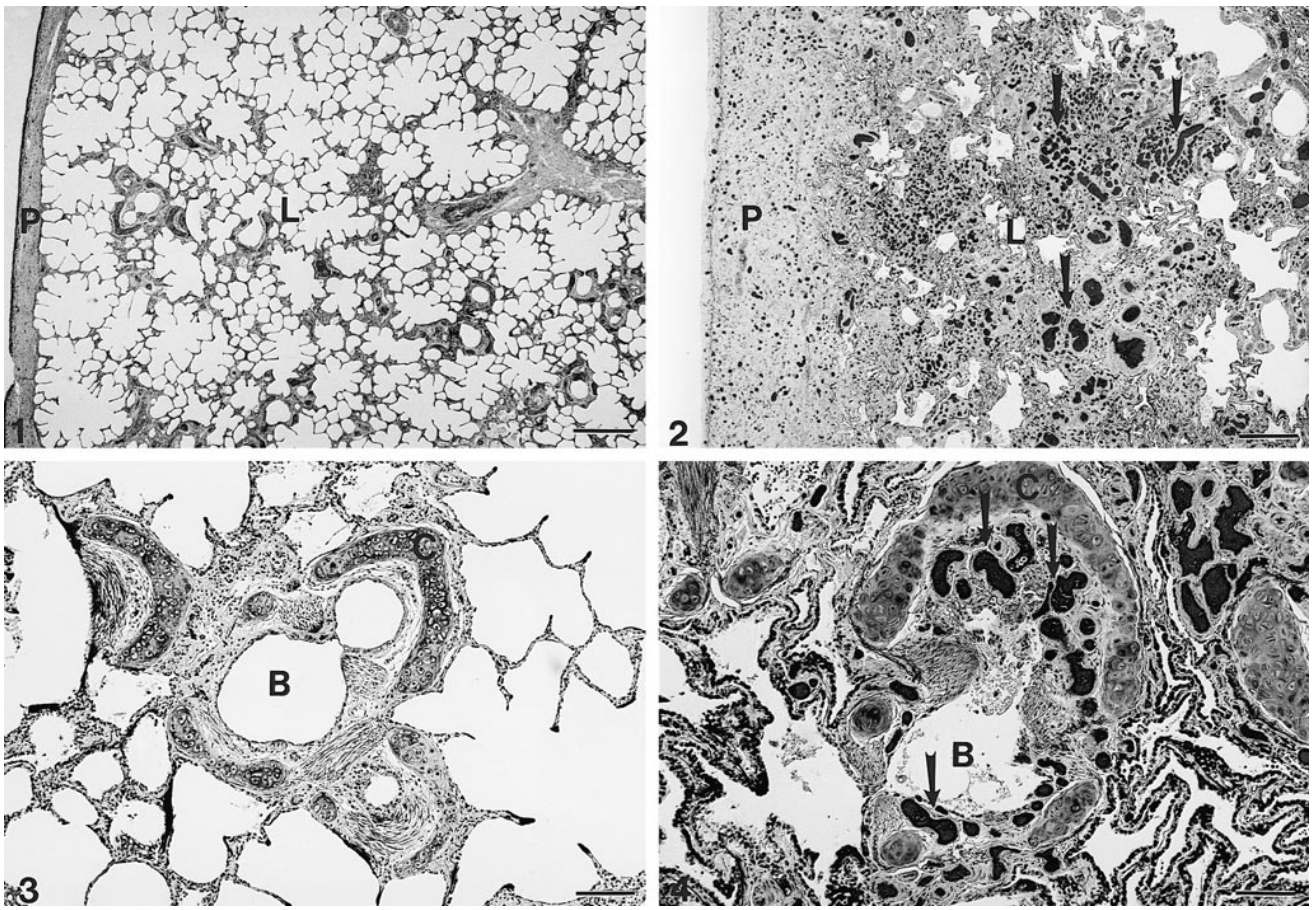

Fig. 1. Lung; normal *T. truncatus.* The pleura (P) is thin with an occasional vessel. The lung parenchyma (L) consists of delicate latticework of alveolar ducts and an occasional vessel. Movat pentachrome stain. Bar = 500 μm.

