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Budd-Chiari-like pathology in dolphins

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brief communications

Gas-bubble lesions in stranded cetaceans

Was sonar responsible for a spate of whale deaths after an Atlantic military exercise?

There are spatial and temporal links between some mass strandings of cetaceans — predominantly beaked whales — and the deployment of military sonar[1,2]. Here we present evidence of acute and chronic tissue damage in stranded cetaceans that results from the formation *in vivo* of gas bubbles, challenging the view that these mammals do not suffer decompression sickness. The incidence of such cases during a naval sonar exercise indicates that acoustic factors could be important in the aetiology of bubble-related disease and may call for further environmental regulation of such activity.

Fourteen beaked whales were stranded in the Canary Islands close to the site of an international naval exercise (Neo Tapon, 2002) held on 24 September 2002. Strandings began about 4 hours after the onset of mid-frequency sonar activity. We necropsied eight Cuvier's beaked whales (*Ziphius cavirostris*), a Blainville's beaked whale (*Mesoplodon densirostris*) and a Gervais' beaked whale (*Mesoplodon europaeus*), six of which were very fresh. These animals showed severe, diffuse vascular congestion and marked, disseminated microvascular haemorrhages associated with widespread fat emboli within vital organs. Intravascular bubbles were present in several organs, although definitive evidence of gas embolism *in vivo* is difficult to determine after death[3]. No pathogenic bacteria were isolated from the carcasses.

These lesions are consistent with acute trauma due to *in vivo* bubble formation resulting from rapid decompression (as occurs in decompression sickness)[4,5]. Bubble formation in response to sonar exposure might result from behavioural changes to normal dive profiles (such as accelerated ascent rate), causing excessive nitrogen supersaturation in the tissues (as occurs in decompression sickness); alternatively, bubble formation might result from a physical effect of sonar on *in vivo* bubble precursors (gas nuclei) in nitrogen-supersaturated tissues[6,7].

The beaked whales found in the Canary Islands are not the only stranded cetaceans to provide evidence of bubble-associated tissue injury. In strandings that occurred in Britain between October 1992 and January 2003, three out of 24 Risso's dolphins (*Grampus griseus*), three out of 342 common dolphins (*Delphinus delphis*) and one out of 1,035 harbour porpoises (*Phocoena phocoena*) necropsied, as well as the only Blainville's beaked whale studied, contained gas bubbles in their blood vessels and gas-filled cavities in their parenchymous organs.

The livers of these animals were the most consistently affected organ, with macroscopic gas-filled cavities (diameter, 0.2–6.0 cm) occupying 5–90% of the volume (Fig. 1) and having variable degrees of fibrotic encapsulation. Intrahepatic spherical non-staining cavities (gas bubbles) of diameter 50–750 μm were associated with compression of hepatic tissue, distension of portal blood vessels, and sometimes with haemorrhage, acute hepatocellular necrosis or fibrosis, indicating that this damage was inflicted before death. One of the *D. delphis* specimens also had bilateral acute renal infarcts associated with gas bubbles.

The cavitary lesions described here are new to marine-mammal pathology. Their presence in fresh carcasses in the absence of bacterial isolates, and the apparent progression through the stages of pericavitary fibrosis, are inconsistent with decompositional bubbles from bacterial activity. The coexistence of ante mortem gas bubbles and gas-filled fibrosed cavities suggests that *in vivo* bubble formation and expansion is the proximate aetiology of this disease process. Bubble formation may either have initiated in the liver and kidneys ('autochthonous' bubble formation) or in other tissues (fatty tissue, for example) before haematogenous transfer to the liver and kidneys as gas emboli.

Nitrogen bubbles and emboli can develop in decompression sickness in humans and experimental animals as a result of expansion of pre-existing gas nuclei within nitrogen-supersaturated tissues[8]. Anatomical, physiological and behavioural adaptations may mitigate against *in vivo* formation of nitrogen bubbles in marine mammals[9–11], although there is empirical evidence of nitrogen supersaturation in cetaceans[7]. Some deep-diving species are predicted to undergo up to 300% nitrogen tissue supersaturation[7]. Static diffusion in nitrogen-supersaturated tissues is therefore a plausible mechanism for bubble development and is consistent with a greater prevalence of cases in deep-diving species such as Risso's dolphins and beaked whales.

