

# POST-MORTEM FINDINGS IN BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) IN THE SLOVENE SEA

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**Summary:** Between 1996 and 2012, a total of eleven bottlenose dolphins (*Tursiops truncatus*) were found dead in the Slovene sea. Only nine animals were dissected, since two were unsuitable for further examination due to severe post-mortal decomposition. Of the dissected dolphins, six were female and three were male; seven animals were adults, one subadult and one was a calf. At the dissection, pathological lesions in the lung prevailed; we established verminous pneumonia (N=5), lung worms in the bronchi (N=3), and multifocal nodular calcifications, probably calcified parasitic nodules (N=2). Among other pathological lesions, erosive and ulcerative oesophagitis (N=3); ulcerative gastritis (N=2); cutting injuries, excoriations and cicatrizations of the skin (N=2) were the most numerous. The most common histopathological lesions were also associated with parasites; we diagnosed parasitic nodules (N=3), verminous pneumonia (N=3) and bronchitis (N=1), and parasites in the lumina of bronchi (N=1). In six cases, parasitological examination revealed a presence of lung worms of the *Metastrongyloidea* superfamily.

We concluded that five bottlenose dolphins died of verminous pneumonia caused by nematodes of the order *Strongylida*, superfamily *Metastrongyloidea*; one animal died of chronic ulcerative oesophagitis and gastritis; one died after having been caught in a fishing net; one died due to endoparasitosis and emaciation, whereas the exact cause of death of one bottlenose dolphin could not be established due to severe post-mortal decomposition.

**Key words:** bottlenose dolphin; *Tursiops truncatus*; pathology; verminous pneumonia; Slovene sea

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## Introduction

The Slovene sea is the northernmost part of the Adriatic Sea with a surface area of 180 km<sup>2</sup>, the average depth of 17 metres (1) and the deepest point – the Piran Punta – of 37.25 metres (3). The entire Slovene coast is 46.6 km long, and is composed of Eocene sedimentary flysch (1,4). The Slovene sea is shallow, it has a relatively high average sea temperature (15.8°C) (1), average

salinity between 37 and 38‰ (2), average oxygen concentration between 6 mg/l and 9 mg/l (3) and a great amount of nutritive substances introduced by several large and small rivers, making it a rich habitat for numerous marine organisms (2).

Dolphins (*Delphinidae*) are the most numerous and diverse family of marine mammals, belonging to the order *Cetacea* and suborder *Odontoceti*. There are 36 genera in the *Delphinidae* family, of which several genera live in the Mediterranean Sea (5). Only one dolphin species, the bottlenose dolphin (*Tursiops truncatus*, Montagu 1821), lives in the Slovene sea (6-8), where one hundred

and one dolphins living in groups of up to 43 individuals, have recently been identified. Annual mark-recapture density estimates 0.069 dolphins/km<sup>2</sup> (8). Other species of cetaceans, which are considered occasional in the region, are fin whale (*Balaenoptera physalus*), Risso's dolphin (*Grampus girseus*) and the striped dolphin (*Stenella coeruleoalba*) (9).

Bottlenose dolphins are prone to numerous infectious diseases. The most common are parasitic invasions, mostly in gastrointestinal and respiratory tracts, followed by bacterial, viral and fungal infections (10-16). Non-infectious diseases rarely occur: poisoning with biotoxins, sporadic neoplasia and congenital defects, organic disorders, mechanical injuries and suffocations have been reported (11, 16-17).

Between 1996 and 2012, eleven bottlenose dolphins were found dead in the Slovene sea. This study is the first to present the pathomorphological, bacteriological and parasitological findings discovered at the dissection of bottlenose dolphins found in the Slovene sea.

## Materials and methods

### *Anamnestic data*

Between October 1996 and August 2012, eleven bottlenose dolphins (*Tursiops truncatus*) were submitted for necropsy to the Institute of Pathology, Forensic and Administrative Veterinary Medicine at the Veterinary Faculty, University of Ljubljana. Five bottlenose dolphins had stranded on the coast of the Slovene sea, five were found dead in the open sea, one had been caught in a fishing net and managed to escape, but afterwards died in shallow water. Only nine animals were dissected - two carcasses were unsuitable for

further examination due to severe post-mortal decomposition.

