Proceedings of the Third ECS Workshop on Cetacean Pathology

Lung Pathology

Held in Lisabon, 14 March 1996
Editor: Manuel García Hartmann

European Cetacean Society Newsletter no. 37, Special issue

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May 2001

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INTRODUCTION

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The Pathology Working Group of the European Cetacean Society was established in 1991 by a handful of enthusiastic pathologists within the society. The aim of this group was to learn more about the diseases and (patho)physiology of cetaceans. To all veterinarians working in the field of cetacean pathology at that time, it was painfully obvious how little we knew about the diseases of wild dolphins and whales in Europe.

Step by step, the Pathology Working Group has tried to encourage to increase our knowledge of cetacean pathology, by improving communication between pathologists in various European countries and by organising workshops on matters considered of special importance. The first workshop in 1991 established a common minimal dissection protocol for small cetaceans in Europe, which enabled pathologists of different countries to compare their datasets more reliably (Kuiken & García Hartmann, 1993).

The second workshop in 1994 focused on the subject of by-catch pathology, since all pathologists had encountered cases of stranded carcasses which showed some signs of potentially being bycatches. Yet it was difficult to clearly distinguish bycatches -which had been thrown overboard and subsequently drifted ashore- from other causes of death.
The findings of different pathologists recollected and discussed during the workshop led to the compilation of criteria which could be considered "pathognomonic" for such an elusive diagnosis as death by "prolonged submersion in water" in mammals which usually spend all their life in the sea (Kuiken, 1996).

The next subject of importance was the one of lung pathology. The respiratory tract of odontocetes and mysticetes displays a number of unique anatomical and microanatomical features when compared to other mammals. At the same time, this organ system is the one most often affected by disease in stranded cetaceans. The significance of the diseases found, however, often was unclear and remained a matter of speculation as, for example, in the case of heavy lung parasitism in the harbour porpoise. Other lung diseases were considered to be significant or even lethal; it has been reported that for instance bacterial pneumonia is the most common cause of death of dolphins in captivity (Howard *et al.*, 1984) and one of the most common causes of death in wild odontocetes (Baker & Martin, 1992; García Hartmann, 1997).
A more detailed review of current findings of lung diseases and lung pathophysiology seemed warranted; in 1996 a workshop on cetacean lung pathology was organised in Portugal to compile findings from different countries.

It was a special pleasure to have Dr. Daniel Cowan of the University of Texas Medical Branch opening the workshop with a review lecture on lung pathology in cetaceans. Subsequently, he gave an enlightening paper on the lung disease called angiomatosis, which he had discovered in bottlenose dolphins stranded on the coast of Texas (see article 3 below). I would like to use this occasion to express my sincere gratitude to Dr. Cowan for his great contributions to this workshop.

The present compilation of the papers presented in Portugal includes overviews of cetacean lung pathology found by several research groups from England and Wales, Germany, and the St. Lawrence Estuary in Canada; detailed contributions on the role of parasitism in cetaceans from Spain and The Netherlands, and several papers which deal with particular diseases and findings of special interest (like the above-mentioned angiomatosis, pneumothorax, nocardiosis and a previously unreported flagellate parasite), as well as the histopathology and lung ultrastructure of known bycatches.

This compilation of papers provides us with a unique overview of pathological conditions encountered in one organ system in stranded cetaceans and has given us new insights; yet it does leave many questions unanswered. Hopefully, these obvious gaps in our knowledge will serve as an inspiration to veterinarians to continue investigating the diseases of cetaceans in even more depth. Especially in the veterinary field, there still is a lot to be learned about these fascinating and always surprising creatures.

Manuel García Hartmann
Duisburg, October 1999

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LUNGWORM PARASITISM IN CETACEANS

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ABSTRACT

The only nematodes found in the respiratory system of cetaceans belong to the family Pseudaliidae. This paper reviews different aspects on the biology and pathology of these lungworms and discusses several methods to evaluate their impact on cetacean populations. Pseudaliids occur in odontocetes, particularly in the Delphinidae, Monodontidae and Phocoenidae. Their life cycles are unknown, but indirect evidence suggest that prenatal and/or transmammary infections occur, but heteroxenous (indirect) cycles seem more likely. The family Pseudaliidae is formed by seven genera but only four are common in the lungs of cetaceans: *Pseudalius, Torynurus, Halocercus* and *Skrjabinalius,* the species of each genus differing in their site and fixation mode in the lung. Lungworm pathology has been considered as a common cause of death in several species. However, it is difficult to establish whether lungworm infections have any significant impact on cetacean populations. Several methods to tackle this problem are proposed. Surveys based on lung slicing can provide quantitative information amenable to statistic analyses at the population level. Although such techniques should be used with caution because identical results can be explained by acquired immunity or sampling biases, they are useful exploratory tools which should be used more often.

THE CETACEAN LUNGWORMS

The only nematodes found in the lungs and air sinuses of cetaceans are nematodes of the family Pseudaliidae. This paper reviews different aspects on the biology and pathology of these lungworms and discusses several methods to evaluate their impact on cetacean populations.

The pseudaliids belong to the superfamily Metastrongyloidea, which is formed by several common lungworm families of mammals. Odontocetes are the only cetacean hosts of the Pseudaliidae, most records being from species of the families Delphinidae, Monodontidae and Phocoenidae. Currently, seven genera are recognised within this nematode family, but only four are common in the lungs of cetaceans: *Pseudalius, Torynurus, Halocercus* and *Skrjabinalius.* The species of each genus differ in their site and fixation mode in the lung. Worms of the genus *Torynurus* are usually found free in the main bronchi and bronchioles. *Pseudalius* species have a similar location but their head is commonly attached to peripheral parenchyma. Occasionally, *Torynurus* and particularly *Pseudalius* can also occur in the pulmonary blood vessels and heart. *Halocercus* and *Skrjabinalius* occur encapsulated in the lung parenchyma, outside the bronchial walls although the caudal end of the latter hangs freely in the air passages. Members of an additional genus, *Stenurus,* can occur in the main bronchi and trachea but their usual location is in the air sinuses (Delyamure, 1955).

The biology of the pseudaliids is poorly known. Different lines of evidence indicate that transplacental or transmammary infections might be an important feature in their transmission. First, these are fairly common ways of infection in mammal nematodes, and particularly among the Metastrongyloidea (Miller, 1981; Anderson, 1992). Second, a recent study showed that harbour porpoise (*Phocoena phocoena*) calves seem readily infected with lungworms (Balbuena *et al.,* 1994). And third, the pseudaliid *Halocercus lagenorhynchi* has been recorded infecting a newborn bottlenose dolphin (*Tursiops truncatus*) (Dailey *et al.*, 1991). Although as early as late 1940s, monoxenous (one-host) life cycles involving autoinfections and prenatal transmission were hypothesised (Wesenberg-Lund, 1947), heteroxenous (several-host) cycles seem more likely because this is the common way of transmission of the Metastrongyloidea (Anderson, 1971; 1982; 1992). Besides, field evidence indicated that the number of lungworm species infecting Norwegian harbour porpoises increases with host age, suggesting that ways of transmission other than prenatal or transmammary infections should exist (Balbuena *et al.*, 1994). According to Anderson (1982), the life cycles of the pseudaliidae may be similar to those of metastrongyloids of terrestrial carnivores involving intermediate and paratenic hosts.

PSEUDALIID PATHOLOGY

There is little doubt that lungworms can cause severe pulmonary diseases in cetaceans. Atlantic harbour porpoises can harbour up to four species of lungworms, there always being some associated pathological conditions (Geraci & St. Aubin, 1987). Pseudaliid infections can be related with granulomatous inflammation in the lungs. Woodard *et al.* (1969) described the pneumonic process as being characterised by exudation of neutrophilic and eosinophilic polymorphonuclear leucocytes and macrophages although the damage was generally limited to the area immediately surrounding the parasitised air passage. Additional problems may result from mechanical obstruction of the bronchi or bronchioles (Clausen & Andersen, 1988; Baker & Martin, 1992).

Verminous pneumonia can ultimately produce the host's death either directly, or indirectly by facilitating bacterial and cardiovascular complications, or hypersensitivity to the parasites (Geraci & St. Aubin, 1987). Stroud & Roffe (1979) reported pneumonia caused by *Halocercus invaginatus* as the primary cause of death of three subadult harbour porpoises stranded in the Oregon coast. Likewise, Baker & Martin (1992) considered parasitic bronchopneumonia as a common cause of mortality of harbour porpoises found dead in British waters.

IMPACT ON CETACEAN POPULATIONS

Whether parasites play a role in regulating wild populations is a key question for management and conservation. In spite of the above evidence, it is difficult to establish whether lungworm-induced mortality plays a significant role in the dynamics of cetacean populations. Many surveys are based on stranded animals, which are assumed to be diseased, and therefore may not be representative of the population, and quantitative information of the lungworm infections is often missing, hampering statistical analysis of the populations. Poor knowledge of the host and parasite biology and the impossibility of experimental work also account for this situation (see Raga *et al.*, 1997, for details).

To obtain quantitative data, methods based on lung slicing can be used. For instance, Onderka (1989) described a technique consisting in sectioning the lungs transversely to study the degree of lungworm pathology in ringed seals (*Phoca hispida*). The author used a grid to estimate the extent of non-respiratory parenchyma (bronchi, connective tissue and consolidating lesions caused by lungworms) related to that of healthy tissue. Then, the data can be related to host body condition or other parameters of interest.

Quantitative information on parasite infections affords the use of techniques to evaluate the incidence of parasite-induced host mortality at the population level (see Lester, 1984). For instance, a decrease in the variance to mean number of parasites ratio with host age might be indicative of mortality of the oldest individuals of the population (Figure 1).

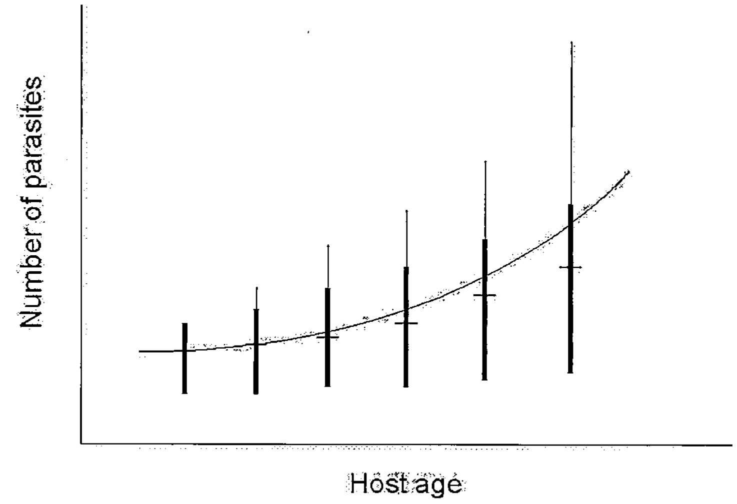

Figure 1: A decrease of the variance to mean abundance ratio with host age can be indicative of parasite-induced mortality. Vertical thick bars represent the variance of parasite abundance observed for each host age class; vertical thin lines, the hypothetical variance if no mortality occurs; horizontal bars, the observed mean parasite abundances; the curve connects the hypothetical means if no mortality occurs. Redrawn from LESTER (1984).

Or the comparison between the frequency distribution of the parasites over the entire host sample and that over the lightly infected hosts may reveal higher mortality among those heavily infected (Figure 2).

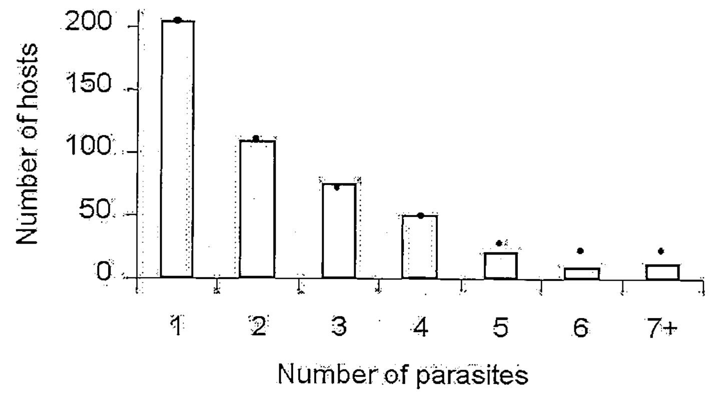

Figure 2: Method of the truncate negative distribution. The bars represent a hypothetical frequency distribution of parasites over a sample of hosts. The points are from the negative binomial distribution that best fits the first four bars of the histogram. The difference between the expected values (points) and those

observed in hosts harbouring ≥ 4 parasites (bars) might be accounted for by mortality of the heavily infected hosts. Redrawn from LESTER (1984).

However, statistical techniques should be used with caution because identical results can be caused, for example, by acquired immunity or higher parasite mortality in the oldest animals; besides random effects or sampling biases can be critically important when sample sizes are small, which is usually the case when working with marine mammals (Raga *et al.*, 1997). Therefore, these methods alone may not provide clear and unambiguous evidence of cetacean mortality induced by lungworm infections but, still, they are useful exploratory tools which can be used to substantiate additional data. Therefore, we encourage their use in further studies.

ACKNOWLEDGEMENTS

This work was supported by grants of the Spanish Government (DGES project PB96-0801 and ICI project) and a postdoctoral fellowship awarded to F.J.A. from the Ministry of Education and Science of Spain.

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ANGIOMATOSIS IN THE ATLANTIC BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*) IN THE WESTERN GULF OF MEXICO

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During the course of post-mortem examinations of cetaceans strandings along the beaches of the Gulf of Mexico, from western Louisiana to south Texas, we have observed an apparently new disease, wich has increased in incidence from 0 in 1991 to 100% of adult *Tursiops truncatus* in 1996. The cumulative incidence over the six year observation period is 46% (25 of 54 animals), with cases being distributed approximately equally between males (55%) and females (41%), and occuring in sub-adults (28% of cases) as well as adults. The disease was not observed in individuals of nine other cetacean species stranding in the same region over the same time period.

This disease, which we named "angiomatosis", may be characterised as a diffuse proliferation of small blood vessels in the lungs, associated in its more severe stages with the appearance of hemangiomas in lungs-associated lymph nodes, and occasionally in the lung (fig. 1 and 2). The earliest lesions of angiomatosis are small caliber, thin-walled vessels budding off the venules. These progress to form discrete clusters or nodules, which in severe cases, coalesce to involve the entire lung. The pleura becomes progressively thickened and opacified, and lesions in the mucosa of small airways thicken the mucosa to the point of airway occlusion and may erode bronchial cartilage. The process begins diffusely throughout both lungs, and lesions in all parts of the lungs are consistent in degree of severity. That is, "early" and "advanced" lesions are not found in the same lung. Because of this, extensive histological sampling is not necessary either to diagnose the condition, or to rate its severity. Pleural opacification is similarly diffuse and even, and when advanced, is a reliable indicator of the disease. Only the visceral, and not the parietal pleura is involved.

At no stage is angiomatosis associated with other than incidental inflammation, and it lacks the characteristic features of morbillivirus disease. Other disease associations (e.g., acute pneumonitis, parasitism) are inconstant, and apparently unrelated. Lesions in the lymph nodes are best characterized as discrete nodular venous, thick walled hemangiomas. They are relatively infrequent in cases in which lung lesions are mild, and become more common as the lung lesions become more severe. While most often limited to lymph nodes associated with the lungs, occasionally an abdominal node is involved.

We interpret this process as evidence of an acquired field defect of vascular endothelium, by which we mean a diffuse genotypic alteration in that tissue, with phenotypic expression of lesions. In this location, we are inclined to attribute the cause to a virus, although we have no direct evidence to support that hypothesis.

