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Spontaneous neoplasms in harbour porpoises *Phocoena phocoena*

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ABSTRACT: Harbour porpoises are widely distributed in the North Atlantic and represent the most abundant cetacean species in the North and Baltic Seas. Spontaneous neoplasms are relatively rarely reported in cetaceans, and only little is known about neoplasia in harbour porpoises. Thus, archival material was reviewed for spontaneous neoplasms in harbour porpoises recorded during post-mortem examinations between 1999 and 2018. Neoplasms were identified in 7 adult porpoises: 6 animals originating from the North and Baltic Seas and investigated as part of German and Dutch systematic health monitoring programs, and 1 porpoise from Greenlandic waters. The tumours were of different histogenetic origins and further characterised by histology and immunohistochemistry. One individual had a neoplasia in the digestive tract (adenocarcinoma, n = 1); 4 animals, in the genital tract (Sertoli cell tumour, n = 1; genital leiomyoma/fibroleiomyoma, n = 3); and 2 porpoises, in endocrine organs (adrenal adenoma, n = 2). This is the first report of an adenocarcinoma in the liver, a testicular Sertoli cell tumour and adrenocortical adenomas in harbour porpoises. The cause of the tumorigenesis in examined cases remains undetermined. The involvement of endogenous factors, including mutation of cell cycle regulating genes, such as the tumour-suppressor gene p53, cannot be ruled out. The aetiopathogenetic significance of exogenous factors, such as infectious agents like liver flukes or anthropogenic factors, including persistent organic pollutants, should be the subject of future investigations.

KEY WORDS: Harbour porpoise · *Phocoena phocoena* · Cetacean · Odontoceti · Spontaneous neoplasms · Environmental effects

1. INTRODUCTION

Harbour porpoises occur throughout all oceans of the Northern Hemisphere. They are the only resident cetacean species in German waters inhabiting both the North and Baltic Seas (Benke et al. 1998, Siebert et al. 2006, Viquerat et al. 2014, Gilles et al. 2016). In the North Sea and adjacent waters they are classified as Least Concern, while the subpopulation in the central Baltic Sea is Critically Endangered (Hammond et al. 2008, HELCOM 2013, ASCOBANS 2020a,b).

The coastal habitat of the harbour porpoise is affected by anthropogenic activities including offshore wind farming (Gilles et al. 2009, Brandt et al. 2011), shipping, fishery-associated bycatch (Gislason 1994, Read 2008), habitat loss, noise (Erbe et al. 2019) and chemical pollution (Weijs et al. 2009, van den Heuvel-Greve et al. 2021). Environmental chemicals such as persistent organic pollutants (POPs) have been implicated in the impairment of the health of harbour porpoises (Das et al. 2006, Weijs et al. 2010, Murphy et al. 2015, Desforges et al. 2016, Williams et al. 2020a, van den Heuvel-Greve et al. 2021). In addition to detrimental effects on the immune, endocrine and reproductive systems, POPs may also promote cancer, as has been implicated in St. Lawrence Estu-

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ary belugas *Delphinapterus leucas* (De Guise et al. 1994b, 1995, Norman et al. 2017). Besides dolphins, this beluga population has the highest tumour incidence among wild marine mammals (Martineau et al. 2002, Albuquerque et al. 2018).

Spontaneous neoplasms are relatively rarely reported in cetaceans (Newman & Smith 2006), and only little is known about neoplasia in harbour porpoises. A comprehensive overview of neoplasms in toothed whales (Odontoceti) is given in Tables S1–S6 in the Supplement at www.int-res.com/articles/suppl/d149p145_supp.pdf. The limited number of published cases in harbour porpoises include a cutaneous squamous papilloma, a penile papilloma, an ovarian granulosa cell tumour, a genital leiomyoma, a renal teratoma, a gastric adenocarcinoma, a gastric squamous cell carcinoma, a metastasising B-cell lymphoma as well as a plasmacytoma (Geraci et al. 1987, Van Bressem et al. 1999, Newman & Smith 2006, Siebert et al. 2010, Seibel et al. 2012, Murphy et al. 2015, Norman et al. 2017, Albuquerque et al. 2018, Rickman et al. 2018). The aim of this study was to compile the morphological, histological and immunohistochemical findings of spontaneous neoplasms that were diagnosed in harbour porpoises between 1999 and 2018 based on necropsy cases from the south-eastern North Sea, the western Baltic Sea and Greenlandic waters.