Fig. 2. Lung; dolphin with angiomatosis (No. 19). The pleura (P) is thickened and hypervascularized. The lung parenchyma (L) is replaced with confluent clusters of collagenized vessels (arrows). Movat pentachrome stain. Bar = 500 μm.

Fig. 3. Bronchiole; normal *T. truncatus.* Normal cartilage (C) surrounds a bronchiole (B). Movat pentachrome stain. Bar = 100 μm.

Fig. 4. Bronchiole; dolphin with angiomatosis (No. 19). Vascular lesions (arrows) encroaching on bronchiole (B), eroding cartilage (C), and reducing airway diameter. Movat pentachrome stain. Bar = 100 μm.

lungs. In advanced stages, the pleura was dense, thickened up to 2 mm, grayish white, and opaque. Lymphatic channels were obscured. This appearance was typical, and extensive angiomatosis could be diagnosed by the appearance of the pleura with a high degree of confidence. Pleural vascularization was not apparent in the gross specimen because the vessels were small and the surrounding tissue dense.

As with all Delphinids, the normal *T. truncatus* lung has several unusual features in comparison with terrestrial mammals, including cartilage plates and bars in the wall of bronchi and bronchioles extending to the beginning of the alveolar duct, muscular rings or sphincters in the distal airways, and a double layer of capillaries in the alveolar wall (Fig. 1). On routine histologic examination, angiomatosis was readily apparent. The minimal detectable lesion was a cluster of small, discrete, round vessels containing red blood cells, always in relation to a larger vessel, and without associated inflammation or exudation. Because the vessels were convoluted, a single section displayed round, elliptical, and irregular vascular profiles. In mild cases, one or two vascular clusters, measuring about 0.17 mm × 0.15 mm, were present per 10× power field. The vessel walls tended to be thin, and the external diameters of new vessels ranged from 23 to 49 μm. In moderate cases, the number and size of vascular clusters increased, distorting the normal lung architecture. The vessel walls thickened owing to the appearance of smooth muscle and collagen. The external diameters of vessels remained about the same, and wall thickness often came to exceed lumen diameter. Eventually, the new vessels became solid and cordlike. In severe cases, the clusters were confluent

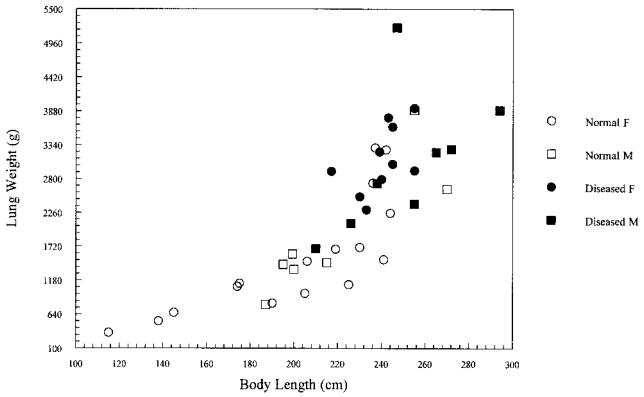

Fig. 5. Lung weight versus body length of dolphins with and without angiomatosis. Lung weight is the average weight of both lungs.

and measured up to 0.5 mm wide, and little to no normal lung parenchyma remained (Fig. 2). The clusters became increasingly collagenized so that vessels appeared as small collagenous or muscular islands in a fibrotic nodule. Airway diameters were reduced by mucosal thickening from the neovascularization (Fig. 3, 4). In 2 of 25 cases, hemangiomas were present in the lungs. In all cases, the neovascularization within the lung pleura and parenchyma was distinct from the normal rete mirabilis structures, which occurred under the dorsal parietal pleura and in the adjacent superior mediastinum.