LUNG PATHOLOGY IN ST. LAWRENCE BELUGA WHALES

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ABSTRACT

The different lung pathologies encountered in necropsies of stranded beluga whales from the St. Lawrence estuary are reviewed. Parasites were most often associated with lung lesions. *Halocercus monoceris*, *Halocercus taurica* and *Stenurus arctomarinus* are the nematodes that were identified in lungs of St. Lawrence beluga whales. Minimum inflammation was associated with the adult parasites, while neutrophils and eosinophils were associated with larvae. A severe and extensive pneumonia with unidentified large ciliated protozoa was observed in a single whale. Opportunistic bacteria were cultured from lung lesions, often in association with dissiminated infections and/or with lung parasites. Viruses were not found in association with lung lesions in our study. Neonatal atelectasia was observed in the lungs of a neonate. Significant pneumonia, defined by the severity and extensiveness of the lesions in the lungs, was observed in 50% of the belugas examined. The clinical significance of the lesions observed is unknown, but it was felt that the histological examination of several sections of lungs greatly helped to evaluate the importance of these lesions.

INTRODUCTION

A small population of beluga whales (*Delphinapterus leucas*) resides in the St. Lawrence estuary. From 5,000 animals at the beginning of the century (Reeves & Mitchell, 1984), the population has been reduced to approximately 500 (Michaud, 1990) and has been listed as an endangered population (Pippard, 1985). After the decline initiated in the early 20th century by overhunting, several hypotheses have been put forward to account for the failure of this population to recover during the last 40 years.

High concentrations of organochlorines, as well as benzo-a-pyren (BaP) exposure, have been demonstrated in the tissues of these animals (Martineau *et al.*, 1987; Martineau *et al.*, 1988); the concentrations of polychlorinated biphenyls (PCBs), DDT, Mirex, mercury and lead were much higher than those found in Arctic belugas (Muir *et al.*, 1990; Wagemann *et al.*, 1990).

Postmortem examination of carcasses retrieved from the shores of the St. Lawrence since 1982 has shown a high prevalence of degenerative, infectious, hyperplastic, or necrotic lesions often associated with mildly pathogenic organisms, in addition to a very high prevalence of neoplasms (Martineau *et al.*, 1990; De Guise, Lagacé & Béland, 1994; De Guise *et al.*, 1995). The frequency and severity of the lesions described in this population were considerably higher than what has been found in marine mammals elsewhere. Consequently, a link was suggested between toxic contaminants in the St. Lawrence basin food web and the precarious state of the population. The present paper describes the lung lesions encountered during our studies, and discusses their clinical significance.

RESULTS AND DISCUSSION

As a prerequisite for the study of pathology of lung lesions in cetaceans, it is interesting to study the anatomy of the respiratory system to better understand the pathophysiological processes. It is generally agreed that there is no communication between the upper respiratory system and the oral cavity, the larynx being inserted into an opening in the palate and opening through the blow hole. Such anatomical features would prevent accidental pneumonia through aspiration of food into the airways under normal circumstances. However, such a problem was observed in a newborn orphan beluga calf that had been found stranded alive along the shores of the St. Lawrence which we were trying to rehabilitate. The animal died immediately after an episode of regurgitations a few minutes after a meal of artificial milk formula. Necropsy showed milk into the lungs in addition to a defect (probably congenital) of the insertion of the larynx into the palate. The opening of the palate was approximately twice as big as the diameter of the larynx, allowing the latter to move easily in and out of its insertion into the palate.

In belugas as in other cetaceans, each lung consists of a single lobe, which is fairly uniform and relatively firm compared to most land mammals. The firmness of the lungs is due to the relatively large amount of cartilage throughout the bronchi and bronchioli and to the thick and highly collageneous interalveolar septae. Those characteristics are probably derived from the physiological requirement of deep diving and the need to exhale and then inhale within a relatively short period of time. Therefore, the firmness of the lungs in belugas do not allow the easy detection of foci of pneumonia by palpation as in most land mammals. In order to accurately assess the presence or absence of lesions, each beluga lung was sampled as follows for histopathology: a superficial, intermediate, and a deep sample in each of the cranial, middle and caudal third of each lung, for a total of 18 lung sections per animal. Sections of the mediastinal lymph nodes, which seem to react like the lungs do in belugas, were also examined. Histologically, the thick and highly collageneous interalveolar septae described above are covered by a capillary on each side (rather than one capillary in the middle of interalveolar septae as in land mammals), and very thin epithelial cells (pneumocytes). While this thin air-blood barrier probably allows for rapid and efficient gas exchange, it also provides abundant blood supply to the lungs and may facilitate the inflammatory response to pathogens.

The lung lesions most often observed in belugas stranded on the shores of the St. Lawrence estuary were bronchopneumonia, most often of parasitic origin, but often complicated with bacterial infections. The gross lesions sometimes observed in animals with pneumonia were reddish to dark red areas seen from the the surface of the lungs and on cut sections, as well as whitish, reddish and greenish areas that were often seen around bronchi. Small nematodes were occasionally seen in small bronchi and large bronchioli on cut sections. The detection and characterization of the lesions was best achieved through histopathology. The most common histopathological lesions were larvae and degenerated adult nematodes surrounded by leukocytes (neutrophils and eosinophils) into the bronchial or alveolar walls and lumina, and lymphocytes infiltrated the adjacent interstitial tissues, while intact adult parasites caused little or no inflammatory response. Cuticle debris elicited the formation of granulomas, sometimes with giant cells, and often with calcified centers. Accumulations of leukocytes into the bronchial or alveolar walls and lumina were also found without parasites, and probably represent primary bacterial pneumonia. Lesions were observed with no particular or specific distribution in the lungs, and were relatively randomly distributed. *Halocercus monoceris, Halocercus taurica* and *Stenurus arctomarinus* were the nematodes commonly recovered from the lungs of St. Lawrence beluga whales (Measures *et al.*, 1995). One animal was also observed with a severe pneumonia associated with an unidentified ciliated protozoan (De Guise *et al.*, 1995). The bacteriae isolated from lungs of St. Lawrence belugas were: *Escherichia coli, Pseudomonas putrefaciens, Vibrio parahaemolyticus, Edwardsiella tarda, Aeromonas hydrophila, Klebsiella sp., Clostridium tertium, and Kingella kingae* (De Guise *et al.*, 1995). All of these bacteria are opportunistic and none has demonstrated a specific pathogenicity for beluga lung. In fact, it was not unusual to also culture those bacteria in several tissues and organs other than the lungs. Virus and fungus were not cultured from lungs of belugas from the St. Lawrence.

Other lesions observed in St. Lawrence beluga whales respiratory system included an aspiration pneumonia in a newborn animal (discussed above), neonatal atelectasis in another newborn animal (less than 10% of the lungs had been inflated in an animal that had been in captivity for 10 days after being rescued as a newborn), and diffuse alveolar damage in one adult whale (thick hyaline membrane as well as a moderate amount of filamentous fibrin were found in alveoli), a condition usually associated with acute respiratory distress syndrome in humans (De Guise *et al.*, 1995).

It is difficult to assess the importance of lung lesions observed in belugas from the St. Lawrence estuary. Although significant lesions were observed in approximately 50% of the animals examined, the severity and extensiveness of those lesions varied considerably. The pathophysiological consequensces of the lesions observed grossly and microscopically are very hard to assess because those animals could not be clinically examined or even remotely observed before their death, but were found dead. In addition, the proportion of lung integrity necessary to perform vital functions (such as feeding) in a deep diving animal is unknown.

In conclusion, significant lung pathologies are frequently observed in beluga whales from the St. Lawrence estuary. Our studies demonstrated the importance of proper sampling and histopathological examination for accurate detection and characterization of the lesions. Although the clinical significance of the lesions observed is still difficult to assess, the information gathered up to date justifies lung lesions to be further investigated in these animals.

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NOCARDIOSIS IN A STRIPED DOLPHIN (*STENELLA COERULEOALBA*)

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INTRODUCTION

A case of a striped dolphin (*Stenella coeruleoalba*) with a disseminated infection by the bacterium *Nocardia asteroides* is presented. The dolphin was found dead at the beach of Begur (Girona, Spain) in January of 1996. It was a female, non-pregnant, weighing 70 kg, and of 188 cm length. The dolphin was in good body condition, as judged by the blubber thickness (14 mm, measured behind the lateral flipper). The condition of the carcass for necropsy was moderate fresh. Necropsy was done immediately after finding.

MACROSCOPIC FINDINGS

Macroscopic lesions attributable to *Nocardia* are summarised in table 1. No external skin parasites were found. However, over 25 crustacean organisms were found attached to the surface of the tongue. It could not be determined exactly, even by histopathological study of the tongue, if they were attached to the dolphin when it was alive or after death. Infestation of the subcutaneous tissue at the anogenital area by *Phyllobotrium delphini* -a common finding- was present. A fibrinous pleuritis was found in the thoracic cavity, with approximately 750 ml of blood tinged exudate and many fibrin floccules in suspension and adhered to the pleural surface. The lung showed multifocal areas of consolidation, with a dark red colour at the cut surface (Fig. 3), distributed mainly in the cranial portions of the lung. Trachea and bronchia had a fibrinous exudation covering their mucosa, and were filled by a hemorrhagic fluid (Fig. 4). The mediastinic lymph nodes were enlarged, with many necrotic areas as large as 10 mm of diameter (Fig. 5). Other lymphoid organs, like mesenteric and preescapular lymph nodes, laryngeal tonsil, and spleen, as well as liver, kidney, adrenal, heart, and pancreas, showed several white foci, of 1 mm of diameter. The meningeal vessels showed small whitish areas up to 1 mm in diameter.

The keratinized and the secretory stomach chambers were empty, and the pyloric chamber contained bile-stained liquid. Some crustacean like those found in the tongue, were found free in this chamber. Trematoda were found in the pancreatic duct.

Table 1: Macroscopic lesions in a case of Nocardiosis
Fibrino-haemorraghic pleuritis Pyogranulomatous multifocal pneumonia Fibrino-haemorrhagic tracheitis and bronquiitis Pyogranulomatous multifocal lymphadenitis

MICROSCOPIC FINDINGS

The lung showed a pyogranulomatous necrotizing pneumonia, distributed in part as foci around blood vessels, but also in an acinar distribution with bronchiolar dependence (Fig. 6). Some vessels showed thrombosis. With the Gram stain many bacillary forms growing in ramified colonies were observed. These bacteria were also seen inside thrombotic vessels (Fig. 7).

Microscopic appearance of lesions in many other organs, including the brain, suggested an haematogenous spread of the infection, with thrombi in vessels, and outgrowth of bacterial branches in the affected tissue. However, in some organs, as heart and adrenal, foci of ischemic necrosis due to septic thrombi were more prominent.

DIAGNOSIS AND COMMENTS

An actinomycetal bacteria, classified by biochemical tests in the genus *Nocardia* was isolated from the lung. This bacteria was unequivocally classified by DNA sequencing as *Nocardia asteroides*. The severe pneumonia and pleuritis due to this bacteria, with haematogenous dissemination to other organs, including the brain, was considered to be the cause of the death of this dolphin.

The portal of entry of the infection was probably the aerogenous route, based on the magnitude of the lesions in the lung and mediastinal lymph nodes. Although the lesions were intense in the respiratory system, dissemination to the nervous tissue and heart, with a multifocal embolic encephalitis and multiple myocardial microinfarcts was probably critical for the fatal outcome of the disease. The fibrino-haemorrhagic tracheitis was an obstructive process of the airways, and might have hampered respiration.

Pathogenic species of the genus *Nocardia* (*N.asteroides, N.braziliensis, N.caviae*) are known to be saprophytic bacteria, found in the normal soil flora. Studies of marine sediment suggest that Actinomycetal bacteria (which include the genus *Nocardia*), are found normally in marine ecosystems (Weland, 1969, Nature 223:858, cited by Pier *et al.*, 1970).

Nocardia sp. can cause disease in animals and humans as primary agents or as secondary, opportunistic invaders in debilitated organisms (Pier & Fitchner, 1985). Main forms of Nocardiosis in mammals are:

(1) respiratory, pleural, or peritoneal infections,
(2) subcutaneous granulomata, and
(3) mastitis in cattle.

Nocardia infections have been described frequently in cetaceans (Pier *et al.*, 1970; Jasmin *et al.*, 1972; Sweeney *et al.*, 1976; MacNeill *et al.*, 1980), causing mainly respiratory disease, and sometimes also pleuritis.

Factors that favour pulmonary *Nocardia* infections in marine mammals have not been clearly identified, but debilitation and immunsupression have been proposed (Dailey, 1985). No special cause of debilitation was observed in this dolphin.

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TENSIONAL PNEUMOTHORAX IN SMALL CETACEANS

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INTRODUCTION

Cetaceans dive in apnoea, and most species are able to dive well under 100 m deep, equivalent to ≥11 bar atmospheric pressure. Adaptation to high pressures during diving include a highly collapsible chest cavity, reinforcement of the bronchial and bronchiolar walls by cartilage, and well developed muscle sphincters along the small airways (Britt & Howard, 1983).

Pulmonary barotrauma arising from sudden elevation of air pressure in the lung has been described in sport scuba divers, associated to emergency ascends without expelling previously inhaled compressed air. Clinical manifestations of pulmonary barotrauma may include mediastinal emphysema, pneumothorax, pulmonary bullous emphysema or life-threatening gas embolism (Raymond, 1995). Apnoeic divers are usually protected from barotrauma, as the amount of air contained in the lung and air passages does not increase during diving.

Pneumothorax has been reported in small cetaceans incidentally, but has not been described in detail. We describe five cases of tensional pneumothorax in two Mediterranean striped dolphins (*Stenella coeruleoalba*), and three harbour porpoises (*Phocoena phocoena*) from the southern North Sea, as well as one case of solitary pulmonary bulla in another Mediterranean striped dolphin, and postulate that these cases originate from pulmonary barotrauma.

CASE HISTORY

In case 1 (code 960111), a male striped dolphin of 199 cm length, found dead at Viladecans (Barcelona, Spain), a pneumothorax of the left hemithorax was discovered at necropsy while opening the pleural cavity (done by the left side), due to the outflow of large amounts of foul-smelling air from the inside. The left lung was collapsed, of minor size than the right one, and a section was unable to float in formalin. In its dorsal aspect, about 2 cm caudal from the tracheal bifurcation, there was a bulla of about 3 cm in diameter, with a large fibrin clot near the bulla (Fig. 8). The inner surface of the bulla was of grey colour, due to compactation of connective tissue. A rough area was present on the pleural surface at the base of the bulla, which was interpreted as a possible point of perforation. Small thin lung nematodes, probably *Skrjabinalius guevarai*, were observed, in low numbers, at the cut surface of both lungs. One nematode was located at approximate 1 cm of the pleural perforation.

The pleural cavity of the left hemithorax showed a granulomatous pleuritis, with many white-yellow nodules from 2 to 5 mm in diameter, firmly adhered to both the parietal and visceral pleural surfaces, and approximately 100 ml of a sero-haemorrhagic exudate.