2. MATERIALS AND METHODS

2.1. Animals and post-mortem examination

In a retrospective review of archival material from necropsy cases between 1999 and 2018, spontaneous neoplasms were identified in 7 adult harbour porpoises (Table 1). Six animals originated from the North and Baltic Seas as part of the German and Dutch systematic health monitoring programs (Schleswig Holstein coast, Germany, and South Holland coast, the Netherlands). One individual was killed in West Greenlandic waters by Greenlandic hunters and brought to Maniitsoq in September 2009 (Heide-Jørgensen et al. 2011). The samples were acquired with permission from the Greenlandic Central Government. The porpoises were in different stages of decomposition and stored at −20°C until necropsy or autopsied within 24 h after finding according to the standardised European protocol (Siebert et al. 2001, IJsseldijk et al. 2019). The status of decomposition was classified as 1 (freshly dead) to 5 (macerated) (Siebert et al. 2001, IJsseldijk et al. 2019). Weight and length measurements were taken in addition to 4–6 teeth from the mandible for age determination by annual growth layers (Lockyer 1995). Nutritional status was determined based on the state of longissimus dorsi muscles, presence of visceral fat and blubber thickness (Siebert et al. 2001, IJsseldijk et al. 2019).

2.2. Histology and immunohistochemistry

Respective tissue samples were taken and fixed in 10% neutral buffered formalin. After routine processing, specimens were embedded in paraffin wax, sectioned at 2 µm and stained with haematoxylin-eosin (H&E). Pathomorphologic evaluation of each neoplasia was performed by 2 pathologists, with the mean mitotic rate determined in 10 high-power fields (field of view: 0.045 mm^2). In addition, selected cases were stained with Heidenhain's azan and Gomori's methenamine-silver.

Sections of all neoplasms were subjected to immunohistochemistry. Briefly, after dewaxing and blocking of endogenous peroxidase, antigen re-

Table 1. Harbour porpoises with neoplasms investigated in this study

No.	Year	Retrieval location	Water body	Sex	Age (yr)	Affected organs	Diagnosis
Alimentary system							
1	2018	Amrum, Germany	North Sea	Male	12	Liver, periaortic lymph nodes	Adenocarcinoma
Genital tract							
2	1999	Sylt, Germany	North Sea	Male	25	Testis	Sertoli cell tumor
3	2012	Heringsdorf, Lübecker Bucht, Germany	Baltic Sea	Female	5	Uterus	Leiomyoma
4	2013	Langevelderslag paal 74.250, Netherlands	North Sea	Female	13	Cervix	Fibroleiomyoma
5	2016	Eckernförder Bucht, Germany	Baltic Sea	Female	Adult	Vagina	Fibroleiomyoma
Endocrine system							
6	2009	Maniitsoq, Greenland	North Atlantic	Male	Adult	Adrenal gland	Cortical adenoma
7	2012	Sylt, Germany	North Sea	Female	14–15	Adrenal gland	Cortical adenoma

trieval was performed using simmering citrate buffer (pH 6.0). In the case of anti-cytokeratin (CK) 14, tissue sections received no pretreatment. All sections were blocked with normal goat serum to prevent non-specific protein binding and were subsequently incubated overnight at 4°C with primary antibodies. Antisera used are listed in Table 2. Incubation with the primary antibody was followed by the application of a secondary, biotin-labelled goat-anti-mouse (1:200, #BA-9200, VECTOR®, Biozol Diagnostica Vetrieb) or goat-anti-rabbit (1:200, #BA-1000, VECTOR®, Biozol Diagnostica Vetrieb) antibody for 30 min at room temperature. Visualisation was performed by means of the avidin-biotin per-

Table 2. Antibodies tested to characterize spontaneous neoplasms in harbour porpoises. α-sma: α-smooth muscle actin; CK: cytokeratin; EMA: epithelial membrane antigen; GAM: biotin-labelled goat-anti-mouse antibody; GAR: biotin-labelled goat-anti-rabbit antibody; mAb: monoclonal antibody; NSE: neuron-specific enolase; pAb: polyclonal antibody; p53: tumour-suppressor gene