The lung weights of dolphins with and without angiomatosis are shown in Fig. 5. Although lungs with angiomatosis tended to be heavier at a given body size, the weight, even in advanced cases, did not increase to an extent where weight alone could be used as a reliable diagnostic criterion.

The abnormal vessels appeared de novo; that is, there was no prior proliferation of muscle or fibroblasts, and there was no antecedent or concurrent inflammation or exudation. This suggests that the initial event was proliferation of endothelium, later followed by the appearance of thickening walls. The walls of new vessels were composed of smooth muscle and collagen but not elastin, as suggested by the staining reaction on HPS, Masson's trichrome, and Movat pentachrome stains, and supported by binding of antibody against smooth muscle actin. It has been shown previously that this antibody, prepared for use in humans, reacts with *T. truncatus* smooth muscle actin.[8] However, the amount of smooth muscle in association with lesions was no more than expected from the number of vessels. That is, the lesions were not vascularized smooth muscle.

Angiomatosis in lymph nodes

Lymph nodes occur at three sites in direct association with the lung of *T. truncatus:* at the hilus (hilar), at the junction of the free anterior margin of the lung with the base of the lung (marginal), and on the medial diaphragmatic surface (diaphragmatic) (D. F. Cowan, unpublished observations). Diffuse, nonnodular proliferative hypervascularity was present in lymph nodes of 19 of 22 (86.4%) dolphins with lung lesions (the lymph nodes of dolphin Nos. 2, 15, and 16 were not sampled). Table 3 shows the distribution of lymph node involvement. Lung-associated lymph nodes (hilar, marginal, and diaphragmatic) and mesenteric lymph nodes were most commonly affected, although cervical (prescapular), pancreatic, and pelvic lymph nodes were occasionally involved. In advanced cases, the normal nodal architecture was distorted (Fig. 6). Thick-walled hemangiomas were found in 9 of 19 cases (47.4%) with affected lymph nodes. One hemangioma in a pancreatic lymph node measured 6 × 7 × 5 cm.

Diffuse and localized lymph node enlargement was present in cases with and without angiomatosis. This enlargement was regarded as a reaction to general or regional infection. The majority of lymph nodes in animals in this study appeared reactive with mature lymphocytes; however, the lymph nodes of a few animals with angiomatosis showed marked depletion of lymphocytes, suggesting viral infection, although no cause has been demonstrated.

Discussion

Angiomatosis in dolphins is a newly recognized disease. An increase in incidence from 0 to 100% over the 6-year study period, combined with a definite trend toward more advanced lesions over the same time period, implies that angiomatosis is an active, progressive disease process. It is characterized by proliferation of small-caliber vessels in clusters in the lungs and by proliferative hypervascularity in the visceral pleura and lung-associated and other visceral lymph nodes. In the lung, the walls of new vessels thicken as smooth muscle and collagen appear. This thickening is at the expense of the lumenal diameter and tends to progress to occlusion. The combination of progressive, dense fibrosis of pleura, addition of dense blood vessels that thicken alveolar walls and interstitium, and reduction of lumens of peripheral airways must be reflected in impaired compliance, ventilation, and gas exchange. Hemangiomas, sometimes multiple and very large, form frequently in the lymph nodes and occasionally in the lungs. Although these hemangiomas are lesional, it seems unlikely that they would have a significant effect on morbidity or mortality.

A large majority of the dolphins with angiomatosis also had other lung conditions, such as amyloidosis, acute bacterial pneumonia, empyema, or verminous pneumonitis. However, the distribution, severity, and

Table 3. Distribution of lung and lymph node involvement associated with angiomatosis.

