Comparative histopathology of lungs from by-caught Atlantic white-sided dolphins (Leucopleurus acutus)

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Abstract
During 1993 and 1994, carcasses of by-caught dolphins and porpoises were brought ashore by the Dutch fishing fleet for scientific studies. The lungs of 44 Atlantic white-sided dolphins (Leucopleurus acutus), 2 common dolphins (Delphinus delphis) and 4 harbour porpoises (Phocoena phocoena) were examined histologically and results were compared with findings from drowning and asphyxia of other mammals and humans. The lungs of all dolphins and porpoises showed severe oedema within the alveolar spaces, rupture of alveolar walls and myosphincters of the bronchioli, combined with intraalveolar haemorrhages. Our investigations were supplemented with Gomori silver stain to demonstrate the fibre structure of the lungs. In all cases, the alveolar walls showed slight to maximal distension, with stretching of the capillaries and distinct fibre ruptures. Using the criteria of Reh (1969), who classified four stages of distension of the reticulum fibre in human lungs of drowned, we found the histological changes of the reticulum fibre structure in the dolphin lungs to be equivalent to stages two and three. Hence, our findings for known by-caught dolphins and porpoises coincided with the description of ‘atypical drowning lung’ in humans and other terrestrial mammals.

Key words: by-catch, Atlantic white-sided dolphins, Leucopleurus acutus, lung histology, Gomori silver stain

Introduction
Death caused by accidental entrapment in fishing gear (‘by-catch’) occurs world-wide in dolphins and porpoises and has drawn considerable attention due to its implications for species conservation (Perrin, 1991). The pathophysiology of drowning has been studied in humans, but veterinary literature on this subject is scanty (Schoon & Kikovic, 1989). Recent efforts to identify pathognomonic characteristics of death by accidental net entrapment in dolphins led to a list of potentially useful pathological criteria (Kuiken, 1996). These criteria, however, can only provide indirect proof, because most findings are of a general nature, e.g., non-pathognomonic (García Hartmann et al., 1996). In-depth studies on histological and pathomorphological changes in the lungs of known by-catches have not been reported to the knowledge of the authors.

Drowning in humans causes a series of complex pathophysiological and pathomorphological changes, which develop either during or following the process of drowning. In general pathomorphological changes depend on whether water enters the lung, so called ‘wet drowning’, or whether there is no water penetration, so called ‘dry drowning’ (Yagil et al., 1983). The different mechanisms leading to death should be clearly distinguished: death by ‘drowning’ describes the process of dying after aspiration of fluid, whereas death after laryngospasms or vagus caused cardiac arrest should be named as ‘death after submersion’ (Modell, 1978). In ‘wet drowning’, the fluid medium with all its components and contents, enters the air passages and is inhaled under pressure into the alveoli, where the fluid causes local damage, and rupture of the alveolar walls, as well as biochemical reactions in the adjacent lung tissue and other organs. Schwann & Spafford (1951) noted considerable differences between fresh-water and sea-water drowning. The latter brings about severe haemococoncentration and pulmonary oedema because water and secondary proteinaceous fluid stream from the blood into the alveolar spaces according to the osmotic gradient.

The aim of the present study is to compare the histopathological changes in the lungs of
known by-caught dolphins with findings in human drowning pathology.

**Materials and Methods**

During 1993 and 1994, carcasses of accidentally by-caught dolphins and porpoises were brought ashore by the Dutch fishing fleet for scientific examination. 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*). Additionally, lung tissue of 4 Atlantic white-sided dolphins were sampled directly onboard and preserved in 10% buffered formalin. All other carcasses were frozen onboard the ship and later thawed in water immediately prior to a complete dissection following the protocol of Kuiken & García Hartmann (1993).

For examination of the lung tissues, 5 samples were taken of each lung respectively: three samples from the central lung (from the deep region, from the thin ventral margin, and from the most dorsal convex lateral surface), as well as two samples from the cranial and the caudal regions of the lobe. These samples were fixed in 10% buffered formalin and stained according to standard methods (Haematoxilin-Eosin; AbPAS, van-Gieson, Weigert’s Elastica-stain). Additionally, the same samples were stained with the Gomori silver stain. Samples of four lungs were prepared for and examined by electronmicroscopy. One sample of gastric fluid of a live adult bottlenose dolphin (*Tursiops truncatus*) at Duisburg Zoo was gained by stomach tube 4 hours after feeding mackerel (*Scomber scombrus*); the fluid was fixed in 10% buffered formaline and the deposited particles were embedded in paraffin and processed with the same stains mentioned above.