Of the nine dissected bottlenose dolphins, six were female and three were male. Seven of them were adults, one was subadult and one was a calf. One dolphin (case 6) was estimated to be 15 years old (18, 19). There are no exact data about the age of other dolphins. Four animals died in autumn, two in winter, two in summer and one in spring.

Data on sex, age and body weight of dissected animals, as well as the month and year of death and the site of their finding, are listed in Table 1.

### *Histopathological, bacteriological and parasitological examination*

During the dissection, samples were taken for histopathological, bacteriological and parasitological examinations.

Samples for histopathological examination were fixed in 10% buffered formalin and embedded in paraffin. Four- $\mu$ m-thick tissue sections were deparaffinised, stained with hematoxylin and eosin (HE) and examined under a light microscope.

Samples of the lung and intestine were taken for parasitological examination, and samples from several parenchymatous organs were taken for bacteriological examination. The parasitological and bacteriological examinations were performed at the Institute for Microbiology and Parasitology at the Veterinary Faculty in Ljubljana.

## Results

### *Gross pathology*

In all the examined bottlenose dolphins, pathological lesions in the lung prevailed: in five animals (cases 1- 5), multifocal verminous

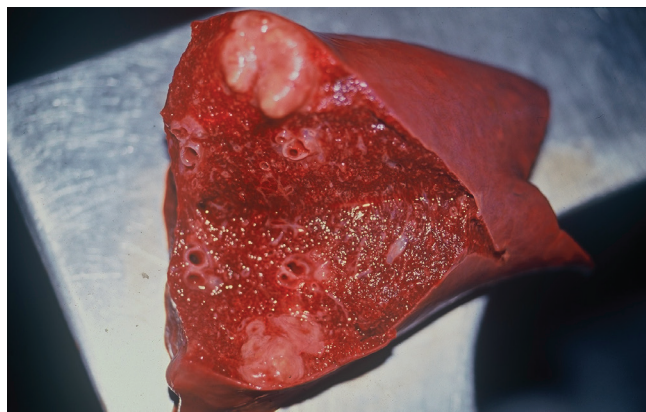
**Table 1:** Characteristics of dissected bottlenose dolphins

Case	Dolphin sex, body length and age	Month and year of death	Site of finding
1	♂, approximately 300 kg	October 1996	Izola, Slovenia
2	♀, 226 cm	May 2001	Izola (Cape Ronek), Slovenia
3	♀, 297 cm	September 2002	Izola, Slovenia
4	♂, calf, 191 cm	January 2003	Strunjan, Slovenia
5	♀, 255 cm	October 2004	Piran, Slovenia
6	♀, 265 cm, 15 years (18, 19)	January 2005	Izola (White Rocks), Slovenia
7	♀, 265 cm	August 2007	Piran, Slovenia
8	♀, 272 cm	October 2008	Piran, Slovenia
9	♂, 297 cm	July 2011	Piran, Slovenia