Dolphin No.	Lung	Hilar Node	Mesenteric Node	Marginal Node	Diaphragmatic Node	Prescapular Node	Pancreatic Node	Pelvic Node
Mild disease								
1	+	−	+	−	−	−	−	−
2	+	NS*	NS	NS	NS	NS	NS	NS
3	+	−	−	−	−	−	−	−
4	+	−	+	−	−	+†	−	−
5	+	−	−	+	+	−	−	−
6	+	+	−	−	−	−	−	−
Moderate disease								
7	+	+†	+	+	+	−	−	−
8	+	−	−	+	−	−	−	−
9	+	−	−	−	−	−	−	−
10	+†	−	+†	+	−	+	−	−
11	+	−	+	−	−	−	−	−
12	+	+	−	−	−	−	−	−
13	+	−	+†	−	−	+†	−	−
14	+	−	−	−	−	−	−	−
15	+	NS	NS	NS	NS	NS	NS	NS
16	+	NS	NS	NS	NS	NS	NS	NS
17	+	+	−	+	+	−	−	−
Severe disease								
18	+	+	+	+†	−	+	−	−
19	+	+	−	+	−	−	−	+
20	+†	+†	−	+†	+	−	+†	−
21	+	+	−	+†	+†	−	−	−
22	+	+†	+†	+†	+†	−	+†	−
23	+†	+	+	+	+†	−	+	−
24	+	−	−	+	+	+	−	+
25	+	+	+	+	+	+	+	+

* NS = not sampled.
† Hemangiomas present in the lung or lymph node.

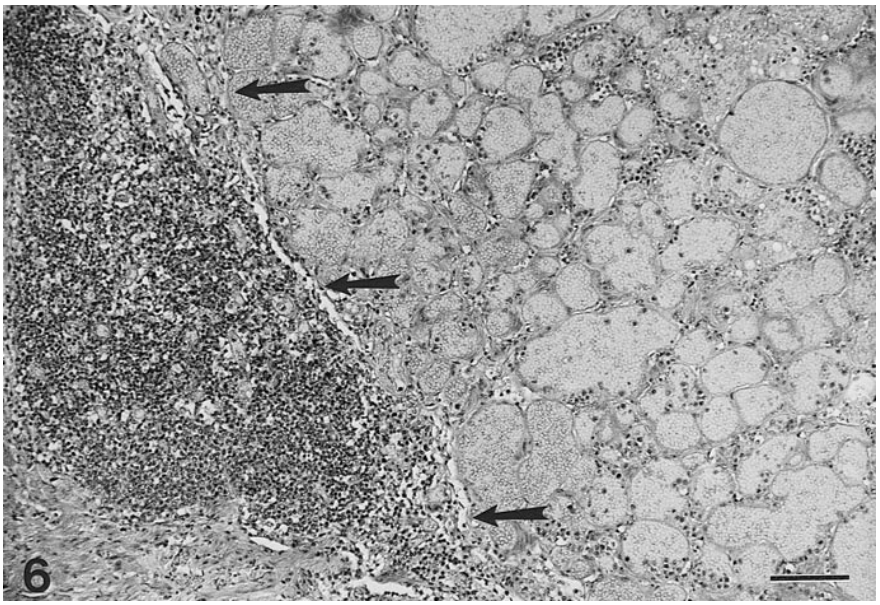

Fig. 6. Lymph node; dolphin with angiomatosis (No. 19). Hemangioma (outlined by arrows) in lymph node (N). HPS stain. Bar = 100 μm.

histologic characteristics of angiomatosis were the same in all cases; that is, the other conditions identified were coincidental with no apparent etiologic or pathogenetic relationship to angiomatosis. For example, empyema, which was found in five dolphins with angiomatosis, was associated with a heavy fibrinous exudation over both visceral and parietal pleurae with subsequent organization. This process was quite different from the symmetric bilateral visceral pleural thickening characteristic of angiomatosis. In addition, none of the lung conditions found in animals with and without angiomatosis could explain the presence of lymph node and lung hemangiomas, which were found only in animals with angiomatosis.