**Results**

With conventional staining, the lungs of all dolphins and porpoises showed histologically severe, proteinaceous oedema and haemorrhage within the alveolar spaces and, to a lower degree, the interstitium (Fig. 1). The degree, localization and proteinaceous character of the oedema varied among individuals.

Distension of the alveoli, with ruptures of alveolar walls and of the myosphincters of the bronchioi, combined with intraalveolar haemorrhages and compression of capillaries by distension, were found regularly (Fig. 2). The degree of emphysema also varied between individuals, as well as among different localizations of each lung. Most ruptures were seen in the subpleura or located between the bronchiolar sphincter and alveoli.

Histology furthermore revealed prominent bronchioalveolar, interstitial and subpeural haemorrhages. The ruptures of bronchiolar sphincters often led to an important dilatation of the corresponding alveolar passages, creating large distended spaces filled with oedematous,  

![Figure 1. Severe oedema and haemorrhage, multiple ruptures of septae in a drowned Atlantic white-sided dolphin. Stain: H/E, magnification: 10 ×.](image-url)
eosinophilic fluid. Adjacent alveoli often were atelectatic.

Contents of the alveolar lumina
The lumina of the alveoli contained large amounts of eosinophilic and rather homogenous proteinaceous fluid (see Fig. 1). In the intraalveolar fluid, foreign particles were found in 14 cases in samples from both central, as well as subpleural areas. These loose particles varied in size, with some of them completely filling the alveolus; at least in ten animals these particles resembled the muscle tissue found in the gastric fluid sample taken from the live bottlenose dolphin, yet were in various stages of decomposition and/or digestion (Fig. 3). Furthermore, amorphous, often round, basophilic particles were found. Intraalveolar haemorrhage appeared in all samples, with large individual differences in amount, shape, and staining characteristics of the erythrocytes. Desquamated epithelial cells of the bronchioli and alveoli, eosinophilic and PAS-positive detritus and fragments of erythrocytes, mononuclear cells and occasional macrophages were seen in the alveolar spaces as well. Several multifocal histologically ‘empty’ spaces, partly confluent, consisted of cavities created by the emphysema.

Alveolar septae and blood vessels
In areas of alveolar oedema, interstitial oedema with considerable widening of the septa was also seen, with diapedesis of erythrocytes into the septal and perivascular connective tissue. This perivascular oedema and diapedesis often was accompanied by dilation of the lymphatic vessels. Mostly, the lumen of the veins was partially or completely collapsed.

The nuclei of epithelial, sepal, and endothelial cells showed karyorhexis and pycnosis, and these changes were interpreted as the beginning of hydropic necrosis of the lung parenchyma with extensive vacuolar degeneration of all compartments.

Bronchial tree and peribronchial tissues
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 epithelium of the bronchi, as well as submucosal and peribronchial connective tissue were oedematous. Disrupted bronchioli usually led to damage of the surrounding tissue as well, i.e. cavities of different sizes in the lung parenchyma.

Damage to the interstitial matrix of the lungs
The Gomori silver stain revealed significant damage to the reticulum fibre structure of the lungs. In all animals, stretching and thinning of the alveolar septae was observed of different grades, varying from slight distention to rupture (Figs 4–6). The alveolar walls showed slight to maximal distensions, leading to distended, threat-like capillaries. In some
cases, the capillaries had separated from the alveolar walls and layed parallel to the remains of the septum, and their oval lumina were reduced in size. Distinct reticular fibre ruptures were observed in both oedematous and emphysematous areas; in the emphysematous parts, usually the damage to the reticulum fibres of the entire septum was uniformly distributed.

**Ultrastructure (electronmicroscopy)**

The epithelia showed rough surfaces with irregular, partially thin, finger-like protrusions. In emphysematous areas, the septae were thinned by traction, with capillaries being completely occluded (Fig. 7). Nuclei of such septae often were stretched. The knobs (terminal ends) of the septae contained capillaries with unobstructed lumina. A number of

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**Figure 3.** Fragments of striated muscle in the oedematous alveolar fluid, with rupture of alveolar walls and loss of general lung structure in an Atlantic white-sided dolphin. Stain: H/E, magnification: 400 ×.