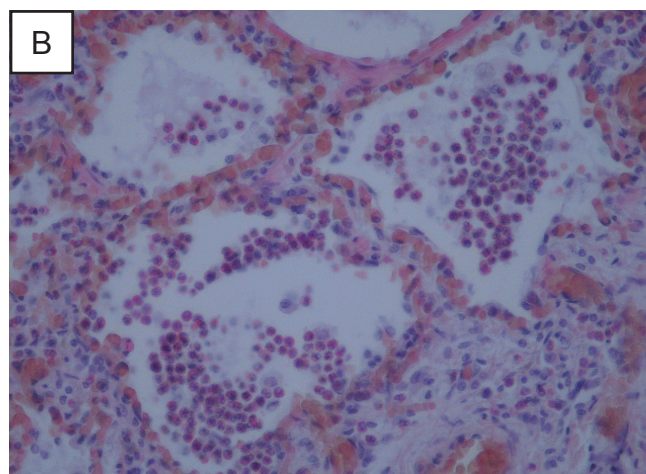
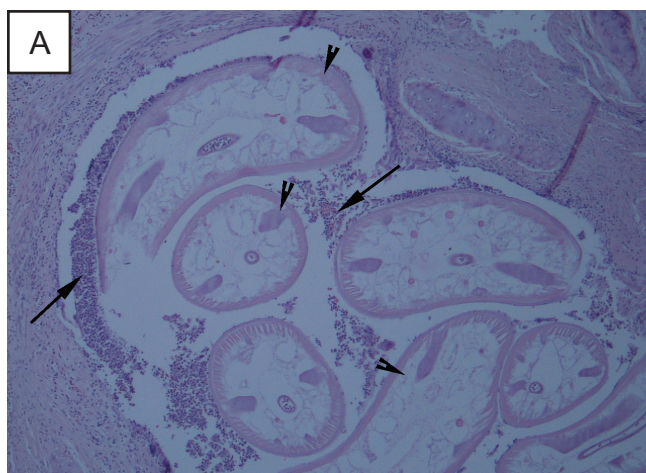
pneumonia was detected (Fig. 1) and in two (cases 6 and 7), multifocal nodular calcifications, probably calcified parasitic nodules, were found. In the bronchi of three bottlenose dolphins (cases 2, 7 and 9), numerous or a few lung worms were found. In case number 7, numerous thread-like parasites were detected in the small intestine and in case number 3, parasitic nodules were also found in the stomach wall. In eight cases, a dilatation of the right or both ventricular chambers was determined. Transudate was found in body cavities of five bottlenose dolphins. In one animal, erosive and ulcerative stomatitis was established; in three cases erosive and ulcerative oesophagitis; in two, ulcerative gastritis; and in two cases cutting injuries, excoriations and cicatrizations of the skin, mostly in the region of fins, were detected. Lung adhesions were noted in one animal. The necropsy findings are presented in Table 2.

#### *Histopathological examination*

The most common histopathological lesion was parasitic bronchopneumonia. Numerous adult nematodes were found in the bronchi of two bottlenose dolphins (cases 2 and 3) (Fig. 2A). In case number 3, a few desquamated epithelial cells and numerous degenerated neutrophil granulocytes were found in the bronchial lumina. There was significant thickening of the bronchial walls, with formation of granulation tissue, infiltrated with several eosinophil granulocytes, macrophages and lymphocytes. The infection spread through the interstitium into the walls of the surrounding alveoli. In the bronchial lumina, calcified adult parasites and amorphous calcifications were found; several small calcifications were observed also in the propria of these bronchi. In the lung parenchyma of two bottlenose dolphins (cases 1 and 5), there were multifocal parasitic nodules of various sizes, with adult parasites in the centre surrounded by a fibrous capsule; in two bottlenose dolphins (cases 6 and 7), round calcified nodules were found in the lungs and were most likely of the same aetiology. In case 4, nematodes in the lung parenchyma caused severe pyogranulomatous inflammation with abundant proliferation of the fibrous tissue, dense infiltration of eosinophil granulocytes, macrophages and lymphocytes in the alveolar walls and their lumina and extensive hyperplasia of the bronchus-associated lymphoid



**Figure 1:** Verminous pneumonia in a bottlenose dolphin (case 4): greyish-white multifocal granulomas at the cut surface of the lung



**Figure 2:** Verminous pneumonia in a bottlenose dolphin. A: Case 3. Numerous cross-sections of adult nematodes (arrowheads) and desquamated epithelial cells (arrows) in the bronchial lumina. HE staining, x 40; B: Case 4: Dense eosinophilic infiltrate in alveolar lumina and walls, thickened due to fibrous tissue proliferation. HE staining, x 200