Further investigation is needed into the physical and behavioural effects on cetaceans exposed to sonar, and the relation of these effects to bubble growth *in vivo* and to strandings. Necropsies should aim to compare fat and gas emboli in stranded cetaceans suspected of having been exposed to sonar with results from unexposed stranded controls. In a wider conservation sense, our findings need to be taken into account in considering the regulation and limitation of the adverse impact of anthropogenic sonar on cetaceans.

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Figure 1 Gas-filled cavities in the liver of a stranded common dolphin (*Delphinus delphis*). **a**, Cut surface of the liver, showing that cavitary lesions have extensively replaced the normal tissue. Scale bar, 10 mm. **b**, Photomicrograph of liver section, showing multiple cavities (gas bubbles) within the portal tracts and hepatic parenchyma. Scale bar, 750 μm.

Two decades ago, acute decompression-like sickness related to acoustic factors such as mid-frequency active naval military sonar (MFAS) was described in different cetaceans (Jepson et al., 2003; Fernández et al, 2004; Fernández et al., 2005). Different pathological changes were proposed to be correlated with the decompressive event:

Systemic acute gas embolism lesions
(intravascular gas bubbles, disseminated microvascular haemorrhages, fat embolism)
**First observed in beaked whales*

Gas filled cavities in parenchymous organs
(cystic liver lesions)
**Observed in other cetacean species, mainly dolphins*

directly related to mid frequency military sonar ?

- YES → In vivo gas bubble formation due to acute rapid decompression (consistent with human decompression sickness) (Fernández et al., 2005).
- ? → but lack of comparable CLL in human decompression sickness. (Piantadosi et al., 2004)

OBJECTIVE

Twenty years later, after 1200 cetacean necropsies, cetacean CLL and their etiopathogenesis were reassessed.

RESULTS

After the pathologic investigations (n=1200), only 4 male striped dolphins (Stenella coeruleoalba) showed these cystic liver lesions (CLL), with a low prevalence (2%) (n= 172)

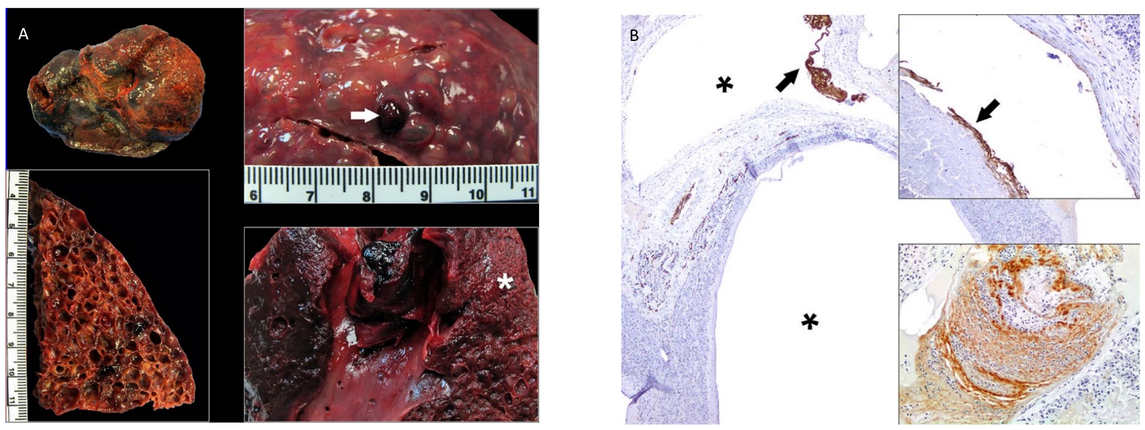

Figure 1. (A) Macroscopic features of CCL in dolphins. (B) Immunohistochemistry features of CCL in dolphins. Factor VIII is expressed in endothelial cells and some fibrin (arrow and right upper inset) clusters lining hepatic venous cystic dilatations (*). Right lower inset: Partially organized fibrinous thrombus exhibits Factor VIII labelling