Antigen	Clone	Target	Clonality/ host species	Dilution antibody	Secondary	Source
Cytokeratins	AE 1/3	CK 1-8, CK 10, CK 14-16, CK 19	Mouse, mAb	1:500	GAM	Agilent Dako, #M3515
	MNF 116	CK 5, CK 6, CK 8, CK 17 and probably CK 19	Mouse, mAb	1:1000	GAM	Agilent Dako, #M0821
	34βE12	CK 1, CK 5, CK 10, CK 14	Mouse, mAb	1:500	GAM	Agilent Dako, #M0630
	D5/16B4	CK 5/6	Mouse, mAb	1:100	GAM	Agilent Dako, #M7237
	OV-TL 12/30	CK 7	Mouse, mAb	1:20	GAM	Agilent Dako, #M7018
	DE-K10	CK 10	Mouse, mAb	1:100	GAM	Agilent Dako, #M7002
	CK 14	CK 14	Rabbit, pAb	1:500	GAR	Fisher Scientific, #12623327
	5D3	CK 8/18	Mouse, mAb	1:200	GAM	Novocastra, #NCL-L-5D3
	C-04	CK 18	Mouse, mAb	1:500	GAM	Abcam, #ab668
	Ks20.8	CK 20	Mouse, mAb	1:100	GAM	Agilent Dako, #M7019
Melan A	A103	Melan A	Mouse, mAb	1:500	GAM	Agilent Dako, #M7196
Synaptophysin	DAK-SYNAP	Synaptophysin	Mouse, mAb	1:500	GAM	Agilent Dako, #M7315
Chromogranin A	DAK-A3	Chromogranin A	Mouse, mAb	1:100	GAM	Agilent Dako, #M0869
NSE	BBS/NC/VI-H14	NSE	Mouse, mAb	1:100	GAM	Agilent Dako, #M0873
Vimentin	V9	Vimentin	Mouse, mAb	1:100	GAM	Agilent Dako, #M0725
α-sma	1A4	α-sma	Mouse, mAb	1:200	GAM	Agilent Dako, #M0851
c-kit	CD117	c-kit	Rabbit, pAb	1:100	GAR	Agilent Dako, #A4502
p53	DO-7	p53	Mouse, mAb	1:50	GAM	Agilent Dako, #M7001
EMA	–	Mucin-1	Rabbit, pAb	1:300	GAR	Biozol, #ARP41445_P050

oxidase complex (#PK 6100, Vectastain elite ABC kit, Vector Laboratories) using 3.3′-diaminobenzidine tetrahydrochloride as chromogen. Finally, sections were counterstained with Mayer's haematoxylin. Normal skin, liver, adrenal gland, testis and uterus of harbour porpoises served as representative tissue controls. For negative controls, primary antibodies were substituted with ascites fluid from non-immunised BALB/c mice (1:1000; #BL CL8100, Cedarlane®, Biologo) or rabbit normal serum (1:3000; #R4505, Sigma-Aldrich Chemie), respectively.