Histopathologic examination of the inner wall of the bulla at the rough area showed a perforation that was covered by a fibrin layer (Fig. 9). A low grade mixed inflammatory infiltration was detected in the lung parenchyma at the margins of the bulla, *but no specific*

microscopic lesion could be associated with the fibrin layer covering the perforation. The pleural surface of the left hemithorax showed a chronic granulomatous mycotic pleuritis, with fungal hyphae in the centre of the granulomata, and a pyogranulomatous inflammatory infiltration (Fig. 10), with some giant cells. No signs of mycotic pneumonia could be found. The latero-ventral pulmonary lymph node of the left side had a single parasitic granuloma of about 2 mm in diameter, at the subcapsular zone. An apparently adult parasite, probably a nematode, was in the centre of the granuloma. Other microscopic lesions were a mild haemosiderosis in the liver Kupffer cells, signs of cerebral perivascular and submeningeal oedema, and a granuloma in response to larvae of parasites in the tracheal tonsil.
The microbiologic study of a swab from the thoracic cavity yielded *Aspergillus* sp., *Penicillium* sp., *Aeromonas hydrophila* and *Pseudomonas* sp.
Other findings were infestation of the anogenital subcutaneous tissue by *Phyllobothrium delfini*, of the pancreatic ducts by trematodae, and presence of one *Xenobalanus* sp. attached to the skin.

Case 2 (code 960122) was a male striped dolphin, of 209 cm length, found dead in Pineda de Mar (Barcelona, Spain). It had pneumothorax on the right side. While opening the pleural cavity (done by the left side), displacement of the mediastinic wall to the left side of the thorax, with severe compression of the left lung against the chest wall, was observed (Fig. 11). At the opening of the right hemithorax, outflow of air from the inside was noted. The right lung was compressed, and sunk in formalin. A pleural fissure of about 2 mm was found at the pulmonary hilum behind the bronchial lymph node. Small subpleural bullae were seen near the fissure. A mild purulent pleuritis, with dry yellowish exudate over the dorsal mediastinum was present. Other alterations were infestation of the anogenital subcutaneous tissue by *Phyllobothrium delfini*.
Histopathologic examination of the lung revealed a mild, focal inflammatory infiltration in alveolar lumen, in the areas near the pleural fissures. No other significant lesions were observed. The cause of the pleural fissure could not be determined.

Case 3 was an adult male harbour porpoise (code PP90-THEO) from the North Sea, of 150 cm length and 47 kg weight, found dead on the beach in Renesse, province of Zeeland, The Netherlands, on 9 March 1990. The carcass did not undergo a complete pathological dissection, but was used for an anatomical research project in 1990 which studied the relation between anatomy and ultrasound examination (Manuel García Hartmann, Ursula Siebert & Wim Kersten, unpublished data).
The animal was examined by ultrasound with a 5,0 kHz probe, frozen, and later sawn with a band saw transversally into 1 cm sections in the head region and into 3 cm sections for the rest of its body. For this purpose, parallel lines had been drawn on the carcass to provide correct anatomical relationships. To prevent thawing, the sections and the remaining intact carcass were replaced into the freezer periodically. Each sections was numbered, cleaned dry and photographed from both sides.
During ultrasound examination, the findings in the thorax were difficult to interpret due to postmortem artifacts, lack of experience with this species and the evident but unexpected changes in location of the thoracic organs. A diagnosis could not be made at that point. Only after cross-sectioning the whole animal's thorax it became evident that this male had suffered from a pneumothorax of the right hemithorax. In the thorax, the heart was deviated to the right side. The left lung was significantly smaller than the right one (Fig. 12).
Extending the diaphragm into the abdomen, the right hemithorax extended caudally into the abdomen, deviating the cranial visceral organs e.g., the liver and the stomach compartments and their associated organs, latero-caudally to the left (Fig. 13).

The trachea, bronchi and bronchioli contained nematodes of the species *Pseudalius inflexus* and *Torinurus convolutus*. The pulmonary veins contained several specimen of *Pseudalius inflexus*. The degree of parasitism of the lung and lung vessels was considered high. No origin of the pneumothorax could be established.

Other findings of pathological interest included skin ulcers and severe infestation of wounds with *Isocyamus delphinii*, severe liver infestation and pericholangitis caused by *Campulla oblonga*, fatty liver, an acute arthritis of the humero-scapular joint and fracture of the lower jaw, the latter being considered to be post mortem in origin.

No histopathological investigation of the tissues was carried out, and the bacteriological findings were unrewarding.

Case 4 was a juvenile male harbour porpoise (code PP970704-2), which stranded on the Dutch coast in 1997 and was brought to the National Museum of Natural History in Leiden, The Netherlands, without further details about its origins or exact date of stranding. The porpoise was 123 cm long, weighted 27,5 kg and was reasonably fresh (condition code 2-3). On external examination, the animal seemed tympanic, more pronouncedly so in the left abdomen. On opening the carcass from the left side according to our routine procedure following the ECS "Standard protocol for the basic postmortem examination and tissue sampling of small cetaceans" (Kuiken & García Hartmann, 1993), it became obvious that the tympany was caused by the caudally protruding diaphragm. On incising the diaphragm air escaped and a small amount (some 30 ml) of sero-haemorrhagic fluid was found; the heart, right lung and mediastinum were displaced to the right, with the shape of the dorsal right lung visibly protruding dorsal of the thoracic aorta under the rete mirabile thoracicum. On the cranial side of the left diaphragm, a nematode parasite was found laying loosely. The left lung was approximately half the size of the right one and of a denser consistency; a tissue sample of this lung sank in formalin. On its surface, several nematode parasites were clearly visible through the thin serosa of the lung -both on the lateral as well as the medial side of the lung-, most often assuming an "U"-shape with their body, and on at least three locations protruding over the lung surface under the tensely stretched serosa. One parasite next to the hilus of the main bronchus had just perforated the serosa and was extracted without resistance nor damage to the serosa. A heavy parasitic load was also found in the upper bronchial tree and in the pulmonary veins of the left lung, while the right lung showed fewer subserosal parasites, and no parasites at all in its blood vessels.

All nematodes mentioned above were identified as *Pseudalius inflexus*; the only other lung parasites found were few encapsulated *Halocercus* sp.. The right lung was not collapsed, but oedematous and emphysematous, and in most areas severely congested. It showed focal subserosal haemorrhages and some abscesses of ≤ 5 mm diameter containing the firmly attached cranial parts of *Pseudalius inflexus*. Overall, the porpoise was well nourished, as documented by a thick blubber and a subcutaneous fat layer beneath the blubber in the dorsal neck. Other findings included a light ascites, presence of a male external "pseudo-uterus", focal fat necrosis, large thymus cysts, liver infestation with *Campulla oblonga* associated with marked pericholangitis, and heavy infestation of the periotic spaces with *Stenurus minor*.

Case 5 was a juvenile male harbour porpoise (code PP910204), stranded dead at Kalansoog, The Netherlands on 28 December 1990, being 107 cm long and 24 kg in weight. The animal was in a moderate state of decomposition (condition code 3) and remained frozen until the dissection. The subjective impression from the exterior was of a well nourished and slightly "bloated" animal. While the carcass was opened from its left side, a pneumothorax of the right hemithorax was detected: the diaphragm severely protruded caudally on the right side while the left side was in expiratory position; large amounts of air escaped upon incision of the diaphragm of the right thorax. The right lung was significantly smaller than the left one; on its surface, miliar caseous abscesses ≤ 8 mm protruded and contained parts of *Pseudalius inflexus* which were rolled up; furthermore, the bronchi and bronchioli contained *Pseudalius inflexus, Torinurus convolutus* and *Halocercus sp.* in large amounts; miliar abscesses caused by *Halocercus sp.* were equally seen. Several specimen of *Pseudalius inflexus* were found in the pulmonary blood vessels of the right lung but not in the left one. Histology of both lungs revealed a severe verminous pneumonia with larvae and adult forms, as well as alveolar oedema. The cause of the pneumothorax could not be identified.

The left lung was oedematous, severely congested and highly infested with the same parasites as the right one, to the point that the sheer number of parasites seemed to occlude all main bronchioli completely.

Further findings in this porpoise included skin ulcers. The finding of a good nutritive state (thick blubber and subcutaneous fat beneath the blubber in the dorsal and lateral neck) and of food remains in the stomachs suggest that the pathological process(es) which led to the death of this animal were not long-standing.

Case 6 (code 960421) was a female, non pregnant striped dolphin of 202 cm length, found alive, but which died before rescue could be attempted. Necropsy was done less than 24 hr after dead. Macroscopic examination revealed a prominent bulla in the caudal third of the left lung. The bulla was fixed in formalin; it had approximately 4 cm of diameter and contained no liquid (Fig. 14). Its wall was of grey colour. A small hard nodule of approximately 1 mm of diameter, of yellow colour was attached to the internal surface of the bulla.

Other findings were empty gastric compartments, with multiple ulcerations of the mucosa of the keratinized compartment, and a erosive-ulcerative glossitis.

DISCUSSION

Pneumothorax is defined as the presence of air or gas in the pleural cavities. In domestic mammals, pneumothorax can be spontaneous or traumatic in origin (Dungworth, 1993). In humans, pulmonary barotrauma (PBT) causing pneumothorax is a frequent accident associated to scuba diving activities (Richardson, 1997).

It is thought that diving mammals like odontocetes are resistant to expansive PBT because they hold their breath when diving, and therefore the same or even less amount of gas fills the lungs when the animal returns back to the surface. The collapse of the dolphin lung under increased pressure of depth is well documented (Ridgway, 1972) and the rete mirabile -mainly the thoracic one- are assumed to function as buffers: under the pressure of depth, they are believed to distend and occupy the thoracic space created by the created lung volume (Simpson & Gardner, 1972).

However, and despite their physiology being adapted to diving, pneumothorax has been already described incidentally in cetaceans: Greenwood & Taylor (1977) observed a tension pneumothorax in one dolphin, attributed to a severe lung nematode infestation, but do not give more details about the lesion. Andersen (1982) mentions a pneumothorax in a harbour porpoise (*Phocoena phocoena*), which died during transport to an experimental pool, without giving more details.

Additionally, Jepson *et al.* (this volume) describe one case out of 133 harbour porpoises from British waters examined from 1990 to 1996.

We present five cases of tensional pneumothorax, and one case of an uncomplicated pulmonary bulla. Tensional pneumothorax is defined as accumulation of air in the pleural cavity at a higher pressure than the atmospheric pressure, then compressing the lungs.

In the striped dolphins no. 1 and 2, escape of air to the pleural cavity occurred throughout a fissure in the pleural serosa.

In the harbour porpoise no.3 , the freezing of the carcass and the anatomical preparation by transversal slicing precluded the detection of a possible communication between the lung and the pleural space.

In the harbour porpoise no. 4, several nematodes *Pseudalius inflexus* were located immediately under the pulmonary pleura and in one case perforating the pleura. This is the only time that this parasite has been found being located immediately under the pleura in this way; equally, this is the only case of a harbour porpoise where a nematode was found lying freely in the thoracic cavity. In all other harbour porpoises from the Dutch coast dissected in the time period from 1990 to 1996, the characteristic presentation of *Pseudalius* infestation were small thick-walled abscesses located several millimetres or even centimetres under the serosa and typically containing the curled-up and strongly attached cranial part of the nematode.

In case no. 5, no cause of the pneumothorax could be identified although, similar to the findings of Greenwood & Taylor (1977) and Anderson (1982), the lungs of this animal and of all other harbour porpoises described in this paper were heavily parasitised.

It is interesting to take special notice of the asymmetry of the infestation: no *Pseudalius inflexus* were found in the blood vessels of the contralateral, non-collapsed lung of the harbour porpoises no. 4 and 5. Moreover, both animals were well nourished, indicating a rather quick (peracute to acute) development of the condition.

The female striped dolphin (case no. 6) represents a case of uncomplicated, stable, parenchymatous pulmonary bulla.

The pathogenesis of tensional pneumothorax in cetaceans is unknown. During apnoeic diving of cetaceans, the amount of air in the respiratory spaces does not change. The volume of air in the airspaces is reduced as the pressure increases with depth (law of Boyle-Mariotte), and at the same time, the pulmonary volume is reduced, leading to the collapse of the chest (Ridgway, 1972). A rupture of the pleura under pressure e.g., entry of air into the pleural space during diving, with the physiological increase of air volume when rising to the surface to atmospheric pressure level, is suggested as the mechanism of formation of tensional pneumothorax.

The following may occur:

(1) at first, pleural fissures or perforations in the lung pleura may form, allowing the escape of air from the lung and airways into the pleural cavity. The role of lung parasites in the genesis of this lesion is unknown, but lesions by nematode parasites may be implicated.

(2) air must be introduced into the pleural cavity -through the above mentioned fissures or perforations- to create a tensional pneumothorax at water surface level. To achieve this, it is most likely that air may be introduced in the pleural cavity during diving because at some point during diving, e.g. descent or ascent, air pressure in the lungs must be greater than in the pleural cavity.

(3) hypothetically, the perforation or fissure of the lung pleura may act as a valve, only allowing air to move in one direction, e.g. into the pleural cavity. If such is the case, those pleural lesions without this "valve" character may not lead to pneumothorax.

The role of the bullae encountered in two of the dolphins is unknown. In a study of pneumothorax in professional divers the presence of lung bullae was a constant feature (Calder, 1985). In the two cases described here the bullae may have been caused by barotrauma, as can be found in humans as well (Richardson, 1997). In PBT in humans, such bullae may play a role in the development of tensional pneumothorax or be an independant lesion.

The clinico-pathological consequences of a tension pneumothorax for the dolphin or porpoise are unknown. Dyspnoea (by compression), thoracic pain, circulation impairment by abnormal venous return and pressure on the heart; abnormal buoyancy, and impaired floating or diving skills may be anticipated. In one case reported in a captive bottlenose dolphin (*Tursiops gili*), a pneumothorax of unknown origin led to an excessive buoyancy which made it difficult for the animal to dive; the clinician in charge felt that her breathing was compromised. The pneumothorax was confirmed by radiology (Dr. Jim McBain, Sea World USA, personal communication, 1996). Without human assistance, dolphins and porpoises with pneumothorax presumably are bound to eventually drown, die of exhaustion from trying to keep an upright position for breathing, or from emanciation due to their inability to capture sufficient prey.

ACKNOWLEDGEMENTS

We would like to thank Dr. Jim McBain for generously sharing his experience and for providing the details of the clinical case of a bottlenose dolphin with pneumothorax.

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THE PULMONARY PATHOLOGY OF HARBOUR PORPOISES STRANDED BETWEEN 1990 AND 1996 FROM ENGLAND AND WALES

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INTRODUCTION

In 1990 a research programme was initiated to investigate the diseases and causes of mortality of marine mammals stranded in England and Wales. Selected pathological findings and causes of death of subsets of this dataset have been published previously (Baker & Martin 1992; Kennedy *et al.* 1992; Kuiken *et al.* 1993, 1994a; Kuiken & Law 1994; Kirkwood *et al.* 1997). The aim of this report is to review the pathological findings from the lungs of harbour porpoises stranded between October 1990 and December 1995 and for which detailed pathological investigations were conducted.

MATERIALS AND METHODS

Between September 1990 and December 1995, 133 harbour porpoise carcasses, ranging from freshly dead to moderately decomposed condition, were examined in detail at necropsy using standardised protocols (Kuiken & Baker 1991, 1993). Samples were taken, depending on carcass condition, for microbiology, histology, parasitology, and other studies. Parasites were collected and preserved in 70% alcohol for identification.

Multiple samples of lungs tissue were preserved in 10% neutral buffered formalin and cut into 2-4μm paraffin wax sections and stained with haematoxylin and eosin (H&E) for histological examination. Age category (neonate, juvenile, adult) was estimated from body length and gonadal appearance (Lockyer 1995).