Dolphin angiomatosis can be distinguished from other superficially similar but rare pulmonary diseases in humans. Lymphangiomyomatosis is reported almost exclusively in women of reproductive age, with estrogen stimulation considered to be a contributing factor.[5,12] The main lesion of lymphangiomyomatosis, diffuse proliferation of smooth muscle along lymphatic pathways extending into small blood vessels, small air spaces, and bronchiole walls, is not a feature of dolphin angiomatosis. Another disease, lymphangiomatosis, is characterized by dilated lymphatic channels, usually filled with chyle.[5] In dolphin angiomatosis, the vessels are unambiguously blood, not lymph, channels. In contrast to these two diseases, dolphin angiomatosis occurs not only in females but in a large proportion of males with large testes and active spermatogenesis, which is indicative of physiologic hormone status.

Dolphin angiomatosis also differs from pulmonary angiomatosis in children and pulmonary capillary hemangiomatosis in adults. These two diseases are associated with intra-alveolar hemorrhage and hemosiderosis.[1,6,11] In adults, a secondary veno-occlusive disease results from capillary invasion of vascular walls and pulmonary interstitium.[6] Lymph nodes are not involved in either disease. In dolphin angiomatosis, alveolar hemorrhage and hemosiderosis is not present, whereas extensive lymph node involvement is typical.

A case of lymphangiomyomatosis was reported by Rawson et al.[10] in a male Atlantic bottlenose dolphin from the Gulf coast of Florida. Despite an age determination of 20 years, the dolphin had a low serum testosterone level, and the authors concluded that this may have contributed to the animal's disease. Although the testes appeared normal on gross examination, their weight and histologic appearance were not reported. The gross and histologic lung lesions described and illustrated are different from those of the angiomatosis reported here because they are described as nodular, not diffuse, and are composed primarily of proliferated, vascularized smooth muscle.

Dolphin angiomatosis is vaguely similar to Kaposi's sarcoma (KS) in that it appears to be a field defect of endothelium and can produce a variety of vascular patterns. A field defect is a genotypic alteration of an expanse of cells of a particular type, with multifocal phenotypic expression of lesions. Round, thick-walled vessels and cavernous hemangioma-like lesions may occur in visceral KS as in angiomatosis.[7] Here, the similarity ends. In KS, these features occur in the context of typical Kaposi lesions, which are absent in dolphin angiomatosis. KS also produces slit vessels, which are not seen in dolphin angiomatosis.

The cause of angiomatosis in *T. truncatus* has not been determined. However, because of the prevalence of this disease in sexually mature animals (with active spermatogenesis in the males), an association with estrogen excess is unlikely. The appearance of this disease in lungs, with involvement of lymph nodes and hemangiomas, suggests a broad field defect of vascular endothelium, possibly associated with viral infection, although none has been identified.

During the 6-year period of this study, we examined nine other species of cetaceans in the same study area: a melon-headed dolphin (*Peponocephalus electra*), a Fraser's dolphin (*Lagenodelphis hosei*), two striped dolphins (*Stenella coeruleoalba* and *S. attenuata*), a short-finned pilot whale (*Globicephala macrorhynchus*), a rough-toothed dolphin (*Steno bredanensis*), a pygmy sperm whale (*Kogia breviceps*), a dwarf sperm whale (*K. simus*), an infant sperm whale (*Physeter catodon*), and an Antillean beaked whale (*Mesoplodon europaeus*). None had evidence of angiomatosis. However, T. Lipscomb of the Armed Forces Institute of Pathology (personal communication) recently noted this disease in *T. truncatus* and *Delphinus delphis* stranded in other regions of the United States, indicating that angiomatosis is limited neither to the Gulf of Mexico nor to the Atlantic bottlenose dolphin. Further study of this morbidity factor in cetaceans is necessary.

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