3. RESULTS

3.1. Adenocarcinoma

In 2018, 12 yr old male harbour porpoise stranded alive on the North Sea island Amrum, Germany (Case #1). It had dyspnoea and died shortly after stranding. The animal was moderately underweight. Numerous white to grey-white, 1–3 cm diameter, firm, well-demarcated nodules were dispersed across the liver (Fig. 1a). The nodules were caseous on cut sections (Fig. 1b). A cyst (1 × 3 × 3 cm) containing black mucus was present in the liver at its ventral margin. The abdominal periaortic lymph nodes had white, caseous foci in cortex and medulla on cut surface. Histology revealed an infiltrative, poorly differentiated adenocarcinoma (Fig. 1c). Neoplastic cells were arranged in packets, islands and cords supported by fine strands of fibrovascular stroma to abundant fibrous connective tissue, infiltrating and effacing the liver parenchyma. The cells were medium-sized, cuboidal to polygonal with moderate amount of pale eosinophilic, granular cytoplasm and variably distinct cell borders. They contained a central, round to ovoid nucleus with vesicular, coarse or marginated chromatin and 1–2 variably distinct, approximately 1–3 µm diameter eosinophilic nucleoli. Marked anisocytosis and anisokaryosis and a mitotic count of approximately 9 per high-power field were present (Fig. 1d). The tumour had multiple large areas of coagulative necrosis, multifocal infiltration of surrounding tissue and vascular invasion. In the periphery, the neoplasia sporadically had a scirrhous response, while adjacent liver parenchyma was compressed and had multiple areas of mild to moderate haemorrhage. Other hepatic microscopical findings consisted of a lympho-histiocytic and eosinophilic hepatitis with fibrosis and bile duct proliferation. Metastases with severe coagulative necrosis were visible in the abdominal periaortic lymph nodes and the right adrenal gland. In addition, lymphatics near the affected lymph nodes had intraluminal neoplastic cells. Further lesions independent of the neoplasia are listed in Table S7. Immunohistochemistry revealed expression for MNF116 (Fig. 1e), AE1/3, 34βE12, CK 14 (Fig. 1f) and CK 18 (Fig. 1g) in addition to pronounced p53 immunolabelling (Fig. 1h) in tumour cells. Tumour cells were immuno-negative for CK 5/6, 7, 10, 8/18 and 20, as well as Melan A, synaptophysin, chromogranin A and neuron-specific enolase, with no immunoreactivity detected for CK 7, CK 8/18, and Melan A in representative tissue controls (Table 3). Furthermore, no argentaffin or argyrophilic granules were detectable in tumour cells using Gomori's methenamine-silver staining. The spectrum of morphological findings was consistent with a metastasising adenocarcinoma.

Table 3. Comparative cytokeratin expression in normal harbour porpoise liver parenchyma and adenocarcinoma in the liver of an affected harbour porpoise (Case #1). CK: cytokeratin; + / ++ / +++: low / moderate / high numbers of immunopositive cells; (–) no reaction

Marker/clone	Cytokeratins	Liver tissue control		Case #1		
		Hepatocytes	Bile ducts	Hepatocytes	Bile ducts	Tumour cells
AE 1/3	CK 1-8, CK 10, CK 14-16, CK 19	–	+++	++	+++	+++
MNF 116	CK 5, CK 6, CK 8, CK 17 and probably CK 19	++	+++	++	+++	+++
34βE12	CK 1, CK 5, CK 10, CK 14	–	–	–	–	+/++
D5/16B4	CK 5/6	–	–	–	–	–
OV-TL 12/30	CK 7	–	–	–	–	–
DE-K10	CK 10	–	–	–	–	–
CK 14	CK 14	–	–	–	–	+/++
5D3	CK 8/18	–	–	–	–	–
C-04	CK 18	+++	+++	+++	+++	+
Ks20.8	CK 20	–	–	–	–	–