RESULTS

Pulmonary parasitic disease

The pseudaliid nematodes *Pseudalius inflexus* and *Torynurus convolutus* were commonly found within harbour porpoise lungs. Marked variation existed in the apparent abundance of these two nematode species across individuals. *Pseudalius inflexus* were found within bronchi and/or pulmonary blood vessels and many individuals were parasitised at both sites. The heads of *P. inflexus* were typically embedded in the bronchial mucosa whereas *T.convolutus*, where present, were tangled freely within bronchial lumina. Almost all animals estimated to be over 1 year old were infected by one or both of these nematode species. The youngest animals with gross nematode infections were estimated from body length and date of stranding to be 7-10 months old, although 3 neonatal harbour porpoise had histological evidence of pulmonary nematode infection. *Stenurus minor, Halocercus invaginatus* and *Halocercus taurica* were very rarely recovered from the bronchi or pulmonary parenchyma.

Gross pathology

The nature and severity of the pathological changes associated with pulmonary nematode infections varied widely across individuals. Pulmonary congestion and serous (often frothy) fluid within the trachea and bronchi were common to many parasitised and non-parasitised animals. Extensive focal pulmonary haemorrhage was rare and was considered actually fatal in 1 individual. Multiple firm pulmonary nodules, typically 1-3 mm in diameter (sometimes larger), were frequently distributed throughout the lungs. Foci of parenchymal consolidation and diffuse or localised emphysema were also seen. Emphysematous bullae were rare.

Intravascular *P. inflexus* were distributed within the pulmonary blood vessels (mainly pulmonary arteries) of many individuals and were normally unattached to the vascular endothelium. In heavy infestations they sometimes appeared to at least partially occlude the parasitised vessel. There was little host reaction to these intravascular nematodes in most cases, although occlusive and sometimes necrotic thrombi (infarcts?) and aneurysms were recorded. Despite some quite heavy intravascular parasitoses, cor pulmonale was never seen.

Bronchial and pulmonary associated lymph nodes were often grossly enlarged in animals with *extensive* parasitic and other pulmonary lesions.

Histopathology

Changes associated with bronchial nematode infections included chronic lymphocytic/eosinophilic bronchitis, hyperplasia or squamous metaplasia of the bronchial or bronchiolar epithelium, mucus gland hyperplasia and mild to severe submucosal fibrosis. Microfoci of mucosal necrosis and calcification in small bronchi and bronchioles were probably associated with mucosal attachment of *P.inflexus.*

Changes involving the alveolar parenchyma were found in almost all pulmonary nematode infections. The most common findings were subacute-chronic, multifocal granulomatous interstitial pneumonia associated with interstitial thickening due to variable lymphocytic/plasmocytic infiltration and mild-extensive interstitial fibrosis. Type II pneumocytosis, (multi)focal accumulation of intra-alveolar mononuclear cells (mainly macrophages) and (occasional) multinucleate giant cells , localised atelectasis and (micro)granulomas centred on parasite remains or calcified cores were also commonly found. Small nematodes located within thin fibrous capsules in the lung parenchyma (including the lungs of 3 neonates) tended to elicit only mild chronic inflammatory responses. In contrast, foci of severe mixed inflammatory cell reactions within alveoli, bronchioles and alveolar interstitium were frequently associated with the presence of free nematode larvae.

Proliferative arteritis and occlusive or necrotising thrombovasculitis were occasionally seen in pulmonary blood vessels parasitised with *P.inflexus.* Milder vascular changes included intimal sclerosis of pulmonary arteries and veins. In a case of fatal acute pulmonary haemorrhage, histological examination of the suspected origin of the haemorrhage revealed an adult nematode that had simultaneously ruptured through a pulmonary arterial vessel and a small bronchus or bronchiole.

Enlarged bronchial and pulmonary associated lymph nodes typically showed mild lymphoid hyperplasia, occasional (possible parasitic) granulomatous foci and infiltration with relatively large numbers of eosinophils.

Bacterial pneumonia

There were a few cases of primary bacterial pneumonias, and most (necro-)suppurative (broncho)pneumonias or pulmonary abscesses were considered to have developed as opportunistic infections secondary to severe parasitic disease or as part of generalised bacterial septicaemias. Bronchiectasis was rarely seen. The most frequently isolated organism from the lungs of harbour porpoises in the study was *Aeromonas hydrophila.* Other common bacterial isolates included *Vibrio* spp., *Pasteurella* spp., *Pseudomonas* spp., *Aeromonas* spp., *Escherichia coli*, *Clostridia* spp., *Streptococcus* spp., *Proteus* sp., *Staphylococcus* spp., *Edwardsiella* spp., *Bacillus* spp. and *Morganella morgani*.

Necro-suppurative bronchopneumonia was associated with septicaemia in 6 individuals. Three were infected with ß-haemolytic *Streptococcus canis* (2 with pulmonary abscessation), 1 with *Streptococcus* lactis and 2 with Group B *Salmonella* spp. (1 with pulmonary abscessation).

Apparently asymptomatic pulmonary Group B *Salmonella* spp. infections were also recorded.

Specific cultures for mycoplasmas were not routinely undertaken, although mycoplasmas have been isolated from the lungs of harbour porpoises in other studies (Clausen & Andersen 1988).

Viral pneumonia

One case of generalised morbillivirus infection with secondary pulmonary *Aspergillus* sp. infection was recorded during the period of this report and has been reported previously (Kennedy *et al.* 1992).

Mycotic pneumonia

Three cases of mycotic pneumonia were characterised by variable sized caseous nodular masses within the affected lung(s) and grossly enlarged bronchial and pulmonary associated lymph nodes. Chronic (often extensive) necropurulent bronchopneumonia associated with the presence of multinucleate giant cells and widespread branching septate hyphae typical of *Aspergillus sp.* were seen histologically. Necropurulent lymphadenitis with multinucleate giant cell formation and infiltration of branching, septate fungal hyphae were also seen within the enlarged lymph nodes. Fungal organisms were not cultured from these individuals.

Pleuritis

Lesions associated with the pleurae were rare. One case of acute-subacute fibrinous pleuritis associated with roughened, greyish visceral and parietal pleura, (sero)fibrinous exudation and histological evidence of diffuse fibrino-purulent pleuritis was recorded. A *Proteus* sp. was isolated from both lungs. A small number of cases of chronic pleuritis were associated with mild serous pleural effusion and pleural thickening/opacity associated with ragged fibrinous-fibrous pleural tags or adhesions. Histological examination showed variable fibrinous exudation, fibrous thickening of the pleura and chronic mononuclear cell infiltration.

Entrapment in fishing gear (by-catch)

In the vast majority of animals known or diagnosed by-caught in this study the lungs were diffusely dark red with copious quantities of fine persistent whitish or slightly blood-tinged froth within the trachea and bronchi. Occasionally this whitish froth exuded through the blowhole. Incomplete collapse of the lungs and alveolar emphysema were more infrequent findings. Food remains were found within the airways of one individual. Histologically, diffuse congestion, alveolar and interstitial oedema and multifocal intra-alveolar haemorrhage were routinely seen. Constriction of the smooth muscle sphincters of terminal bronchioles also appeared to be common to a number of by-caught individuals. Gross or histological evidence of inhalation of seawater was never found.

Neoplasia

Two cases of pulmonary neoplasia were described during the period of the report. Both cases had multiple, pink-cream, 1-6 cm wide fleshy pulmonary masses that were sharply demarcated from the surrounding tissue. In one individual the masses were unilateral and in the other the masses were bilateral and had caused displacement of bronchi and pulmonary blood vessels. The pulmonary associated lymph nodes of the affected lung(s) were massively enlarged and resembled the pulmonary masses in appearance. The tissues from both animals were too autolysed for more specific diagnosis.

Neonatal lung pathology

Neonatal animals were typically found to have non-specific, possibly agonal pulmonary changes including pulmonary oedema, congestion and (multifocal) haemorrhage. Encysted nematodes were seen histologically within the pulmonary parenchyma of 3 individuals.

Pulmonary lesions associated with live stranding

A number of animals necropsied in this study were known or suspected to have live-stranded prior to death. The pulmonary changes considered to be associated with live-stranding included unilateral severe (hypostatic) congestion with incomplete collapse and emphysema of the contralateral lung. In one individual aspirated sand was noted within the upper respiratory tract.

Pulmonary lesions associated with physical trauma

A number of individuals were diagnosed to have died due to physical trauma. In most cases the thoracic cavity and lungs were not directly traumatised. Traumatic lesions included fractured ribs, perforations of the parietal pleura, lung puncture or laceration, haemorrhagic pleural effusion and a pneumothorax.

DISCUSSION

The relative absence of lungworms in neonatal or pre-weaning harbour porpoises in this study supports the suggestion that lungworms in harbour porpoises may have heteroxenous lifecycles similar to other metastrongyloids (Balbuena *et al.* 1994). Lactation is thought to last no more than 8 months in harbour porpoises (Klinowska 1991) and the earliest infections were observed grossly in animals estimated to be 7-10 months old. The 3 neonatal individuals with histological evidence of pulmonary nematode infection may have been infected by the transplacental or transmammary route. There is evidence for prenatal infection of *Halocercus sp.*in bottlenose dolphins (Caldwell 1968; Dailey *et al.* 1991) and the parasites found in the lungs of neonatal porpoises in this study may also have been *Halocercus sp.*.

Indeed, it is possible that an artificially low prevalence of *Halocercus sp.* infection is recorded in harbour porpoises due the relatively small size of these nematodes and the inherent difficulty in their detection on gross necropsy. Small adult nematodes, possibly *Halocercus sp.*, were frequently seen histologically within the lungs parenchyma of a large number of animals in this study.

The interpretation of the relative pathogenicity of the individual lung parasite species found in harbour porpoises is complex. Although lesions in pulmonary blood vessels appear to be exclusively associated with *P.inflexus*, parasitic bronchitis, bronchiolitis and subacute-chronic interstitial pneumonia may be attributable to adult and larval stages of any of the nematode species found in the lungs. Anatomically, harbour porpoise lungs do not possess a prominent lobular septal structure and pneumonic processes appear readily able to extend into adjacent areas of the parenchyma. What is clear, particularly from examination of many by-caught animals in good nutritive status, is that harbour porpoises appear able to tolerate relatively heavy pulmonary parasite burdens without incurring significant negative effects to their overall health status.

The histological lesions associated with pulmonary parasite infections found in this study are not dissimilar to those already described for cetacean morbillivirus infection (Kennedy *et al.* 1991, 1992, Domingo *et al.*, 1992; Libscomb *et al.*, 1994) and it is clear that parasitic pneumonia in harbour porpoises may mask the signs of morbillivirus infection. Therefore, if intranuclear or intracytoplasmatic inclusion bodies are not seen histologically (and these may be difficult to identify in autolysed tissue), then morbillivirus infection may be difficult to rule out on H&E stained lung sections. Duingan *et al.* (1992) suggests that the presence of multinucleate syncytial cells in the lungs is indicative of morbillivirus infection. However, the frequent observation of

multinucleate (and Langerhans-type) giant cells in this study would appear to contradict the suggestion that multinucleate syncytia are pathognomonic for morbillivirus infection.
A detailed review of the pathology and diagnosis of cetacean bycatch, including some harbour porpoises, has already been published (Kuiken, 1996). Diagnostic criteria pertaining to the lungs include the presence of fine persistent froth within the airways, oedematous lungs, and bullae within the lung parenchyma (Kuiken *et al.* 1994b; Kuiken 1996). Although fine persistent froth within the trachea and bronchi and oedematous lungs were common findings in most bycaught (and some non-bycaught) individuals in this study, bullae within the lung parenchyma were very rare.

ACKNOWLEDGEMENTS

The pathological investigations of stranded cetaceans in England and Wales was funded under contract to the UK Department of the Environment as a part of their research commitment to the ASCOBANS agreement. Dr. Peter Bennet and Prof. James Kirkwood are gratefully acknowledged for their invaluable support throughout the strandings project and Ms Gill Bell and Mrs Charlotte Iskjaer Ackley are acknowledged for their help in collecting data. We are also particularly grateful to the staff of the Natural History Museum, Mrs. Stell Turk and Mrs. Pamela Thompson and Dr. Nick Tregenza (Cornish Wildlife Trust) and Mr. Rod Penrose (Marine Environment Monitoring) for the coordination of stranding reports and collection of cetacean carcasses for necropsy. In addition, we gratefully acknowledge a number of individuals and organisations that have assisted with reporting and retrieving carcasses including the Sea Mammal Research Unit, Environmental Agency, HM Coastguard, RSPCA, and the National Trust.

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Table 1: Relative frequency of lesions recorded in the lungs of 133 harbour porpoises

Table 1: Relative frequency of lesions recorded in the lungs of 133 harbour porpoises

Cause	Lesion	Relative frequency
Parasitic	parasitism bronchial tract	80% (juveniles and adults only)
	bronchitis/bronchiolitis	30%
	hyperplasia/metaplasia bronchial tract	15%
	chronic granulomatous interstitial pneumonia	75%
	parasitism pulmonary blood vessel	60% (juveniles and adults only)
	proliferative arteritis/thrombvasculitis	15%
	pulmonary artery aneurysm	5%
	fatal pulmonary haemorrhage (probably parasitic origin)	<1%
Bacterial	suppurative (broncho)pneumonia (+/- abscessation)	35%
	pneumonia/abscessation associated with septicaemia	<5%
	bronchiectasis	<5%
Mycotic	chronic necropurulent pneumonia	<5%
Viral	broncho-interstitial pneumonia	<1%
Physical trauma	haemorrhagic pleural effusion	<1%
	pneumothorax	<1%
Neoplasia	pulmonary tumors	<2%
Foreign body	multifocal acute alveolitis and proteinaceous alveolar fluid	<1%
	aspirated sand	<1%
	aspirated food material	<1%
Not determined	chronic fibrinous pleuritis	5%
	fibrinous pleuropneumonia	<1%
Non-specific	fine persistent froth in trachea and bronchi	40%
	pulmonary oedema	60%
	pulmonary congestion	60%
	multifocal intra-alveolar haemorrhage	30%
	atelectasis	20%
	emphysema (alveolar)	15%

PATHOLOGY OF THE RESPIRATORY TRACT OF HARBOUR PORPOISES (*PHOCOENA PHOCOENA*) FROM GERMAN WATERS OF THE NORTH AND BALTIC SEAS

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INTRODUCTION

Since 1991 all harbour porpoises (*Phocoena phocoena*) by-caught or stranded on the German coast of the North and Baltic Sea were dissected. The investigations were part of national research projects on small cetacean populations in German waters founded by the German government.

MATERIAL AND METHODS

From 1991 to 1995 post mortem examinations on 326 harbour porpoises in various states of preservation were performed. Of these, 276 animals originated from the North Sea and 50 from the Baltic Sea; 277 animals were stranded and 49 by-caught animals. A total of 133 harbour porpoises were preserved well enough to allow complete sampling for histological, microbiological and parasitological investigations.

Pathological examinations of the cetaceans were carried out according to the recommendations of the First European Cetacean Society Workshop on Cetacean Pathology (Kuiken & García Hartmann, 1993). From the lung, parasites were removed, fixed in 70% ethanol and identified. Parasitic infestation was graded semi-quantitatively. The lung was completely cut into 2 cm thick slices and lesions were described by distribution-pattern, type and grade. Tissue and swabs from the lungs were submitted for routine bacteriological examination.

Representative samples from changed and unchanged tissue were collected for routine histology, fixed in 10% formalin and embedded in parafin. Sections of 5 µm were stained by Hematoxylin-Eosin, Elastica van Giemson and PAS staining. In 52 cases the lung was examined immunohistologically for the presence of morbillivirus by means of the ABC-technique. A polyclonal antibody against canine distemper virus, kindly provided by Örvell (Örvell & Norrby, 1980) was used.

RESULTS AND DISCUSSION

The respiratory tract was the organ system with the highest incidence of pathological lesions. Marked pulmonary edema was found equally frequent in by-catches as in strandings, whereas pulmonary congestion occurred more often in by-caught animals. Thirteen stranded newborn harbour porpoises showed aspiration of placental fluid. It remains unclear whether these findings can be attributed to dystocia.