**Table 2:** Results of the necropsy and additional examinations of dissected bottlenose dolphins

Case	Necropsy findings	Findings of histopathological examination	Findings of parasitological examination	Findings of bacteriological examination	Cause of death
1	chronic multifocal verminous pneumonia, focal adhesion of the left pulmonary lobe with pleura	encapsulated and incalcinated parasitic nodules in the lung; other organs were unsuitable for examination due to autolysis	lung: lung worms of the <i>Metastrongyloidea</i> superfamily	intestine: <i>E. coli</i> , <i>Pleisiomonas shigelloides</i> ; spleen, liver, kidney and lung: negative	verminous pneumonia
2	chronic multifocal verminous pneumonia; obstruction of bronchi with numerous lung nematodes; hydrothorax; severe dilatation of ventricular chambers	parasites in the lumina of bronchi; other organs were unsuitable for examination due to autolysis	lung: lung worms of the <i>Metastrongyloidea</i> superfamily; intestine: negative	brain, lung, liver, spleen, kidney: unspecific microflora (contaminants); intestine: <i>Clostridium perfringens</i>	verminous pneumonia
3	severe pulmonary oedema and emphysema, severe dilatation of the right ventricle chamber, congestion of the lung and liver, multifocal verminous pneumonia, parasitic nodules in the stomach wall, erosive and ulcerative stomatitis, a cutting injury on the left pectoral fin and several smaller cuts and cicatrices of the skin	congestion of the liver, disseminated myocardial fibrosis, chronic verminous bronchitis, chronic hyperplastic gastritis and a parasitic nodule in the stomach wall; chronic plasmacytic enteritis	lung: lung worms of the <i>Metastrongyloidea</i> superfamily; intestine and liver: negative	intestine: undetermined pleomorphic gram-negative rod-shaped bacteria; liver, spleen and kidney: sterile; lung: numerous undetermined pleomorphic gram-negative rod-shaped bacteria	suffocation
4	chronic multifocal verminous pneumonia; dilatation of ventricular chambers; cutting injury on the right side of the lower jaw and bruises on the dorsal and caudal fin	pyogranulomatous verminous pneumonia, congestion of the parenchymatous organs	lung: negative	intestine, spleen, lung and kidney: negative	verminous pneumonia
5	chronic multifocal verminous pneumonia, pulmonary congestion and oedema, severe dilatation of ventricular chambers, hydrothorax, erosive and ulcerative oesophagitis, incapsulated abscess in the subcutis of the abdominal region	incapsulated and partially incalcinated parasitic nodule in the lung, numerous calcifications in the lung, alveolar pneumonia, incapsulated abscess in the subcutis; other organs were unsuitable for examination due to autolysis	lung: lung worms of the <i>Metastrongyloidea</i> superfamily; intestine: negative	lung: negative; liver and spleen: <i>Pleisiomonas shigelloides</i> (single colonies), intestine: <i>Pleisiomonas shigelloides</i>	verminous pneumonia
6	small multifocal calcifications, congestion and pulmonary oedema, severe dilatation of the right ventricle chamber, ascites, hemorrhagic oedema in the subcutis of the right pectoral fin	multifocal calcifications in the lung; other organs were unsuitable for examination due to autolysis	lung and intestine: negative	intestine and peritoneal smear: negative; spleen: <i>Enterococcus faecalis</i>	exact cause of death could not be determined due to a severe autolysis of the body
7	emaciation, severe enteroparasitosis, multifocal nodular calcifications, congestion and pulmonary oedema, thread-like parasites in the bronchi, congestion of the tracheal mucosa, severe dilatation of ventricular chambers, hydrothorax, ascites, liver congestion, erosive and ulcerative oesophagitis numerous thread-like parasites in the intestine	focal verminous pneumonia and disseminated calcified nodules in the lung, nephrocalcinosis	lung: lung worms of the <i>Metastrongyloidea</i> superfamily; intestine: thread-like unidentified parasites	liver, kidney, lung and spleen: negative	verminous pneumonia
8	chronic multifocal ulcerative oesophagitis and gastritis, pulmonary oedema, hydrothorax, ascites, dilatation of ventricular chambers	two chronic gastric ulcers and gastritis, nephrocalcinosis, other organs were unsuitable for examination due to autolysis	lung and intestine: negative	liver, kidney, lung: <i>Enterococcus faecalis</i> , <i>E. coli</i> , <i>proteus</i> sp.; intestine: <i>E. coli</i> , <i>Enterococcus</i> sp.; spleen: negative	chronic ulcerative gastritis and oesophagitis
9	emaciation, erosive gastritis, thread-like parasites in the bronchi, lung adhesions, severe dilatation of ventricular chambers	organs were unsuitable for examination due to autolysis	lung: lung worms of the <i>Metastrongyloidea</i> superfamily; intestine: undetermined trematode eggs	organs were unsuitable for examination due to autolysis	endoparasitosis emaciation

tissue (BALT) (Fig. 2B). The pleura was diffusely thickened due to proliferation of the fibrous tissue and infiltration of inflammatory cells.