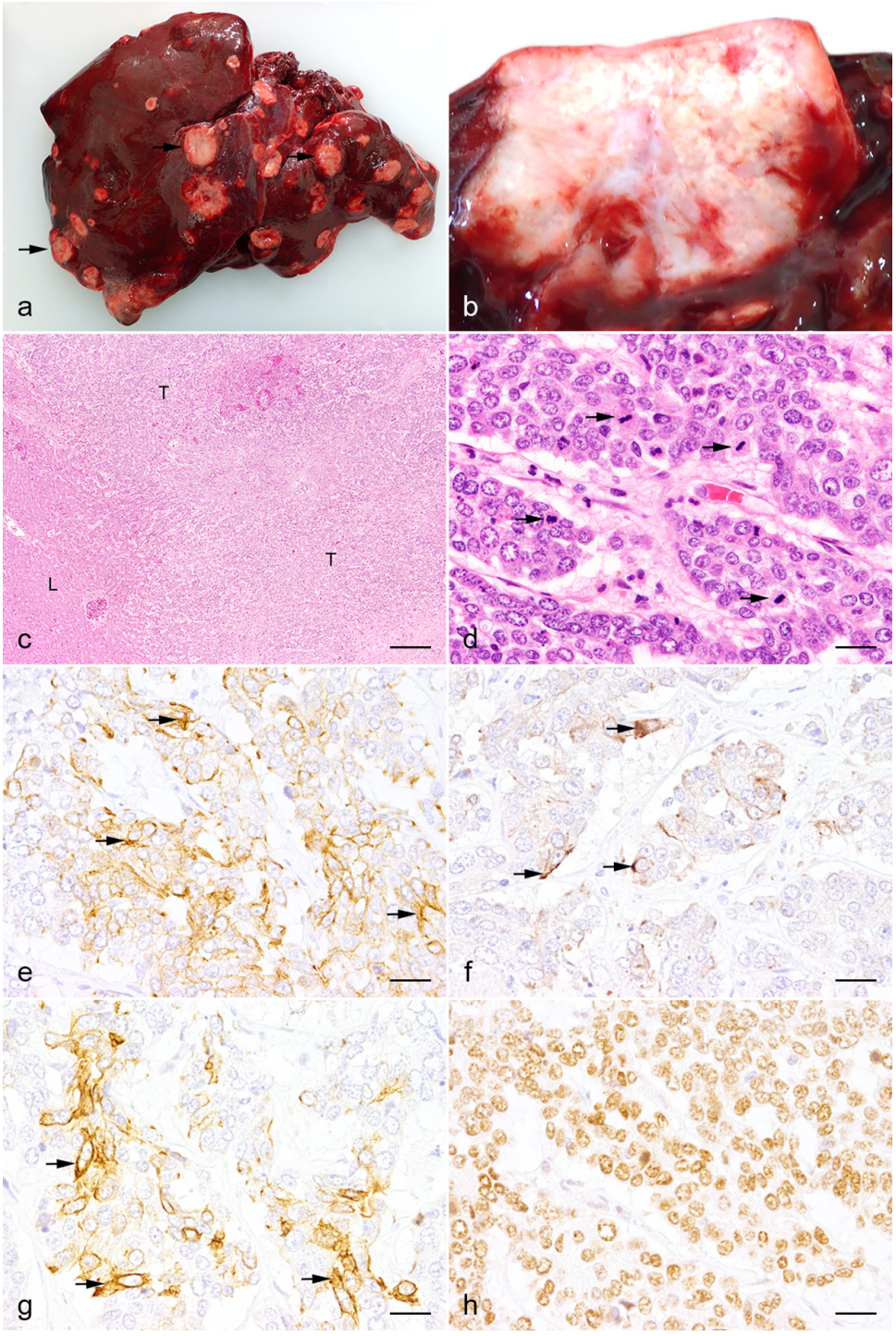

Fig. 1. Gross, histological and immunohistochemical findings in an adult male harbour porpoise *Phocoena phocoena* with an adenocarcinoma of probable bile duct origin. (a) Gross presentation of multiple variably sized, grey-white, raised, well-demarcated, nodular masses with central depression in the liver (arrows). (b) Caseous cut surface of one white, firm, well-demarcated nodule. (c) Microscopically, a densely cellular poorly differentiated neoplasia of epithelial origin (T) infiltrates and effaces the liver parenchyma (L). H&E stain. (d) Neoplastic cells are arranged in packets, islands and cords supported by fine strands of fibrovascular stroma. Mitotic figures are common (arrows). H&E stain. (e) Immunolabelling for cytokeratin with MNF116 in numerous tumour cells (arrowheads). (f) Immunolabelling for cytokeratin with CK 18 in low numbers of tumour cells (arrowheads). (g) Immunolabelling for cytokeratin with CK 14 in low to moderate numbers of tumour cells (arrowheads). (h) Numerous tumour cells express p53 in nuclei. Scale bars = (c) 100 µm, (d–h) 20 µm

3.2. Sertoli cell tumour

In 1999, a 25 yr old male harbour porpoise was found on the island of Sylt (North Sea, Germany; Case #2). The animal was in good post-mortem preservation. It was moderately underweight. Grossly, a firm, well-demarcated, expansile, white-yellow nodule of about 4.0 cm diameter was present in the right testis (right testis: 194 g; left testis: 244 g). Similarly, the right epididymis (right epididymis: 89 g; left epididymis: 44 g) had several approximately 1.0 cm diameter, white-yellow nodules with a greasy consistency on cut surface. The left testis and epididymis did not have any abnormal findings. Histologically, the masses in testis and epididymis were densely cellular. The tumour cells were arranged in nests. Some of the nests were within the confines of the seminiferous tubules. The cells were medium-sized, polygonal to elongate with moderate amount of eosinophilic, homogenous cytoplasm and distinct to indistinct cell borders. They contained a peripherally located, small to medium-sized, round to elongate nucleus with finely stippled chromatin and indistinct nucleoli. There was moderate anisocytosis and -karyosis and a mitotic rate of 0–2 per high-power field. Frequently, tumour cells were palisading along basement membranes. Moreover, the tumour had multiple areas of coagulative necrosis. Additional pathological findings independent of the neoplasia are listed in Table S7. While neoplastic cells stained immunohistochemically for vimentin, no immunolabelling was observed for the antibodies AE1/3 and MNF116, epithelial membrane antigen, Melan A and CD117 (Fig. S1). The findings were consistent with Sertoli cell tumour with metastases to the epididymis.