Lungworms and associated lesions seem to be the most important pathological changes in the harbour porpoises. Two thirds of the animals were affected by lungworms. This is in accordance with the results of Baker & Martin (1992) from harbour porpoises from British waters. The majority had nematodes in the bronchial tree and in the pulmonary blood vessels.

Often there was complete obstruction of the bronchial lumina or larger branches of the pulmonary artery by large (*Pseudalius inflexus*) and small lung nematodes (*Torynurus convolutus*). The life cycle of both nematodes in unknown. Lesions primarily induced by parasitic infestation were bronchitis, peribronchitis, and usually mild peribronchial interstitial pneumonia. In 90 cases the parasites were associated with catarrhal-suppurative or suppurative-necrotizing pneumonias with or without abcessation. They were caused most probably by secondary infections with various bacteria, among which β-haemolytic streptococci were prevalent.

In most cases the nematodes caused chronic thrombangitis partly with organization and calcification of the thrombotic material. The pathologic importance of vascular lesions, especially thrombotic obstruction, ist still unclear. Morphological evidence for increased pulmonary blood pressure and disturbance of lung perfusion, e.g. formation of cor pulmonale, was not observed in this material.

Infections by streptococci were associated in 11 cases with lung abscesses of the size of up to half of the whole lung. This is contrary to the results of Baker & Martin (1992) who identified *Pasteurella haemolytica* and L-streptococci as the main secondary infectious agents.

In some cases the inflammatory lesions penetrated the pleura causing diffuse pleuritis or septicaemia.

Being aware of the importance of morbillivirus infections in harbour porpoises from Great Britain (Kennedy *et al.*, 1991, 1992), special attention was given to the possible occurence of this virus in harbour porpoises from the North and Baltic Seas. However, there were neither gross pathological nor histopathological lesions indicative for morbillivirus infections known from marine mammals. The immunocytochemical essays for morbillivirus antigen were also negative in 52 cases.

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LUNGWORMS IN HARBOUR PORPOISES (*PHOCOENA PHOCOENA*) FROM DUTCH WATERS

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ABSTRACT

Detailed parasitological investigation of the lungs of 16 harbour porpoises stranded on the Dutch coast revealed the presence of five species of lungworms: *Pseudalius inflexus, Torynurus convolutus, Halocercus invaginatus, Halocercus tauricus* and *Stenurus minor.*

Only for *Halocercus tauricus* -a nematode previously unreported from Dutch waters- there was a significant negative correlation between the host's body condition and its parasitic burden.

Indications for a migration route of *Pseudalius inflexus* from the blood vessels into the lungs were found. The presence of *Halocercus tauricus, Torynurus convolutus* and *Stenurus minor* in an unweaned *Phocoena phocoena* indicates a possible prenatal or lactogenous infestation by these nematodes.

INTRODUCTION

The role of lung parasitism in the health status of harbour porpoises has long been a matter of speculation. The degree of parasitism found in stranded harbour porpoises of European and other waters appears to be extraordinarily high (García Hartmann, 1997). Whether lungworms do or do not debilitate the harbour porpoise remains to be elucidated, since some by-caught and otherwise healthy individuals suffer from equally heavy infestations as stranded individuals (García Hartmann, unpublished data).

Five species of lungworms are known to occur in the lungs of harbour porpoises. These are all nematodes of the family of *Pseudaliidae* (*Metastrongyloidea*) (Arnold & Gaskin, 1975). *Pseudalius inflexus* (Rudolphi, 1808) is mostly found with the head fixed in lung tissue and the body floating in the bronchial tree, but it can also be found freely in the blood vessels of the lungs and occasionally in the right heart.

Torynurus convolutus (Kühn, 1829) and *Stenurus minor* (Kühn, 1829) are found in the bronchial tree, but with no fixation to the tissue. However, *Stenurus minor* is more frequently found in the middle ears and periotic cavity than in the lungs.

Halocercus invaginatus (Quekett, 1841) and *Halocercus tauricus* (Skrjabin, 1942, cited in Delyamure, 1955) are found buried in the lung tissue.

In previous research, all these parasites, except *Halocercus tauricus,* were found in harbour porpoises stranded on the Dutch coast (Borgsteede *et al.*, 1991).

In this study, lungworms were identified to the species level and their numbers and sex ratio were determined, in order to test the existence of any correlation between lungworm infestations and body condition of the host.

MATERIALS AND METHODS

Material for this research consisted of the infested lungs of sixteen harbour porpoises stranded on the Dutch coast between 1990 and 1993. The lungs were examined only superficially at necropsy and frozen for parasitological investigation. In three cases, only a right lung was available for parasitological research.

After thawing of the lungs, first the large blood vessels were investigated using a pair of tweezers, then the bronchial tree and the blood vessels were cut open. The tissue was squeezed by hand, in order to collect a maximum number of tissue-embedded worms.

After washing over a 63 µm strainer, the species, numbers and sex of all worms was determined under the microscope. The smaller worms were viewed with a magnifying factor of 100x, 250x and 400x. For the higher magnifications, the nematodes had to be treated with chlorallactophenol, in order to increase their transparency and make their internal organs visible ("parasite clearing"). Species identification followed the "Key to the Genera of *Pseudaliinae*" by Arnold & Gaskin (1975). Both species of *Halocercus* were counted as "*Halocercus* sp.", because the female worms are very difficult to distinguish at the species level. By identifying the males, the presence of each species was confirmed.

The body condition of the porpoises was judged with the two parameters used by Read (1990): the blubber thickness (in mm) and the "weight/length ratio" (WLR = W/L, with W=weight in kg, and L=length in cm).

Sexual maturity was defined by gross and histological examination of the gonads, and age by tooth growth layer groups. These parameters were determined as part of the research programme on stranded cetaceans of the Natural Museum of Natural History, Leiden, and were kindly provided for this study, together with all other basic data of the animals, by Drs. Marjan Addink.

RESULTS

Pseudalius inflexus

This parasite was found in all lungs examined. The mean amount of worms per host was 59 (0-254), with a sex ratio (males/females) of 0.81 (standard deviation: 0.88). However, when not taking into account one extreme ratio of 3.8 found in animal 921016, the sex ratio became 0.58 (s.d. 0.18). A normal distribution pattern could then be established. In all porpoises but one, all larvae found were stage L5. In most cases, however, no larvae were present.

The porpoises 930724 and 910122 were exceptional, since no *Pseudalius* at all was found in the air passages, though they were present in the blood vessels of the lungs. In the blood vessels, the females outnumbered the males slightly: the mean ratio males/females was 0.55 (s.d. 0.46). With a paired T-test, a significant difference in sex ratios of air passages and blood vessels was shown. The same holds true for the percentages of larvae vs. adults, with significantly more larvae in the blood vessels. The only encounter of larvae younger than stage L5 in the blood vessels of porpoise 910122 is unique in our material. In these larvae, the sex organs could be differentiated, but were not fully developed: vulvae were not opened and spiculae had yet to stretch. This differentiates these larvae from stage L5, which has fully developed sexual organs.

Torinurus convolutus

In fifteen of the sixteen examined harbour porpoises, *T. convolutus* was found in the bronchial tree. The mean sex ratio was 0.50 (s.d. 0.22), and the distribution of sex ratios was normal.

Halocercus invaginatus and Halocercus tauricus

Twelve out of the sixteen animals examined were found to be infested by *Halocercus*. Three of them showed mixed infestations of *H. invaginatus* and *H. tauricus*. In three other porpoises, *H. invaginatus* was the only lung parasite found; in another five porpoises, only *H. tauricus* was present. In one further porpoise, the only, single *Halocercus* worm found could not be further identified. The mean sex ratio for both *Halocercus* species together was 0.71 (s.d. 0.64), with a normal distribution.

Halocercus was found in so-called "Halocercus knots", i.e. little white knots of ≤ 2 mm diameter in the lung tissue which, once cut open, appeared to contain curled-up *Halocercus* worms. Using Fisher's Exact Test, an significant correlation was shown between the presence of these knots and infestation with *H. tauricus*. Such correlation could not be shown for *H. invaginatus*.

Stenurus minor

This nematode was found in the lungs of four out of the sixteen harbour porpoises examined.

In general, the amounts of parasites were very small: in porpoise 920229-2 one female was found, in 921016 two males, in 930724 one male and in 910122 three males and eight females. Fisher's Exact Test could not prove any relation between the presence of *St. minor* in the lungs and their presence in the middle ears (not shown here).

Lungworms and host body condition

A correlation was found between infestation by *Halocercus* species (without differentiating the two *Halocercus* sp.) and the WLR of the porpoises (independent W=0.43, p=0.02). Unpaired T-tests showed a significant negative correlation of the WLRs and the infestation by *H. tauricus*. The same was not found for *H. invaginatus*, indicating that only *H. tauricus* may be responsible for the above-mentioned significant correlation of overall Halocercus infestation and reduced WLR.

By multiple regression, no correlation was found between any other lungworm infestation and blubber thickness or the WLR of the porpoises.

Lungworms and maturity of the host

In sexually mature animals, significantly fewer *Halocercus* were found in comparison to the group of non-mature animals (unpaired T-test). When distinguishing between the two *Halocercus* species using Fisher's Exact Test, an association was found between non-mature animals and higher degree of infestion by *H. tauricus*, but not between non-mature and higher degree of infestation by *H. invaginatus*.

No significant differences were found in burden of *P. inflexus* and *T. convolutus* between the mature and immature porpoises (unpaired T-test). Also, no difference was found in the presence or absence of *S. minor* in lungs or middle ears between the mature and immature porpoises (Fisher's Exact Test).

DISCUSSION

Lungworm species found

Previous research on lungs of harbour porpoises from Dutch waters had shown the presence of four species of lungworms: *P. inflexus*, *T. convolutus*, *H. invaginatus* and *S. minor* (Borgsteede *et al.*, 1991). This study reveals the additional presence of *Halocercus tauricus* in porpoises stranded on the Dutch coast between 1990-1993.

Due to the location of the nematode embedded in the tissue, and the difficulty of distinguishing females of *H. tauricus* and *H. invaginatus* at a species level, we believe that the presence of *H. tauricus* is Dutch waters is not new. Rather than that, its proper identification requires a very meticulous examination of a large number of parasites, which is not routinely done for normal diagnostic purposes.

Pseudalius inflexus

The largest of the lung nematodes, *P. inflexus,* is found in the bronchial tree, but also in the blood vessels of the lungs. It was present in all animals examined in this study. Significantly more larvae were present in the blood vessels than in the lung tissue and bronchi; in two immature animals, PP930724 and PP910122, *P. inflexus* was exclusively found in the vessels and not in the bronchial tree, and in both animals, the high percentage of larvae found (in one of them even larvae younger than stage L5) indicates that the nematode infestation had happened relatively recently. In analogy to the situation of other lung nematodes in horses and cows, we conclude that in the harbour porpoise, the blood vessels are the "port de entree" for the infestation of the lungs by *P. inflexus.*

Clausen & Andersen (1988) mention that infestation with *P. inflexus* occurrs during the first twelve months of life, after which the animals remain infested during the rest of their lives. This could be the explanation for finding no significant differences in the older animals of our sample.

Torinurus convolutus

In this study, *T. convolutus* was found in the bronchi of fifteen out of sixteen animals. Although Kontrimavichus *et al.* (1976) record these worms for the bronchi and the blood vessels of the lungs, in none of the porpoises of this study was *T. convolutus* found in the latter. No connection could be found between age of the harbour porpoises and their worm burden of *T. convolutus.*

Animal 930724, which was just 3 months old, provides an example of a harbour porpoise infested with *T. convolutus* in its bronchi at a very young age. Since porpoise calves start ingesting fish from an age of 4 months onwards (Santos, 1999), a prenatal or lactogenous infestation seems probable.

***Halocercus* sp.**

Unfortunately, the lack of reliable identification criteria to distinguish between females of *H. tauricus* and *H. invaginatus* makes the interpretation of our findings less straight-foreward than in the other lung nematodes found. *Halocercus sp.* were found in the lung tissue of twelve of the sixteen animals examined: three had mixed infestations, three only *H. invaginatus* and five had only *H. tauricus* infestations; in one animal, the infestation could not be identified to the species level.

Of all parasite species, only the presence of *Halocercus* sp. appeared to correlate negatively with host body condition: a negative correlation was proven to exist between the burden of *Halocercus* and the WLR, as well as between the burden of *Halocercus* and the blubber thickness. A significant difference in WLR between animals infested by *H. tauricus,* and those which were not, was found. This was not true for *H. invaginatus,* indicating that it is *H. tauricus* which causes the negative correlation between Halocercus infestation and host body condition.

A significant difference in *Halocercus* burden was also found between mature and immature porpoises: a lower burden in mature animals might be explained by the development of immunity, with subsequent elimination of parasites. Some evidence for such elimination does exist, since calcified parasite remains embedded in lung tissue are regularly found in histological examination of harbour porpoises (García Hartmann, unpublished data).

The lower prevalence of *H. tauricus* in mature animals, and the absence of such a negative correlation for *H. invaginatus* suggests that this aquired immunity may be especially related to *H. tauricus.*

Also, an association was found between *H. tauricus* and the presence of so called *Halocercus* knots, which were not found in *H. invaginatus.* If the isolation of a parasite by "encapsulation" is interpreted as an immune reaction of the host, *H. tauricus* causes a more severe reaction than *H. invaginatus*. This supports the hypothesis of *H. tauricus* clearly causing an efficient immune reaction in the host, eventually leading to the described reduction of the numbers of this parasite in mature porpoises.

Dailey *et al.* (1991) described heavy infestations of the lungworm *Halocercus lagenorhynchi* in four young *Tursiops truncatus*, and gave evidence for a prenatal infestation. Although this study does not present evidence for prenatal infestation of *Halocercus* in harbour porpoises, it is remarkable that the only 3 months old porpoise was already heavily infested by *H. tauricus*. This suggests that either a prenatal or lactogenous infestation is probable.

Stenurus minor

S. minor was found in the lungs of four animals and in the middle ears of nine. A relation between the presence on both these locations was not found. Kontrimavichus *et al.* (1976) mention heart and veins as other locations of these nematodes. These could indicate a hematogenous migration of this species in the host, but this was not confirmed by our study. The fact that *Stenurus* is more often found in the middle ears than in the lungs (Delyamure, 1955) could indicate that the middle ears are the target organ of *S. minor*. The specimen found in the lungs could then be considered "lost". The aerobic climate of the bronchi, being similar to the one in the middle ears, could explain why the worms can grow up to the adult stage in the lungs.

The presence of *S. minor* in porpoise 930724 indicates the probability of an early, either prenatal or lactogenous, infestation by this species.

Simultaneous presence of several parasite species

BALBUENA *et al.* (1994) mention that when one species of lungworm is present in a harbour porpoise, the chances of finding another lungworm species increase significantly. Their explanation for this phenomenon is that possibly related species may have the same or very similar life cycle, with the same host and the same intermediate host. Such associations of different species of lungworms could not be established in this study. By examining a larger number of harbour porpoises, a smaller spreading of the data and more reliable conclusions can be expected. This, of course, holds true for all other conclusions of this study as well.

ACKNOWLEDGEMENTS

We would like to thank Chris Smeenk and Marjan Addink, National Museum of Natural History, Leiden, The Netherlands for providing the material and data for this research project, and for the important review of the manuscript.

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HISTOPATHOLOGY OF THE LUNGS OF BYCAUGHT WHITE-SIDED DOLPHINS (*LEUCOPLEURUS ACUTUS*)

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INTRODUCTION

Death caused by accidental entrapment in fishing gear ("bycatch") occurs worldwide in dolphins and porpoises and has drawn considerable attention in recent years, due to its implications for species conservation (Perrin, 1991).