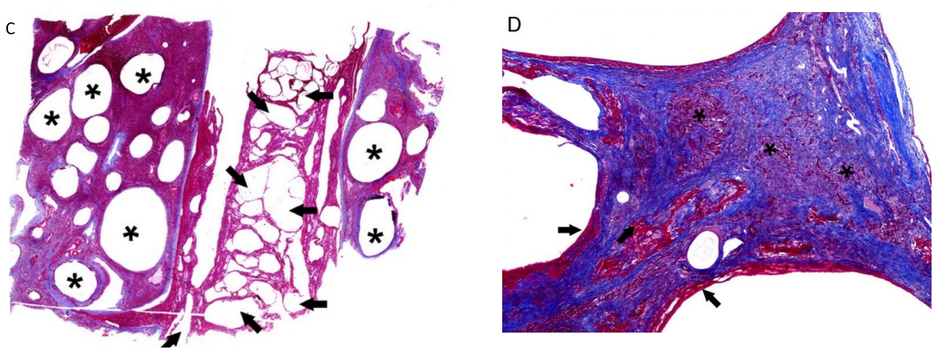

Figure 2. Histochemical features of cystic liver lesions in dolphins. (C)Moderate calibre hepatic vein with an obliterative thrombus. The latter contains numerous cystic spaces (arrows). Adjacent hepatic veins are cystically dilated (*). (D) Chronic cystic liver lesion characterized by fibrous connective tissue (blue), minimal marginal fibrin (arrows), and atrophic hepatic cords (*).

Case	Gas composition
1	Intestinal gas: 60.7% CO_2; 39.3% H_2
2	Not performed.
3	Cystic liver lesions: **83.7 ± 2.1% N_2**; 2.7 ± 0.5% O_2; 13.5 ± 2.4% CO_2
4	Cystic liver lesions: **85.2 ± 2.2% N_2**; 4.8 ± 2.3% O_2; 10.1 ± 0.3% CO_2

Table 1. Gas composition analysis results

DISCUSSION

1. These stranded dolphins were not associated temporally nor spatially with any primary anthropic acoustic activities.
2. CLL were primarily confined to the right liver lobe with a dorso-caudal to cranio-ventral progression and they were associated with phlebothrombosis, intravascular gas bubbles and moderate-severe trematodal cholangiohepatitis.
3. The macroscopic features of CLL were similar among all individuals but the extent of the parenchyma involved varied. The histologic features of CLL were similar in all cases with slight variations, primarily relating to their chronicity, and along with their distribution, revealed a ordered progression from hepatic veins into the adjacent hepatic parenchyma.
4. There was a clear progression from cystic spaces partially lined or circumferentially lined by fibrin ("acute") and erythrocytes towards cystic spaces bound by variably dense bands of connective tissue ("chronic").
5. Cysts are not of epithelial origin as cysts described in Polycystic Kidney Disease in humans.

Thrombosis in hepatic veins (Budd-Chiari-like Pathology) + Decompressive Nitrogen Bubbles
Dolphin Polycistic Liver Pathology

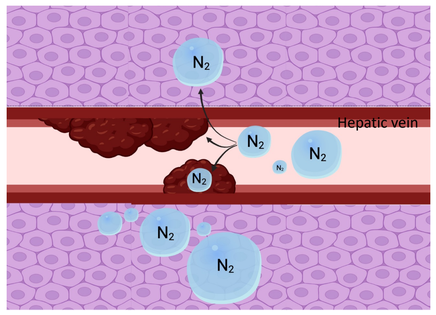

Figure 3. Thrombi within hepatic veins and nitrogen gas bubbles.

Further research is needed to determine precise etiopathogenesis(es) and contributing factors for CLL in cetaceans.

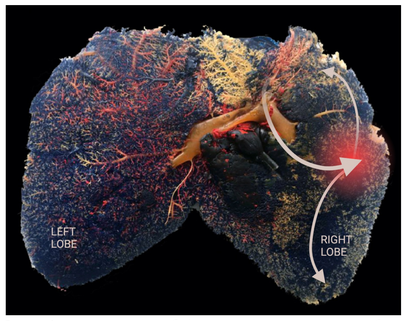

Figure 4.Corrosion cast model of the complex vasculature of a striped dolphin's liver. Arrows indicate the CLL distribution, from the right liver lobe with a dorso-caudal to cranio-ventral progression

Our data strongly suggests that CLL are the result of the combination of a pre-existing hepatic vascular disorder superimposed and exacerbated by ex novo, in situ or circulating decompressive related gas resulting in hepatic cysts, and clearly differ from acute systemic gas embolism in beaked whales linked to MFAS.

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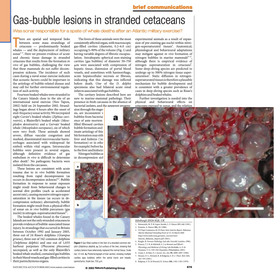
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Dolphin's CLL directly related to mid frequency military sonar ? → NO

BUT

Can Dolphin CLL be associated with a decompressive pathology in cetaceans? → YES

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