3.3. Leiomyomas and fibroleiomyomas

Three female adult harbour porpoises with a neoplastic growth in the genital tract were investigated between 2012 and 2016. The first case was a 5 yr old individual stranded at Lübecker Bucht (Baltic Sea, Heringsdorf, Germany) in 2012 (Case #3). Its status of decomposition was classified as 2 and it was in a good nutritional condition. At necropsy, the animal had a 7.5 × 5.2 × 3.4 cm expansile, firm nodule in the uterine wall adjacent to the bifurcation. The mass protruded into the uterine lumen. On cut surface, the mass was pink to white.

A 13 yr old harbour porpoise was found dead at Langevelderslag paal 74.250 (North Sea, The Netherlands) in 2013 (Case #4). The status of decomposition at necropsy was rated as 3. The animal was emaciated. Macroscopically, the left ovary was 1.5 times larger than the right ovary and had multiple corpora scars. In addition, the cervix had multiple round, well-demarcated, firm nodules ranging from 1.0–5.0 cm in diameter. These nodules were green to beige on cut section.

The third adult harbour porpoise was found in 2016 in the Eckernförder Bucht (Baltic Sea, Germany; Case #5). The animal was in good post-mortem preservation. It was emaciated. At necropsy, abdominal organs and diaphragm were displaced cranially by an expansile, multinodular neoplastic mass of 23.5 × 16 × 16 cm originating in the dorsal vaginal wall between the vaginal vestibule and the closed cervix. The mass was protruding into the vaginal lumen and consisted of 6 firm, well-demarcated nodules ranging in diameter from 0.8–8.6 cm, with a white to ivory, coarse cut surface and lobular texture.

Histologically, the uterine, cervical and vaginal masses were non-encapsulated, moderately to well demarcated and moderately cellular. The tumours were composed of interlacing bundles of well-differentiated smooth muscle cells and had a variable amount of streams of collagenous stroma. The presence of smooth muscle fibres and collagenous tissue was confirmed using Heidenhain's azan stain. In Cases #4 and #5, the blue-stained fibrous portion was predominant. Neoplastic cells were fusiform with eosinophilic, fibrillary cytoplasm and indistinct cell borders. Hyperchromatic nuclei were elongate with blunt ends and indistinct nucleoli. Fewer than one mitotic figure per high-power field was present. Additional pathological findings independent of the neoplasms in all 3 cases (Cases #3–#5) are listed in Table S7. All leiomyomas expressed α-smooth muscle actin (α-sma) as well as vimentin. In Case #3, more than 50% of neoplastic cells consisted of α-sma-positive cells, indicating a leiomyoma. In Cases #4 and #5, the ratio of α-sma-positive and vimentin-positive tumour cells was reversed, as fewer than 50% of α-sma-positive cells were present. This is consistent with the results of azan staining indicating fibroleiomyoma (Fig. S2).

3.4. Adrenal adenoma

An adult male harbour porpoise originating from the North Atlantic (Maniitsoq, Greenland) was found dead and subjected to macroscopic examination in 2009 (Case #6). Its status of decomposition was classified as 4. It was in a good nutritional state. Grossly, an approximately 1 cm in diameter, firm, moderately

well-demarcated and expansile mass of homogenous brown colour was observed in the cortex of the right adrenal gland (10 g). The left adrenal gland (9 g) was without abnormal findings.

A 14 to 15 yr old female harbour porpoise was found on a beach on the island of Sylt (North Sea, Germany) in 2012 (Case #7). The nutritional status could not be determined due to its advanced state of decomposition of 5. Grossly, a 3 × 3 × 4 cm, firm, well-demarcated mass of a homogeneous red to brown colour was present in the cortex of the left adrenal gland (28.9 g). The right adrenal gland (13.03 g) was without any morphological changes.