The pathophysiology of drowning has since long been studied in humans, but veterinary literature on this subject is scanty (Schoon & Kikovic, 1989). Recent efforts to identify pathogical characteristics of death by accidental net entrapment in dolphins have led to a list of potentially useful pathological criteria (Kuiken, 1996). Yet, these criteria can only provide indirect proof, as most findings are of a general, non-pathognomonic, nature.

The aim of the present study is to compare the pathomorphological changes in the lungs of known bycaught dolphins with forensic findings in human pathology of drowning cases.

MATERIAL AND METHODS

The material of this study consisted of 40 Atlantic white-sided dolphins (*Leucopleurus acutus*), 4 harbour porpoises (*Phocoena phocoena*) and 2 common dolphins (*Delphinus delphis*) which were retrieved from nets during fishing operations and brought ashore by Dutch midwater trawlers (common and white-sided dolphins) and coastal fishing vessels (harbour porpoises) in 1993/1994. All carcasses were frozen onboard and later thawed in water immediately preceeding a complete dissection following the protocol of Kuiken & García Hartmann (1993). Additionally, lung tissue of 4 Atlantic white-sided dolphins was sampled onboard and material preserved in 10% buffered formalin.

RESULTS

With conventional staining, microscopic examination of the lungs of all dolphins and porpoises showed severe, proteinaceous and haemorrhagic edema within the alveolar spaces and -to a lower degree- the interstitium. Yet, the localisation and proteinaceous character of the edema varied between individuals to a certain degree. Distension of the alveoli, with ruptures of alveolar walls and of the myosphincters of the bronchioli, combined with intra-alveolar haemorrhages and compression of capillaries by distension, was found regularly. Histology furthermore revealed prominent bronchioalveolar, interstitial and subpeural haemorrhages. The ruptures of bronchiolar myosphincters had often led to an considerable dilatation of the corresponding alveolar passages, creating largely distended spaces filled with edematous, eosinophilic fluid. Adjacent alveoli often were atelectatic. The lumina of the alveoli contained large amounts of acidophilic and rather homogeneous proteinaceous fluid. In the intra-alveolar fluid, foreign particles were found in 14 cases, in samples from both central as well as subpleural areas. In 10 of these cases, the foreign bodies could be determined as particles of striated muscles in various degrees of digestion, most probably originating from fish. Furthermore, amorphous -often round- basophilic particles were found. Intra-alveolar bleeding appeared in all samples, with great individual differences in the amount, shape and staining characteristics of the erythrocytes.

All animals showed multifocal ruptures of the myosphincters in the bronchioli; the sphincters were mostly found closed but also -in some cases- in various stages of opening.

The Gomori silver stain was used to reveal the reticulum fiber structure of the lungs. In all animals, a varying degree of stretching and thinning of the alveolar septae was seen. The alveolar walls showed slight to maximal distensions, with thread-like capillaries.

Independent of any pathomorphological changes caused by or associated with the death of these animals, in 25 cases a purulent bronchopneumonia was found. In 5 cases, this histological finding was clearly associated with a parasitic infestation of the lung; in the other cases, the origin of the inflammatory reaction could not be determined histologically.

DISCUSSION

The diagnosis of drowning has long been the subject of controversial discussion in human forensic pathology (Schoon & Kikovic, 1989) and, owing to the lack of clear pathognomonic criteria, only a summary of typical findings is available (Giertsen, 1977).

In view of the macroscopic absence of water in the lungs of bycaught cetaceans and a variety of other necropsy findings, there has been some discussion in the past whether odontocetes actually drown or suffocate, i.e. die without inhaling water (for details, see García Hartmann *et al.*, 1994).

In human forensic pathology, the amount of water in the lung is not regarded to be a significant criterium of drowning (Pearn, 1985), especially because of the occurrence of so-called "dry drowning": a situation in which laryngospasm may avoid the entrance or aspiration of water into the lungs and death is caused by cerebral hypoxia. Such "dry drowning" is known to occur in 10-20% of drownings in humans (Plueckhahn, 1984; Rivers *et al.*, 1970). It is also known to occur in other mammals.

Drowning fluid causes typical osmotic and hydrostatic changes in lung tissue and other organs: in salt water, severe proteinaceous edema in the alveolar spaces is characteristic (Tonner, 1971). To differ between post-mortem and intra-vitam reactions, signs of respiration and haemodynamic changes should be examined. In all animals studied, proteinaceous edema, dilated venes and lymphatic vessels were observed in various organs and tissues. Several cases of foreign bodies and, in 10 cases, parts of fish muscles within the intra-alveolar spaces clearly point at the intra-vitam respiration of fluid.

All animals showed histological changes, ranging from distension to rupture of the reticular fibres, which coincide with category 2 and 3 of the classification of "atypial drowning lung" according to Reh (1969).

In agreement with other authors, who have found most myosphincters of the lungs of known bycaught dolphins to be closed (Simpson & Gardner, 1972; Ridgway, 1972, Kooyman & Sinnett, 1979), we found that in the animals studied by us the majority of sphincters were closed, though various stages of opening and ruptures of myosphicters were seen as well. Therefore the state of the myosphincters may not be a reliable diagnostic criterium.

In summary, the Gomori silver stain of reticular fibres used in this study is a method which proved useful for detecting histological changes in the lungs of bycaught dolphins which are considered typical for drowning in humans. We propose the use of the classification by Reh (1969) for the description and quantification of these changes, as additional criterium in combination with other typical findings in the lungs of drowned dolphins.

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ACKNOWLEDGEMENT

The authors are endebted to the RIVO-DLO, IJmuiden, The Netherlands, for providing material of the Dutch fishing fleet for this research project, with special thanks for the good collaboration to Bram Couperus and Henk Heesen. The help and continuing support of Chris Smeenk and Marjan Addink, National Museum of Natural History, Leiden, The Netherlands, was -once again- essential and is whole-heartedly appreciated.

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THE STRUCTURE OF THE LUNG IN CETACEA: A SUMMARY

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INTRODUCTION

Cetacea are highly specialized mammals that show numerous anatomical and physiological adaptations that can be correlated to the choice, recent from an evolutionary point of view, of a totally aquatic life. Cetacea lungs are nonseptated and nonsegmented; they are positioned dorsally to a diaphragm more horizontal than in terrestrial mammals, allowing the expulsion of a high percentage of air in a relatively short period. The air-conducting pathways of Cetacea show several features that are suited for a vigorous and fast air exchange, as for instance the larynx is connected to the lungs through the trachea with a cartilaginous armature that extends into the smaller bronchioles, provided sometimes with myoelastic sphincters. These sphincters are possibly an adaptation to the fluctuations of air pressure in the lungs during quick and frequent diving and surfacing, and especially during the "violent" inspiration and expiration of the smaller toothed whales (Goudappel & Sliffier, 1958). In this brief account the general features of the lungs of Cetacea are discussed.

GENERAL FEATURES OF THE RESPIRATORY SYSTEM

Blowhole and nasal sacs

In Cetacea the external nares have migrated on top of the head forming an opening called the blowhole, single in toothed whales and bipartite in baleen whales. Four pairs of sacs are associated and continuous with the nasal passageways:

1) vestibular sacs: proximal to the blowhole,
2) tubular sacs: the nasal plug has a node which enters these sacs,
3) connecting sacs: in communication with the tubular sacs,
4) premaxillary sacs: underneath the melon and over the premaxillary bones.

Larynx

The rostral larynx is formed by the epiglottal and the arytenoid cartilages that elongate to form a tube, the so called "beak" that projects anterio-dorsally from the pharynx floor. Its caudal end rests within the internal nares. This beak-like tube has been described in several species and has been called the "aryteno-epiglottideal" tube (for a review see Green, 1972).

In the larynx few mucous glands can be found between the squamous epithelium and the cartilage. The larynx is connected to the lungs through the trachea with a cartilaginous armature that generally extends into the smaller bronchioles provided sometimes with myoelastic sphincters.

Trachea

In Cetacea the trachea is rather short, only about 5 cm in some dolphins, while its diameter may be more than 30 cm in some whales. It is robust and has a large lumen with numerous complete, heavy rings that may anastomose at irregular intervals. No membranous portion is present in the trachea.

Bronchial tree

The bronchial tree in all Cetacea is surrounded by fused links of cartilage which make it relatively rigid. It can be generally divided into two parts:

a) the conducting division (provided with a high columnar epithelium) (trachea, main bronchi, stem bronchi and their division down to the bronchioles of 0.5 mm in diameter); the size diminishes from the bronchi to the bronchioles but the structure is nearly uniform as far as the mucosa, tunica propria and adventitial coat are concerned.
b) the respiratory division (provided with a flattened respiratory epithelium)

On the contrary bronchioles distal to the conducting division are completely different. The respiratory division starts in the bronchioles of 0.5 mm in diameter where a fundamental change in the bronchiole's morphology takes place with the presence of myo-elastic sphincters. These sphincters occur up to the ends of these terminal bronchioles where they enter a series of air sacs. Once the first sphincter appears, others succeed in rapid succession so that a series of compartments -from six to eighteen depending upon the lenght of the bronchiole- is therefore produced (Wislocki, 1929) (see below).

Bronchi and bronchioles

In Cetacea they generally show the following structure:

i) stratified or pseudo-stratified epithelium;
ii) a tunica propria of loose collagenous connective tissue with a number of blood vessels of a small caliber, a few small mucous glands (not observed at all in the bronchioles), and a robust band of circular smooth muscle that increases close to the terminal bronchioles.
iii) a strong cartilaginous armature extending down to the openings of the bronchioles into the air sacs in all Cetacea so far examined with the exception of the Baird's beaked whale, *Berardius bairdii* (Murata, 1951).

In Cetacea just prior to the bifurcation of the trachea in the main bronchi (approximately 5 cm) branches a separate right bronchus, called accessory bronchus. Some whales lack this bronchus but when this happens the right main bronchus is larger than the left one. No membranous portion is present in the main bronchi.

All bronchi show a ciliary epithelium and are surrounded by a layer of irregular shaped cartilage pieces. A layer of elastic tissue, full of arteries, can be found on the inner side of this cartilaginous layer. These vessels form plexuses that can be found even in the smallest bronchioles and may act as shock-absorbers as well as for heating the air. In a few species (*Delphinus delphis, Phocoena phocoena*) a venous or capillary plexus has been described. Practically no lymphoid tissue is present and the bronchial epithelium and their glands contained almost no mucoid cells. These features could be linked to the absence of dust in the aspired air as suggested by Slijper. Lastly the terminal bronchioles do not arise by dichotomous division but always as side branches of a main bronchiole.

Lungs

Cetacea lungs are nonseptate, nonsegmented, elongated and positioned in the thorax dorsally to a diaphragm more horizontal than in terrestrial mammals allowing the expulsion of a high percentage of air in a relatively short period. The majority of the lung tissue is located dorsally and therefore ventral margins of the lungs are thin. Partly due to the accessory bronchus the lungs are somewhat asymmetrical.

They show the following features:

a) no distinct lobulation (with abundant dense collagenous tissue that surrounds air sacs, air ducts and blood vessels);
b) the presence of an extensive cartilaginous armature that encircle the bronchi and bronchioles;
c) the presence of a double capillary bed, one on each side of the interalveolar septum;

One of the most striking features of this septum is the general presence of heavy bundles of tissue composed of both muscular and elastic fibers. Important differences in the manner in which the myo-elastic tissue is arranged to produce closure of the respiratory portion of the lung have been found within existing sub-orders:

- Mysticeti (Baleen whales)

In the large whales heavy myo-elastic bundles have been described supporting tubular alveolar ducts, around the interalveolar septa at their free ends and projecting into the proximal portion of the air sacs (For these bundle a sphincter-like function has been suggested at this site). In these bundles the elastic component appears to predominate.

- Odontoceti (Toothed whales)

On the other hand, in a number of species belonging to the sub-order of Odontoceti the presence of a system of myo-elastic sphincters, the last one of them at the entrance of the alveolar complex has been described.

Myo-elastic sphincters

In these circular smooth muscle sphincters, as opposed to the situation found within the bundles, the muscular component is more abundant and provides a powerful mechanism for closure of the air sacs whereas the elastic component contributes, it would seem, to rapid evacuation of the air during expiration (Wislocki & Belanger, 1940). The sphincters close at the end of inspiration when the lungs are filled and remain closed until the onset of expiration (Wislocki, 1929) and serve the function of maintaining the air in contact with the respiratory mucosa while the animal is submerged.

Such closure, imprisoning the air, could help in preventing the alveolar collapse with the increasing pressure on the thoracic wall during trained experimental dives that have reached a depth of 535 m in the bottlenose dolphin, *Tursiops truncatus*, and a depth of 646 m in the beluga whale, *Delphinapterus leucas* (Shippee *et al.*, 1993). These myoelastic sphincters seem to appear in the order Cetacea in relation to the adopted diving method which is likely to reflect feeding behaviour. However this is not simply in relation to the "accentuated power of submerging" as previously suggested by Wislocki & Belanger (1940) but possibly with the frequency of diving. These sphincters have been described in the harbour porpoise, *Phocoena phocoena*, the common dolphin, *Delphinus delphis*, the striped dolphin, *Stenella coeruleoalba*, the tucuxi, *Sotalia fluviatilis*, the Atlantic spotted dolphin, *Stenella frontalis*, the long-finned pilot whale, *Globicephala melas*, the beluga whale, the Bairds's beaked whale (for a review see Goudappel and Sliffier, 1958; Kooyman and Andersen, 1969) and the Risso's dolphin, *Grampus griseus*, (Bortolotto, 1994 a).

They are totally lacking in the baleen whales (i.e.: fin whale, *Balaenoptera physalus*, blue whale, *Balaenoptera musculus*, humpback whale, *Megaptera novaeangliae*, bowhead whale, *Balaena mysticetus*) and in the bigger among the toothed whales such as the sperm whale, *Physeter macrocephalus*, and some Ziphiidae (Beaked whales), such as the northern bottlenose whale, *Hyperoodon ampullatus* (Belanger, 1940; Goudappel & Sliffier, 1958; Kooyman & Andersen, 1969).

Those Cetacea which possess myo-elastic sphincters in the bronchioles also possess traces of myo-elastic bundles. In those Cetacea in which the bundles are hypertrophied no traces of sphincters could be demonstrated. Thus it seems that the bundles are a more generalized feature whereas the sphincters appear to be a specialization restricted to a few species. It seems furthermore likely that the myo-elastic bundles are homologous to the extremely delicate strands of muscle described in the ductus alveolaris of many mammals (Wislocki & Belanger, 1940).

Alveolar (or air) sacs

The only communication of the bronchiole with alveolar sacs is via openings provided with complete myo-elastic sphincters. The air sacs are provided with a very thick wall and are very large but variable in size among species while the alveolar component is relatively constant in size. The terminal openings of the bronchioles into the air sacs are always surrounded by a myo-elastic sphincter. As seen above the number of air sacs with a single segmented bronchiole and its branches might be safely placed as between six and eighteen.

Lung volume

There are big differences in lung volumes among Cetacea. Cetacea with typical long-duration dives have a small lung volume with a consequent lung collapse during the dive while seals for instance reach the same result by diving after a partial expiration. On the contrary Cetacea that normally have short-duration dives (i.e., dolphins and porpoises) have a greater lung volume, comparable to terrestrial mammals. They dive after inspiration and possibly their lungs work as an oxygen store (Snyder, 1983). Furthermore it seems that adaptations in the blood oxygen affinity parallel the modifications on lung volume: low affinity where the lungs seem not to represent an oxygen store (i.e., whales), high affinity where the lungs do represent such a store. In this case the uptake of oxygen from the alveolar space is therefore maximized.