Uteri of many adult parasites from the bronchi and lung parenchyma were filled with embryonated eggs and larvae. According to their morphological characteristics, the parasites were classified into the superfamily *Metastrongyloidea* and order *Strongylida*.

In case 3, chronic hyperplastic gastritis and a parasitic knot in the stomach wall were established. In case 8, two chronic gastric ulcers and chronic nephrocalcinosis were diagnosed. In case 4, besides pneumonia, congestion of all other organs was established.

All other organs were severely autolytic and therefore unsuitable for histopathological examination.

The results of the histopathological examination are presented in Table 2.

#### *Parasitological examination*

In six cases (cases 1, 2, 3, 5, 7 and 9), parasitological examination established a presence of lung worms of the *Metastrongyloidea* superfamily. A more exact determination was not possible due to extensive post-mortal changes. In three cases (cases 4, 6 and 8), the parasitological examination of the lungs was negative. In cases 3, 5, 6 and 8, the parasitological examination of the intestine was negative. In case 7, determination of intestinal parasites was not possible due to severe post-mortal decomposition of the parasites. The results of the parasitological examination are presented in Table 2.

#### *Bacteriological examination*

Bacteriological examination was performed in eight cases. Organs of case 9 were unsuitable for examination due to autolysis. The results are presented in Table 2. Single bacterial colonies of *Plesiomonas shigelloides* were isolated in the intestines of cases 1 and 5 and in the liver and spleen of case 5. Numerous undetermined pleomorphic gram-negative rod-shaped bacteria were isolated in case 3. All other organs were negative or overgrown with putrefactive microflora.

On the basis of the dissection and the results of additional examinations we conclude that five

bottlenose dolphins died of verminous pneumonia, one bottlenose dolphin, which had been caught in a fishing net, died of suffocation, one died of chronic ulcerative oesophagitis and gastritis, one died due to endoparasitosis and emaciation, whereas the exact cause of death of one bottlenose dolphin could not be established due to severe post-mortal decomposition.

## Discussion

Only sporadic deaths of the bottlenose dolphins from the Slovene sea were recorded during our study: in 1996 and between 2001 and 2012 (except in 2006, 2009, 2010 and 2012) one dolphin per year was found dead. The annual average of stranded bottlenose dolphins in the Croatian Adriatic is 13.1 animals (20). Croatian scientists established an increased death rate among bottlenose dolphins in summer and autumn (20). Most deaths among dolphins in the Slovene sea also occurred in autumn, but the sample size was too small to draw any conclusions. Seven of nine bottlenose dolphins found in the Slovene sea were adult animals; however, we do not have the exact information about their age, except in case 6 (18, 19). The majority of bottlenose dolphins found on the Croatian coast were between six and seven years old (20).

The most common cause of death of the examined bottlenose dolphins was verminous pneumonia. Due to strong post-mortal decomposition, we could only partially determine the parasites, which were then classified into the superfamily *Metastrongyloidea*. The genera *Halocercus*, *Pharurus*, *Stenurus* and *Pseudalius* of the *Metastrongyloidea* superfamily often cause bronchitis and pneumonia in bottlenose dolphins (11). Italian researchers determined chronic bronchopneumonia caused by *Halocercus* sp. parasites in 85.7% of cases of death of bottlenose dolphins (13). *Halocercus lagenorhynchi* parasites were found in the lungs of numerous bottlenose dolphins with verminous pneumonia and bronchiolitis in Florida (10); Dailey *et al.* (21) found these parasites even in the lung abscess of dead new-born bottlenose dolphins.

One dolphin most probably died of chronic ulcerative oesophagitis and gastritis. Reidarson (11) reported that *Anisakis*, *Contracaecum* and *Pseudoterranova* nematodes, which inhabit the

gastrointestinal tract, predominantly cause gastritis and ulcerations, although in our case the parasitological examination yielded negative results.