Histologically, the densely cellular masses were composed of broad trabeculae and nests (Case #6) or sheets (Case #7) of medium-sized neoplastic cells, supported by either extensive (Case #6) or small (Case #7) amounts of fibrous stroma. The resulting loss of the normal tissue architecture and the compression of the adrenal medulla are consistent with a cortical adenoma. The cells had a moderate amount of eosinophilic, homogenous cytoplasm and distinct cell borders. They contained a small, round nucleus that was variably located in the centre or periphery of the cells. The nuclei were hyperchromatic or had stippled chromatin. The tumour was encapsulated in Case #6, while no capsule was identified in Case #7. Mitotic figures were not detected in either of the 2 masses. Mineral deposits were present within the tumour of Case #7. Further lesions independent of the neoplasms (Cases #6 and #7) are listed in Table S7. In both cases, immunolabelling of neoplastic cells was negative for Melan A, AE1/3, synaptophysin and chromogranin A, with no immunoreactivity detected for Melan A in representative tissue controls. Nonetheless, immunohistochemical labelling of chromogranin A in cells of the adrenal medulla visualised the strong compression by the mass (Fig. S3). These findings were consistent with adrenocortical adenoma.

4. DISCUSSION

The retrospective case study revealed 4 types of spontaneous neoplasms affecting various organ systems in harbour porpoises investigated between 1999 and 2018. This is the first report of an adenocarcinoma in the liver, a testicular Sertoli cell tumour and adrenocortical adenomas in this species. Additionally, one leiomyoma and 2 fibroleiomyomas were presented within the genital tract, located in vagina, cervix and uterus.

Hepatic neoplasms have rarely been observed in cetaceans. The few known case reports include adenocarcinoma, adenoma and reticuloendotheliosis in bottlenose dolphins *Tursiops* sp. (Geraci et al. 1987, Newman & Smith 2006) as well as hepatocellular carcinoma and cholangiocarcinoma in St. Lawrence Estuary beluga whales (De Guise et al. 1994b, Lair et al. 2014). Based on the morphological and immunohistological features, the present case is consistent with metastasising adenocarcinoma. Considering the macroscopic and microscopic features and the fact that no extrahepatic neoplastic growth was detected except for the metastases in the abdominal periaortic lymph nodes and the right adrenal gland, a metastasising adenocarcinoma originating from the bile duct epithelium should be considered. However, no definite final statement can be made on the histogenesis of the tumour.

Interestingly, previous studies discussed liver flukes to be involved in hepatic carcinogenesis in humans (Chaiyadet et al. 2015, Zheng et al. 2017), cats (Andrade et al. 2012) and cattle (Anderson & Sandison 1968). Although no liver flukes were detected at necropsy, the harbour porpoise with the metastasising adenocarcinoma had circumstantial evidence of *Campula oblonga* infection. This interpretation was based on the presence of the large mucus-containing liver cyst, the lympho-histiocytic and eosinophilic hepatitis with fibrosis and bile duct proliferation, but also the hyperplasia of the pancreatic duct with luminal black debris (Siebert et al. 2001, Lehnert et al. 2005). Chronic inflammation and irritation of the bile ducts caused by fluke infections induce epithelial hyperplasia. Furthermore, immune cells produce nitric oxide during the inflammatory process, which is not only cytotoxic, but also genotoxic and therefore can promote neoplastic transformations (Khurana et al. 2005). Thus, liver flukes may be involved in the development of the carcinoma in our present case, although genetics and pollution have to be considered additionally and alternatively.

The adenocarcinoma also presented a prominent expression of the transcription factor p53. Mutation of this protein is implicated in the development of cancer in humans and dogs (Goh et al. 2011, Alsaihati et al. 2021). As in the present case, pronounced p53 expression was also observed in a harbour porpoise with gastric squamous cell carcinoma (Siebert et al. 2010), which could indicate a potential involvement of p53 in tumourigenesis in harbour porpoises. Finally, yet importantly, environmental pollutants should also be considered in carcinogenesis, as postulated for St. Lawrence Lake whitefish *Coregonus*

clupeaformis (Mikaelian et al. 2002). However, further studies on liver concentrations of carcinogenic contaminants are warranted to elucidate their contribution to tumourigenesis in harbour porpoises.