DEEP DIVING ADJUSTMENTS

Cetacea show several adjustments to deep diving:

i) their thoracic cavity is quite flexible and can collapse when under pressure;
ii) they possess retia mirabilia and a complex system of veins and venous sinuses;
iii) their lungs are very elastic;
iv) their trachea is resilient, short, robust and surrounded by heavy cartilaginous rings that may anastomose and form an armature extending into the smaller bronchioles.

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A NEW FLAGELLATE PROTOZOAN LIVING AS A COMMENSAL OR OPPORTUNIST IN THE BLOW HOLE OF A STRANDED PYGMY SPERM WHALE (*KOGIA BREVICEPS*)

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INTRODUCTION

Pygmy sperm whales, *Kogia breviceps* (Physeteridae) have a wide geographical range in the warmer waters of the world, where they live beyond the edge of the continental shelf, usually individually, or in small schools of up to six animals. Much of our knowledge of them comes from individuals that are taken in fisheries, or that strand.

Although numerous helminths and crustaceans have frequently been reported from cetaceans, including *Kogia* spp., very little is known of protozoan infections (Dailey, 1985).

To the best of our knowledge, reports are confined to ciliates from the blow hole and skin of dolphins (Woodard *et al.*, 1969, Sniezek *et al.*, 1996, and Howard *et al.*, 1983 respectively), and the apicomplexan Sarcocystis from muscles of dolphins and whales (Cowan, 1966, Dailey & Stroud, 1978; Owen & Kakulas, 1967).

The paucity of information on protozoan infections in cetaceans can be attributed to the following: cetaceans are not readily accessible in the wild, most stranded animal are dead or in poor condition, protozoa are delicate and do not survive long after the host has died, infections may not be grossly visible, and specialized knowledge and facilities for studying protozoa may not be available at the stranding site.

However, between November 1993 and May 1994, a stranded juvenile pygmy sperm whale was successfully rehabiliated at the National Aquarium in Baltimore. During this time, intensive medical care and effort afforded us a unique opportunity to study aspects of her biology, including observing flagellates in her blowhole.

MATERIALS AND METHODS

The juvenile female whale stranded in New Jersey in November 1993 and was transported to the National Aquarium in Baltimore, where she remained for care until her rehabilitation and release of the coast of Florida in May 1994. Although she was in poor condition on arrival at the National Aquarium, she was successfully treated for dehydration, bacterial pneumonia, and gastroenteritis. Following the removal of plastic from her stomach using endoscopy, she made a full recovery.

Routine health monitoring included the periodic collection of blood and blow hole samples. The latter were collected when the whale was out of the water for a physical examination or transport. Mucus from the respiratory tract was collected in a clean plastic petri dish, held approximately 6 cm above the blow hole, when the whale exhaled. Five to eight exhalations together yielded enough material for examination (care was taken to prevent the sample from drying out). The fresh material was examined microscopically, and then two different types of stained preparations were made. Some samples were fixed in Bouins fixative and stained with silver protein (protargol) to demonstrate the flagella and nuclear membranes. The protargol staining proceedure followed the filter method of Lynn (1992) and Montagnes & Lynn (1987). Other samples were air dried, fixed in methanol and stained by Wrights Giemsa (see Appendix).

RESULTS

Flagellates were routinely detected in fresh wet mounts of mucus, immediately after the whales arrival in Baltimore, throughout the rehabilitation period, and until release. The flagellates were elongate, with an undulating membrane, and one anterior flagellum and one posterior flagellum. They moved very actively through the mucus. Flagellates were frequently associated with epithelial cells in the mucus, and were attached to clumps of epithelial cells by one of their two flagella. We have tentatively identified these flagellates as belonging to the flagellate order Kinetoplastida.

DISCUSSION

Although kinetoplastids have not been previously reported from cetaceans, flagellates with similar relationships to their hosts are known from fishes. Members of the genus *Cryptobia* with two unequal flagella and sometimes also with a narrow undulating membrane, occur as both endozoic and ectozoic commensals of invertebrates and fish (Lom & Dykova, 1992). Those species that are ectozoic are transmitted by direct contact. Although the life cycle of endozoic species is not known, it is known that they can be regurgitated, and can survive in the water for up to 72 hours, (presumably then to be swallowed again). Our knowedge of kinetoplastids from fishes thus suggests that the flagellates from the pygmy sperm whales could be transmitted to other whales directly, i.e. a vector may not be needed. We suggest that transmission could take place between the mother and the calves, and when these usually solitary animal gather in schools.

The attachment of the flagellates to the epithelial cells in the blow hole mucus, is also reminiscent of the attachment of *C. branchialis* (a species from the gills of many species of fish). The *C. branchialis* flagellates adhere to the substratum with their recurrent flagellum while feeding on bacteria and detritus particles. According to Lom (1980), there is no evidence of any pathogenic action of cryptobias, and the adherence of the recurrent flagellum inflicts no damage to the hosts epithelial cells.

The presence of the epithelial cells in the blow and the attachment of the flagellates to them, suggest that the protozoa inhabit the upper respiratory tract of the whale, such as the trachea, rather than the lower parenchymus tissue or the sinuses. The continuous presence of the flagellates, even when the whale returned to good health, suggest to us that they are probably normal components of the fauna of the whale, and are harmless commensals.

It is also possible, though we believe less likely, that the flagellate from the pygmy sperm whale is an opportunistic symbiont, the infection perhaps having been acquired from fish or squid, the latter being the whales primary food. As mentioned above, *Cryptobia* spp. have been reported from fish, and several ectoparasitic flagellates, tentatively identified as members of the blastidinid and bodonid groups, can infect octopus (Hochberg, 1990).

We would like to encourage other aquatic animal health specialists to examine the mucus from the blow hole of cetaceans, to determine which protozoa are present. In this way we can build up host/symbiont geographic distribution records, and thus better understand the relationships between the flagelates and their hosts.

ACKNOWLEDGEMENTS

We extend our sincere thanks to the Marine Animal Rescue Program at the National Aquarium in Baltimore, and to the dedicated team of staff and volunteers who made it possible for us to work with this whale. In particular we would like to thank David Schofield, Christine Steinert, Jill Arnold, Jenni Jenkins, Jennifer Fasick and Dr. Andrew Stamper for their conscientious and enthusiastic observations of the whale and her flagellates.
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APPENDIX

A GOOD GIEMSA STAIN FOR FLAGELLATES
(protocol frown Professor Woo, Guelph)

1. Make smear or impression prep
2. Air dry slide
3. Fix in 100% methanol for 1 min.
4. Air dry slide
5. Fix in 10% formalin for 2 mins.
6. Air dry
7. Stain with Wright's Giemsa

BOUINS FIX FOR PROTARGOL SILVER PROTEIN STAIN

Add specimen to 20 ml vial containing:
2 ml Bouins fixative
7 drops of glacial acetic acid
18 ml diluent to match source of sample
e.g.

- if ectoparasite: filtered water from tank or pool,
- if endoparasite: appropriate saline or ringers

ILLUSTRATIONS

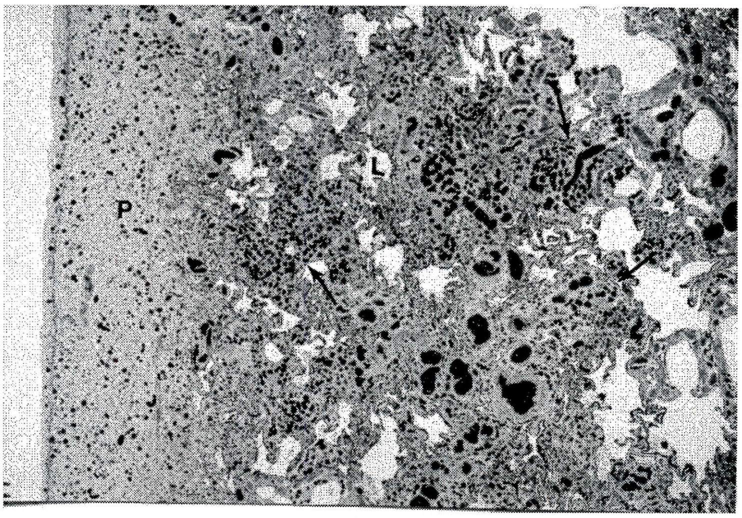

Figure 1: Angiomatosis. Low power view of the lung (L) and pleura (P) of an adult *Tursiops truncatus* with an advanced case of angiomatosis. Arrows point to nodular proliferations of small vessels, which have become confluent. Note the marked thickening and vascularization of the pleura. 20x magnification, hematoxylin, phloxine and saffranin (HPS) stain.

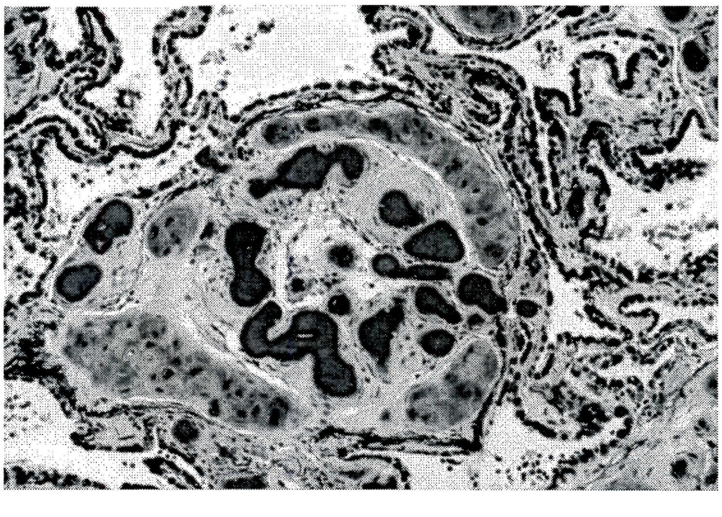

Figure 2: Angiomatosis. High power view of a small airway of figure 1. Notice the marked proliferation and engorgement of the vessels (irregular dark staining objects) of the mucosa within the encircling cartilage plates of the airway. This may progress to occlusion in exceptional cases. 400 x magnification, HPS stain.

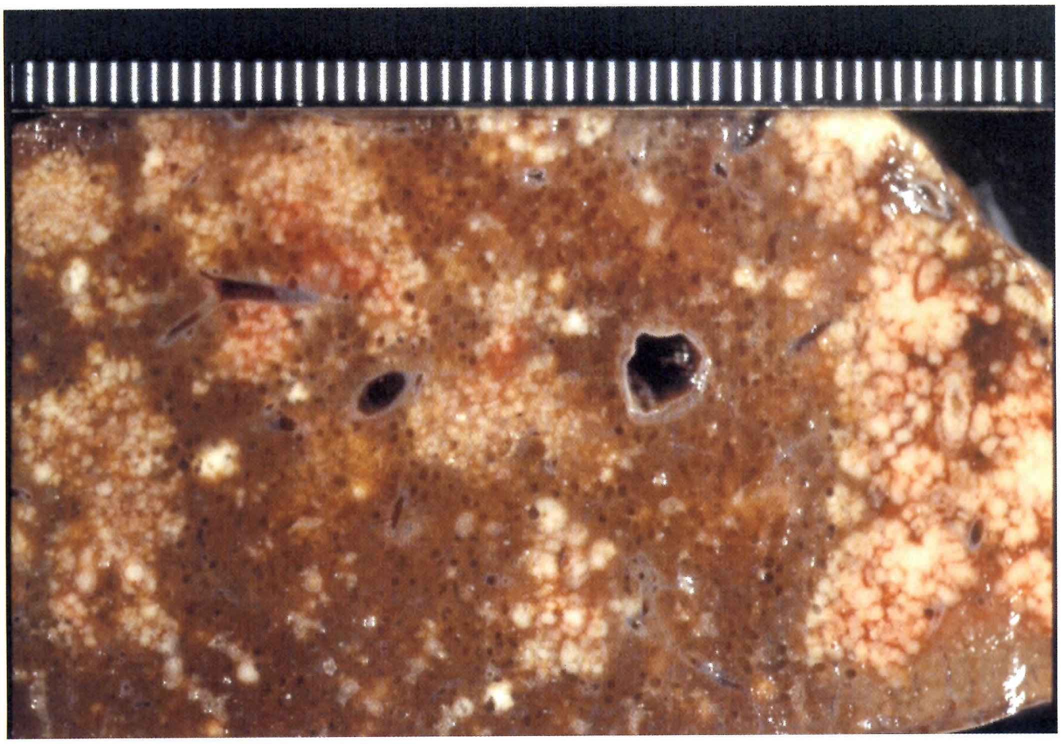

Figure 3 : Nocardiosis. Lung, multifocal consolidations ; *Stenella coeruleoalba*.

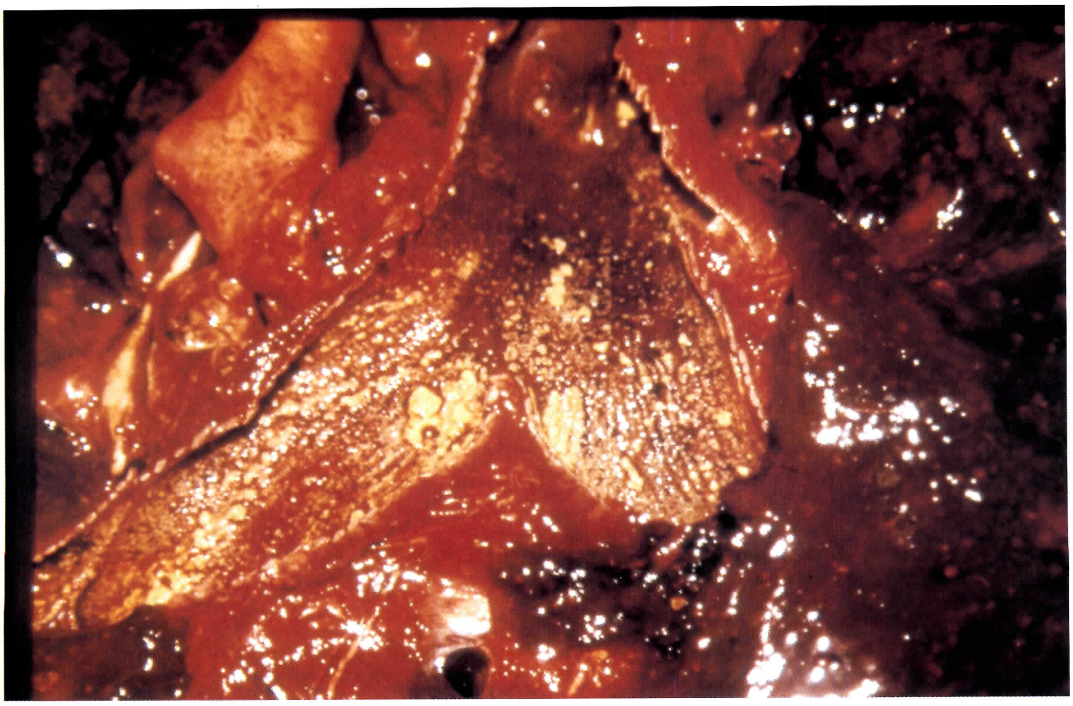

Figure 4 : Nocardiosis. Trachea and main bronchi filled with fibrinous exsudate; *Stenella coeruleoalba*.

Figure 5: Nocardiosis. Mediastinal lymph node, necrotic areas; *Stenella coeruleoalba*.