Two of the examined animals had numerous skin injuries, which were most likely caused during their attempt to escape from fishing nets. Gomerčič *et al.* (16) established in seven out of 17 dissected bottlenose dolphins that they died as a consequence of entanglement in fishing nets.

In the intestines of two bottlenose dolphins and in the liver and spleen of one, single colonies of the bacterium *Plesiomonas shigelloides* were isolated. *Plesiomonas shigelloides* is widely spread in the aquatic environment (22) and causes several diseases in animals and humans with a weakened immune system (23, 24). We could find no reference in literature that the isolated bacterium could cause any diseases in bottlenose dolphins.

Establishing the cause of death of bottlenose dolphins is hindered or even prevented by strong post-mortem decomposition of their bodies, because there is usually a substantial time lapse between the actual death and the stranding of the body. The most common cause of death of bottlenose dolphins found in the Slovene sea was verminous pneumonia; however, skin lesions are also very common and are most likely the consequence of entanglement in fishing nets. We found no bacterial or fungal diseases or neoplasia in the examined bottlenose dolphins.

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## PATOMORFOLOŠKE UGOTOVITVE PRI POGINULIH VELIKIH PLISKAVKAH (*TURSIOPS TRUNCATUS*) IZ SLOVENSKEGA MORJA

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**Povzetek:** Delfini (*Delphinidae*) so najštevilčnejša in najbolj raznolika družina morskih sesalcev. Spadajo v red Cetacea, podred Odontoceti. V družini je 36 rodov, ki naseljujejo vse oceane planeta. Številne rodove najdemo tudi v evropskih morjih, vendar le ena vrsta iz rodu *Tursiops*, velika pliskavka (*Tursiops truncatus*, Montagu 1821), živi v slovenskem morju, kjer so našli sto eno žival. Zaradi svoje lege, plitkosti in stalnih dotokov celinskih voda predstavlja slovensko morje zelo specifično in bogato, vendar tudi zahtevno življenjsko okolje, zaznamovano z velikimi nihanji temperature, slanosti in vsebnosti kisika ter največjim plimovanjem v Jadranu.

Med letoma 1996 in 2012 smo v slovenskem morju našli enajst poginulih delfinov vrste velika pliskavka (*Tursiops truncatus*). Raztelesili smo jih devet, saj sta bili dve zaradi močne posmrtno razpadlosti neprimerni za preiskave. Šest pliskavk je bilo ženskega spola, tri pa moškega. Sedem pliskavk je bilo odraslih, ena je bila juvenilna, en pa je bil mladič. Pri vseh pliskavkah so prevladovala patološke spremembe na pljučih: diagnosticirali smo verminozno pljučnico (N=5), pljučne črve v bronhih (N=3) in multifokalne vozličaste poapnitve, najverjetneje poapnele parazitne vozličke (N=2). Pogosto smo ugotovili tudi erozivno in ulcerativno vnetje sluznice požiralnika (N=3) in ulcerativno vnetje sluznice želodca (N=2) ter vreznine, odrgnine in brazgotine po koži (N=2). Najpogostejše patohistološke spremembe so bile prav tako povezane s pljučnimi paraziti: ugotovili smo parazitne vozličke (N=3), verminozno vnetje pljuč (N=5) in bronhov (N=1) ter zajedavce v svetlini bronhov (N=1). S parazitološko preiskavo smo pri šestih pliskavkah našli pljučne črve iz naddružine *Metastrongyloidea*.

Na osnovi opravljenih preiskav smo ugotovili, da je pet pliskavk poginilo zaradi verminozne pljučnice, ki so jo povzročili nematodi iz reda *Strongylida*, naddružina *Metastrongyloidea*, po ena pliskavka pa je poginila zaradi kroničnega vnetja sluznice požiralnika in želodca, zaradi zapleta v ribiško mrežo ter zaradi endoparazitoze in posledične shujšanosti. Zaradi močne posmrtno razpadlosti trupla ene pliskavke nismo mogli ugotoviti vzroka njenega pogina.

**Ključne besede:** velika pliskavka; *Tursiops truncatus*; patologija; verminozna pljučnica; slovensko morje