**Figure 4.** Alveoli completely filled with fluid, only little air remains in the alveolar spaces. Some septae were stretched-out and thinned, some ruptured, while general structure was still present in an Atlantic white-sided dolphin. Stain: Gomori silver, magnification: 100 ×.
Figure 5. Detail of similar finding as in Figure 4 with a selective stain; septae are stretched-out and thinned, some remains of fibre ruptures are visible. In the right lower-corner a collapsed vein can be distinguished in an Atlantic white-sided dolphin. Stain: Gomori silver, magnification: 200 ×.

Figure 6. The alveolar lumen was mainly filled with air, septae were severely stretched-out and ruptured, corresponding to class 3 according to the classification of Reh (1969). Veins were collapsed in this Atlantic white-sided dolphin. Stain: Gomori silver, magnification: 100 ×.
cell nuclei, devoid of any cytoplasm, were seen intravascularly.

*Shape and staining of erythrocytes*

The material obtained from those 4 by-caught dolphins of which lung material was sampled and stored in buffered formaline, differed from temporarily frozen material. The most obvious changes were seen in the erythrocytes, which stained better in non-frozen material and showed a larger variety of shapes, deformations as well as erythrocyte sludges.

*Other histological findings*

Independent of any pathomorphological changes caused by or associated with the death of these animals, in 25 cases a purulent bronchopneumonia was found (Fig. 8). In 5 cases, this histological finding clearly was associated with parasitic infestation of the lung; in the other cases, the origin of the inflammatory reaction could not be determined histologically.

**Discussion**

*Drowning vs. suffocation*

Based on the macroscopic absence of water in the lungs of by-caught cetaceans and a variety of other pathological findings, there has been some discussion in the past whether by-caught odontocetes actually drown or suffocate, e.g., die without inhaling water into the lungs (for details see García Hartmann et al., 1996).

All dolphins examined in this study showed clear histological changes in the lung: the distention and rupture of reticular fibres, which coincided with the 'atypical drowning lung' of Reh (1969), and the other histological and ultrastructural findings suggest that animals of this study drowned, rather than suffocated.

Similar to the mentioned scientific discussion in dolphins, the diagnosis of drowning has long been controversial also in human forensic pathology (Brinkmann et al., 1983) and in lack of clear pathognomonic criteria, some authors consider that only a summary of typical findings is available (Giertsen, 1977). In human pathology, the amount of water in the lungs is not regarded to be a significant criterion of drowning (Pearn, 1985), especially because of the existence of so called 'dry drowning': in a drowning situation, laryngospasm may prevent 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 human drownings (Plueckhahn, 1984; Rivers et al., 1970). It is equally known to occur in other mammals, as for instance Gilbert & Gotfon (1982) reported that not all beavers (*Castor fiber*) which were experimentally drowned inhaled water.
Typical findings in human drowning are severe acute vesicular emphysema, often combined with interstitial and alveolar haemorrhage (Janssen, 1977). This ‘emphysema aquosum’ caused by drowning is described as causing an acute distension of the alveoli, stretching of the septae, and compression of the septal capillaries (Müller, 1975; Reh, 1966, 1969; Janssen, 1977). According to extensive experimental and histological studies of Reh (1969), the structure and damage to the reticular fibres of the lungs can be differentiated into 4 categories. All potential differential diagnosis given by Reh (1969) e.g., diseases causing similar histological damages, such as aspiration of blood, bronchitis, and asthmatic death were highly unlikely in the dolphins and porpoises of this study which were retrieved from fishing nets. The animals in this study showed histological changes ranging from distension to rupture of the reticular fibres, which coincide with category 2 and 3 of the classification of the ‘atypical drowning lung’ according to the description of Reh (1969). It seems possible that category 4 was not encountered because the dolphin lung is much stronger and at the same time more elastic compared to humans and other terrestrial mammals mainly due to its strong pulmonary pleura and enhanced cartilage support (Berlanger, 1940; Green, 1972; Kooyman & Sinnet, 1979).

The ‘typical drowning lung’ in humans, also described as ‘ballooning lung’, was not found in the odontocetes. We believe that this could be caused by a major difference between humans and dolphins in the drowning process. Drowning humans often have prolonged periods of drowning with repeated breathing at the surface, as well as swallowing and inhalation of water, while the entangled dolphins of this study died in nets set at depths (≥30 m and therewith had no access to air at the surface).