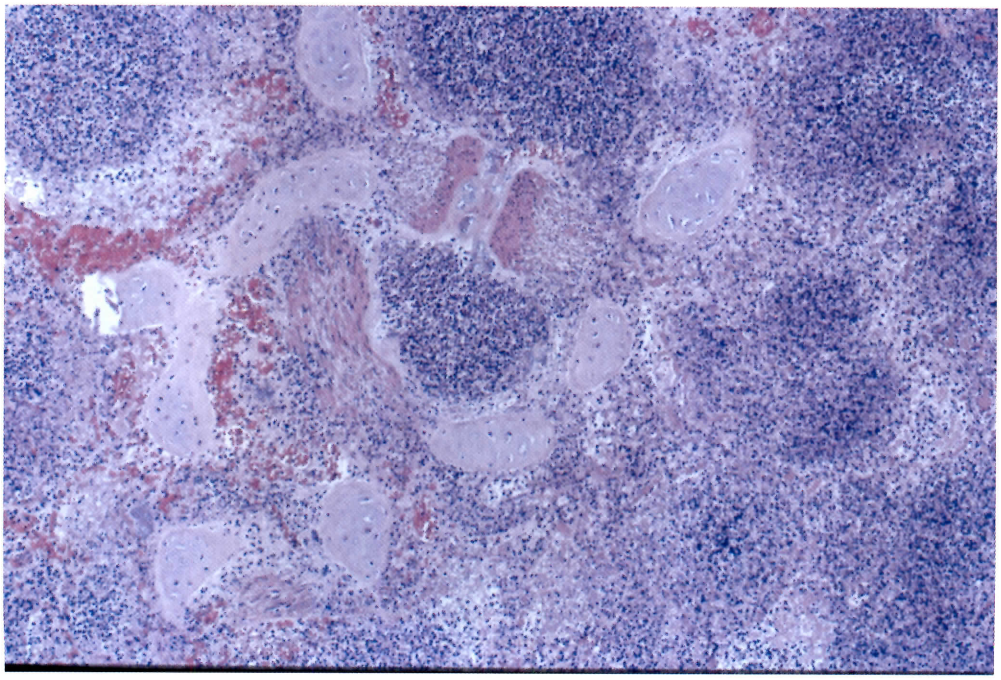

Figure 6: Nocardiosis. Pyogranulomatous nectrotising pneumonia, H/E stain; *Stenella coeruleoalba*.

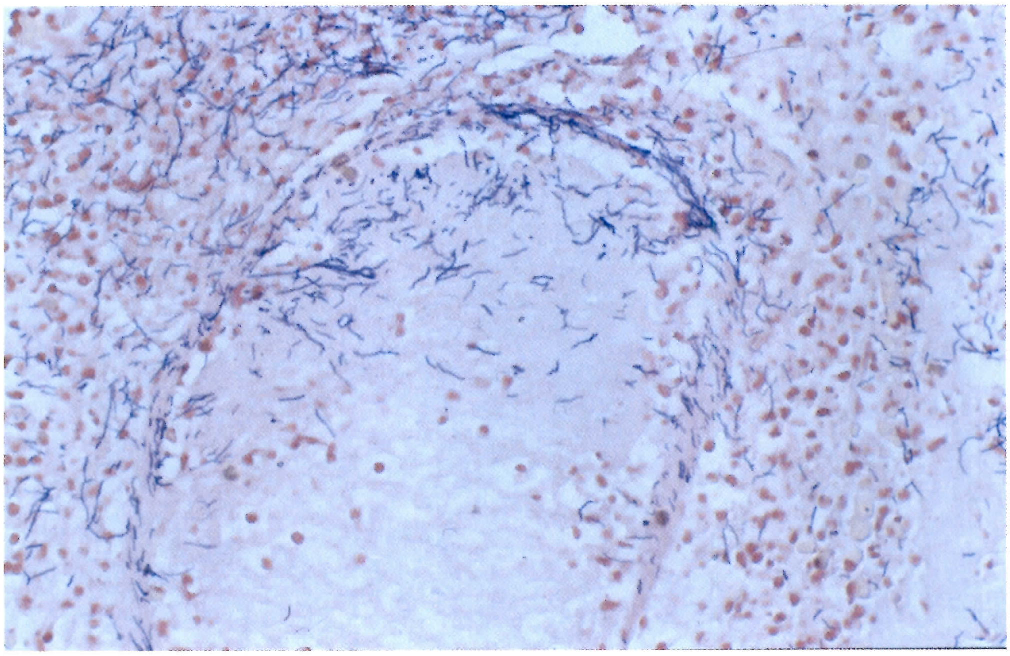

Figure 7: Nocardiosis. Bacteria growing in ramified colonies, thrombotic blood vessels, Gram stain; *Stenella coeruleoalba.*

Figure 8: Pneumothorax/Bullae: Bulla in the collapsed lung of a dolphin with pneumothorax, *Stenella coeruleoalba*, case 1.

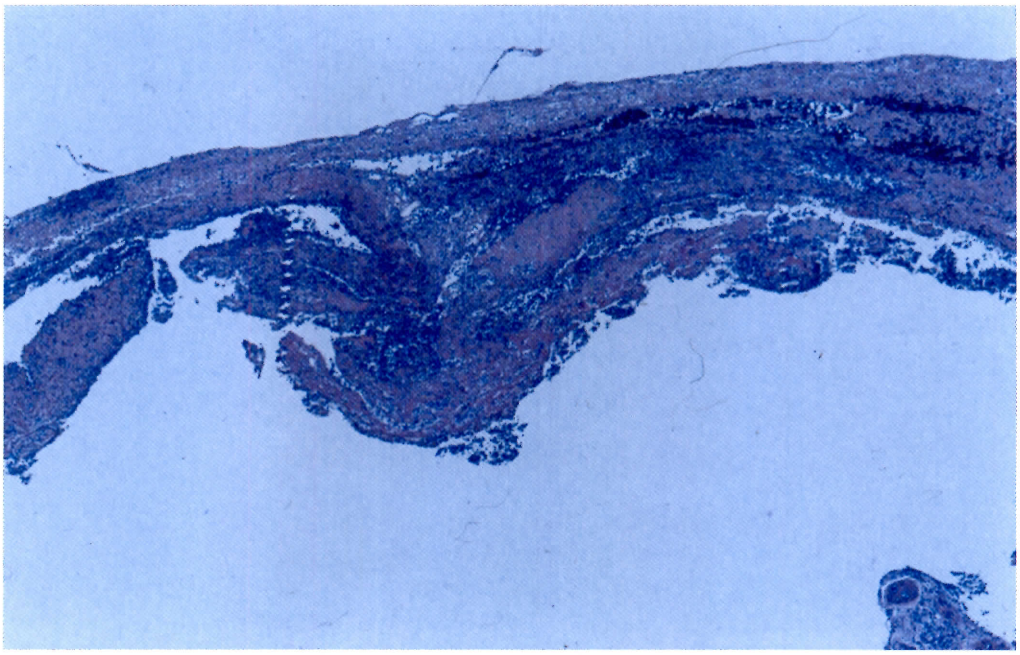

Figure 9: Pneumothorax/Bulla: Bulla of Fig. 1, fibrin layer, H/E stain, *Stenella coeruleoalba*, case 1.

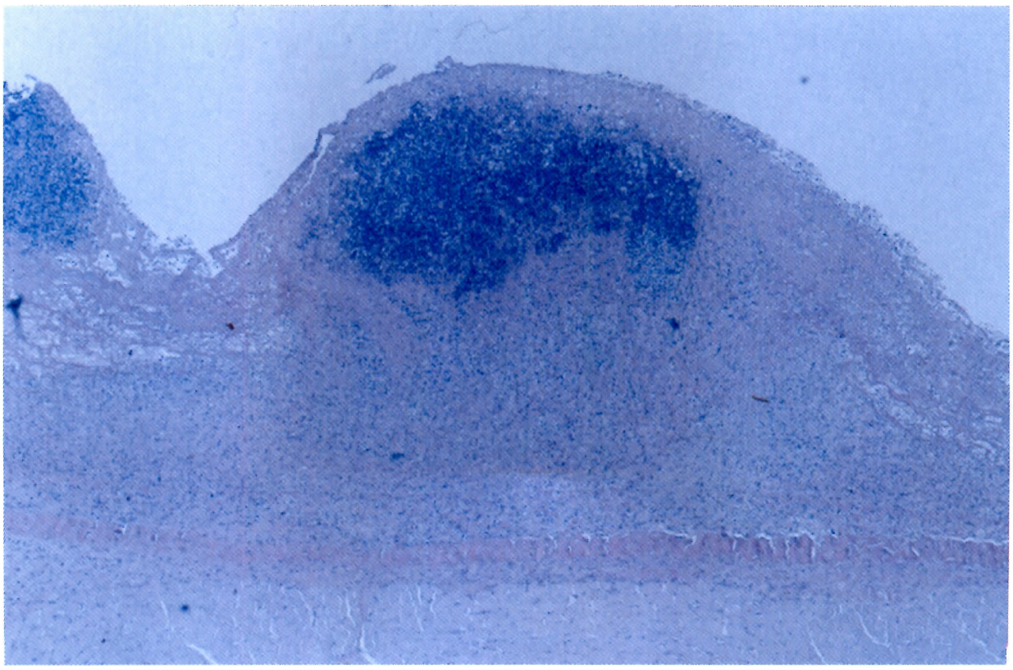

Figure 10: Pneumothorax. Pleura of the left hemithorax, chronic granulomatous mycotic pleuritis, H/E stain, *Stenella coeruleoalba*, case 1.

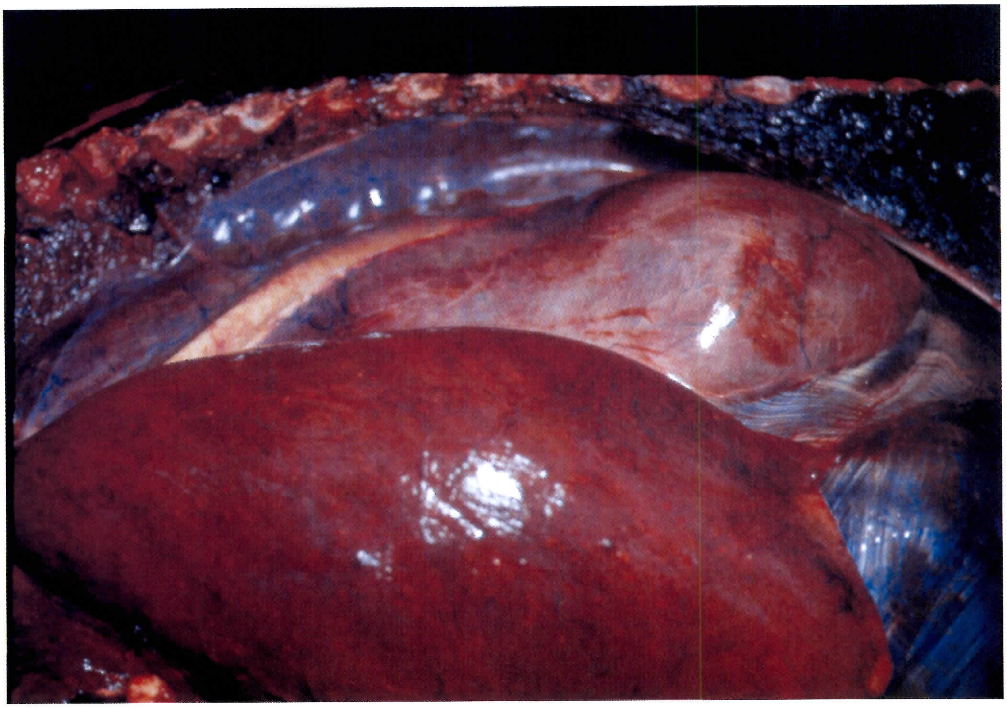

Figure 11: Pneumothorax. Displacement of the mediastinic wall, *Stenella coeruleoalba*, case 2.

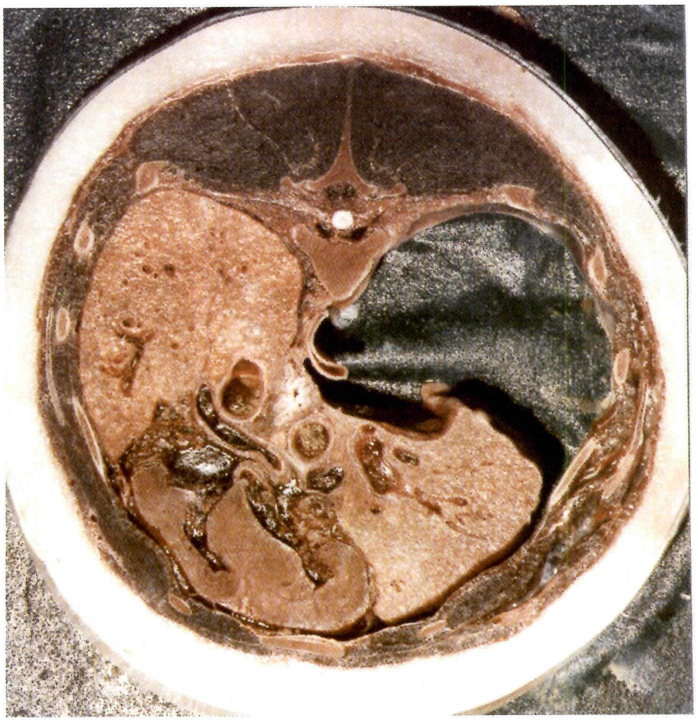

Figure 12: Pneumothorax. Cross section of the thorax, at height of the heart, *Phocoena phocoena*, case 3.

Figure 13: Pneumothorax. Cross section, at the height of cranial abdomen, *Phocoena phocoena*, case 3.

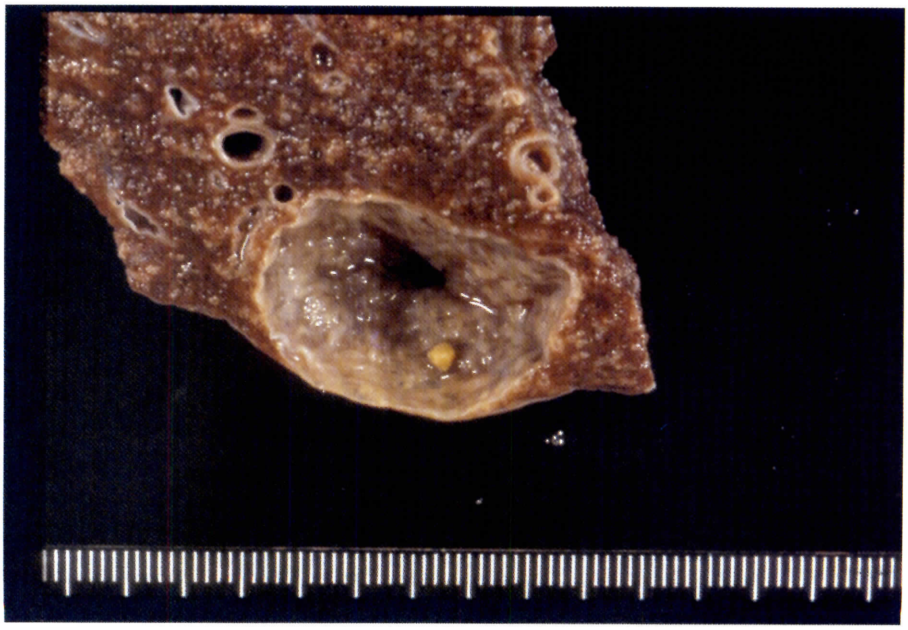

Figure 14: Pneumothorax/Bulla: Bulla in the lung of a dolphin without pneumothorax, formalin fixed, *Stenella coeruleoalba*, case 6.

The publication of these proceedings was sponsored by

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