Few reports exist regarding testicular neoplasms in cetaceans (Geraci et al. 1987, Van Bressem et al. 2000, Estep et al. 2005, Díaz-Delgado et al. 2012, Díaz-Santana et al. 2020, Page-Karjian et al. 2020), with a Sertoli cell tumour reported in a spotted dolphin *Stenella frontalis* (Estep et al. 2005) and a short-beaked common dolphin *Delphinus delphis* (Díaz-Delgado et al. 2012). Metastases are rare and indicate a malignant transformation (Littleton et al. 1981), as previously described in a common dolphin (Díaz-Delgado et al. 2012) and observed in this case. Although Sertoli cell tumours can be functional, there was no evidence of hormonal activity in the form of feminisation manifested by gynaecomastia, contralateral testicular or bone marrow atrophy (Post & Kilborn, 1987). Measuring the concentration of free hormones in the blood would be of great interest in determining functional activity of the Sertoli cell tumour, but is not feasible using post-mortem specimens.

Leiomyomas have been observed repeatedly among various cetaceans in recent decades (Geraci et al. 1987, Van Bressem et al. 2000, Mikaelian et al. 2000, Newman & Smith 2006, Díaz-Delgado et al. 2015, Page-Karjian et al. 2020) and have also been reported for harbour porpoises (Murphy et al. 2015). They are most frequently observed in adult individuals in the myometrium, as in Case #3. However, the presence of a leiomyoma in a 5 yr old animal is rather peculiar, especially considering that harbour porpoises in German waters do not reach sexual maturity until 4.95 yr of age (Kesselring et al. 2017). The most discussed causes are imbalances of endogenous hormones (oestrogen, progesterone) and growth factors as well as genetic predispositions, while age, nulliparity or increased menstrual cycles are considered contributing risk factors (Flake et al. 2003). Another possible cause for tumourigenesis could be the previously indicated association with POPs and reproductive failure as reported in Baltic harbour porpoises, Baltic grey seals *Halichoerus grypus* and polar bears *Ursus maritimus* (Bäcklin et al. 2003, Murphy et al. 2015, Dietz et al. 2018).

Adrenal gland tumours are rare entities and have only been documented sporadically in cetaceans (Geraci et al. 1987, De Guise et al. 1994b, Estep et al. 2005, Newman & Smith 2006, Lair et al. 2014). Besides the present cases, cortical adenomas have only been described in white-sided dolphins *Lagenorhynchus acutus* (Geraci et al. 1987) and implicated in beluga whales (De Guise et al. 1995) as well as in a bottlenose dolphin (Geraci et al. 1987). In addition, adenocarcinoma, adrenocortical nodules, and cortical cysts have been described in St. Lawrence Estuary beluga whales (De Guise et al. 1995, Lair et al. 2014). Adrenal adenomas may be functional in animals (Beuschlein et al. 2012), although there was no evidence of hormonal activity in the cases investigated. The extent to which genetic and epigenetic alterations are involved in tumour development, as has been implicated for humans and domestic animals (Bielinska et al. 2009), or whether chemical pollutants play a role in proliferative adrenal lesions in harbour porpoises, as has been indicated for Baltic grey seals (Brandt et al. 1992) and St. Lawrence Estuary belugas (De Guise et al. 1995), remains to be elucidated.

In conclusion, harbour porpoises do not show a high tumour rate compared to other Odontoceti, such as St. Lawrence Estuary beluga whales or dolphins. Although harbour porpoises can reach an age of 24–26 yr, the majority of animals in the German North and Baltic Seas die before or shortly after reaching sexual maturity (Kesselring et al. 2017), most likely as a result of direct or indirect pressure from human activities. Nonetheless, in view of reported neoplasms in harbour porpoises, including this report, the following becomes clear: (1) tumours were only described in adult individuals and (2) there seems to be an increased incidence in the genital tract (Table S1). However, the aetiology of tumourigenesis in the present cases remains unknown. Whether endogenous factors, including mutation of the tumour-suppressor gene p53, or exogenous factors, such as infectious agents or environmental pollutants, have an aetiopathogenic significance cannot be conclusively clarified in the herein investigated cases and therefore warrant further studies.

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