The other histological characteristics found, like erythrocyte sludge, intraepithelial vacuoles, a variable epithelial surface and densification of the matrix, have been reported by Brinkmann et al. (1983) in rat lungs which were in contact with a hypertonic fluid medium like salt water. All characteristics described by this author were found in the lungs of the drowned dolphins of this study, being more distinctive in the lungs which had not been frozen. The electronmicroscopic findings coincide with the ones reported in drowned rats by Brinkmann & Buthenuth (1982) and in humans by Reidbolz & Spitz (1966) and Noyanitaya et al. (1974).

Lung-penetrating drowning fluid causes typical osmotic and hydrostatic changes in lung tissues and other organs. In saltwater, severe proteinaceous oedema in alveolar spaces is characteristic (Tonner, 1971). Therefore, if by-caught dolphins drown, the diagnosis of by-catch can be established by the presence of typical intra vitam reactions, e.g., signs of respiration and haemodynamic changes. In all animals, proteinaceous oedema, as well as dilated veins and lymphatic vessels were observed in various other organs and tissues (not shown here). Those ‘vital reactions’ and several cases of foreign bodies within intraalveolar spaces remarkably
In at least 10 animals, muscle particles in various stages of decomposition/digestion were part of the alveolar content. A sample of stomach fluid of a live animal confirmed the histomorphological resemblance of these muscle particles with semi-digested fish muscle. With no other explanation available, we assumed that these muscle structures constituted partially digested fish originating from the first stomach compartment, e.g., evidence for regurgitation. In humans, it is well known that vomiting occurs during drowning, with the possibility of small particles of stomach contents becoming trapped in the terminal alveoli (Müller, 1975).

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Diagnostic use of the Gomori silver stain
The Gomori silver stain used, which specifically stains reticular fibres, proved to be useful for detecting specific histological changes of the lung in dolphins which we considered to be most likely caused by drowning. We propose the use of the classification by Reh (1969) for description and quantification of these changes. Therefore, the Gomori stain potentially is useful to determine whether a stranded carcass is, in fact, an animal which has drowned. Because reticular fibres are relatively resistant to decomposition, this method might provide evidence of reticular changes even for carcasses in advances stages of decomposition, e.g., condition codes 2 and 3 according to the European Cetacean Society (ECS) standard dissection protocol for small cetaceans (Kuiken & García Hartmann, 1993).

Before issuing a definitive diagnosis, however, it should be considered that drowning also could be the immediate cause of death in all odontocetes which are severely weakened or have an impairment in movement. A complete and detailed dissection could be necessary to rule-out other causes of drowning (Kuiken & García Hartmann, 1993).

Other diagnostic criteria for by-catch
In the past, the (increased) presence of diatoms in the lungs has been associated by some authors with death by drowning (Auer, 1991), but in the literature ‘the use of diatoms as a diagnostic tool in drowning cases is a confusing and contradictory subject’ (Peabody, 1980). Varying amounts of diatoms in drowned, as well as non-drowned humans, has led to the assumption that diatoms may not be a reliable indicator for death by drowning (Foged, 1983; Reh, 1969, Spitz & Schneider, 1961). In the present study, a limited number of diatoms was discovered histologically, but no attempt of quantification or identification was made.

Other criteria of by-catch, such as the examination of bronchial fluid for chemical or protein markers (García Hartmann et al., 1996) or biological markers originating from the sea water (Larsen & Holm, 1996) and the pathological findings mentioned in Kuiken (1996), are all potentially useful, but do not give reliable results in all cases and are subject to the state of decomposition of the carcass.

Other findings
Next to the findings associated with or caused by the accidental entrapment in fishing gear, the high percentage of bronchopneumonias in the dolphins and porpoises examined is worth noting. In only five of 25 cases of pneumonia could parasitic lung infestation, a potential cause of bronchopneumonia, be confirmed by histology. Similarly, during the gross pathological examination of the dolphins, the occurrence of visible lung parasites was infrequent. It is assumed that at least in some cases, a bacterial (or combined bacterio-parasitic) infection was the cause of the bronchopneumonia. This finding warrants further bacteriological and parasitological investigation of the lungs of by-caught odontocetes in the future, as potentially the ‘by-catch associated’ changes observed in the lungs of by-caught dolphins and porpoises could mask subtle signs of infection and infestation.

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